Molecular Biology of the Gene Draft: Final Copy: Chapter 4

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CHAPTER IV: THE IMPORTANCE OF WEAK CHEMICAL BONDS

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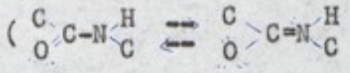
Figure IV-2 The planar shape of the peptide bond. Here is shown a portion of an extended polypeptide chain. Virtually no rotation is possible about the peptide bond because of its partial double bond character (). All the atoms in the grey must lie in the same plane. Rotation is possible, however, around the remaining two bonds which make up the polypeptide backbone. Hence a polypeptide chain can assume many configurations.

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THE IMPORTANCE OF WEAK CHEMICAL BONDS

Until now we have focused our attention on the existence of discrete organic molecules and, following classical organic chemistry, have emphasized the covalent bonds which hold them together. It takes little insight, however, to realize that this type of analysis is inadequate for describing a cell, and that we must also concern ourselves with the exact shape of molecules and with the several factors which bind them together in an organized fashion. The distribution of molecules in cells is not random, and we must ask ourselves which chemical laws determine this distribution. Clearly covalent bonding is not involved; by definition, atoms united by covalent bonds belong to the same molecule.

The arrangement of distinct molecules in cells is controlled instead by chemical bonds much weaker than covalent bonds. Atoms united by covalent bonds are capable of weak interactions with nearby atoms. These interactions, sometimes called "secondary bonds," occur not only between atoms in different molecules, but also between atoms in the same molecule. Weak bonds are important not only in deciding which molecules lie next to each other, but also in giving shape to flexible molecules like the polypeptides and polynucleotides. It is, therefore, very useful to have a feeling for the nature of weak chemical interactions and to understand how their "weak" character makes them indispensable to cellular existence. The most important include van der Waals bonds, hydrogen bonds and ionic bonds.

A definition of and some characteristics of a chemical bond

A chemical bond is an attractive force which holds atoms together. Aggregates of finite size are called molecules. Originally it was thought that only covalent bonds held atoms together in molecules, but now, as we shall show later in this chapter, weaker attractive forces are known to be important in holding together many important macromolecules. For example, the four polypeptide chains of hemoglobin are held together by the combined action of several weak bonds. It is thus now customary to also call weak positive interactions chemical bonds, even though they are not strong enough to effectively bind two atoms together when present singly.

Chemical bonds are characterized in several ways. A most obvious characteristic of a bond is its strength. Strong bonds almost never fall apart at physiological temperatures. This is why atoms united by covalent bonds always belong to the same molecule. Weak bonds are easily broken, and when they exist singly, they exist fleetingly. Only when present in ordered groups do weak bonds exist for a long time. The strength of a bond is correlated with its length, so that two atoms connected by a strong bond are always closer together than the same two atoms held together by a weak bond. For example, two hydrogen atoms bound covalently to form a hydrogen molecule (H:H) are 0.6 Å apart, while the same two atoms, when held together by the van der Waals forces instead, are held 1.2 Å apart.

Another important bond characteristic is the maximum number of bonds which a given atom can make. The number of covalent bonds an atom forms is called its valence. Oxygen, for example, has a valence of two: it can never form more than two covalent bonds. There is more variability in the case of van der Waals bonds, where the limiting factor is purely steric: the number of possible bonds is limited only by the number of atoms which can simultaneously touch each other. The formation of hydrogen

bonds is subject to more restrictions. A covalently bonded hydrogen atom usually participates in only one hydrogen bond, while an oxygen atom seldom participates in more than two hydrogen bonds.

The angle between two bonds originating from a single atom is called the bond angle. The angle between two specific covalent bonds is always approximately the same. For example, when a carbon atom has four single bonds, they are directed tetrahedrally (bond angle = 109°). In contrast, the angles between weak bonds are much more variable.

Bonds differ also in the freedom of rotation they allow. Single covalent bonds permit free rotation of bound atoms (Figure IV-1), while double and triple bonds are quite rigid. For example, the carbonyl (C=O) and imino (N-H) groups bound together by the rigid peptide bond must lie in the same plane (Figure IV-2) because of the partial double bond character of the peptide bond. Much weaker, ionic bonds show completely opposite behavior: they impose no restrictions on the relative orientations of bonded atoms.

Chemical bonds are explainable in terms of quantum mechanics

The nature of the forces, strong as well as weak, which give rise to chemical bonds remained a mystery to chemists until the Quantum Theory of the atom (quantum mechanics) was developed in the 1920's. Then, for the first time, the various empirical laws about how chemical bonds are formed were put on a firm theoretical basis. All chemical bonds, weak as well as strong, were realized to be based on electrostatic forces. Quantum mechanical explanations were provided not only for covalent bonding by the sharing of electrons but, in addition, for the formation of weaker bonds.

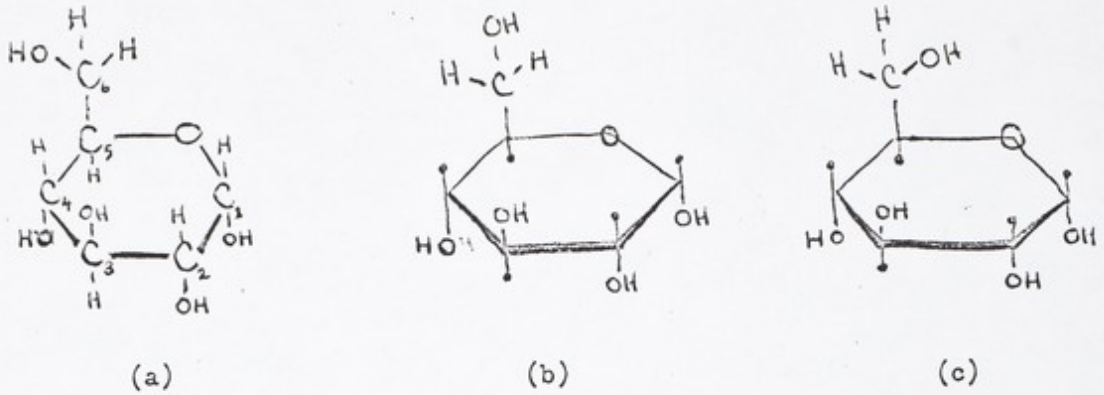


Figure IV -4. Rotation about the C_5-C_6 bond in glucose. This carbon-carbon bond is a single bond and so any of the 3 configurations shown in (a), (b), and (c) may occur.

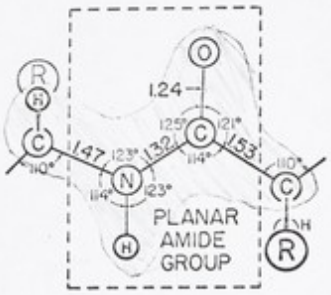


Figure IV-2 The planar shape of the peptide bond. Here is shown a portion of an extended polypeptide chain. Virtually no rotation is possible about the peptide bond because of its partial double bond character.

$$\begin{matrix} C \\ O \end{matrix} \quad C\text{-}N \begin{matrix} H \\ C \end{matrix} \quad = \quad \begin{matrix} C \\ O \end{matrix} \quad C{=}N \begin{matrix} H \\ C \end{matrix}$$

All the atoms in the grey must lie in the same plane. Rotation is possible, however, around the remaining two bonds which make up the polypeptide backbone. Hence a polypeptide chain can assume many configurations.

The formation of a chemical bond involves a change in the form of energy

The spontaneous formation of a bond between two atoms always involves the release of some of the internal energy of the unbonded atoms and its conversion to another energy form. The stronger the bond, the greater the amount of energy which is released upon its formation. The bonding reaction between two atoms A and B is thus described by:

(eq IV-1) $A + B \rightarrow AB + \text{energy}$

where AB represents the bonded aggregate. The rate of the reaction is proportional the the frequency of collision between A and B. The unit most commonly used to measure energy is the calorie, the amount of energy required to raise the temperature of one gram of water from 14.5° C to 15.5° C. Since thousands of calories are usually involved in the breaking of a mole of chemical bonds, most chemical energy changes in chemical reactions are expressed in kcal/mole.

Atoms joined by chemical bonds, however, do not forever remain together. There also exist forces which break chemical bonds. By far the most important of these forces arises from heat energy. Collisions with fast moving molecules (atoms) can break chemical bonds. During a collision, some of the kinetic energy of a moving molecule is given up as it pushes apart two bonded atoms. The faster a molecule is moving (the higher the temperature), the greater is the probability that, upon collision, it will break a bond. Hence, as the temperature of a collection of molecules is increased, the stability of their bonds decreases. The breaking of a bond is thus always indicated by the formula:

(eq IV-2) $AB + \text{energy} \rightarrow A + B$

The amount of energy which must be added to break a bond is exactly equal to the amount which has been released upon its formation. This equivalence follows from the First Law of Thermodynamics, which states that energy (except insofar as it is interconvertible with mass) can be neither made nor lost.

Equilibrium exists between bond making and breaking

Every bond thus is a result of the combined actions of bond making (arising from electrostatic interactions) and bond breaking forces. When an equilibrium is reached in a closed system, the number of bonds forming per unit time will equal the number breaking. Then the proportion of bonded atoms is described by the following Mass Action formula:

$$(\text{eq IV-3}) \quad K_{eq} = \frac{\text{conc}^{AB}}{\text{conc}^{A} \times \text{conc}^{B}}$$

(where K_{eq} is the equilibrium constant, and conc^{A}, conc^{B}, and conc^{AB} are the concentrations of A, B, and AB in moles/liter). Whether we start with only free A and B, with only the molecule AB, or with a combination of AB and free A and B, at equilibrium the proportions of A, B, and AB will reach the concentration given by K_{eq}.

The concept of free energy

There always exists a change in the form of energy as the proportion of bonded atoms moves toward the equilibrium concentration. The biologically most useful way to express this energy change utilizes the physical chemists' concept of free energy (G).* Here we will not give a rigorous description of free energy and show how it differs from the other ways of measuring energy. For this, the reader must refer to a chemistry text which discusses the Second Law of Thermodynamics. We must suffice by saying that free energy is energy which has the ability to do work.

The Second Law of Thermodynamics tells us that there always occurs a decrease of free energy (ΔG is negative) in spontaneous reactions. When equilibrium is reached there is no further change in the amount of free energy ($\Delta G=0$). The equil-

*It was the custom in the United States until recently to refer to free energy by the symbol F. Now, however, most new texts have adopted the international symbol G which honors the great nineteenth century physicist Gibbs.

ibrium state for a closed collection of atoms is thus that state which contains the least amount of free energy.

The free energy lost as equilibrium is approached is transformed either into heat or used to increase the amount of entropy. Here, we shall not attempt to define entropy (again this task must be left to a chemical text), except to say that the amount of entropy is a measure of the amount of disorder. The greater the disorder, the greater the amount of entropy. The existence of entropy means that many spontaneous chemical reactions do not proceed with an evolution of heat. For example, in the dissolving of Na Cl in water, heat is adsorbed. There is, nonetheless, a net decrease in free energy because of the increase of disorder of the Na^+ and Cl^- ions as they move from a solid to a liquid phase.

K_{eq} is exponentially related to ΔG

It is obvious that the stronger the bond, and hence the greater the change in free energy (ΔG) which accompanies its formation, the greater the proportion of atoms which must exist in the bonded form. This common sense idea is quantitatively expressed by the physical chemical formula:

$$\text{(eq IV-4)} \quad \Delta G = -RT \ln K_{eq} \quad \text{or} \quad K_{eq} = e^{-\frac{\Delta G}{RT}}$$

(where R is the universal gas constant, T the absolute temperature, e = 2.718, ln the logarithm of K to the base e, and K_{eq} the equilibrium constant when all reactants are present at molar concentrations).

Insertion of the appropriate values of R (= 1.987 cal/degree/mole) and T (= 298 at 25°C) tells us (Table IV-1) that ΔG values as low as 2 kcal/mole can drive a bond-forming reaction to virtual completion if all reactants are present at molar concentrations.

Covalent bonds are very strong

The ΔG values accompanying the formation of covalent bonds from free atoms like hydrogen or oxygen are very large and negative in sign, usually -50 to -110 kcal/mole. Application of eq IV-4 tells us that K_{eq} of the bonding reaction will be correspondingly large, and so the concentration of hydrogen or oxygen atoms existing unbound will be very, very small. For example, a ΔG value of 100 kcal/mole tells us that, if we start with one mole/liter of the reacting atoms, only one in 10^{40} atoms will remain unbound when equilibrium is reached.

Weak bonds have energies between 1 and 7 kcal/mole

The main types of weak bonds important in biological systems are the van der Waals bonds, hydrogen bonds, and ionic bonds. Sometimes, as we shall soon point out, the distinction between a hydrogen bond and an ionic bond is arbitrary. The weakest of the bonds are the van der Waals bonds. These have energies (1-2 kcal/mole) which are only slightly greater than the kinetic energy of heat motion. The energies of the hydrogen and ionic bonds range between 3 and 7 kcal/mole.

Table IV-1

The numerical relationship between the equilibrium constant and ΔG at 25°C

K_{eq}	ΔG kcal/mole
0.001	4.089
0.01	2.726
0.1	1.363
1.0	0
10.0	-1.363
100.0	-2.726
1000.0	-4.089

In liquid solutions, almost all molecules are forming a number of weak bonds to nearby atoms. All molecules are able to form van der Waals bonds while hydrogen and ionic bonds can also form between molecules (ions) which have a net charge or in which the charge is unequally distributed. Some molecules thus have the capacity to form several types of weak bonds. Energetic considerations, however, tell us that molecules will always have a greater tendency to form the stronger bond.

Enzymes are not involved in the making (breaking) of weak bonds

The energy of the strongest weak bond is only about 10 times larger than the average energy of kinetic motion (heat) at 25°C (0.6 kcal/mole). Since this is a significant spread in the energies of kinetic motion, there always exist many molecules with sufficient kinetic energy to break the strongest weak bond. Weak bonds therefore are constantly being made and broken at physiological temperatures. The average lifetime of a single weak bond is only a very small fraction of a second. Cells thus do not need a special mechanism to speed up the rate at which they are made and broken. Correspondingly, enzymes never participate in reactions of weak bonds.

The distinction between polar and non-polar molecules

All forms of weak interactions are based upon attractions between electric charges. These electric charges can be permanent or very temporary, depending upon the atoms involved. For example, the oxygen molecule (O:O) has a symmetrical distribution of electrons between its two oxygen atoms, and so each of its two atoms is uncharged. In contrast, there is a non-uniform distribution of charge in water (H:O:H) where the bond electrons are unevenly shared (Figure IV-3). They are held more strongly by the oxygen atom, which thus carries a considerable negative charge, while the two hydrogen atoms together have an equal amount of positive charge. The center of the positive charge is on one side of the center of the negative charge. Such a combination of separated positive and negative charges is called an electric dipole moment. Molecules like H_2O, which contain a dipole moment,

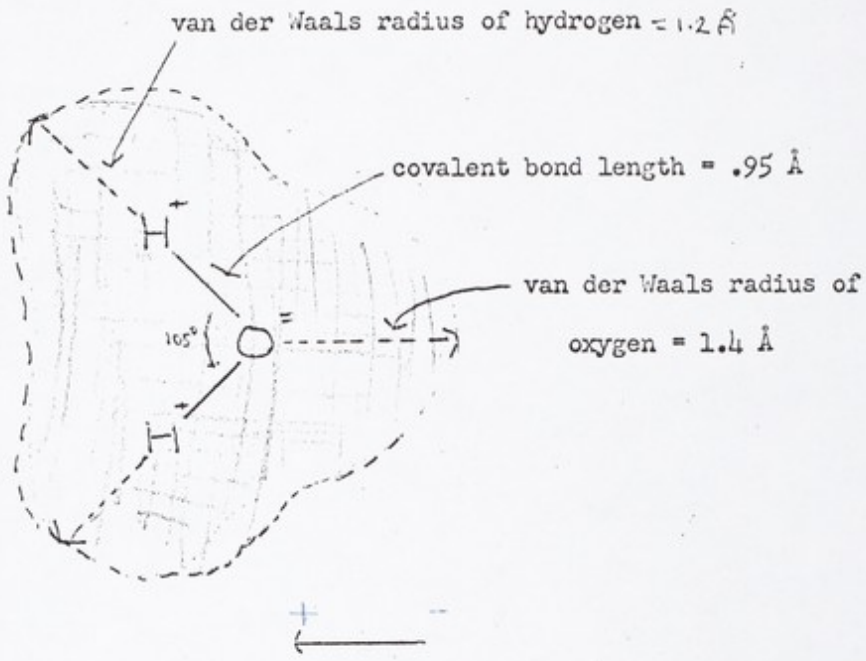


Figure IV - 3 The structure of a water molecule

are called polar molecules (a category which includes molecules with a net permanent charge, such as $CH_3C(=O)O^-$).

Non-polar molecules are those with no effective dipole moments. In CH_4 (methane), for example, the carbon and hydrogen atoms have very similar affinities for their shared electron pairs, and so neither the carbon nor the hydrogen atom is strongly charged.

The distribution of charge in a molecule can also be affected by the presence of nearby molecules, particularly if it is a polar molecule. This may cause a non-polar molecule to acquire a slight polar character. If the second molecule is not polar, its presence will still alter the non-polar molecule, establishing a fluctuating charge distribution. Such induced effects, however, give rise to a much smaller separation of charge than is found in polar molecules, thus resulting in smaller interaction energies, and, correspondingly, weaker chemical bonds.

Van der Waals forces

Van der Waals bonding arises from a non-specific attractive force originating when two atoms approach very close to each other. It is based not upon the existence of permanent charge separations, but rather upon the induced fluctuating charges caused by the nearness of molecules. It therefore operates between all types of molecules, polar as well as non-polar. It depends heavily upon the distance between the interacting groups, since it is inversely proportional to the sixth power of distance (Figure IV-4).

There also exists a more powerful van der Waals repulsive force which comes into play at even shorter distances. This repulsion is caused by the overlapping of the outer electron shells of the atoms involved. The van der Waals attractive and repulsive forces balance at a certain distance specific for each type of atom. This distance is the so-called van der Waals radius (Table IV-2 and Figure IV-5). The van der Waals bonding energy between two [redacted] atoms separated by the sum of their van der Waals

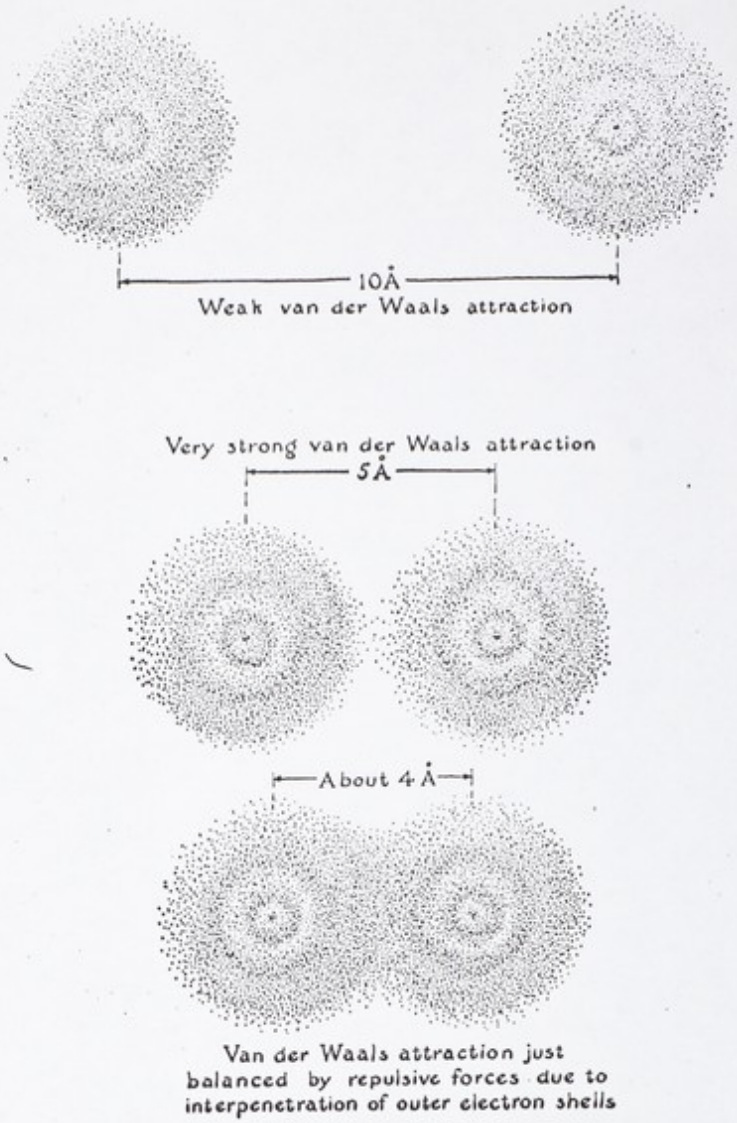


Figure IV - 4 Diagram illustrating van der Waals attraction and repulsion forces in relationship to electron distribution of monoatomic molecules of the inert rare gas argon. (from Pauling, General Chemistry, 2nd edition, p322)

Table IV-2 Van der Waals radii of the atoms in biological molecules

atom	Van der Waals radius
H	1.2Å
N	1.5Å
O	1.4Å
P	1.9Å
S	1.85Å
CH_3 group	2.0Å
half thickness of aromatic molecule	1.7Å

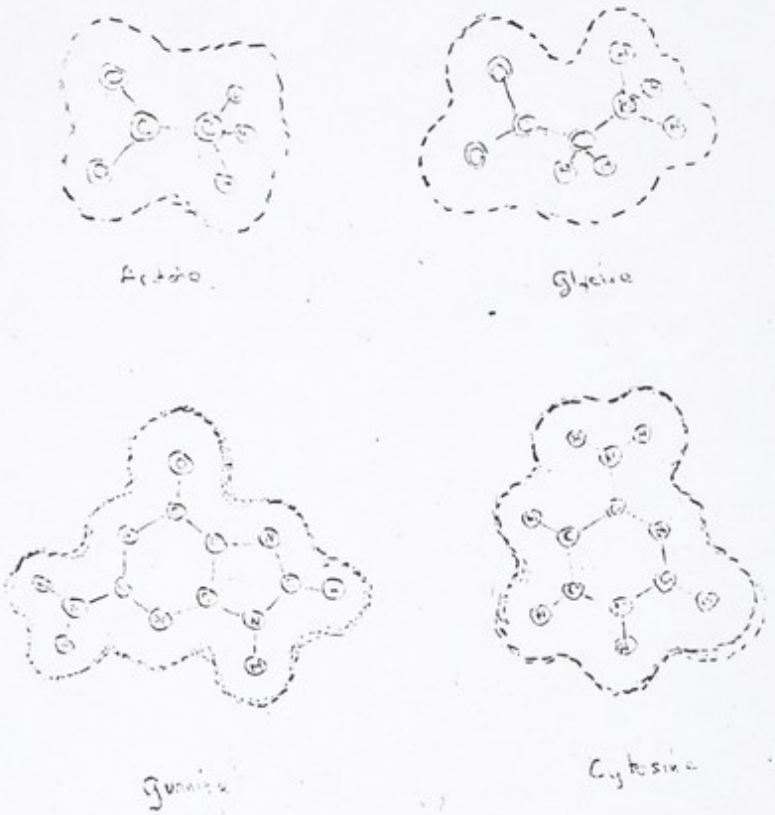


Figure IV- 5 Drawings of several molecules with atoms shown as spheres with radii equal to their van der Waals radii.

increases with the size of the respective atoms. For two average atoms it radii is only about 1 kcal/mole, which is just slightly more than the average thermal energy of molecules at room temperature (0.6 kcal/mole).

This means that van der Waals forces are an effective binding force at physiological temperatures only when several atoms in a given molecule are bound to several atoms in another molecule. Then the energy of interaction is much greater than the dissociating tendency due to random thermal movements. In order for several atoms to interact effectively, the molecular fit must be very good, since the distance separating any two interacting atoms must not be much greater than the sum of their van der Waals radii (Figure IV-6). The strength of interaction rapidly approaches zero when this distance is only slightly exceeded. Thus, the strongest type of van der Waals contacts arise when a molecule contains a cavity exactly complementary in shape to a protruding group of another molecule (Figure IV-7). This is the type of situation thought to exist between an antigen and its specific antibody (see Chapter XV). In this instance, the binding energies sometimes can be as large as 10 kcal/mole, so that the antigen-antibody complexes seldom fall apart. Many polar molecules are but seldom affected by van der Waals interactions, since such molecules acquire a lower energy state (lose more free energy) by forming other types of bonds.

Hydrogen bonds

A hydrogen bond arises between a covalently bound hydrogen atom with some positive charge and a negatively charged covalently bound acceptor atom (Figure IV-8). For example, the hydrogen atoms of the imino group (N-H) are attracted by the negatively charged keto oxygen atoms (C=O). Sometimes the hydrogen bonded atoms belong to groups with a unit of charge (e.g., NH_3^+ or $C(=O)O^-$). In other cases, both the donor hydrogen atoms and the negative acceptor atoms have less than a unit of charge.

The biologically most important hydrogen bonds involve hydrogen atoms covalently bound to oxygen (O-H) or nitrogen atoms (N-H). Likewise, the negative

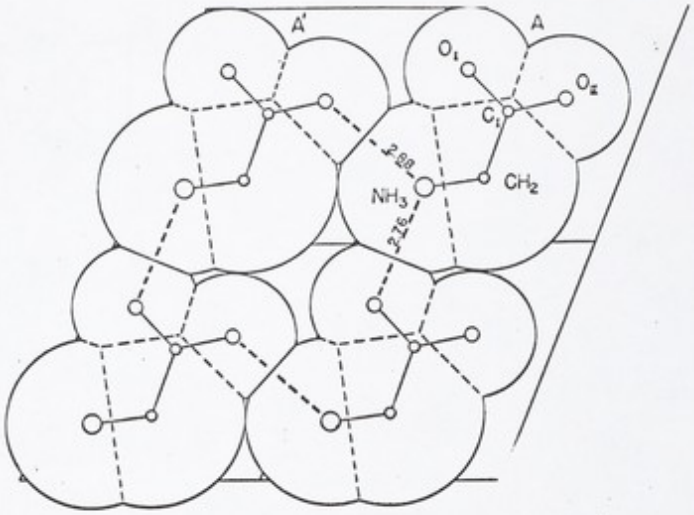
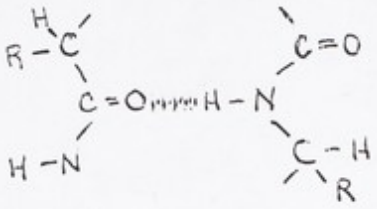
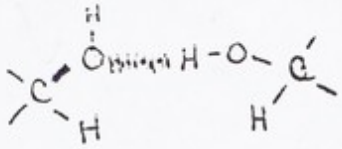


Figure IV- 6 The arrangement of molecules in a layer of a crystal formed by the amino acid glycine. The packing of the molecules is determined by the van der Waals radii of the groups, except for the N-H···O contacts which are shortened by the formation of hydrogen bonds.

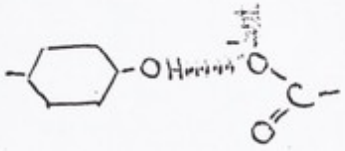
Figure IV-7 Schematic drawing showing the complementary relation between the surface configurations of an antigen and an antibody.

a) 

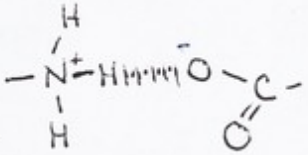
Hydrogen bond between peptide groups

b) 

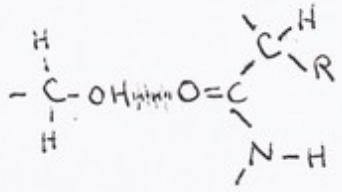
Hydrogen bond between two hydroxyl groups

c) 

Hydrogen bond between uncharged carboxyl group and the hydroxyl group of tyrosine

d) 

Hydrogen bond between charged amino group and a charged carboxyl group

e) 

Hydrogen bond between hydroxyl group of serine and peptide group

Figure IV - 8. Examples of cellular hydrogen bonds

acceptor atoms are usually nitrogen or oxygen. In Table IV-3 are listed some of the most important hydrogen bonds. Bond energies range between 3 and 7 kcal/mole, the stronger bonds involving the greater charge differences between donor and acceptor atoms. Hydrogen bonds are thus weaker than covalent bonds, yet considerably stronger than van der Waals bonds. A hydrogen bond, therefore, will hold two atoms closer together than the sum of their van der Waals radii, but not so close together as a covalent bond would hold them.

Hydrogen bonds, unlike van der Waals bonds, are highly directional. In optimally strong hydrogen bonds, the hydrogen atom points directly at the acceptor atom (Figure IV-9). If it points indirectly, the bond energy is much less. Hydrogen bonds are also much more specific than van der Waals bonds, since they demand the existence of molecules with complementary donor hydrogen and acceptor groups.

Some ionic bonds are in effect hydrogen bonds

Many organic molecules possess ionic groups which contain a unit or more of net positive or negative charge. The negatively charged mononucleotides, for example, contain phosphate groups ($PO_3^{=}$) with three units of negative charge, while each amino acid (except proline) has a negative carboxyl group ($C\overset{O}{\underset{O}{<}}^{-}$) and a positive amino group, both of which carry a unit of charge. These charged groups are usually neutralized by nearby, oppositely charged groups. The electrostatic forces acting between the oppositely charged groups are called ionic bonds. Their average bond energy in an aqueous solution is about 5 kcal/mole. In many cases, either an inorganic cation like Na^+, K^+, or Mg^{++}, or an inorganic anion like Cl^- or $SO_4^{=}$, neutralizes the charge of the ionized organic molecules. When this happens in aqueous solution, the neutralizing cations and anions do not occupy fixed positions, because inorganic ions are usually surrounded by shells of water molecules and so do not directly bind to

Table IV-3 Approximate bond lengths of biologically important hydrogen bonds

Bond	Approximate Bond Length
O-H - - - O	2.70 ± .10Å
O-H - - - O^-	2.63 ± .10Å
O-H - - - N	2.88 ± .13Å
(amino) N-H - - - O	3.04 ± .13Å
(amide) N^+H - - - O	2.93 ± .10Å
N-H - - - N	3.10 ± .13Å

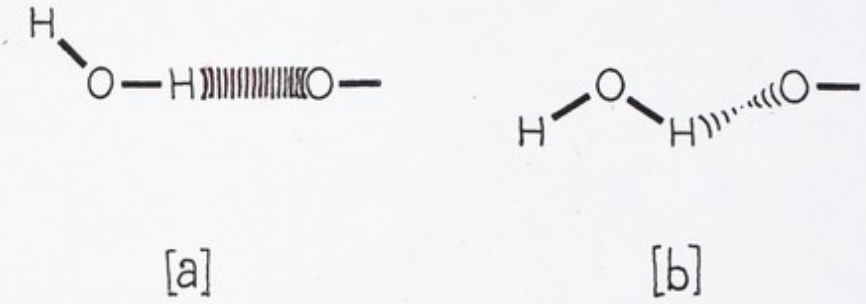


Figure IV- 9 Directional properties of hydrogen bonds. In (a) the vector along the covalent O-H bond points directly at the acceptor oxygen, thereby forming a strong bond. In (b) the vector points away from the oxygen atom, resulting in a much weaker bond.

oppositely charged groups. Thus it is now believed that, in water solutions, electrostatic bonds to surrounding inorganic cations or anions are not of primary importance in determining the molecular shapes of organic molecules.

On the other hand, highly directional bonds result if the oppositely charged groups can form hydrogen bonds to each other. For example, both the $C\leq^{O}_{O}$ and NH_3^+ groups are often held together by strong hydrogen bonds. Since these hydrogen bonds are stronger than those which involve groups with less than a unit of charge, they are correspondingly shorter. A strong hydrogen bond can also form between a group with a unit charge and a group having less than a unit charge. For example, a hydrogen atom belonging to an amino group ($-NH_2$) bonds strongly to an oxygen atom of a carboxyl group ($C\leq^{O}_{O}$).

Weak interactions demand complementary molecular surfaces

Weak binding forces are effective only when the interacting surfaces are very close. This is possible only when the molecular surfaces have complementary structures, so that a protruding group (or positive charge) on one surface is matched by a cavity (or negative charge) on another, i.e., the interacting molecules must, in a sense, have a lock and key relationship. In cells this requirement often means that some molecules hardly ever bond to other molecules of the same kind, because such molecules do not have the properties of symmetry necessary for self-interaction. For example, some (polar) molecules contain donor hydrogen atoms, and no suitable acceptor atoms, while others can accept hydrogen bonds, but have no hydrogen atoms to donate. On the other hand,, there do exist many molecules with the symmetry to permit strong self-interaction in cells, water being the most important example.

H_2O molecules form hydrogen bonds

Under physiological conditions, water molecules rarely ionize to form H^+ and OH^- ions. Instead, they exist as polar H-O-H molecules. Both the hydrogen and oxygen atoms form strong hydrogen bonds. In each H_2O molecule, the oxygen atom can bind to two external hydrogen atoms, while each hydrogen atom can bind to an adjacent oxygen atom. These bonds are directed tetrahedrally (Figure IV-10), so that in its solid and liquid forms, each water molecule tends to have four nearest neighbors, one in each of the four directions of a tetrahedron. In ice the bonds to these neighbors are very rigid and the arrangement of molecules fixed. Above the melting temperature (0°C) the energy of thermal motion is sufficient to break the hydrogen bonds and to allow the water molecules continuously to change their nearest neighbors. Even in the liquid form, however, at a given instant most water molecules are bound by four strong hydrogen bonds.

Weak bonds form between almost all the molecules in aqueous solutions

The average energy of a secondary bond, though small compared to that of a covalent bond, is nonetheless strong enough compared to heat energy to insure that most molecules in aqueous solution will form secondary bonds to other molecules. The proportion of bonded to non-bonded arrangements is given by eq IV-4, corrected to take into account the high concentration of molecules in a liquid. It tells us that interactions energized as low as 2-3 kcal/mole are sufficient at physiological temperatures to force most molecules to form the maximum number of good secondary bonds.

The specific structure of a solution at a given instant is markedly

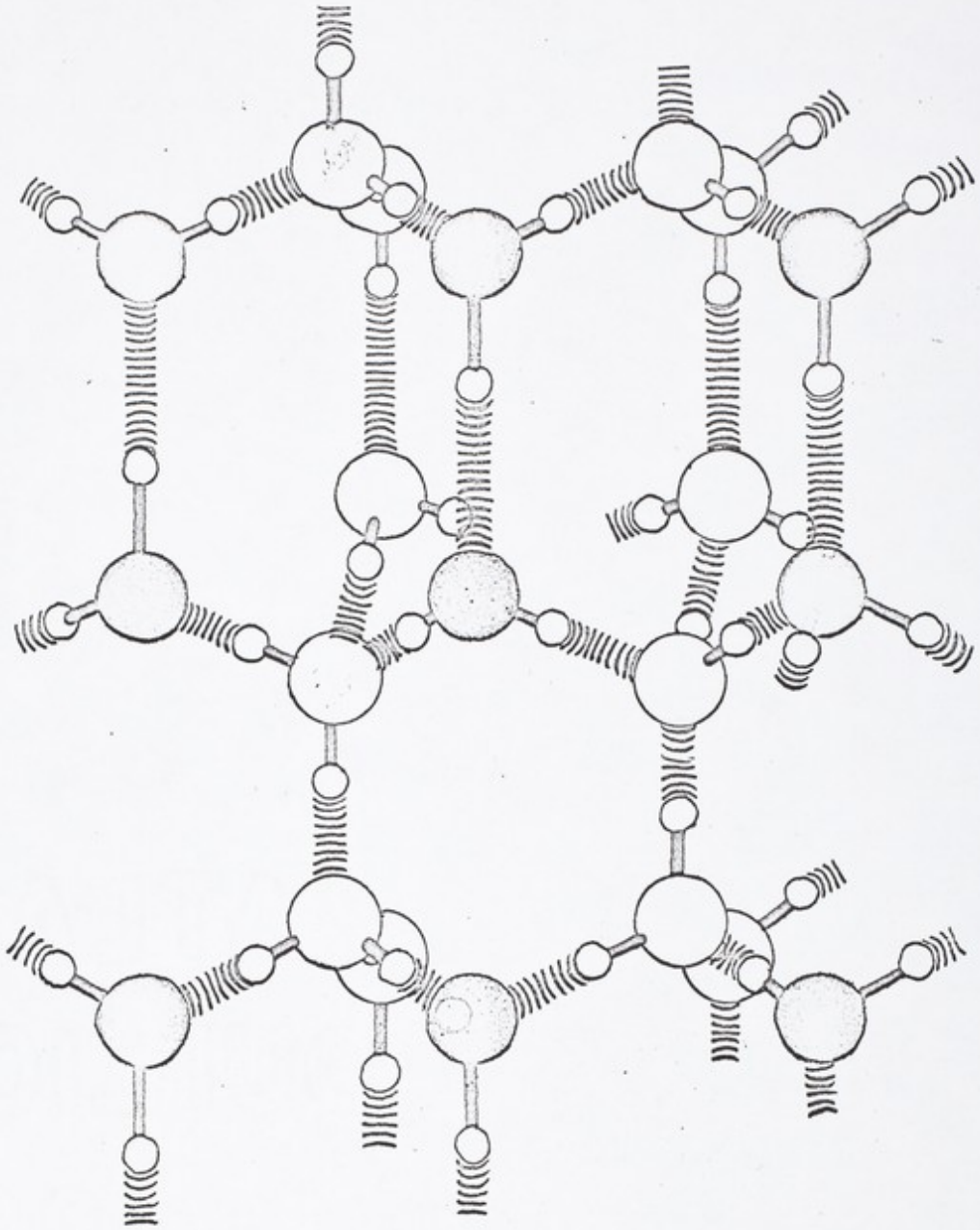
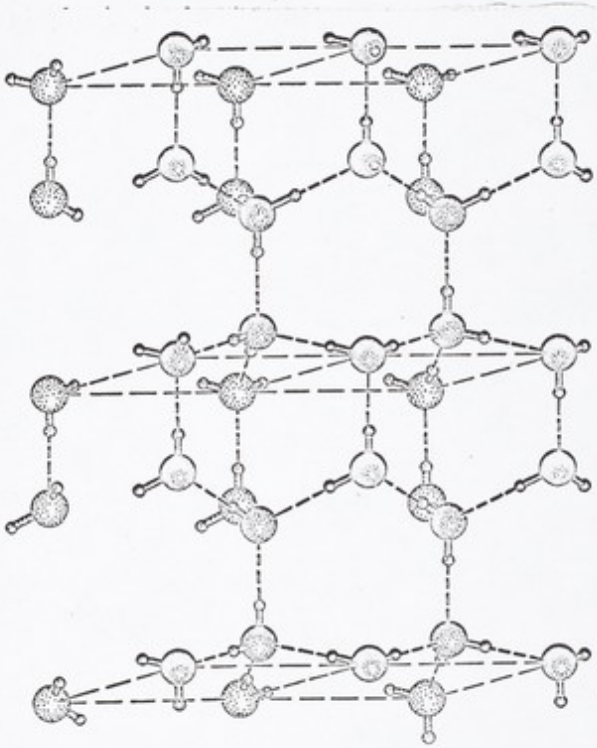


Figure IV- 10 Schematic diagram of a lattice formed by H_2O molecules. The energy gained by forming specific hydrogen bonds (HHHH) between H_2O molecules favors the arrangement of the molecules in adjacent tetrahedrons. Oxygen atoms are indicated by large circles, colored differently according to planes, and hydrogen atoms by small circles. Although the rigidity of the arrangement depends upon the temperature of the molecules, the pictured structure is, nevertheless, predominant in water as well as in ice.



influenced by which solute molecules are present. This is not only because molecules have very specific shapes but also because the molecules differ in which types of secondary bonds they can form. This means that a molecule will tend to move until it is next to a molecule with which it can form the strongest type of secondary bond.

Solutions of course are not static. Because of the disruptive influence of heat, the specific configuration of a solution is constantly changing from one into another arrangement of approximately the same energy content. Equally important in biological systems is the fact that the metabolism is continually transforming one molecule into another and so automatically changing the nature of the secondary bonds which can form. The solution structure of cells is thus being disrupted not only by heat motion but also by the metabolic transformations of its solute molecules.

Organic molecules which tend to form hydrogen bonds are water soluble

The energy of hydrogen bonds per atomic group is much stronger than that of van der Waals contacts. Thus those molecules which can form hydrogen bonds will form them in preference to van der Waals contacts. For example, if we try to mix water with a compound which cannot form hydrogen bonds, such as benzene, the water and benzene molecules rapidly separate from each other, the water molecules forming hydrogen bonds among themselves while the benzene molecules attach to each other by van der Waals bonds. The amount of free energy (ΔF, kcal/mole) released with a hydrogen bond [illegible] the probability of finding a non-bonded arrangement in H_2O is approximately 1,000 to 1. Thus it is effectively impossible to insert a non-hydrogen bonding molecule into water.

On the other hand, polar molecules like glucose and pyruvate, which contain a large number of groups (e.g., =O, or -OH) that form excellent hydrogen bonds, are somewhat soluble in water (hydrophyllic as opposed to hydrophobic). This is because, while their insertion into a water lattice breaks water-water hydrogen bonds, it results simultaneously in hydrogen bonds between glucose and water. These alternative arrangements, however, are not usually as satisfactory as the water-water arrangements, so that even the most polar molecules ordinarily have only limited solubility.

Thus almost all the molecules which cells are constantly acquiring (either through food intake or biosynthesis) are somewhat insoluble in water. These molecules, by their thermal movements, randomly collide with other molecules until they find complementary molecular surfaces on which to attach and thereby release water molecules for water-water interactions.

The uniqueness of molecular shapes -- the concept of selective stickiness

Even though most cellular molecules are built up from only a small number of groups such as OH, NH_3, CH_3, great specificity exists as to which molecules tend to lie next to each other. This is because each molecule has unique binding properties. One very clear demonstration comes from the specificity of sterioisomers. For example, proteins are always constructed from l-amino acids, never from their mirror images, the d-amino acids (Figure IV-11). Though the d and l amino acids have identical covalent bonds, their binding properties to assymetrical molecules are often very different. Thus most enzymes are specific for l-amino acids. If an l-amino acid is able to attach to a specific enzyme, the d-amino acid is unable to bind.

The general rule exists that in cells most molecules can make good "weak" bonds with only a small number of other molecules. This is partly because

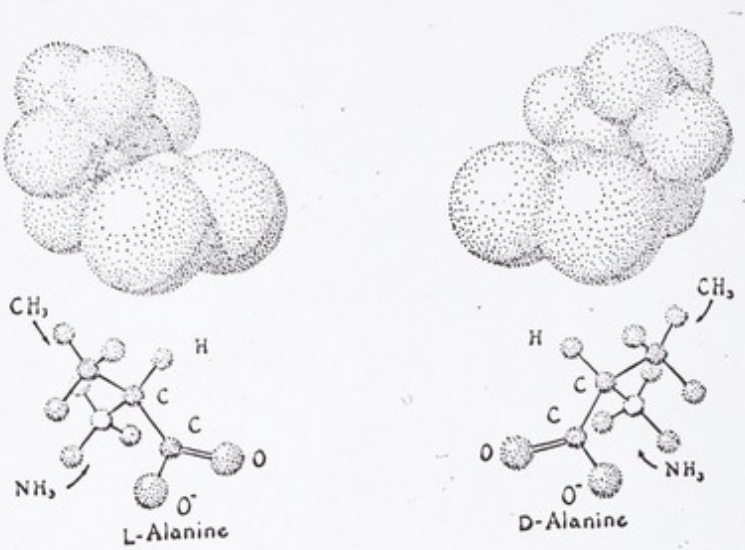


FIG. 29-2 *The two stereoisomers of the amino acid alanine.*

Figure IV - 4 The two stereoisomers of the amino acid alanine. (redrawn from Pauling, General Chemistry 2nd edition, Freeman 1958)

all molecules in biological systems exist in an aqueous environment. The formation of a bond in a cell depends not only upon whether or not two molecules bind well to each other, but also upon whether or not the bond will permit their water solvent to form the maximum number of good hydrogen bonds.

The strong tendency of water to exclude non-polar groups is frequently referred to as hydrophobic bonding. Some chemists like to call all the bonds between non-polar groups in a water solution hydrophobic bonds (Figure IV-12). In a sense this term is a confusing misnomer, for the phenomenon which it seeks to emphasize is the absence, not the presence, of bonds. (The bonds which tend to form between the non-polar groups are due to van der Waals attractive forces.) On the other hand, the term hydrophobic bond is often useful since it emphasizes the fact that non-polar groups will try to arrange themselves so that they are not in contact with water molecules.

Consider, for example, the energy difference between the binding in water of the amino acids alanine and glycine to a third molecule which has a surface complementary to alanine. Alanine differs from glycine by the presence of one methyl group. When alanine is bound to the third molecule, the van der Waals contacts around the methyl group yield 1 kcal/mole of energy. This energy is not released when glycine is bound instead. This small energy difference alone would give (using equation IV-4) only a factor of 6 between the binding of alanine and glycine. This calculation does not take into consideration, however, the fact that water is trying to exclude alanine much more than glycine. The presence of alanine's CH_3 group upsets the water lattice much more seriously than does the hydrogen atom side group of glycine. At present it is still difficult to predict how large a correction factor must be introduced for this disruption of the water lattice by the hydrophobic side groups. A current guess is that the water tends to exclude alanine, thrusting it toward a third molecule with a hydrophobic force 2-3 kcal/mole larger than the force which excludes glycine.

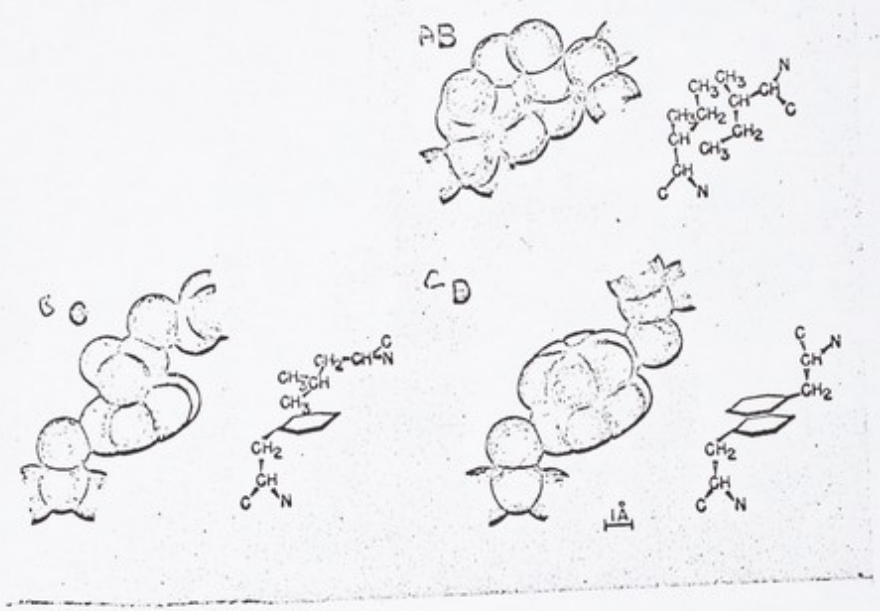


Figure IV - 12. Illustrative examples of van der Waals (hydrophobic) bonds between the non-polar side groups of amino acids. The hydrogens are not indicated individually. For the sake of clarity, the van der Waals radii are reduced by 20%. The structural formulas to the right of each space-filling drawing indicate the arrangement of the atoms. A) isoleucine-isoleucine bond; B) phenylalanine-leucine bond; C) phenylalanine-phenylalanine bond. (Redrawn from Neurath and Scheraga, The Proteins, Vol. I, Academic Press 1963.)

We thus arrive at the important conclusion that, in the aqueous interior of cells the energetic difference between the binding of even the most similar molecules to a third molecule is at least 2-3 kcal/mole (when the difference involves a non-polar group). Frequently it is 3-4 kcal/mole, since the differences between many molecules involve polar groups which can form hydrogen bonds.

The advantage of ΔG's between 2 and 5 kcal/mole

We have seen that the energy of just one secondary bond (2-5 kcal/mole) is

often sufficient to insure that a molecule preferentially binds to a selected group of molecules. Equally important, these energy differences are not so large that rigid lattice arrangements develop within a cell -- the interior of a cell never crystallizes, as it would if the energy of secondary bonds were several times greater. Larger energy differences would mean that the secondary bonds only seldom broke, resulting in diffusion rates incompatible with cellular existence.

Weak bonds attach enzymes to substrates

Secondary forces are necessarily the basis by which enzymes and their substrates initially combine with each other. Enzymes do not indiscriminantly bind all molecules, but in general have noticeable affinity only for their own substrates.

Since enzymes catalyze both directions of a chemical reaction, they must have specific affinities for both sets of reacting molecules. In some special cases it is possible to calculate an equilibrium constant for the binding of an enzyme and one of its substrates, which consequently enables us (eq. IV-4) to calculate the ΔG upon binding. This calculation, in turn, gives hints about which types of bonds may be involved. ΔG values ranging between 5-10 kcal/mole suggest that from one to several good secondary bonds are the basis of specific enzyme-substrate interactions. Also worth noting is that the ΔG of binding is never exceptionally high: thus enzyme-substrates complexes can be both made and broken apart rapidly as a result of random thermal movement. This explains why enzymes can function so quickly, sometimes as often as 10^6 times/second. If enzymes were bound to their substrates by more powerful bonds, they would act much more slowly.

The shapes of most molecules are determined by weak bonds

The shapes of numerous molecules are automatically given by the distribution of covalent bonds. This inflexibility occurs when groups are attached by covalent bonds about which free rotation is impossible. Rotation is only possible when atoms are attached by single bonds. (For example, the methyl groups of ethane H_3C-CH_3 rotate about the carbon-carbon bond.) When more than one electron pair is involved in a bond, rotation does not occur; in fact, the atoms involved must lie in the same plane. Thus the aromatic purine and pyrimidine rings are planar molecules 3.4Å thick. No uncertainty exists about the shape of any aromatic molecules. They are always flat independent of their surrounding environment.

On the contrary, for molecules containing single bonds, the possibility of rotation around the bond suggests that a covalently bonded molecule exists in a large variety of shapes. This theoretical possibility, however, is seldom in fact realized, because the various possible 3-D configurations differ in the number of good weak bonds which can be formed. Generally there exists one configuration which has significantly less free energy than any of the other geometrical arrangements.

Two classes of secondary bonds may be important in determining 3-D shapes. One class is internal, forming between atoms connected by a chain of covalent bonds (Figures IV-(3)). Internal bonds often cause a linear molecule to bend back upon itself, allowing contacts between atoms separated by a large number of covalent bonds. The other class of bonds is external, forming between atoms not connected by covalent bonds. In some cases the optimal 3-D configuration is achieved by forming external bonds. Molecules formed in this manner usually have extended (fibrous) configurations. There are other cases where many of the weak bonds are formed internally. In such molecules the final shape is often more

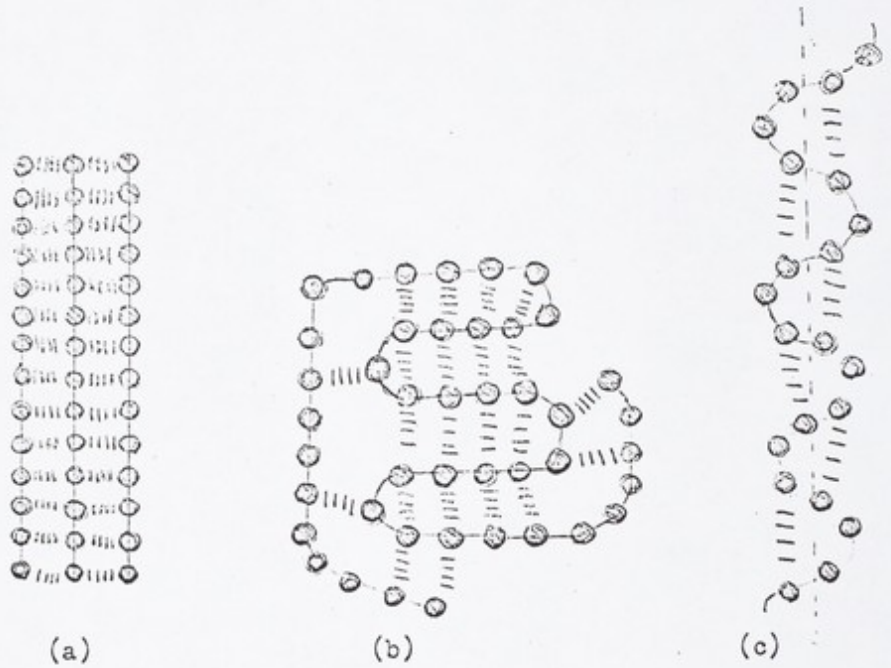


Figure IV-13 The distinction between internal and external weak bonds. The external bonds in (a) lead to extended fibrous molecules which often associate in sheet-like structures. The silk proteins are an example. When internal bonds are formed, the structure is often much more compact. The various globular proteins, like hemoglobin (see Chapter VI), belong to this type of structure. Sometimes, however, internal bonds hold together long, thin helical molecules (c). The α helix conformation of the polypeptide chain (see Chapter VI) is an example.

compact (globular).

It is no simple matter to guess the correct shape of a large molecule from its covalent bond structure; although one configuration for a protein or a nucleic acid may be energetically much more suitable than any other, we cannot yet derive it from our knowledge of bond energies. There are two reasons for this difficulty. One is purely logistical. The number of possible configurations of a nucleic acid or even a small protein molecule is very, very large. Given present techniques for building molecular models, a single person (or even a small group of people) cannot rapidly calculate the sum of the energies of the weak bonds for each possible configuration. At our present pace, years would be required. Future work using electronic computers, however, should simplify some of this task. The second reason for our present inability to guess protein and nucleic acid structures is that our knowledge about the nature of weak bonds is still very incomplete - in many cases, we are not sure either about the exact bond energies or of the possible angles they form to each other.

Today, protein and nucleic acid shapes can be revealed only by x-ray diffraction analysis. Fortunately these experimental structure determinations are beginning to give us some general rules that tell us which weak chemical interactions are most important in governing the molecular shapes of large molecules. In particular, these rules emphasize the vital importance of interactions of the macromolecules with water, which is by far the most common molecule in all cells.

Polymeric molecules are sometimes helical

Earlier we have emphasized that polymeric molecules like proteins and nucleic acids have regular linear backbones in which specific groups (e.g., $-\overset{O}{\overset{\|}{C}}-\underset{H}{N}-$)

repeat over and over along the molecule.

Often these regular groups are arranged in helical configurations, held together by secondary bonds. The helix is a natural conformation for regular linear polymers since it places each monomer group in an identical orientation within the molecule. Each monomer thereby forms the same group of secondary bonds. On the contrary, when a regular linear polymer has a non-helical arrangement, different monomers must form different secondary bonds. Clearly an unstable state occurs if any one set of secondary bonds is much stronger than any of the other arrangements. Thus, helical symmetry does not evolve from the particular shape of the monomer, but is instead the natural consequence of the existence of a unique monomer arrangement which is significantly more stable than all other arrangements.

It is very important to remember that most biopolymers are not regular polymers containing identical monomers. Instead they often have irregular side groups attached to a regular backbone. When this happens, as in the case for both nucleic acids and proteins, we need not necessarily expect a helical structure. This is because a 3-D arrangement which is energetically very satisfactory for the backbone groups often produces very unsatisfactory bonding of the side groups. The 3-D structure of many irregular polymers is thus a compromise between the tendency of regular backbones to form a regular helix and the tendency of the side groups to twist the backbone into a configuration which maximizes the strength of the secondary bonds formed by the side groups.

Protein structures are usually irregular

In the case of proteins, the compromise between the side groups and the backbone groups usually is decided in favor of the side groups. Thus, as we shall present in much greater detail in Chapter VI, most amino acids in proteins are not part of regular helices. This is because almost one half of the side

groups are largely non-polar and can be placed in contact with water only by a considerable input of free energy. This conclusion was at first a surprise to many chemists, influenced by the fact that backbone groups could form strong internal hydrogen bonds while the non-polar groups could form only the much weaker van der Waals bonds. Their past reasoning was faulty, however, because it did not consider either the fact that the polar backbone can form almost as strong external hydrogen bonds to water or the equally important fact that a significant amount of energy is necessary to push non-polar side groups into a hydrogen bonded water lattice.

This argument leads to the interesting prediction that in aqueous solutions macromolecules containing a large number of non-polar side groups will tend to be more stable than molecules containing mostly polar groups. If we disrupt a polar molecule held together by a large number of internal hydrogen bonds there is often only a small change in free energy since the polar groups can also hydrogen bond to water. On the contrary, when we disrupt molecules having many non-polar groups, there is usually a much greater loss in free energy because the disruption necessarily inserts non-polar groups into water.

DNA can form a regular helix

At first glance, DNA looks even more unlikely to form a regular helix than does an irregular polypeptide chain. It not only has an irregular sequence of side groups, but in addition, all its side groups are hydrophobic. Both the purines (adenine and guanine) and the pyrimidines (thymine and cytosine), even though they contain polar C=O and NH_2 groups, are quite insoluble in water because their flat sides are completely hydrophobic.

Nonetheless, most DNA molecules have regular helical structures. This is because most DNA molecules contain two polynucleotide strands which have complementary structures (see Chapter IX for more details). Both internal and external secondary bonds stabilize the structure. The two strands are held together by

hydrogen bonds between pairs of complementary purines and pyrimidines (Figure IV-14). Adenine (amino) is always hydrogen bonded to thymine (keto), while guanine (keto) is hydrogen bonded to cytosine (amino). In addition, virtually all the surface atoms in the sugar and phosphate groups form bonds to water molecules.

The purine-pyrimidine base pairs are found in the center of the DNA molecule. This allows their flat surfaces to stack on top of each other and so have only limited contact with water. This stacking arrangement would be much less satisfactory if only one chain were present. A single chain could not have a regular backbone because its pyrimidines are smaller than the purines and so the angle of helical rotation would have to vary with the sequence of bases. The presence of complementary base pairs in double helical DNA makes a regular structure possible since each base pair is of the same size.

DNA molecules are very stable at physiological temperature

The double helical DNA molecule is very stable at physiological temperatures. This stability is not only because disruption of the double helix breaks the regular hydrogen bonds and brings the hydrophobic purines and pyrimidines into contact with water. It also is because individual DNA molecules have a very large number of weak bonds so arranged that most of them cannot break without the simultaneous breaking of many others. Even though thermal motion is constantly breaking apart the terminal purine-pyrimidine pairs at the ends of each molecule, the two chains do not usually fall apart. This is because the hydrogen bonds in the middle are still intact (Figure IV-15). Once a break occurs, the most likely next event is the reforming of the same hydrogen bonds, to restore the original molecular configuration. Sometimes, of course, the first breakage is followed by a second one, and so forth. These events, however, are very, very rare, and so double helices held together by more than 10 nucleotide

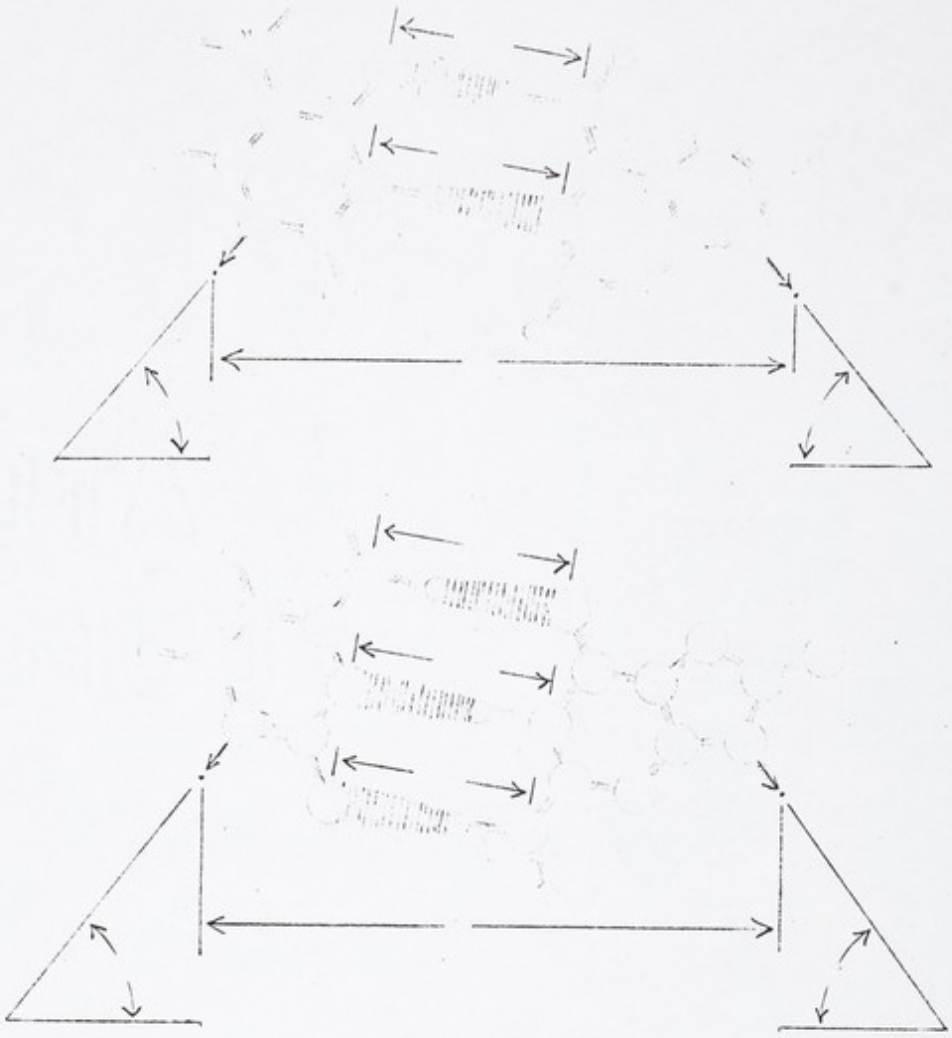
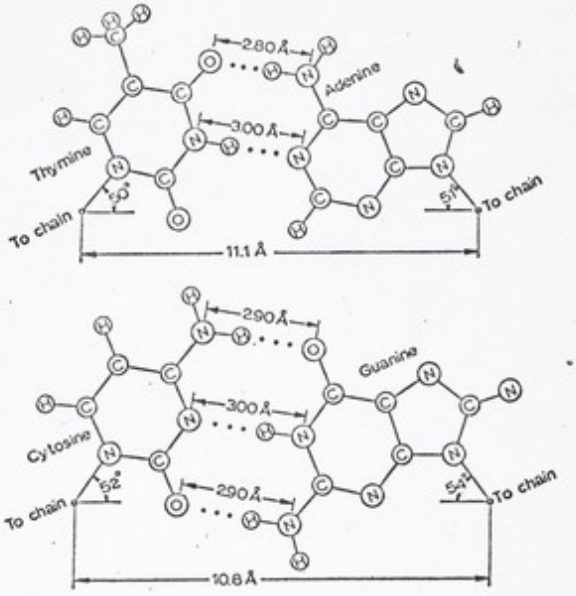
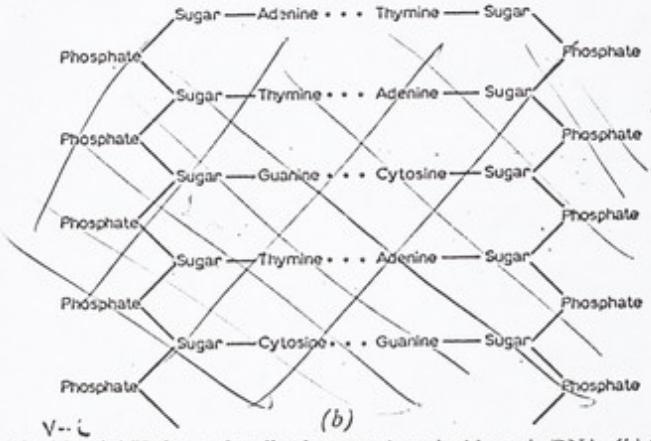


Figure IV- 14 The hydrogen bonded base pairs in DNA. Adenine is always attached to thymine by two hydrogen bonds, while guanine always bonds to cytosine by three hydrogen bonds. The obligatory pairing of the smaller pyrimidine with the larger purine allows the two sugar-phosphate backbones to have identical helical configurations. All the hydrogen bonds in both base pairs are strong, since each hydrogen atom points directly at its acceptor atom (nitrogen or oxygen).



(a)



(b)

Fig. 25. *(a)* Hydrogen bonding between the paired bases in DNA. *(b)* Complementary sequences of bases in the 2 strands of the double helix. Note that there is no preference for the direction of the pairs.

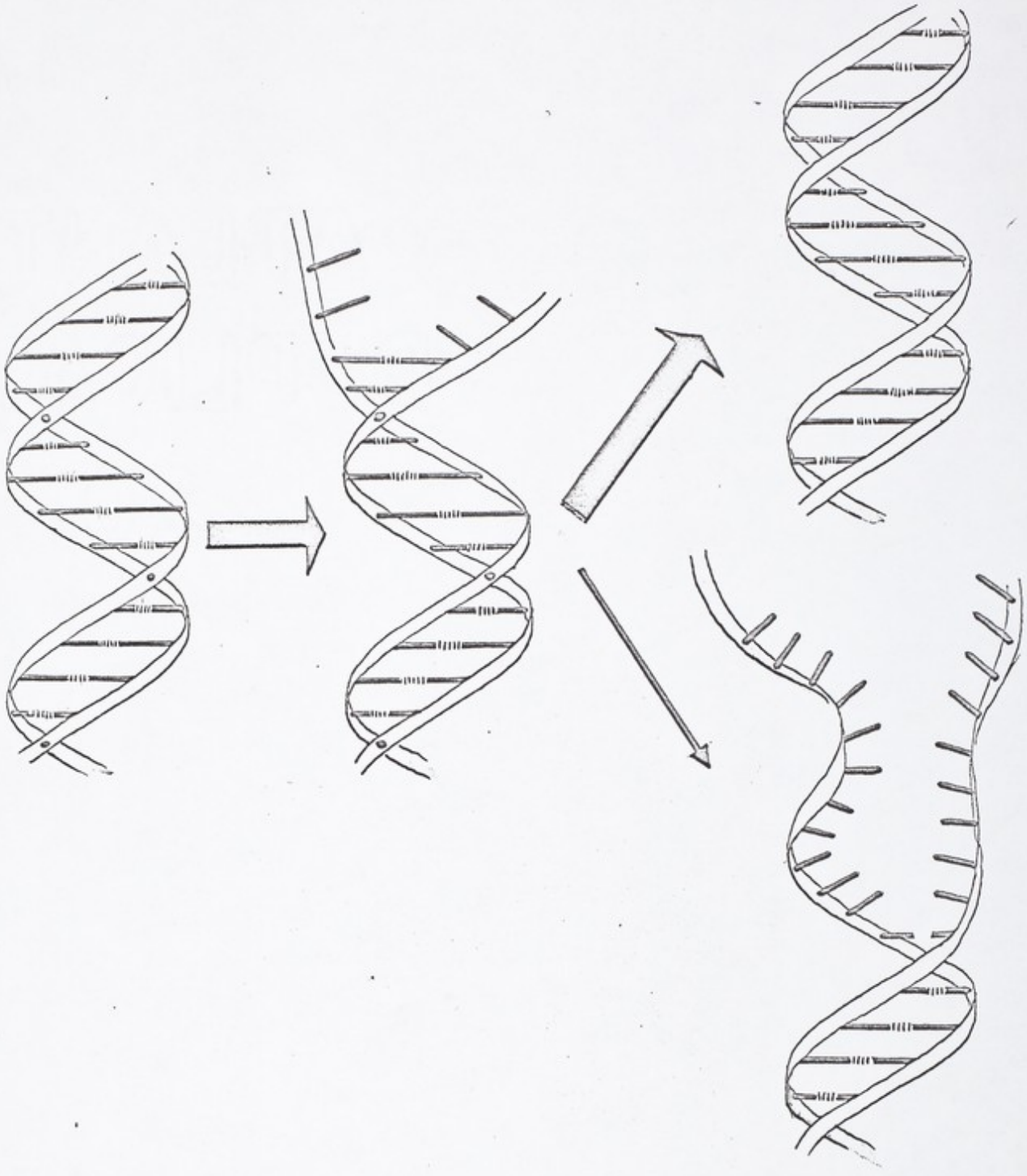


Fig IV - 15

The breaking of terminal hydrogen bonds in DNA by random thermal motion. Because the internal hydrogen bonds continue to hold the two chains together, the immediate reforming of the broken bonds is highly probable. Also shown is the very rare alternative: the breaking of further hydrogen bonds, and the consequent disentangling of the chains.

pairs are very stable at room temperature.

The same principle also governs the stability of most protein molecules. Stable protein shapes are never due to the presence of just one or two weak bonds, but must always represent the cooperative result of a number of weak bonds.

Ordered collections of hydrogen bonds become less and less stable as their temperature is raised above physiological temperatures. At these abnormally high temperatures, the simultaneous breakage of several weak bonds is more frequent. After a significant number have broken, a molecule usually loses its original form (the process of "denaturation") and assumes an inactive ("denatured") configuration.

Most medium size and almost all very large protein molecules are aggregates of smaller polypeptide chains

Earlier we pointed out how the realization that macromolecules are all polymers constructed from regular, small monomers, like the amino acids, greatly simplified the problem of solving macromolecule structure. It has, moreover, recently become clear that most of the very large proteins are regular aggregates of much smaller polypeptide chains containing not more than 400 amino acids apiece. For example, the protein Ferritin, which functions in mammals to store iron atoms, has a molecular weight of about 480,000. It contains, however, not a single polypeptide chain with 4,000 amino acids, but instead 24 identical smaller polypeptide chains of about 200 amino acids each. Similarly, the protein component of Tobacco Mosaic Virus was originally thought to have the horrendous molecular weight of 36,000,000. Most fortunately, it was subsequently discovered (see Chapter XII) that each TMV protein contains 2150 identical smaller protein molecules, each containing 158 amino acids. Even much smaller protein molecules are frequently constructed from a number of polypeptide chains. Hemoglobin, which has a molecular weight of only 64,500, contains four polypeptide chains, 2 α chains and 2β chains, each of which has a molecular weight of about 16,000. (Figure IV-16)

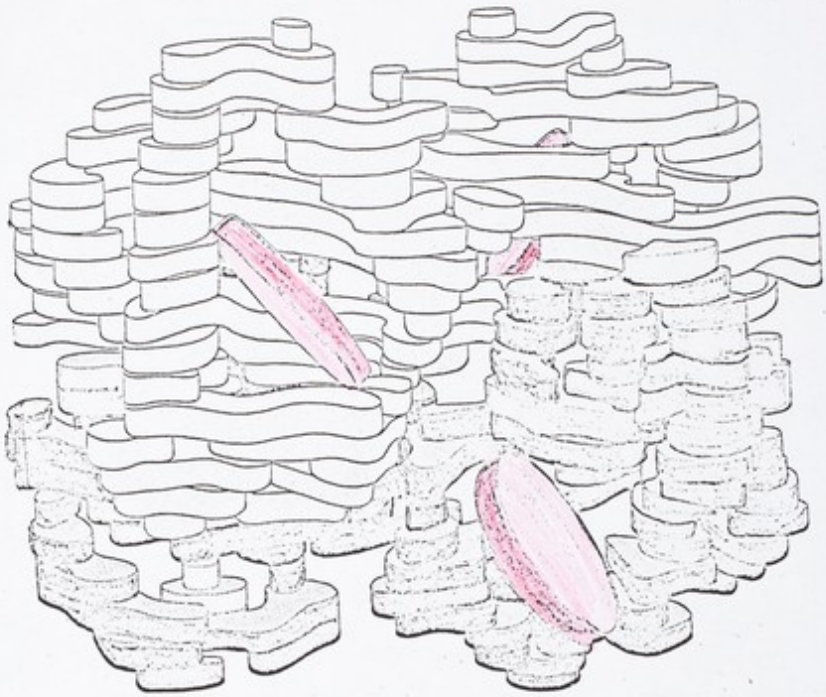


Figure IV- 16 Schematic drawing of the hemoglobin molecule, showing the existence of 4 sub-units (2 α and 2β). The two α chains are shown in light color, and the two β chains in dark color. Each sub-unit contains a heme molecule (red). The iron-containing molecule which bonds oxygen in this drawing is adapted from Perutz (Scientific American: Nov., 1964). The irregular blocks represent regions where X-ray diffraction analysis reveals a high concentration of atoms. No attempt has been made to show individual atoms.

In all three examples, as with most other protein aggregates, the smaller units are held together by secondary bonds. This is shown by the fact that they can be dispersed by the addition of reagents (e.g., urea) which tend to break secondary bonds, but not covalent bonds. Weak bonds are not, however, the only force holding macromolecular units together. In some cases, for example in the protein insulin, disulphide bonds (S-S) between cysteine residues are the main binding force.

Sub-units are economical

Both the construction of polymers from monomers and the use of polymeric molecules themselves as sub-units to build still larger molecules, reflect a very general building principle applicable to all complex structures, non-living as well as living. This principle states that it is much easier to reduce the impact of construction mistakes if we can discard them before they are incorporated into the final product. This is shown by considering two alternative ways of constructing a molecule with 1,000,000 atoms. In scheme (a) we build the structure atom by atom, while in scheme (b) we first build 1,000 smaller units, each with 1,000 atoms, and subsequently put the sub-units together into the 1,000,000 atom product. Now consider that our building process randomly makes mistakes, and inserts the wrong atom with a frequency of 10^{-5}. Let us assume that each mistake results in a non-functional product.

Under scheme (a) each molecule will contain, on the average, 10 wrong atoms, and so there will exist almost no good products. Under scheme (b), however, there will occur mistakes in only 1% of the sub-units. Thus, if a device exists to throw away the bad sub-units, then good products can be easily made and the cell will hardly be bothered by the presence of the 1% of non-functional sub-units. This concept is the basis of the assembly line in which complicated industrial products like radios and automobiles are constructed. At each stage of assembly

there are devices to throw away bad sub-units. In industrial assembly lines, the mistakes were initially removed by human hands; now manual control is often replaced by automated control. In cells, the mistakes are sometimes controlled by the specificity of enzymes: if a monomeric sub-unit is wrongly put together, usually it will not be recognized by the polymer making specific enzyme, and hence not incorporated into a macromolecule. In other cases, the faulty substances are rejected because they are unable to spontaneously become part of stable molecular aggregates.

The principle of self-assembly

ΔG values of 1-5 kcal/mole mean not only that single weak bonds will be spontaneously made, but also that structures held together by several weak bonds will be spontaneously formed. For example, an unstable, unfolded polypeptide chain tends to assume a large number of random configurations as a result of thermal movements. Most of these conformations are also thermodynamically unstable. Inevitably, however, some of these thermal movements bring together groups which can form good weak bonds. These groups tend to stay together because more free energy is lost when they form than is gained by their breakage. Thus, by a random series of movements, the polypeptide chain gradually assumes a configuration in which most, if not all, the atoms have fixed positions within the molecule.

Aggregation of separate molecules also occurs spontaneously. The protein hemoglobin furnishes a clear example. It can be broken apart by the addition of reagents like urea which break secondary bonds to yield half molecules with a 32,000 MW. If however, the urea is removed, the half molecules quickly aggregate with each other to form functional hemoglobin molecules. The surface structure of the half molecules is very specific for they bind only to each other and not with any other cellular molecules.

This same general principle of self-assembly operates to build even larger

and more complicated structures, like the cell membrane and the cell wall. Both are mosaic surfaces containing large numbers of various molecules, some large, like proteins, and others much smaller, like lipids. At present, virtually nothing is known about the precise arrangement of the molecules in these very large, complicated structures. Nonetheless, there is every reason to believe that the constituent molecules form stable contacts only with other molecules in the cell membrane (or wall). This is very easy to visualize in the case of lipids. They are extremely insoluble in water because of their long, non-polar hydrocarbon chains. These newly synthesized lipids will thus have a strong tendency to attach to other lipids in the cell membrane or cell wall by van der Waals forces.

Summary

Many important chemical events in cells do not involve the making or breaking of covalent bonds. The cellular location of most molecules depends on the existence of "weak" (secondary) attractive or repulsive forces. In addition, weak bonds are important in determining the shape of many molecules, especially very large ones. The most important of these "weak" forces are: hydrogen bonding, van der Waals interaction, and ionic bonds. ~~and the electrostatic interaction between molecules (ions) with net charge.~~ Even though these forces are relatively weak, they are still large enough to ensure that the right molecules (groups) interact with each other. For example, the surface of an enzyme is uniquely shaped to allow specific attraction of its substrates.

The formation of all chemical bonds, weak interactions as well as strong covalent bonds, proceeds according to the laws of thermodynamics. A bond tends to form when it results in a release of free energy (ΔG is negative). In order for it to be broken, this same amount of free energy must be supplied. Because the formation of covalent bonds between atoms usually involves a very large

negative ΔG, covalently bound atoms almost never separate spontaneously. In contrast, the ΔG values accompanying the formation of weak bonds are only several times larger than the average thermal energy of molecules at physiological temperatures. Single weak bonds are frequently being made and broken in living cells.

Molecules having polar (charged) groups interact quite differently from non-polar molecules (in which the charge is symmetrically distributed). Polar molecules can form good hydrogen bonds, while non-polar molecules can form only van der Waals bonds. The most important polar molecule is water. Each water molecule can form four good hydrogen bonds to other water molecules. While polar molecules tend to be soluble in water (to various degrees), non-polar molecules are not because they cannot hydrogen bond to water molecules.

Every distinct molecule has a unique molecular shape which restricts the number of molecules with which it can form good secondary bonds. Strong secondary interactions demand both a complementary (lock and key) relationship, between the two bonding surfaces, and the involvement of many atoms. While molecules bound together by only one or two secondary bonds frequently fall apart, a collection of these weak bonds can result in a stable aggregate. The fact that double helical DNA never falls spontaneously apart demonstrates the extreme stability possible in such an aggregate. The formation of such aggregates can proceed spontaneously, with the correct bonds forming in a stepwise fashion (the principle of self-assembly).

The shape of polymeric molecules is determined by secondary bonds. These polymers all contain single bonds about which free rotation is possible. They do not, however, exist in a variety of shapes. This is because, in general, the formation of one of the possible configurations involves a maximum decrease in free energy. This energetically preferred configuration is thus formed

exclusively. Some polymeric molecules have regular helical backbones held in shape by sets of regular internal secondary bonds between backbone groups. Regular helical structures cannot be formed, however, if they place the specific side groups in positions where they cannot form favorable weak bonds. This happens in many proteins where an irregular distribution of non-polar side groups forces the backbone into a highly irregular conformation, permitting the non-polar groups to form van der Waals bonds with each other. Irregularly distributed side groups do not always lead, however, to non-helical molecules. In the DNA molecule, for example, the specific pairing of purines with pyrimidines allows the non-polar aromatic groups to stack on top of each other in the center of the molecule.