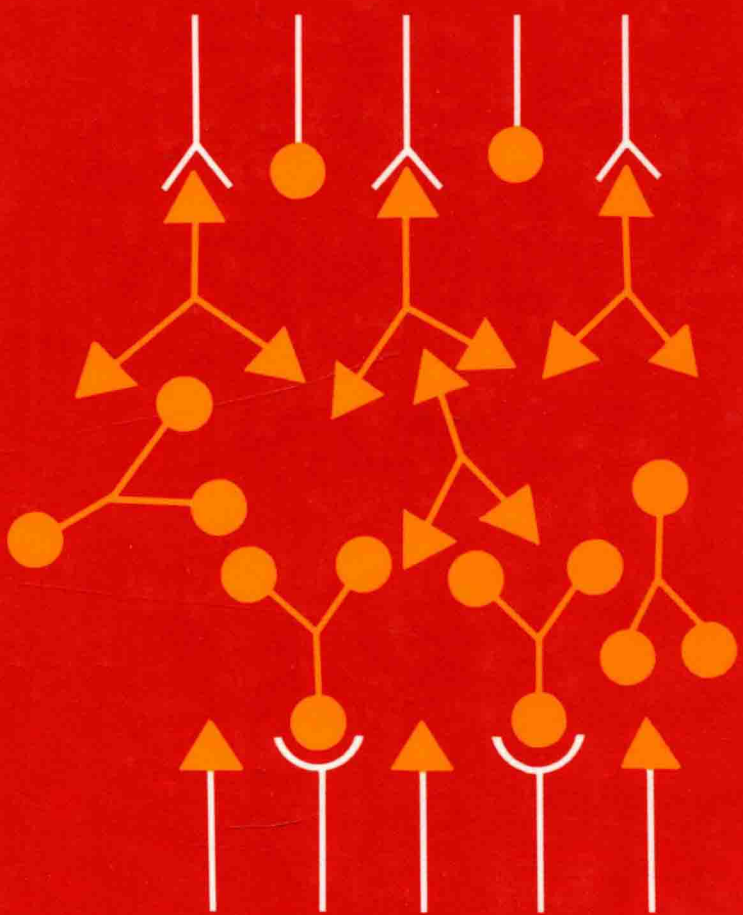
MOLECULAR RECOGNITION MECHANISMS

EDITED BY

Michel Delaage



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Chapter 1

Physico-Chemical Aspects of Molecular Recognition

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Cellular metabolism, the dynamic stability of structures and the development of organisms are all dependent on systems of molecular recognition whose physico-chemical characteristics are in accordance with their physiological role.

Molecular recognition depends on a number of elementary interactions from which are built more complex interactive systems such as an enzyme and its substrate, a hormone and its receptor, antibodies and antigens, etc... Their study allows the pharmaco-chemist to use a rational approach in developing active molecules.

1. Molecular recognition : specificity and information content

Two different scales may be applied to molecular recognition :

- Time scale : in order to speak of recognition, the entities must interact during a relatively long time as compared to the initial collision time. This is generally of the order of at least several microseconds.

- Space scale : the interacting entities represent an association of molecules which maintain their individual structures. Recognition is not the loss of identity even if the ultimate outcome is catalysis, resulting in the irreversible transformation of one of the entities.

Specificity is a term which envelops several different notions. For the chemist, the specificity of a receptor is measured by the sub-group of molecules it recognizes (at a given level of affinity) among a larger ensemble of molecules. For example, among 20 different natural steroids, an antibody or a carrier protein may selectively bind only to testosterone and dihydrotestosterone. According to the formalism of information theory, specificity is measured by the logarithm (base 2) of the ratio of the entire ensemble to the sub-group recognized.

For the biologist, it is more pertinent to define specificity in given situations where the concentrations of the possible ligands intervene. The state of the receptor is defined by a partition function Z, each term of which corresponds to a state. In simple cases, Z takes the form :

$$Z = 1 + Ci / Ki$$

where Ci is the concentration of ligand i and Ki is the dissociation constant.

The constant 1 corresponds to the free receptor. The specificity of the binding of a ligand j, for example, is measured by the logarithm (base 2) of the ratio of the probabilities of binding ligand j rather than an array of other ligands, that is :

$$\frac{Cj / Kj}{Z - 1 - Cj / Kj}$$

Another problem is that of knowing the quantity of information provided by observation of the state of a receptor. From a static point of view, the observation of its free or bound state gives binary information concerning the presence or absence of ligand. A more refined model in which the level of binding is measured may give information regarding ligand concentration. From a dynamic point of view, the receptor transmits variations in hormonal signals to the cell and, in this sense, is a vehicle for information transfer. The role of receptor specificity is to furnish this information faithfully and without distortion. The quantity of information transmitted is irrelevant to receptor specificity and depends only on the variability of the signals.

As defined by the theory of information, the genetic code, as represented by the viral nucleic acids (and which are exploited by the mechanisms of translation) is the basic unit of information. This will not be treated in this course.

On the other hand, sequences which block their complementary sites are, in the full sense of the term, specifically recognized ligands. A large part of this course will be devoted to these (see in particular the chapters by J. Igolen and by C. Helène).

2. Basic elements of molecular recognition

Non-covalent bonds

The bonds implicated in molecular recognition are the same as those which ensure the secondary structure of macromolecules. These are the non-covalent bonds, sadly neglected in chemistry courses, partly due to experimental difficulties and partly to problems of visualization. The possibility of combining within a limited area a large number of non-covalent bonds having complementary elements allows the formation of a specific association whose affinity may be of the same order of magnitude as a covalent bond. To this advantage of specificity may be added that of even more favorable kinetic characteristics since the formation of non-covalent bonds is never hindered by high energy barriers. Classically, the following types of non-covalent bonds may be distinguished :

2.1. Electrostatic bonds

The electrostatic bond is the most familiar and the easiest to evaluate. It contributes enthalpy factors that are easily calculated using Coulomb's law which may be rewritten in the modified form :

$$W = \frac{33.2\ Z_1 Z_2}{D\ r}\ \text{kcal}$$

where Z_1 and Z_2 are the numbers of charges, r is the distance between the charges in Å, and D is the dielectric constant (79 in water, but usually reduced to about 40 in a hydrophobic environment).

The charged functional groups which may be paired are summarized as follows :

	anions	cations
carboxylate	$-COO^-$	ammoniums : $-NH_3^+$, $-NH_2^+$, $-NH^+-$, $-N^+-$
phosphate	$-PO_4^{2-}$	guanidinium : $-NH-C^+(NH_2)_2$
sulfonate	$-SO_3^-$	
sulfate	$-SO_4^-$	metallic : Zn^{++}, Ca^{++}, Mg^{++}, etc...
phenoxide	$\Phi-O^-$	

An important aspect of these bonds is their dependence on pH in the case of ionizable functionalities. This explains, for instance, the affinity changes of transmembrane carriers operating between media of differing pH (see the chapter by D. Sherman).

The essential character of the electrostatic bond is its range since the energy decreases inversely with the distance r. The bond is strengthened if the charges are in a hydrophobic environment and, inversely, the presence of electrolytes weakens the bond energy due to a shielding effect :

$$\frac{33.2\ Z_1 Z_2}{D\ r}\ \exp(-Kr)$$

where K, the Debye-Hückel constant, has a value of 1.25 nm^{-1} for 0.15 M NaCl. Thus, as an example, two unit charges separated by 4 Å in water produce an interaction energy of about 1 kcal / mole, corresponding to a contribution of a factor of 5 to the equilibrium constant. The Debye-Hückel effect is already perceptible at this distance (1).

2.2. Van der Waals interactions

Discovered by Van der Waals in attempting to explain the equation of state of non-ideal gases, this attractive interaction was only interpreted much later as being a function of the dipole moment of molecules which may be :

- permanent (Keesom calculation)
- induced (Debye calculation)
- instantaneous (London calculation)

The sum of these three terms is an enthalpy potential decreasing with distance according to $1/r^6$. It is thus a short range interaction. Van der Waals interactions are difficult to evaluate. In gases, these attain considerable levels (5 kcal/mole) and the interaction between two methylene groups has been evaluated at 0.7 kcal/mole. However, it is probably more realistic to consider much lower values obtained by evaluating the interaction between two plane surfaces in an aqueous medium. The bond energy is thus expressed for a unit surface by :

$$W = \frac{A}{12 \pi r^2} \ J/m^2$$

where $A = 5 \times 10^{-21}$ J in water and r is in meters. For a steroid having a surface of 30 $Å^2$ and separated by 5 Å from a similar type surface in water, W = 22 cal, a very small value (1).

2.3. The hydrogen bond

This is a particular type of dipolar interaction in which one of the dipoles is formed by a bond between an electronegative atom and hydrogen, such that the proton may be approached very closely by an unshared pair of electrons.

Hydrogen tends to draw the atoms it binds closer together. Thus, in O-H···O types of bonds, the O···O distance may vary from 2.45 to 2.85 Å while the distance between non-bonded oxygens is never less than 3 Å.

The intrinsic contribution of a hydrogen bond may attain 5 to 7 kcal and is essentially enthalpy. However, in water, considerably smaller values are concerned since the contribution due to binding with the solvent must be subtracted.

2.4. The " aromatic " bond

This concerns conjugated π systems, the planes of which attract each other when superimposed. This allows for sliding, as is observed in graphite. The potential of this interaction is proportional to $1/r^6$, thus ensuring good definition of intermolecular distances. Its field of application largely surpasses that of aromatic molecules since it also includes heterocycles such as tryptophan and purine and pyrimidine bases whose stacking contributes to the stability of nucleic acids at least as much as the hydrogen bonds between the bases.

Again, this type of binding interaction is difficult to evaluate with precision. As an example, the enthalpy contribution from the stacking of two adenines in water has been evaluated as 8 kcal with a dispersion of 5.3 to 11 kcal depending on the method used to measure.

2.5. Hydrophobic bonds

The stability of binding between hydrophobic molecules is partly the result of Van der Waals interactions but is mainly due to the effects of entropy produced on the water molecules ; a hydrophobic molecule in an aqueous medium restrains the variability of the hydrogen bonding network which binds the water molecules among themselves, entropy thus being diminished locally. When two hydrophobic zones approach each other, water molecules are " liberated ". The resulting increase in entropy helps to stabilize the approach of the hydrophobic zones.

Approaching two methylene groups towards each other contributes up to 0.7 kcal/mole to the binding energy. This contribution is certainly preponderant to that of Van der Waals interactions since it is effective over considerably greater distances.

3. Thermodynamic parameters of a recognition process

These consist of three values, all difficult to calculate :

3.1. The " entropy toll " *

The formation of a complex is necessarily accompanied by a loss of a degree of freedom. The resulting decrease in entropy may be estimated using the classical Sackur-Tetrode equations which allow the entropy of ideal gases to be calculated. The association of 2 atoms represents an entropy loss of the order of 10 cal/°. For two molecules, 20 to 30 atoms may be considered, resulting in a positive contribution to ΔG_0 of +7 to +10 kcal. The free energy of interaction must represent at least twice this value of opposite sign in order to produce an observable reaction.

3.2. Bonding free energy

Non-covalent bonds between two species whose components have been analysed represent the only negative free energy term. The latter compensates for the obligatory entropy value as well as for the positive induced fit value.

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* This term has been borrowed from Claude Hélène.

3.3. Induced fit

The induced fit represents an eventual conformational change imposed upon one or the other of the interacting species as a result of an adjustment between their structures. It may in itself correspond to a loss of conformational entropy. The induced fit is at the heart of the problem, being responsible for the transformation of molecular recognition into an effect : modulation of enzymatic activity, opening of a channel, exposure of reactive functional groups...

To summarize :

$G_0 =$	ΔG_{01}	+	ΔG_{02}	+	ΔG_{03}	< 0
	>0		<0		>0	
	entropy of association		bonding energy		induced fit	

The variation in free energy also depends on enthalpy. The latter influences the variation of the equilibrium constant as a function of temperature :

$$\left(\frac{d \log K}{dT}\right)_p = \frac{\Delta H_0}{RT^2}$$

When, under physiological conditions, the entropy contribution is predominant (hydrophobic bonds), the equilibrium is practically insensitive to temperature. Variations in free energy cover a very large scale of values. In terms of equilibrium dissociation constants it varies from 10^{-3} M, the affinity of carriers, to 10^{-15} M, the affinity of protein inhibitors.

If it is considered that one kcal causes the equilibrium constant to vary by a factor of 5, it is evident that minimal changes in structure may occasion considerable variation in the concentrations of the different species at equilibrium.

4. Multiple bonds and additivity of free energies

The problem may be stated as follows : a ligand is composed of two parts, a - b, interacting with two distinct sites on the receptor. The variations in standard free energy ΔG_{0a} and ΔG_{0b} for the binding of each part is known. As a general rule, the variation ΔG_0 for the binding of the a - b unit is not the sum $\Delta G_{0a} + \Delta G_{0b}$. In the preceding equation, only ΔG_{02} is additive.

The entropy term $\Delta G_{01} = - T\Delta S_{01}$ is not additive. The " entropy toll " is only paid once. However, the number of degrees of freedom lost is not constant from one ligand to another and ΔG_{01} may be greater than each of the corresponding values for a and b. As for the induced fit value, it may be completely different : nil, for example, for ΔG_{0a} and ΔG_{0b} and strongly positive for the species a - b.

Thus :

- in the absence of induced fit

$$\Delta G_0 < \Delta G_{0a} + \Delta G_{0b} < 0$$

- in the presence of induced fit, ΔG_0 increases (algebraically) and may be greater than the sum $\Delta G_{0a} + \Delta G_{0b}$ but generally remains inferior to each of these values. The affinity thus increases as a result of the coupling of the binding sites.

This phenomenon may be profitably used to create powerful pharmacodynamic effects. Thus, DNA intercalating dimers provide an affinity up to 10^5 times higher than that of the monomeric homologues. This increase in affinity is translated almost entirely into an increase of the life-span of the complex (3). The coupling of haloperidol with albumin has allowed us to obtain a ligand capable of binding to several D2 dopaminergic receptors at once (4). Injected into the caudate nucleus of rats, this multiple ligand provokes behavioral perturbations lasting more than eight days, compared to barely three hours for non-coupled haloperidol. The relation between affinity and duration of activity will be clarified below.

5. Kinetic aspects of recognition processes

The lock and key image often used to describe recognition phenomena is the worst possible one ; taken at face value, it means that " successful " encounters would be prodigiously rare, being characterized by a particulary low " statistical factor ", to use the jargon of the kineticists. It is precisely the progressive formation of non-covalent bonds and the configurational multiplicity leading to the final state that ensures that neither " entropy barriers " nor " energy barriers " interferes with the formation of complexes. The second-order rate constant of formation is thus always relatively high, though it never reaches the upper limit of a diffusion controlled process in which all collisions are active.

Several examples :

- Antigen - antibody systems :
. c-AMP : 1.2 - 6.6 x 10^5 M^{-1} sec^{-1} (5)
. vasopressin : 1.7 - 3.1 x 10^7 M^{-1} sec^{-1} (6)
. vinca alkaloid : 0.5 - 2.8 x 10^6 M^{-1} sec^{-1} (7)
. growth hormone : 0.3 - 1.1 x 10^4 M^{-1} sec^{-1} (8)

Other examples showing that rates of association are generally rapid :
the Ti Pti complex : 1 x 10^6 M^{-1} sec^{-1} (9)
the association of leucyl tRNA-tRNA synthetase : 1 - 5 x 10^6 M^{-1} sec^{-1} (10)

- Dissociation rate constants :
The range of dissociation constants is immense, going from 10^4 sec^{-1} for enzymes or the most rapid carriers to 10^{-6} sec^{-1} and less for trypsin-inhibitor complexes (9).