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Enzymes, Drugs and Antibodies, some Chemical Common Factors

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Biological function at the molecular level is fundamentally dependent upon interactions between biological molecules. This is essentially a chemical problem which might be defined by the question: what is the significance of the chemical structure of biological molecules in relation to their activity?

Interactions of Biological Molecules. An approach to this question can be made by considering interactions of three major groups of biological molecules: the enzymes, antibodies and drugs. The function of these molecules is dependent upon reversible interactions of the form:

$$\begin{matrix} A+B \rightleftharpoons (AB) \\ + \\ C \rightleftharpoons (AC) \end{matrix} \qquad (1)$$

$$\text{(a)} \quad \frac{d}{dT} \ln K = \frac{\Delta H^\circ}{RT^2}$$

$$\text{(b)} \quad \Delta F^\circ = -RT \ln K$$

$$\text{(c)} \quad \Delta S^\circ = R \ln K + \frac{\Delta H^\circ}{T}$$

$A = \begin{cases} \text{Enzyme} \\ \text{Antibody} \\ \text{Drug (or hormone)} \end{cases} \qquad B = \begin{cases} \text{Substrate} \\ \text{Antigen} \\ \text{Enzyme (or receptor)} \end{cases}$

$(AB) =$ Molecular complex.

Since ideally these are equilibrium processes to which thermodynamic criteria apply, one can employ the usual equations (1 a, b) relating the equilibrium, constant K, to the standard enthalpy (ΔH°) and free energy changes (ΔF°). The free energy change, which is quite critical since it determines the degree of interaction between A and B, depends upon structural factors governing size, shape, conformation and energy content of A and B. The entropy contribution (ΔS°) to the free energy change as expressed by equation 1 c, is also important. It is characteristic of biological interactions that they are quite strong (ΔF° −5 Kilocals. per mole), at the same time they are reversible and to a considerable degree specific. As a consequence competitive interaction by molecular species C may take place giving rise to equilibrium complexes of the type (AC) with their corresponding free energy changes.

Enzymes. Enzymes and their interactions will first be considered with particular reference to two major groups of enzymes: The esterases (hydrolysing enzymes) and the oxidases (oxidizing enzymes). The esterases characteristically possess no co-enzyme group. They include the enzymes ribonuclease, chymotrypsin, trypsin and the cholinesterases. The oxidases (including the dehydrogenases) possess co-enzyme groups on which their oxidative activity depends. Two examples of oxidases are monamine oxidase and lactic dehydrogenase.

Enzymes are globular proteins consisting of helical[1] peptide chains held in the form of a loose coil by disulphide links. This coil is further folded in a highly specific manner to complete the tertiary structure of the protein. The tertiary structure is maintained in the folded condition by interactions involving aromatic side-chains (for example of tyrosine, tryptophane and histidine) within the molecule. These interactions may take the form of hydrogen bonds with carboxyl groups[2] but dispersion forces between aromatic side chains may contribute significantly to bonding[3–5]. The tertiary structure is dynamic in the sense that it can open, unfold and refold in response to the demands of environment or function. This process of 'reversible denaturation' may be represented by the intramolecular equilibrium $N \rightleftarrows D$, where N is the normal (folded) state of the protein and D is the reversibly denatured (unfolded) form. Thermodynamic considerations show that equilibria of this type are characterized by large positive entropy changes. In this respect the intramolecular interactions which govern tertiary folding of a protein resemble the specific interactions between biological molecules, and similar binding forces may be involved. In reversible denaturation the position of equilibrium is influenced by agents such as urea, urethane, the barbiturates and salicylic acid, which cause unfolding of the tertiary structure[6,7]. Phosphate, pyrophosphate

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[2] S. J. LEACH and H. A. SHERAGA, J. biol. Chem. *235*, 2827 (1960).
[3] E. J. WILLIAMS and J. F. FOSTER, J. Amer. chem. Soc. *81*, 865 (1959).
[4] S. YANARI and F. A. BOVEY, J. biol. Chem. *235*, 2818 (1960).
[5] W. KAUZMANN, in Advanc. Protein Chem. *14*, 54 (1959).
[6] F. H. JOHNSON H. EYRING, and M. J. POLISSAR, *The Kinetic Basis of Molecular Biology* (John Wiley & Sons, New York 1954), p. 284.
[7] M. L. ANSON and A. E. MIRSKY, J. gen. Physiol. *17*, 399 (1934).

and certain other ions appear to restore equilibrium in favour of the folded form of the protein[8]. These effects are particularly important in enzymes (for example trypsin), the activity of which is often critically dependent upon retention of folding[9].

The Action of Enzymes. An enzyme (E) acts by combining with a substrate (S) yielding a complex (ES) which breaks down into E and products (P):

$$E+S \rightleftharpoons (ES) \rightarrow E+P \qquad (2)$$
$$+$$
$$I \rightleftharpoons (EI)$$

The degree of interaction of E with S, or the affinity between them, is the determining factor in the first stage of the enzymic process. This is usually expressed by the Michaelis-Menten kinetic constant (Km) which, with certain approximating assumptions, may be treated thermodynamically as the enzyme-substrate equilibrium constant (K^{-1}). Enzyme-substrate interaction is a specific process which takes place at the *active site* of the enzyme. Most enzymes have only one active site and in that sense are univalent.

The Active Site. Interaction between substrate and enzyme at the active site in an esterase is depicted in Figure 1.

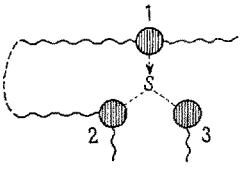

Fig. 1. Amino acid side-chains: 1 = Attacking group, 2 = orienting group, 3 = activating group, S = substrate molecule.

Three amino-acid side-chains (1, 2 and 3) located at different points in the enzyme molecule co-ordinate the substrate (S) in a specific manner by tertiary folding of the protein chain. This 'active' configuration may exist in the enzyme quite independently of the presence of substrate, although the substrate may impose its own requirements on the active site when interaction takes place. If the active configuration is unfolded in the presence of a denaturing agent, specific interaction between enzyme and substrate will not be possible, and the enzyme is inactivated. The amino-acid side-chains 1, 2 and 3 all contribute to the binding affinity of S for the enzyme. In addition they appear to have rather different but complementary functions. Thus the side-chain 1 may attack the substrate, 2 may orientate the process, and 3 may provide activation. Considering the active site of chymotrypsin, the attacking group, 1 is believed to be the iminazole side-chain of histidine (which is strongly nucleophilic), group 2 is a serine hydroxyl, while group 3 may be a tryptophane (or a tyrosine) residue. The interaction of an acyl substrate with these three groups at the active site of chymotrypsin is shown in Figure 2.

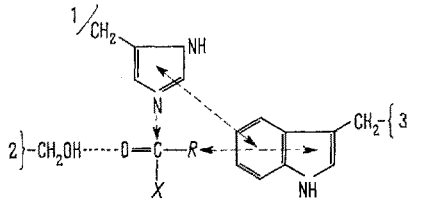

Fig. 2. 1 = Histidine residue, 2 = serine residue, 3 = Tryptophane (?) residue.

The double arrows indicate dispersion (or non-classical) interactions between aromatic residues. A considerable amount of indirect evidence indicates that the esterasic active sites of trypsin, the cholinesterases and certain other enzymes utilize this same combination of histidine and serine with possibly tryptophane (or tyrosine) as the third active residue[10,11].

Inhibition of Enzymes. Competitive inhibition of an enzyme (E) by an inhibitor (I) may be represented by equilibria (2) involving an enzyme-inhibitor complex (EI). The enzyme-inhibitor equilibrium is assigned its own characteristic equilibrium constant ($K_I = K^{-1}$). This is a reversible process in which the inhibitor competes with the substrate for the active site of the enzyme. In general, the inhibitor has a high affinity for the enzyme and is not degraded by it. Competitive inhibition is essentially a physical process which differs from the irreversible inhibition brought about by substances which block the active site of an enzyme by chemical combination. Aromatic carboxylic acids are good competitive inhibitors of chymotrypsin[12]; β-phenyl propionic acid (I) being particularly effective. The low values of the inhibitor equilibrium constant in this case ($K_I = 4.5 \times 10^{-3}$) indicates that the inhibitor is bound quite strongly but reversibly to the active site of the enzyme probably in a similar manner to an ester substrate. This is represented in Figure 3, in

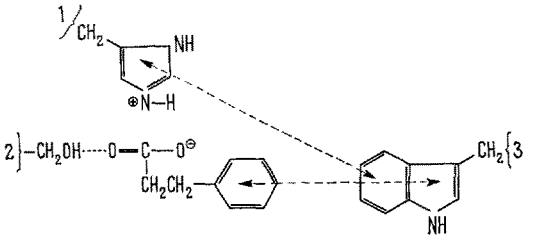

Fig. 3.

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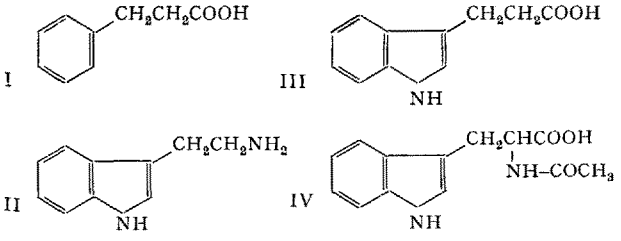
[9] J. I. HARRIS, Nature *177*, 471 (1956).
[10] D. R. DAVIES and A. L. GREEN, Advanc. Enzymol. *20*, 284 (1958).
[11] E. A. BARNARD and W. D. STEIN, Advanc. Enzymol. *20*, 51 (1958)
[12] H. NEURATH and G. W. SCHWERT, Chem. Rev. *46*, 123 (1950).

which β-phenyl propionic acid is bound by histidine-carboxylate interaction, hydrogen bonding to serine, and aromatic dispersion forces to the indole side-chain of tryptophane.

Chymotrypsin is also inhibited by indoles notably tryptamine (II) and its derivatives[13] which may inhibit the enzyme by displacing the tryptophane side-chain from co-ordination at the active site. Such a process would represent inactivation by specific denaturation. Strong competitive inhibitors of chymotrypsin are obtained by combining both carboxyl and indole functions in β-indolyl propionic acid (*III*) and N-acetyl-tryptophane (IV)



Antibodies. Antibodies are soluble proteins of high molecular weight which are associated with the globulin fractions of immune sera. Less is known about their chemical nature than about crystalline enzymes, since antibodies are difficult to isolate as homogeneous substances. The approximate amino acid compositions of several antibody preparations reported[14] appear to conform quite closely to the composition of the serum γ-globulins almost irrespective of the nature of the antigen; tryptophane, tyrosine and histidine being commonly present. Antibody molecules possess in some degree the tertiary coiled and folded structure which is present in the enzymes. The degree of folding in the tertiary structure may be an important factor in determining specificity in the antibody molecule.

The Action of Antibodies. An antibody (A) acts by combining with its antigen (S), yielding an antigen-antibody complex (AS):

$$A + S \rightleftarrows (AS) \rightarrow P \qquad (3)$$
$$+$$
$$I \rightleftarrows (AI)$$

When S is polyvalent, as in the normal precipitin reaction, the complex (AS) may take the form of a precipitate of very high molecular weight. On the other hand (AS) may be a soluble complex, if S is a univalent hapten, or in the case where (A) is a low molecular weight, 'non-precipitating' antibody. In some circumstances (AS) may undergo chemical breakdown into products (P), regenerating (A) as in the enzymic process. An example of this would be the induced breakdown of a toxin (S) by its antitoxin (A). Antibody interactions show a remarkable similarity to enzyme interactions in several respects.

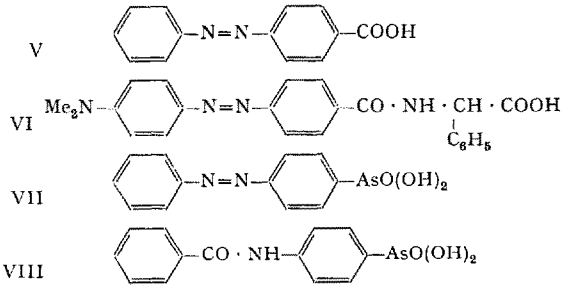
Thermodynamic Considerations. The thermodynamics of antibody interaction at active sites has been studied using diverse antigens (including ribonuclease) and haptens[15]. The data are rather typical of interactions of biological molecules in general[5,16]:

$$\Delta F^\circ \sim -5 \text{ Kilocals. per mole}$$
$$\Delta H^\circ \sim 0 \text{ to } +2 \text{ Kilocals. per mole}$$
$$\Delta S^\circ \sim +20 \text{ E.U.}$$

The large positive entropy change may indicate some unfolding of the tertiary structure brought about by interaction with antigen[5,15,17].

The Active Site. The binding of antigen to antibody is due to specific interaction which takes place at active sites in the antibody molecule. There are two active sites in the antibody molecule which is therefore bivalent. These sites appear to be identical. The highly specific nature of the interaction between antibody and natural antigen (S) is recognized. Where S is a hapten interaction is less specific.

Interaction between antibody and antigen appears to be primarily an acid-base effect, in which a basic, nucleophilic group at an active site in the antibody molecule combines reversibly with a carboxylic acid group in the antigen. In the case of antibody-hapten interaction, the acidic group is provided by the hapten and may be carboxyl, arsonic or other acidic function:



Typical haptens.

The nature of the basic group at an antibody active site has given rise to some speculation. The suggestion has been made that this group is the iminazole side-chain of histidine[18]. A tryptophane or tyrosine residue

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[14] R. R. Porter, in F. Putnam, *The Plasma Proteins* (Academic Press, 1960), p. 251.
[15] S. I. Epstein, P. Doty, and W. C. Boyd, J. Amer. Chem. Soc. *78*, 3306 (1956).
[16] R. F. Steiner, Arch. Biochem. Biophys. *53*, 457 (1954).
[17] M. Winkler and P. Doty, Biochim. biophys. Acta *54* 448 (1961).
[18] J. H. Turnbull, Exper. *15*, 304 (1959). – M. E. Koshland, F. M. Engelberger, M. J. Erwin, and S. M. Gaddone, J. biol. Chem. *238*, 1343 (1963).

may also be involved. On this basis interaction between the active site of antibody and the combining group of antigen may be depicted in Figure 4, which is analogous to the proposed interaction between esterase and substrate or inhibitor (Figures 2 and 3).

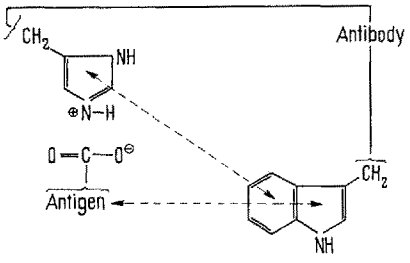

Fig. 4

Inhibition of Antibody Interaction. Antibody systems resemble enzymes in showing competitive inhibition by inhibitor molecules (*I*). In the precipitin reaction, for example, an inhibitor can prevent formation and precipitation of the antigen-antibody complex by competing with the antigen for the antibody active sites. Carboxylic acids are particularly effective inhibitors, consistent with the view that the specific combining groups of antigens are carboxyl groups. From the analogy between the active sites of antibodies and the active sites of esterases, the strongest competitive inhibitors of, for example, chymotrypsin should effectively inhibit the precipitin reaction. This is evident from the data of KLEINSCHMIDT and BOYER[19], who showed that the ovalbumin-antiovalbumin system was strongly inhibited by β-phenyl (I) and β-indolyl propionic acids (III) and N-acetyl tryptophane (IV). The precipitin reaction is also inhibited by certain bases[19] which compete with the antibody sites for the carboxyl group of the antigen. One of the most effective of these inhibitors is histamine itself, which appears to simulate the antibody active sites. This is particularly relevant in the light of the suggestion that histidine side-chains may be the basic combining groups in antibodies.

Another significant phenomenon is the well-known inhibition of the precipitin reaction by excess antigen[20]. This shows an interesting resemblance to the inhibition by excess substrate, which occurs in the cholinesterase[12,21] and in certain flavin systems[22]. The effect may be due to the formation of multiple complexes, and it is conceivable that quenching of active states is involved.

Drugs. Drugs in general are small molecules of very diverse structure designed to produce a determined physiological response in the organism. Hormones are internal factors with a similar function. Many drugs exert their effects by interaction with enzymes controlling physiological responses. This is represented by the equilibrium

$$E+S \rightleftharpoons (ES) \rightarrow E+P \qquad (4)$$
$$+$$
$$D \rightleftharpoons (ED) \not\rightarrow E+P'$$
$$(S = \text{Hormone})$$

The interaction of the drug D with the enzyme is competitive with the substrate (S). In the case where S is a hormone which itself regulates function, D can influence the process indirectly. Cholinesterase interactions provide a familiar example of this process. Here S is the neurohormone acetyl choline which acts at the neuromuscular junction. The transient level of free acetyl choline is regulated by E, the cholinesterase. The drug D (for example eserine or prostigmine) interacts competitively at the active site of the enzyme causing inhibition, this allows acetyl choline to accumulate and block the response.

The active site of cholinesterase is believed to consist of two centres A and B. The A centre has a high affinity for positively charged groups. The B centre appears to function by means of an active histidine combination, in this respect it closely resembles the active site of chymotrypsin. The interaction of the inhibitor prostigmine with the active site of cholinesterase is represented in Figure 5.

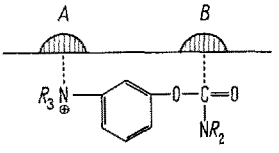

Fig. 5.

Inhibition of controller enzyme systems by drug-enzyme interactions also accounts for the effect of psychotomimetic agents on the central nervous system. Some of these drugs (notably the hydrazides) inhibit the brain monamine oxidase system which controls the level of transmitter amines serotonin and noradrenalin. Although at present little is known about the structure of the active site of monamine oxidase, these drugs most probably inhibit by blocking the

[19] W. J. KLEINSCHMIDT and P. D. BOYER, J. Immunol. *69*, 247 (1952).
[20] W. C. BOYD, *Fundamentals of Immunology* (Interscience, 1956), p. 305.
[21] D. H. ADAMS and V. P. WHITTAKER, Biochim. biophys. Acta *4*, 547 (1950).
[22] L. R. TETHER and J. H. TURNBULL, Biochem. J. 85, 517 (1962).

oxidation-reduction system of a nucleotide co-enzyme group at the active site of the enzyme. If histidine and tryptophane side-chains are associated with the co-enzyme group at the active site (as may be the case in lactic-dehydrogenase[23]) inhibition of the system might also occur with esterase inhibitors. It is particularly interesting to note, in this connection, that indole derivatives such as the drug bufotenine inhibit both monamine oxidase and chymotrypsin, while local anaesthetics of the procaine type combine central nervous effects with antagonism of acetyl choline at the neuromuscular junction[24].

Drugs in general are not highly specific agents. Their ability to interact with enzymes suggests that similar interactions with active sites of antibodies may be an important factor in drug therapy in relation to immunity. It is probably true to say that this is recognized in clinical practice today, where suggestive evidence of drug-antibody interaction is found in the role of cortisone and the salicylates in arthritis; in the mechanism of histamine release of drugs; and in the causal relationship which emerges between central nervous disturbances and allergic reactions.

The basic concept of the molecular complex (AB) in equilibrium with the interacting biological molecules A and B refers to molecules in the ground state. In some circumstances one may have to take account of interactions in the excited state. Suppose, for example, that a drug molecule A interacts with an excited enzyme or receptor molecule B^x, to give a complex $(AB)^x$. If interaction permits energy transfer within the complex, the drug molecule A may be raised to the excited state, leaving the enzyme or receptor molecule in the ground state (B). The result is in effect a deactivation of the molecule B by the drug:

$$A + B^x \rightleftarrows (AB)^x \rightarrow A^x + B$$

This idea is attractive since it could account for the phenomenon of desensitization of receptors by stimulant drugs and related effects in pharmacology[25].

In conclusion then, molecular interactions between enzymes, drugs and antibodies are well-established, and the biological significance of these interactions is becoming clearer. The physical nature of the interaction between biological molecules is still obscure. This is a problem which quantum chemistry applied to energy transfer between biological molecules may be able to solve within the next decade.

Zusammenfassung. Die biologische Tätigkeit von Enzymen, Antikörpern und Pharmaka auf molekularem Niveau wird in bezug auf umkehrbare Molekularwechselwirkungen beschrieben. Diese Prozesse scheinen charakteristische thermodynamische Parameter zu haben, öfters von deutlichen Strukturähnlichkeiten begleitet. Molekularwechselwirkungen, welche in angeregten Zuständen auftreten, können offenbar bedeutend sein.

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Brèves communications – Kurze Mitteilungen – Brevi comunicazioni – Brief Reports

Zur Biochemie autoxydabler, H_2O_2-bildender Verbindungen

Vor einiger Zeit wurden von uns zusammen mit Lehmann et al.[1] Untersuchungen über die Hemmung der «Morphogenese» durch α-Aminoketone durchgeführt. Einige dieser Aminoketone zeigten hierbei eine starke Hemmwirkung in einem Regenerationstest und führten bei lokaler Anwendung, wie Sträuli[2] fand, zu Tumorregressionen.

Wir versuchten seinerzeit abzuklären, welches chemische Verhalten die Ursache der morphostatischen Wirkung dieser Verbindungsklasse ist. Es ergaben sich hierbei Anhaltspunkte dafür, dass nicht eine auf Grund der Strukturähnlichkeit zu vermutende Antimetabolitwirkung zu Aminosäuren für diese Wirkung verantwortlich ist, sondern dass vielmehr die biologische Aktivität dieser Verbindungen mit ihrer Fähigkeit zur Autoxydation verbunden ist[3]. Es wurde festgestellt, dass die Hemmwirkung im Regenerationstest mit zunehmendem pH der wässrigen Lösung der Aminoketone anstieg[3]. In solchen schwach alkalischen Lösungen bildeten sich unter O_2-Aufnahme nicht näher identifizierte Peroxide, aus welchen z. B. mit Katalase O_2 freigesetzt wurde.

[1] H. Erlenmeyer und F. E. Lehmann, Exper. *12*, 472 (1949). – F. E. Lehmann, A. Bretscher, H. Kühne, E. Sorkin, M. Erne und H. Erlenmeyer, Helv. chim. Acta *33*, 1217 (1950).
[2] P. Sträuli, Oncologia *12*, 143 (1959).
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