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Abstract

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FUNDAMENTALS OF THE CHEMISTRY OF ENZYMES AND ANTIMETABOLITES FROM THE STANDPOINT OF THE THEORY OF CATALYSIS

1. Enzymes are colloidal, microheterogeneous catalysts; the theory of catalysis has been applied to them at various stages of its development. It is shown below that the multiplet theory ([1]) is capable of explaining especially characteristic properties of enzymes—their high selectivity and activity. In applying the multiplet theory, one must first of all identify in the reaction its index group, i.e., those atoms that react and, consequently, come into contact with the atoms of the catalyst (in the sequence found by isotope methods ([1])). Table 1 gives the types of enzymes and their indices; from it it is evident that most enzymatic reactions have a doublet or triplet index and that, as a rule, different types of enzymes correspond to different indices. In the indices, −C differs from =C, and therefore the indices, for example, for esterases and carbohydrases are different. The indices in Table 1 naturally fit into the multiplet classification, with which, accordingly, the classification adopted ([2]) in enzymology is in agreement. Classification by indices makes it possible in places to improve the existing classification; for example, aspartase, with its index

$$\begin{matrix} C & C \\ H & N \end{matrix}$$

should be excluded from the amideases with their index

$$\begin{matrix} C & O \\ N & H^{\cdot} \end{matrix}$$

In dehydrogenases, the detached H restores another molecule, for example indigo, and the reactions are triplet, as is the decomposition of H_2O_2.

The methods of consideration in multiplet theory and in enzyme chemistry turn out to be analogous; the indices of Table 1 correspond to the active groups of substrates in enzyme chemistry, or to pharmacodynamic groups; the principal valences participate in them.

2. Each type with the same index includes up to a dozen enzymes of narrower selectivity; this occurs as a result of the influence of extra-index substituents (see §§ 3-4).

3. As in catalysis, in enzymatic reactions substituents influence the energy of the reacting bonds that are included in the index. Thus, the introduction of a methyl group next to the group $> C = O$ increases the energy of this bond by 7 kcal. From the equations of the multiplet theory it follows that, if the bond $> C = O$ is in the index, and next to it, instead of CH_3, H is the substituent, and if at the same time the bond energy of C with the catalyst does not change, then the energy of formation of the multiplet complex decreases by 7 kcal, while the activation energy of the reaction decreases by

$$\frac{3}{4} \cdot 7 = 5.2 \text{ kcal}.$$

Then, according to the Arrhenius equation, the reaction-rate constant increases 4680-fold at 37°C ($\lg 4680 = 5200/4.57\,(273 + 37)$). The energetic influence of substituents consists in the effect of electron displacement on the bond energy in the index.

4. A finer adjustment is provided by the structural correspondence of substituents. This is indicated by the heterogeneous catalysis of optically active substances. A complex of a doublet index group is optically inactive by its symmetry; meanwhile, an optically active catalyst deposited on an optically active support (for example, metal—quartz) selectively accelerates

catalyzes the reaction of one optical antipode from their mixture[3]. Hence we conclude that the asymmetric action of a catalyst or enzyme is concentrated not in the reacting group—the index—but in the non-index substituents, when they are superposed on the carrier near the active center as a result of molecular adsorption. This agrees with the conclusion of Klabunovskii and Patrikeev that the adsorption stage is dissymmetric, whereas the catalysis stage is symmetric[3].

Table 1

Types of enzymes and their indices*

1. **Esterases**, for example (O) = CO/OH cholinesterase, PO/OH phosphatases, SO/OH sulfatases.

2. **Carbohydrases**, for example (C) – CO/OH maltase.

3. **Carbohydrate-metabolism enzymes**, for example –CO – (P)/OH phosphorylase.

4. **Nucleases**, for example CO/NH nucleosidases.

5. **Amidases**, for example CO/NH urease.

6. **Proteolytic enzymes**, for example (O) = CO/NH pepsin.

7. **Oxidative enzymes containing Fe**, for example H · O.O · H catalase, H · O.O · H peroxidase, H · O : O/C : C · H oxidase of dioximaleic acid (complex index), cytochrome C.

8. **Oxidative enzymes containing Cu**, for example CO/HO, etc., tyrosinase.

9. **Dehydrases** H · CO/A.B · H.

10. **Enzymes reducing cytochrome C.**

11. **Yellow enzymes** H · CO/A.B · H.

12. **Nucleindeaminases**, for example CO/NH, etc., guanase.

13. **Various oxidases**, for example C.O/CO lipoxidase, H · O : O/C.O · H fatty-acid dehydrase.

14. **Desmolases**, for example C.O/CH decarboxylase, C.O/OH carbonic anhydrase.

15. **Hydratases and mutases**, for example CO/CH fumarase, CS/OH coglyoxalase.

* Dots are bonds. In a doublet reaction, two vertical bonds pass into two horizontal ones. Some characteristic substituents are placed in parentheses.

In order for such superposition of substituents with their large van der Waals atomic radii to be possible, there must be a sufficient cavity near the active center, on which the atoms of the index have smaller valence-chemical radii. The existence of such cavities is confirmed in the study of the catalytic dehydrogenation of molecules of complex form—unsym. diphenylethane, various secondary alcohols—and the hydrogenation of triptycene derivatives.

The nature of the cavities is explained by the fact that the carrier, the apoenzyme, is a molecular, protein crystal. In a molecular crystal the molecules are in the densest packing, so that the protrusions of one molecule enter the recesses of a neighboring one, being held by van der Waals forces and also by hydrogen bonds, while the bonding hydrogen does not occupy any special place. If one molecule is removed from the surface, a cavity is formed that, to the greatest possible degree (at the points of contact), repeats the shape of the removed

Fig. 1

Figure 1: Fig. 1

molecule. A molecule of another kind can fit into such a cavity, entering the cavity, a part of which is identical or close in shape to that of the removed one. Such surface isomorphism recalls epitaxy[4] (oriented intergrowth of crystals of two substances), but differs from it in that, owing to free rotation about bonds,

C—C and other molecules in a recess on the surface may assume a form different from that required for the construction of a new solid phase during epitaxy.

In catalysis by metals at high temperatures, non-index substituents are oriented perpendicular to the surface of the catalyst; whereas over oxide catalysts, when H-bonding is possible, the carbon chains of the substituents are arranged parallel to the surface (cf. experiments on the dehydrogenation of alcohols on an oxide chromium catalyst and the adsorption of alcohols by aluminosilicates).

Fig. 1

Figure 1 gives a scheme of the energy levels of molecules in solution (*I*), of molecules at an active center without adsorption of non-index substituents on the carrier (*II*), and of the same molecules, but with adsorption of the latter on the carrier (*III*). The energy barrier *I*—*II* is greater than *I*—*III*, and therefore in *I*—*III* the reaction is accelerated. If the adsorbed non-index substituents do not fit completely into their recess on the surface, then, owing to the increase in the distance between the molecules, the heat of adsorption *II*—*III* decreases. The reaction rate will be greatest when the difference between the levels *II*—*III* is maximal, which occurs when the adsorbed part of the molecule fits exactly into the recess. This model explains, in principle, the high selectivity of enzymes. An example of the latter may be the ability of cathepsin I of the spleen, acting on amino acids, to hydrolyze peptide chains according to the scheme:

$$\ldots \mathrm{CO{-}NH \cdot CHR \cdot CO{-}NH \cdot CHR} \ldots + \mathrm{HOH} = \ldots \mathrm{CO{-}NH \cdot CHR \cdot CO \cdot OH} + \mathrm{H_2N \cdot CHR} \ldots$$

(the atoms of the index group are shown in boldface), only on condition that R is $\mathrm{HO{-}}\langle\!=\!\rangle\mathrm{CH_2{-}}$ or $\langle\!=\!\rangle CH_2{-}$. Urease hydrolyzes urea, but not butylurea—the group C_4H_9 is unable to fit where H fits.

In refining the theory, one should take into account the possibility of motion of oriented-adsorbed molecules (the ensemble of which resembles a surface liquid crystal) along the surface.

Over many years of natural selection, an especially exact structural correspondence has been established in enzyme chemistry between apoenzymes and substituents.

Fig. 2

Figure 2: Fig. 2

In homogeneous catalysis, a molecule of the starting substance may form such a molecular compound with the catalyst that the molecules of both in the complex are in contact not only through the reacting atoms, but also through others. This explains the greater rate of reaction of one of the antipodes with the corresponding optically active catalyst.

Molecular adsorption of substituents at the index–surface isomorphism–also provides an explanation of the especially high rate of enzymatic reactions. The molecule is held on the surface of the enzyme in the position required for the reaction (entropic factor). Adsorption of non-index substituents reduces the energy barrier of the reaction and increases the heat of adsorption (energetic factor). Estimating the energy of an H-bond at 7 kcal (on average) from a calculation similar to that given above, we find an acceleration of the reaction by a factor of 4680. Molecules adsorbed by non-index groups prove to be pressed more strongly by their index atoms against the active centers of the catalyst-enzyme, which is analogous to the action of high pressure.

An example of an intermediate complex is given in Fig. 2. In it the substrate (*I*), the protein part of the enzyme (*II*), and the coenzyme (*III*) must be in contact with one another, fitting well to one another with protrusions opposite recesses. In Fig. 2, for simplicity, all atoms are taken to be identical and no distinction is made between valence-chemical and van der Waals radii. Thus the multiplet theory explains Fischer' s "lock-and-key" principle.

5. Enzyme inhibitors act at different stages of the reaction. Strongly adsorbed substances (Hg, HCN, S, etc.) block the active centers of various enzymes independently of their structure. Conversely, antimetabolites can poison, out of hundreds of enzymes in a single cell, only one, because their side chains are adsorbed on structurally similar recesses of the protein part of enzymes. Thus, an antimetabolite must have a structure of the group adsorbed on the protein part of the enzyme close to the structure of the substrate, a stronger adsorbability in the index group, but it may contain various substituents (sulfanilamide preparations). The structure of the poisoning group also must not differ greatly from the structure of the substrate' s index group, so that it can fit into the active center of the enzyme. Thus, in *p*-aminobenzoic acid and in white streptocide the larger parts of the molecules are identical ($NH_2C_6H_5$—). The differing groups (—COOH and —SO_2NH_2) are structurally similar, and their sizes are close (Fig. 3), while —SO_2NH_2 is adsorbed more strongly.

Fig. 2

Our theory of antimetabolism shows that the theory treating an antimetabolite as a heterogeneous catalytic poison (Woolley ([5])), and the theory pointing to

Fig. 3

Figure 3: Fig. 3

a parallelism between antimetabolism and epitaxy (Erlenmeyer ([6])), do not exclude but complement one another.

Fig. 3

Our theory also explains why selective enzyme poisons are often prepared from the same enzymes by weak chemical treatments. In this case the structure of the apoenzyme molecule is preserved, but the molecules acquire a more strongly adsorbed group that is incapable of transformation. Such molecules of "weakened" enzymes especially readily block the surface of the original enzymes, because there is a structural correspondence between the molecules of the antibody and the original enzyme. Such should be the principle for obtaining immune bodies.

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Note: Figure translations are in progress. See original paper for figures.