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Chemical Specificity in Biological Systems

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THE importance of specificity in biology has been mentioned or implied in a great many contributions presented in this volume, and many examples have been discussed. It is worthwhile, however, to examine the concept of specificity by itself, and it is advisable to review some of the more important physical and chemical factors that are currently believed to be involved in the phenomenon.

In this paper are considered some typical examples of biological specificity involving more or less well-defined chemical systems as one of the specific interacting agents. The molecular basis of specificity is discussed with special emphasis on the concept of complementarity. Finally, some words are devoted to a review of the important and old-fashioned idea of specificity as a form of organization.

Serological specificity was defined by Landsteiner[1] as "the disproportional action of a number of similar agents on a variety of related substrata," and this definition is readily extended to other fields in which biological specificity manifests itself. Boyd[2] points out that this definition "would also include many chemical reactions, as might be expected if serological specificity were fundamentally chemical in nature, which we now believe," and again there is no need to hesitate in applying the remark to biological specificity in general. Indeed, specificity is an important aspect of chemistry; qualitative and quantitative analysis in organic and inorganic chemistry depends in a large degree upon "disproportionate actions" of similar agents on "related substrates" (e.g., the precipitation of silver halides in water and the separation of silver chloride from the bromide and iodide by treatment with ammonia, H_2O and NH_3 being regarded as similar agents and the silver halides being related substrates).

It is important to emphasize at the outset, too, that biological specificity is very variable in degree. Certain systems show a wide range of specificity whereas other systems show extremely narrow selectivity. What is likely to impress the chemist about biological specificity is the sharpness with which closely similar substances can be distinguished in some instances, but this sharpness is not at all universal.

It is worth mentioning that the concept of specificity is involved in the actions of inhibitors as well as in those of normal substrates, since many closely related biological systems are poisoned to decidedly different degrees by substances having similar chemical compositions. The drug industry thrives on this fact—as does mankind in general.

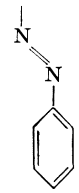
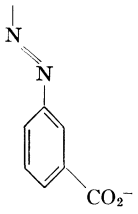
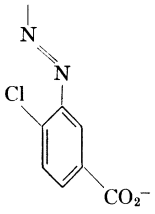
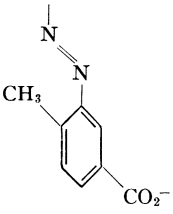
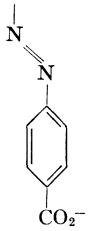
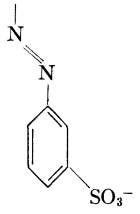
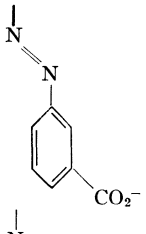
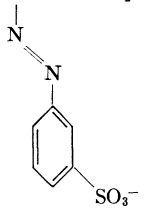
EXAMPLES OF CHEMICAL SPECIFICITY IN BIOLOGICAL SYSTEMS

1. Antibody-Antigen Interactions

Because of the pioneering work of Landsteiner,[1] the field of immunochemistry provides one with probably the most easily understood examples of biological specificity, and it has made possible a fruitful method of attack on the relationship between specificity and chemical structure. When certain foreign substances (antigens) are introduced into an organism, the organism reacts by producing proteins (antibodies) which are capable of reacting specifically with the antigens that gave rise to them. Antigens are invariably substances of rather high molecular weight, so that it is difficult to alter their chemical composition in a systematic fashion. Landsteiner was able to show, however, that new antigens may be prepared by combining small chemical groups (such as substituted aromatic rings) with proteins. These new antigens give rise to antibodies whose specificity depends on the nature of the conjugated groups. The small chemical group is called a hapten. Let w, x, y, z represent a series of chemically related haptens, let Aw, Ax, Ay, Az represent antigens produced from protein A by conjugation with these haptens, and let Bw, Bx, By, Bz represent the antibodies arising from injection of Aw, Ax, Ay, Az into a rabbit. It is clear that a great deal might be learned about the chemical factors involved in immunological specificity by comparing the interactions of the different antibodies with the different antigens. Furthermore, it is found that a low molecular-weight compound, aw, containing the hapten group w, is able to inhibit the interaction between Aw and Bw. Evidently, Bw contains sites which are capable of reacting with the hapten groups on Aw, and the haptens in aw can compete for these sites. The study of this competition using a series of related haptens makes possible a more quantitative study of antibody-antigen specificity.

Some typical results of this general approach are reported in a paper by Landsteiner and Lampl.[3] Metaaminobenzoic acid and metasulfanilic acid were coupled by diazotization of the amino group to the proteins of horse serum, and antibodies to these modified proteins were obtained by injection into rabbits. The resulting rabbit antisera then were mixed with samples of chicken sera which had been diazotized to aniline or to various aniline derivatives. Evidence for interaction consisted in the formation of a precipitate. (The use of horse serum in the production of antibodies and of chicken serum in the antibody-antigen test

TABLE I. Effects of structural changes in the hapten on antibody-antigen interaction.[a]

Hapten present on horse-serum antigen used in preparing antisera.	Hapten present on chicken-serum antigens used to test interaction with antisera.					
						
	0	+++	++++	+++	0	+
	0	0		0	0	++++

[a] 0 = no precipitate; +, +++, ++++ = increasing amount precipitate.

eliminates any contribution of the nonhapten portion of the antigen to the antibody-antigen interaction.) The results are shown in Table I. It is seen that:

1. No interaction will occur if an acidic group is not present on the hapten of the chicken-serum antigen.
2. No interaction will occur unless the acidic group is meta to the amino group (i.e., CO_2^- and SO_3^- must be meta to the point of coupling of the hapten to the protein).
3. Replacement of a hydrogen atom on the benzene ring by a chlorine atom or a methyl group has little effect on the interaction.
4. The antibodies are readily able to distinguish between a carboxyl group and a sulfonic-acid group on the antigen.

On the basis of many experiments of this type, it could be concluded that the nature of the charged groups of the hapten is of decisive importance in determining the specificity. Haptens containing sulfonic-acid groups do not tend to compete effectively for sites on antibodies which are generated by carboxyl-containing haptens, and vice versa. The introduction of an arsenic-acid group into an antigen makes for a particularly strong interaction between the modified antigen and the antibodies which it induces. Introduction of an uncharged methyl, halogen, methoxyl, or nitro group into a hapten has a much smaller effect on the specificity; strong cross reactions are observed between antigens containing a given hapten and antibodies induced from antigens containing haptens that differ from the given hapten merely through the presence of one of these uncharged groups. On the other hand, antibodies are found to be able to distinguish very well between two haptens which are optical isomers or which are (*cis*-)(*trans*-) isomers.

The important inference to be drawn from these studies is that the specificity of the antibody-antigen reaction must depend upon a complementarity between the surfaces of the antibody and antigen molecules. This complementarity presumably involves an apposition of positive and negative electric charges, if a charge is present in the antigen. It also involves a rather close matching of the contours of portions of the surfaces of the antibody and antigen molecules—a lock and key relationship that had been visualized already by Ehrlich early in the development of immunology. Small deviations in the perfection of this complementarity are tolerated (e.g., replacement of a methyl group by a chlorine atom, or even of a hydrogen atom by a methyl group). Forces of all types are presumably in action across the surface of contact of the antibody and antigen, but electrostatic forces seem to be particularly important. One can visualize these relationships by means of semischematic drawings such as Fig. 1, where a portion of the antibody molecule

is supposed to envelop the hapten, with electric charges suitably located on the antibody surface so as to interact favorably with the opposite charge on the hapten.

A paper by Pressman, Siegel, and Hall[4] illustrates the possibility of obtaining quantitative information about complementarity by making use of the hapten-inhibition phenomenon. Samples of ovalbumin were diazotized to ortho-, meta-, and para-aminobenzoic acid. Three antigens were produced in this way, consisting of ovalbumin molecules bristling with azobenzoate groups having the three isomeric configurations shown in Fig. 2. The three antigens are referred to as "X_o-ovalbumin," "X_m-ovalbumin," and "X_p-ovalbumin." The three corresponding antibodies were prepared by injecting these antigens into rabbits, bleeding the rabbits, and separating the globulin fraction (which contains the antibodies) by ammonium-sulfate precipitation. The addition of suitable proportions of X_o-ovalbumin to anti-X_o-globulin gave a visible precipitate, the amount of which could be measured. When low molecular-weight haptens [such as benzoate, *o*-chlorobenzoate, *m*-chlorobenzoate, *p*-chlorobenzoate, *o*- (*p*'-hydroxyphenylazo) benzoate, and various chlorinated derivatives of *o*- (*p*'-hydroxyphenylazo) benzoate] were added, the amount of the precipitate was decreased because of the competition of the small haptens for the complementary site on the antibodies. By varying the concentration of low molecular-weight hapten and observing the change in the amount of precipitate produced, it was possible to compare the affinities of the different hapten groups for the sites on the anti-X_o-globulin.* These relative affinities could be expressed in terms of the differences between the mean free energies of combination of the haptens for the sites on the anti-X_o-globulin.

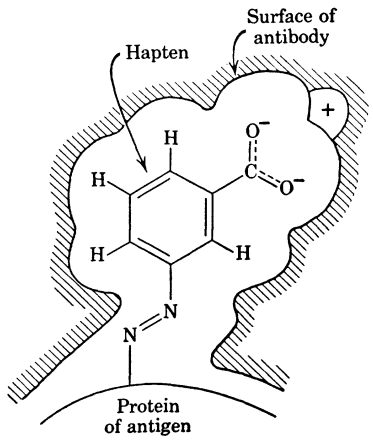


FIG. 1. Diagrammatic representation of complimentarity of the hapten-antibody relationship.

TABLE II. Relative affinities of various haptens for anti-$X_{o,m,p}$-globulins.

Hapten	Δ F_{rel}. cal/mole of hapten
For anti-X_o-globulin:	
Benzoate	(0)
o-chlorobenzoate	−580
m-chlorobenzoate	0
p-chlorobenzoate	430
o-(*p*'-hydroxyphenylazo) benzoate	−1700
3-Cl, 2-(*p*'-hydroxyphenylazo) benzoate	−1100
4-Cl, 2-(*p*'-hydroxyphenylazo) benzoate	−1100
5-Cl, 2-(*p*'-hydroxyphenylazo) benzoate	−1300
6-Cl, 2-(*p*'-hydroxyphenylazo) benzoate	−1500
For anti-X_m-globulin:	
Benzoate	(0)
o-chlorobenzoate	680
m-chlorobenzoate	−300
p-chlorobenzoate	480
m-(*p*'-hydroxyphenylazo) benzoate	−1600
2-Cl, 3-(*p*'-hydroxyphenylazo) benzoate	−560
4-Cl, 3-(*p*'-hydroxyphenylazo) benzoate	−350
5-Cl, 3-(*p*'-hydroxyphenylazo) benzoate	−980
6-Cl, 3-(*p*'-hydroxyphenylazo) benzoate	−500
For anti-X_p-globulin:	
Benzoate	(0)
o-chlorobenzoate	960
m-chlorobenzoate	−300
p-chlorobenzoate	−560
p-(*p*'-hydroxyphenylazo) benzoate	−1700
2-Cl, 4-(*p*'-hydroxyphenylazo) benzoate	−940

Similar experiments were performed on the X_m-ovalbumin-anti-X_m-globulin system and on the X_p-ovalbumin-anti-X_p-globulin system. As a result, it was possible to observe the effects that different groups in different positions in the hapten have on the ability of the hapten to combine with a given antibody. Some results for a series of chlorobenzoates and chloro-*p*-hydroxyphenylazobenzoate haptens are shown in Table II. The numerical values of ΔF_{rel} listed in this table are the differences between the average free energy for the combination of the hapten in question with the antibody sites and the average free energy of combination of the unsubstituted benzoate ion with the same sites. It is seen that, with each of the three globulins, the *p*-hydroxyphenylazo group greatly increases the affinity as compared with the benzoate ion (makes ΔF_{rel} more negative), presumably because it is able to take advantage of some of the binding forces associated with the portion of the antibody site that the organism intended to be occupied by the azo group and by the side chain of the protein to which the azo group is attached (see Fig. 3). This simple picture also shows

* The detailed mathematical analysis by Pauling, Pressman, and Grossberg[5] of the dependence of the amount of precipitate on the concentration of the low molecular-weight hapten showed that there must be a considerable variability in the affinities of the hapten for different sites on the antibody molecules. That is, the antibody sites at which the haptens are bound are not all alike. This heterogeneity can be described adequately by means of a Gaussian distribution of the free energies of binding about a mean value which is characteristic of each hapten, the spread in the free energies on either side of the mean value amounting to 1000 to 2000 cal/mole of hapten. The complication introduced by heterogeneity can be avoided by talking in terms of the mean free energy of binding for a given hapten at the sites on the anti-X_o-globulin.

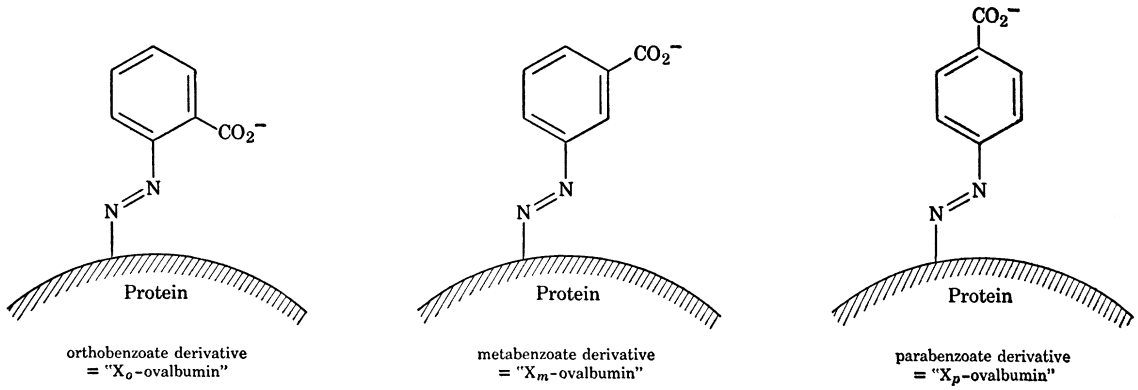


FIG. 2. Configuration of haptens used in studying antibody-antigen complementarity.

why the introduction of a chlorine atom in place of a hydrogen atom at various positions in the benzene ring in the simple benzoate ion tends to be unfavorable (makes ΔF_{rel} more positive by several hundred calories), if the slightly bulky chlorine atom is not able to go into the position intended for the azo group of the original antigen. When the much larger *p*-hydroxyphenylazo group is present in the hapten, it occupies the position intended for the protein of the original antigen; the introduction of a chlorine atom into such a hapten then invariably decreases its affinity for the antibody site. The amount of the decrease in affinity is different, depending upon the location of the chlorine atom on the benzene ring. This reveals something about the nature of different portions of the surface of the hapten-binding site on the antibody. It is clear that experiments of this kind allow one to probe the various regions of the antibody-combining sites and to obtain quite detailed information on the factors that determine antibody-antigen specificity.

2. Enzyme-Substrate Interactions

Enzymes are well known to be rather particular about the substrates whose reactions they choose to catalyze. The degree of this specificity varies widely, however. The enzyme urease evidently catalyzes only the hydrolysis of urea and appears to have no noticeable effect on any urea derivative or other substance that has yet been tested. Fumarase adds water to the double bond of the fumarate ion to produce L-malate ion and no other double-bonded organic molecule has been found which is attacked by this enzyme. On the other hand, certain lipases will hydrolyze ester bonds containing widely different organic groups attached to either side of the bond.

It is generally agreed that enzymes act by adsorbing the substrate molecule or molecules at some "active site" on the enzyme molecule in such a way that the appropriate bonds in the substrate show an increased reactivity. It is difficult to avoid thinking in terms of the hypothesis that the enzyme-substrate interaction, like the antibody-antigen interaction, depends upon a complementarity in structure between the two substances, the substrate molecule fitting into a characteristically shaped impression in the surface of the enzyme molecule. The increased reactivity may arise because in the adsorbed state the bonds involved in the

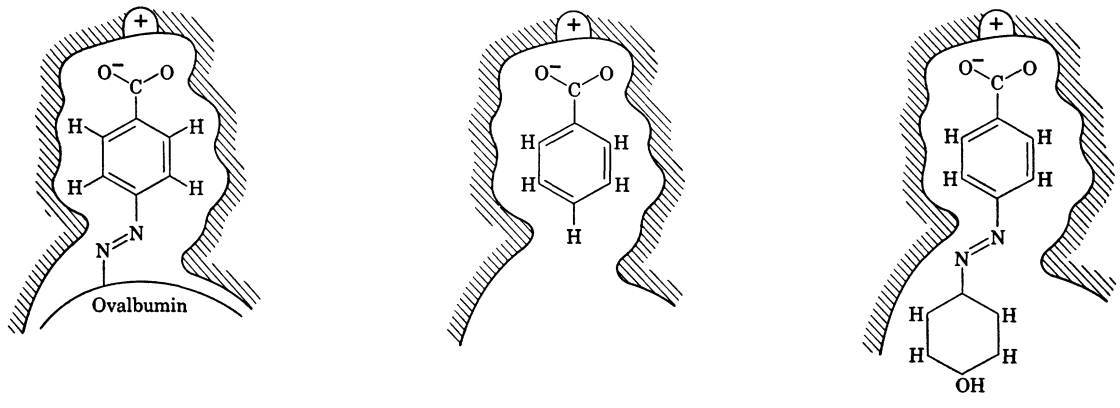


FIG. 3. Hapten inhibition of anti-X_p-globulin.

reaction are placed under strain or because they are brought into a favorable position for reaction. This simple picture seems to be consistent in a general way with the wide range of specificity found among enzymes. Highly specific enzymes presumably contain a highly characteristic impression of the substrate molecule into which it is not possible to fit even closely related molecules capable of undergoing the same reaction as the substrate. Enzymes showing a wide range of specificity, on the other hand, must interact with only the region of the substrates in the immediate vicinity of the bonds that are involved in the reaction. This picture also explains why substances closely related to a given substrate, but incapable of undergoing the reaction that is catalyzed by the enzyme, often act as powerful inhibitors (e.g., malonic acid and malic acid strongly inhibit the conversion of succinic acid to fumaric acid by succinic dehydrogenase). These inhibitors must fit into the site intended for the substrate but, like the dog in the manger, they neither take advantage of the catalytic capabilities of the site nor permit access to the true substrate.

The hypothesis of structural complementarity also explains the high degree of stereospecificity shown by practically all enzyme reactions in which the potential substrates are capable of existing as stereoisomers. (E.g., the dehydration of malic acid in the presence of fumarase to form fumaric acid is absolutely specific for L-malic acid, D-malic acid being unaffected by fumarase to any detectable degree; similarly, maleic acid, the *cis* isomer of fumaric acid, is not hydrated by fumarase to any detectable degree. Most peptidases will not hydrolyze peptide bonds involving D-amino acids.) Suppose that the active center of an enzyme is surrounded by a mold into which all of the chemical groups in the vicinity of a double bond of the *trans* isomer of a compound will fit. It is easy to understand why this mold will not be able to accommodate the *cis* isomer of the compound, which has an entirely different external shape. The specificity to optical isomers is no less easy to understand in terms reflecting the common experience that a left hand will not fit into a glove made to cover its mirror image, the right hand.

It is interesting to examine some typical enzyme systems in order to observe the types of behavior that can be accommodated to the complementarity concept. One finds that many systems can be interpreted very easily in these terms, but one finds also that there are certain instances in which the concept does not present a very convincing picture of what must be going on.

(*a*) *Peptidases*

Bergmann studied the action of various peptidases on simple peptides and was able to show that there is a high degree of specificity among these enzymes which depends on the nature of the groups located on either side of the peptide bond that is split. He was able to formulate certain general specificity requirements for the action of pepsin, trypsin, and chymotrypsin, which are summarized in Table III. Further work has shown, however, that many of these requirements are not met by all substrates of these enzymes, and the specificity picture has become somewhat more complex (see, for example, the discussion in Dixon and Webb[6] and Neurath and Schwert[7]). To illustrate these complexities, the chief points of attack on ribonuclease by various peptidases are shown in Fig. 4. Inspection of this figure and Table III will show that the specificity discovered by Bergmann for trypsin is invariably maintained: the points of attack by trypsin are always at the peptide bond next to either arginine or lysine, with the carbonyl end of the bond that is hydrolyzed belonging to the cationic amino acid. It does appear, however, that the bond adjacent to one of the cationic amino-acid residues of ribonuclease (the lysine at position 44 from the lysine end of the chain) may not be attacked by trypsin. The points of attack on ribonuclease by chymotrypsin are frequently adjacent to aromatic side chains (usually at the carbonyl side), as demanded by the Bergmann rules. Unexpected exceptions are found, however, in the ready opening of the leucine-threonine linkage between positions 35 and 36, and of the alanine-lysine linkage between positions 103 and 104. A weak ability of chymotrypsin to split peptide bonds involving methionine had been noted in simple peptides (chymotrypsin also hydrolyzes a cystine-serine bond in insulin). These exceptions are somewhat puzzling from the point of view of the complementarity concept, because the groups that are involved can hardly be said to resemble in general shape the aromatic groups that the Bergmann rule demands for chymotrypsin. More complex factors seem to operate than the mere binding by the enzyme of the amino-acid residue next to the peptide bond that is being hydrolyzed. The slight ability of methionine and cystine to replace an aromatic ring in some substrates for chymotrypsin is especially puzzling and might suggest that an electronic mode of interaction may be involved rather than one involving steric complementarity.

The specificity rule for pepsin proposed by Bergmann on the basis of its action on simple peptides turns out to be violated frequently when pepsin acts on long

TABLE III. Bergmann's specificity rules for the hydrolysis of the peptide bond in $R_2—NH—CHR_1—CO—NH—R_3$.

Enzyme	R_1	R_2	R_3
Pepsin	glutamic or aspartic	not H	aromatic (tyrosine or phenylalanine)
Trypsin	cationic (lysine or arginine)	not H	nonspecific; may be H
Chymotrypsin	aromatic (tyrosine or phenylalanine)	nonspecific	nonspecific

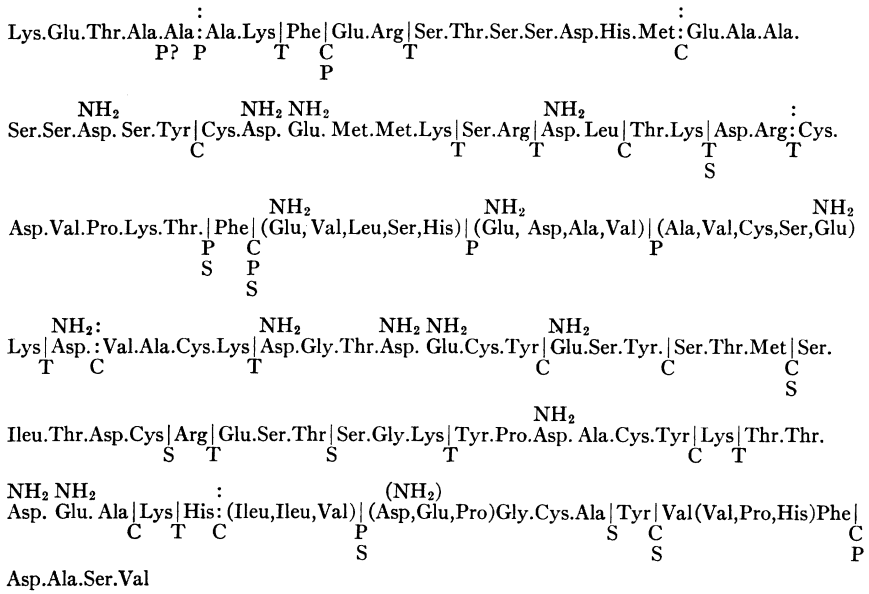


FIG. 4. Points of hydrolysis of ribonuclease by chymotrypsin (C), pepsin (P), trypsin (T), and subtilisin (S). Dotted lines represent points of slower hydrolysis.

polypeptide chains. This is evident from Fig. 4, where the attack on an alanine-alanine bond may be noted. Pepsin also attacks an alanine-serine bond in ACTH and a leucine-valine linkage in insulin. In general, however, pepsin does show a tendency to attack either bonds involving glutamic or aspartic acids by themselves, or bonds involving aromatic amino acids by themselves—in partial agreement with Bergmann's rules. It appears that the true range of specificity of pepsin does not reveal itself until one allows it to act on relatively long polypeptides.

It is interesting that trypsin and chymotrypsin are able to hydrolyze esters and suitably activated carbon-carbon bonds as well as peptides. The specificity requirements on the amino acids adjacent to the ester or carbon-carbon bond are similar to those observed for peptides with the same two enzymes. Here one has a particularly striking example of the importance of the environment of a reacting bond, rather than the bond itself, in manifesting the specificity of the enzyme.

(*b*) *Cholinesterase and Acetylcholinesterase*

The reaction

$$(CH_3)_3N^+-CH_2-CH_2-O-CO-CH_3$$
(acetylcholine)
$$+H_2O \rightarrow (CH_3)_3N^+-CH_2-CH_2-OH$$
(choline)
$$+CH_3-CO-OH$$

is catalyzed by two distinct groups of enzymes which have interestingly different specificity requirements. One of these groups of enzymes, the acetylcholinesterases is inhibited by the substrate acetylcholine at higher substrate concentrations, so that its activity *vs* substrate-concentration curves pass through a maximum. The other group of enzymes, known as cholinesterases, is not so inhibited, and the activity *vs* substrate-concentration curves follow the normal Michaelis-Menton behavior. Both kinds of enzymes can act on many substrates related (in some instances rather distantly) to acetylcholine, but mention is made only of the interesting and opposite effects of replacing the acetyl group with other acyl groups containing longer hydrocarbon chains. In mammalian brain acetylcholinesterase, the replacement of acetyl with butyryl greatly lowers the activity and does not remove the inhibition at high substrate concentrations. In cholinesterase, on the other hand, the same substitution greatly increases the activity of the enzyme. Here one has an example of two enzymes which catalyze the same reaction but whose specificity patterns are markedly different, showing that considerably different types of complementarities must be possible for a given substrate.

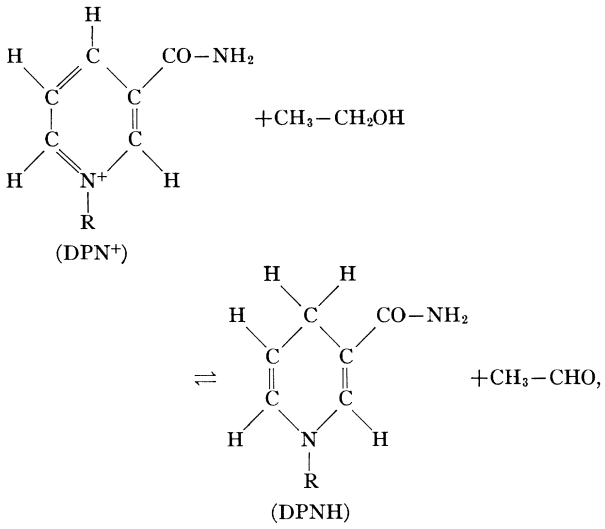
(*c*) *Glycosidases*

The hydrolysis of α- or β-alkyl- or aryl-glycosides to form free sugars plus alcohols or phenols is catalyzed by a group of enzymes that show interesting specificities. In general, a given glycosidase is highly specific to the sugar moeity, and to whether the glycoside has the α- or β-configuration, but it is relatively unconcerned about the nature of the aglucone residue. For instance, α-glucosidase will hydrolyze α-methylglucoside, α-phenylglucoside, and many other α-glucosides, but it will not cause the hydrolysis of any β-glucoside or any α-glycoside of a sugar other than glucose. Here one has

examples of very high specificity toward a group that lies on one side of the chemical bond that is attacked, and a very low specificity to the other side.

(d) Stereospecificity in the Transfer of Hydrogen to DPN in Dehydrogenase Reactions.

Vennesland[8] and Westheimer have shown by means of ingenious experiments with deuterium that, in the reaction



both the addition of the hydrogen atom to the pyridine ring of DPN^+ in the forward direction and the addition of hydrogen to acetaldehyde in the reverse reaction are stereospecific. Thus, if the equilibrium

$$CH_3-CH_2-OD+DPN^+ \rightleftharpoons CH_3-CHO+DPNH+D^+$$

is carried out in D_2O, it is found that no additional deuterium atoms are introduced into either DPN^+, DPNH, alcohol, or aldehyde. Similarly, no normal hydrogen is introduced when the reaction

$$CH_3-CD_2-OH+DPN^+ \rightleftharpoons CH_3-CDO+DPND+H^+$$

is equilibrated in H_2O. This proves that the hydrogen atom is transferred directly from the carbon atom of the alcohol to the DPN^+ (and vice versa) and does not go through the solvent. Furthermore, the hydrogen atom introduced into the DPNH must lie on one definite side of the pyridine ring. The ring must lie, therefore, in a definite orientation on the enzyme surface, and relative to the alcohol molecule, in the complex of the enzyme with its two substrates. That the orientations of the ethanol and aldehyde molecules also must be fixed rigidly relative to the pyridine ring is shown by the fact that the reaction

$$H^+ + DPND + CH_3-CHO \rightarrow DPN^+ + CH_3-CHD-OH$$

produces a single optical isomer of ethanol-1*d*. Here is an especially clear example of the detailed stereochemical specification of the structure of an enzyme-substrate complex. It must take advantage of a large fraction of the possible complementarity that can exist between the substances concerned in the reaction.

(e) The Koshland-Stein Rule

Koshland and Stein[9] have proposed the general principle that "if the enzyme requirements for a substrate having an oxygen bridge, i.e., R−O−Q, show high specificity for R and low specificity for Q, than R−O cleavage occurs in the enzyme reaction." This rule has been found to be valid for invertase, alkaline phosphatase, acid phosphatase, trypsin, and chymotrypsin. The simplest interpretation of the rule in terms of complementarity would be that the group with the highest specificity would be presumed to have the closest contact with the enzyme; the enzyme then would be expected to have the greatest activating effect on the bond adjacent to this group.

(f) Existence of Identical Amino-Acid Sequences in the Active Centers of Enzymes Having Diverse Specificities

It has been found recently that the active centers of thrombin, chymotrypsin, trypsin, and phosphoglucomutase all contain the sequence aspartic acid-serine-glycine. These enzymes have markedly different specificities, the first three catalyzing the hydrolysis of peptide bonds, whereas phosphoglucomutase catalyzes the transfer of a phosphate group from one glucose ring to another. The specificity of these enzymes must clearly reside in regions quite distinct from the center that activates the chemical bonds which are disrupted.

3. Binding of Small Molecules to Proteins (Other Than Hapten-Antibody Interactions)

Serum albumin is remarkable for its ability to adsorb a wide variety of small molecules, particularly (though not at all exclusively) those bearing negative charges (acetate ion, halide ions, methyl orange, and many other anionic dyes, detergents, etc.). The most striking feature of this adsorption, however, is the lack of any reasonably marked specificity. Changes in the structures of the adsorbed molecules do have some effects on the strength of the binding. These changes in affinity are not, however, as large as one would expect to find if the surface of the serum albumin molecule were covered with a mosaic of rigid binding sites having a reasonably high degree of complementarity to the necessarily limited number of types of substances which such a mosaic might potentially bind. (For example, enantiomorphic isomers of a dye are adsorbed to almost the same extent.) In order to account for this, Karush[10] has proposed the concept of "configurational adaptability," according to which the serum-albumin molecule is considered to possess a degree of internal flexibility.

As a result, the surface conformation can be changed so as to establish a measure of complementarity with the surface of almost any molecule that approaches it, and there is no need to deal with a limited number of fixed types of potential binding sites. A strong point in support of this concept is the fact that serum albumin does possess a high degree of internal flexibility as compared with other proteins, which also show a far smaller affinity for small molecules.[11] In a sense, the serum-albumin molecule behaves like a lump of putty onto which one can stick an unlimited number of different shapes.

The concept of configurational adaptability is important because it shows that complementarity need not imply specificity. Furthermore, it raises the possibility that, in some instances, the specific complimentarity structure may be developed only when the substrate is present.

SPECIFICITY AND ORGANIZATION

Green has set forth[12] reasons for believing that, in certain biochemical processes involving a sequence of steps, each catalyzed by a different enzyme, the enzyme molecules must occupy fixed positions in space which are located so as to facilitate the successive reactions. He speaks of "an organized mosaic of enzymes in which each of the large number of component enzymes was uniquely located to permit efficient implementation of consecutive reaction sequences." A primary example of this mosaic is the cyclophorase system, which includes all of the enzymes for the citric-acid cycle, fatty-acid oxidation, oxidative phosphorylation, and terminal electron transport. This system of enzymes has been shown to be located exclusively in the mitochondria. In view of the striking mitochondrial structures which have been revealed by electron microscopy [see the papers by Sjöstrand (p. 301) and Fernández-Morán (p. 319) in this volume], there is a certain temptation to accept this theory.

Dixon and Webb[13] have disputed the necessity for assuming organization in this spatial sense. As an alternative, they go back to the point of view presented twenty-five years ago by Hopkins[14] in these words: "The organizing potentialities inherent in highly specific catalysts have not, I believe, been adequately appraised in chemical thought. Highly specific catalysts determine just what particular materials, rather than any others, shall undergo reaction. They select from their environment. The specific catalyst determines which among possible paths the course of change shall follow. It has directive powers . . . ." Dixon[15] speaks of "organization by specificity" as an alternative to Green's spatial organization of enzyme systems. The concentration of the cyclophorase enzymes into the mitochondria is ascribed to the need to reduce the distance between the enzymes involved in consecutive reactions. The cell is able in this way to reduce the "transit times" required for the products of one enzymatic step to diffuse to the enzyme molecules involved in the next step. According to this view, there is no need to assume any regular arrangement of the different enzymes. It is necessary only to have an arrangement (a random one would do) with a sufficiently small mean distance of separation between the enzymes involved in the different enzymatic steps.

It is interesting in this connection to estimate the order of magnitude of the transit time for a typical metabolite to move a given distance from one enzyme to another by a diffusion mechanism. It is well known that if x^2 is the mean square distance moved in time t by a particle whose macroscopic diffusion constant is D, then

$$x^2 = 2Dt.$$

Typical metabolites have diffusion constants of the order of 1 cm^2/day, or 10^{-5} cm^2/sec. Thus, it is found that, if the enzymes required for two successive steps of a reaction sequence are 1000 A apart, the transit time is 10 μsec. If the separation is 100 A, the transit time is 0.1 μsec. It would appear, therefore, that the arrangement of enzymes of a reaction sequence in layers whose repeat distance is a few hundred Ångström units, without any regard to the serial positions in space of the enzymes involved in successive reaction steps, would give a more than adequately short transit time for most biochemical requirements. Specificity alone should be adequate to cope with problems of organization at this level. This is not to say, of course, that the living cell actually is organized in this manner. For reasons unknown, it may have chosen to solve its transit time problems in a less straightforward manner.

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