Waterlogged Molecules

Richard Wolfenden

When biological compounds combine, react with each other, or change shape in watery surroundings, solvent molecules tend to be reorganized in the neighborhood of the interacting groups. If there were a difference between the substrates and products of a metabolic reaction, in the strength of their interactions with the surrounding solvent, then their relative stabilities would be influenced by changchemical systems have long been recognized in a qualitative sense (1), but quantitative information concerning their actual magnitude has been scarce. Many biological compounds are so polar that their complete removal from water to nonaqueous surroundings, essential in determining the absolute strength of their solvation, has been difficult or impossible. The very difficulty of removing

Summary. Measurements of vapor pressures over their aqueous solutions indicate that organic compounds show profound differences in hydrophilic character. These differences are of such magnitude as to suggest an important role for changing solvation in determining free energy changes associated with metabolic transformations in water, and in governing structural equilibria of proteins and other large molecules in water. When two or more functional groups are present within the same solute molecule, their combined effects on its free energy of solvation are commonly additive. Striking departures from additivity, observed in certain cases, indicate the existence of special interactions between different parts of a solute molecule and the water that surrounds it. Similar considerations presumably apply to activated intermediates in the interconversion of biological materials.

ing solvation (Fig. 1). To that same extent, the position of equilibrium of the reaction would be affected by the presence of solvent water. In a somewhat different sense, the noncovalent binding functions of enzymes, antibodies, and other receptor molecules can be considered to include the cost of removing the interacting molecules, at least the parts that make contact, from the solvent to which they were previously exposed. In seeking to understand the observed affinities of enzymes and other specific receptors, for ligands such as substrates, intermediates, and analog inhibitors, it would be helpful to have experimental information concerning the absolute tendencies of these ligands to leave water and enter a "naïve" or featureless cavity of unit dielectric constant that neither attracts nor repels ligands. It might then be possible to infer the presence of specific attractive or repulsive interactions between these ligands and the sites at which they are bound.

Influences of solvent water on bio-

these molecules from water suggests, however, that differences between their solvation energies might be considerable. In this article, we will examine new experimental evidence bearing on this proposition.

Water Affinities

The affinity of a compound for watery surroundings can be determined by measuring the dimensionless equilibrium constant for its transfer from the dilute vapor phase, in which each molecule exists in virtual isolation, to an aqueous solution so dilute that each solute molecule is completely surrounded by water, and solute-solute interactions can be neglected. This can be accomplished by measuring solubilities of a gas under known pressures or, for less volatile compounds, by determining concentrations of solute in the gas space over solutions of known concentration. In extreme cases involving highly polar solutes, measured volumes of an inert carrier gas can be bubbled through an aqueous solution of known concentration,

and then through an efficient trap that recovers the solute quantitatively from the vapor phase (2). With the use of radioactive tracers and a suitable arrangement of gas washing bottles, it is possible in this way to measure vapor-towater distribution coefficients as large as 10^{10} (3). Low concentrations of strongly colored solutes can sometimes be determined by direct spectrophotometric observation, in the gas space over solutions of known concentration, with gas-tight cuvettes of long light path (4).

Vapor-to-water distribution coefficients have occasionally been calculated simply by combining the vapor pressure of a pure compound with its solubility in water. Self-association is likely to be encountered with polar compounds near their limits of solubility in water, so that such values should be viewed with skepticism. To ensure that self-association of the solute does not occur in the vapor phase, it is important to demonstrate that observed distribution coefficients do not change with changing concentration of the solute. This criterion has been satisfied in most of the work discussed below (5).

Simple Organic Molecules

Empirical affinities of organic compounds and functional groups for watery surroundings are of practical concern to experimentalists, who find it useful to refer to certain compounds or groups as being relatively "hydrophilic" or "hydrophobic." Strictly speaking, virtually all molecules are attracted to water by dispersion forces. A net attractive force exists, for example, at the interface between liquid octane and liquid water (6), and isolated methane and water molecules exhibit a modest affinity for each other in the vapor phase (7).

Not all molecules, however, are attracted strongly enough to overcome the self-cohesive properties of water, as is necessary if they are to enter solution instead of merely adhering to its surface. Figure 2 shows that paraffins and olefins, like the noble gases, have a significant tendency to leave water and enter the vapor phase (8, 9). Increasing chain length of hydrocarbons consistently favors the vapor phase, and this effect is about the same regardless of other substituents that may be present (9). In the six homologous series in which five or more members have been examined, the mean increment in distribution coefficient toward the vapor phase per methylene ($-CH_2-$) group is 28 percent (10).

Small increases in hydrophilic charac-

The author is a professor in the Department of Biochemistry, University of North Carolina, Chapel Hill 27514.

ter accompany replacement of hydrogen by deuterium on carbons α to the carbonyl group in N-methylformamide (11), and β to the carbonyl group in acetone. ethyl acetate, and p-nitroacetanilide (12). Hydrophilic character is considerably enhanced in hydrocarbons by the introduction of double and triple bonds (Fig. 3) (8), and affinities of organic compounds for solvent water are greatly enhanced by polar functional groups (9, 13). In Fig. 3, some representative uncharged aliphatic compounds are arranged on a scale that spans ten orders of magnitude. In attempting to understand the origins of these differences in hydrophilic character, the relative numbers of atoms present in each solute that might be expected to form hydrogen bonds to solvent water should be considered. Ethyl acetate, acetic acid, acetamide, and methylguanidine (with one, two, three, and four such groups, respectively) seem to lie roughly in the order expected on this basis. Almost identical values are observed for acetaldehyde and acetone. Ketones and esters are also similar to each other in hydrophilic character, suggesting that the ester bridge oxygen atom confers little affinity for water. Comparable observations have been made for phosphate esters: the hydrophilic character of triethyl phosphine oxide, in which -CH₂- replaces -O-, is in fact a little greater than that of trimethyl phosphate (14).

Some of the details in Fig. 3 are not readily explained by simply enumerating hydrogen bonding groups. In view of the exceptional water affinity of acetamide, it is interesting to examine the effect of N-methylation, which is expected to reduce the number of possible hydrogen bonds to solvent water. Instead, the hydrophilic character of N-methylacetamide exceeds that of acetamide itself (3). A modest loss of hydrophilic character is observed on introduction of a second methyl group, but even at this degree of substitution, N,N-dimethylacetamide remains substantially more hydrophilic than acetic acid itself (Fig. 3). Evidently the unusual strength of solvation of amides is associated with the carbonyl group or with the dipolar character of the amide group taken as a whole. Amines exhibit comparable behavior; thus methylamine and dimethylamine are similar to ammonia in hydrophilic character, while trimethylamine is a little less hydrophilic. In both the amide and the amine series, maximal hydrophilic character is observed in molecules that can form two kinds of hydrogen bonds with water, one as a hydrogen donor and one as a hydrogen acceptor (3). Provided that this requirement is satisfied, further



Fig. 1. If reactants and products differ in the strength of their interactions with solvent water, then changing solvation influences the position of equilibrium of a reaction. In this case, $K_w/K_v = K_2/K_1$.

changes in *N*-methylation seem to have little effect on solvation. Hydrogen bonds to solvent that might have been eliminated by these methyl substitutions are evidently weak in both series.

If the vapor phase is regarded as a special kind of "solvent," that neither attracts nor repels solutes, do any real solvents exhibit similar properties? Fluorocarbons seem to approach the ideal of "noninteracting" solvents more closely than do hydrocarbons (15), but for practical reasons, water-solvent distributions have usually been measured with hydrocarbons as the second phase. A scale based on solvent "lipophilicity" (inversely related to water content at saturation) has been used for correlating thousands of water-to-solvent distribution coefficients, leading to fragment constants that express the observed tendencies of various organic groups to be transferred from water to organic solvents (16). When these are compared with substituent constants obtained from correlations of vapor-to-water partition coefficients, the observed correlation is not bad (Fig. 4). The slope of a line relating the points in Fig. 4 would be greater than unity, reflecting a net tendency for polar substituents to pass from the vapor phase to a nonpolar solvent. This tendency, increasing with increasing substituent polarity, is expected as a consequence of dipolar interactions with any real solvent that might be used as a reference phase.

Breakdown of Additivity

When two or more moderately polar groups are present within the same molecule, their combined influence on transfer from water to vapor, expressed in terms of free energy, is often found to be approximately additive. The regularity of these effects, first noted by Butler (9), has been confirmed by the results of later investigations. Additivity schemes, permitting rough prediction of free energies of transfer of multifunctional compounds from vapor to water, have been compiled from available data (13, 17).

Departures from additivity would be of special interest, insofar as they might indicate special interactions between different parts of a solute molecule, and different regions of the solvent that surrounds it. A recent study of the vapor pressures of ethylene glycol and related compounds over water shows that their affinities for water, which had been seriously underestimated, are nevertheless considerably lower than would be expected from the behavior of monohydric alcohols (18). This discrepancy can probably be attributed, at least in part, to intramolecular hydrogen-bonding, known to occur in vicinal diols in the vapor phase (13, 19)

Alkyl-substituted pyrazines are also less hydrophilic than expected, with vapor pressures over water comparable with those of pyridines that contain only a single ring nitrogen. This appears understandable in terms of the conflicting electrostatic requirements of hydrogen bonds from solvent water to each of the two nitrogen atoms in pyrazine (13). Hydrogen bonding ability depends on electron density at nitrogen, and interactions at each nitrogen are expected to be weaker than they would be if the other were not present (Fig. 5).

In deviations of the opposite kind, compounds containing several substituents exhibit higher affinities for water than would be predicted from the behavior of their monosubstituted analogs. Imidazoles, for example, are extremely hydrophilic, whereas pyrrole and cyclopentadiene exhibit low boiling points and limited solubility in water (20). p-Nitrophenol is also much more hydrophilic than expected (13). In these compounds (Fig. 5), one polar group presumably acts as a proton donor in hydrogen bonding to solvent water, the other acting as a proton acceptor. These effects are expected to reinforce each other electronically, so that hydrogen bonds at one end are stronger than they would be if those at the other end were not present.

There is reason to believe that drastic departures from additivity of constituent group effects on hydrophilic character may occur in cases where at least one of these groups is very polar or ionic. Ion cyclotron resonance spectroscopy has made possible the determination of numerous equilibria of proton transfer in the vapor phase. By comparing these with proton transfer equilibria in solution, the relative strengths of solvation of conjugate acids and bases can be estimated. Solvation of ammonium ions becomes progressively less favorable with increasing methylation, its free energy changing by approximately 19 kilocalories per mole from ammonium ion to trimethylammonium ion (21). The free energy of solvation of uncharged ammonia is nearly the same as that of methylamine and dimethylamine, and only 2.3 kcal/mole more negative than that of trimethylamine. As a result, very great differences in the intrinsic gas phase basicities of amines are "leveled" almost to the vanishing point in aqueous solution. Pronounced effects of methyl substitution on solvation of ions, far exceeding the moderate effects observed on neutral solutes, have also been unveiled by studies of the gas phase acidities of normal aliphatic carboxylic acids (22) and pyridinium ions (23).

In considering the origins of these effects, it seems reasonable to suppose that alkyl substituents may stabilize ionized groups in the vapor phase by serving as "sinks" for delocalization of charge. Alternatively, alkyl substituents may interfere with access of solvent water to ionized groups, destabilizing them in water as compared with the vapor phase (22). Evidence of a different kind suggests that regions of solvent water, surrounding different parts of solutes, sometimes interact with each other in a way that departs from expectations based on simple additivity relationships. Dilatometry shows that, in a series of normal carboxylic acids, ionization in water is accompanied by decreases in volume that vary over a surprisingly large range; such a result is difficult to explain without supposing that hydrocarbon substituents influence the structure of water in the vicinity of the ionizing groups (24). In a series of normal aliphatic aldehydes, decreases in molar volume that accompany gem-diol formation range from 4 ml for formaldehvde to a limiting value of about 13 ml for isobutyraldehyde. These effects of increasing chain length, not readily explained in steric or inductive terms, are presumably exerted through the solvent (25).

Equilibria of Biochemical Reactions

Along the pathways of metabolism, intermediates usually tend to appear in such an order that their thermodynamic stability increases progressively from starting materials to final products. The concept of group transfer potential (the negative free energy of hydrolysis of a compound in water at pH 7) has proved helpful in organizing these observations (26). Compounds with high group transfer potential, such as phosphoric acid anhydrides and amino acid esters of transfer RNA, tend to serve as biosynthetic precursors of compounds that are more stable thermodynamically. For example, the chemical event in which amino acids are joined together in protein biosynthesis, aminolysis of an ester, is accompanied by release of a large amount of free energy. How much of the free energy of peptide bond formation is associated with a difference in free ener-

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gy of solvation between reactants and products? Given an answer to this question, it would be possible to say how much remained to be explained in terms of the intrinsic chemical properties of reactants and products.

Primary amides are among the most strongly hydrated neutral molecules yet to be encountered (Fig. 3). If one compares the positions on this scale of pri-



amides (for marv example, Nmethylacetamide) and alcohols (for example, ethanol) with those of esters (such as methyl acetate) and amines (such as ethylamine), it becomes evident that the products of ester aminolysis are very much more strongly hydrated than the substrates. This difference is more than sufficient to account for the negative free energy of aminolysis of esters in water (27). In the absence of water, equilibrium would actually favor formation of esters and amines from alcohols and amides. One is led to conclude that peptide polymerization, an essential event in the expression of genetic information, derives its thermodynamic drive from changing solvation.

Phosphoric acid derivatives differ from carboxylic acid derivatives in being very much more strongly hydrated. For example, trimethyl phosphate is about four orders of magnitude more hydrophilic than methyl acetate (Fig. 3). Triethyl phosphine oxide (not shown), in which each methoxyl group of trimethyl phosphate is replaced by an ethyl substituent, is a little more hydrophilic still, so that this stronger affinity for water seems to be an intrinsic property of $\geq P = O$ as compared with > C = O derivatives. In contrast to carboxylic acids, phosphoric acids are even more hydrophilic than their amides (14).

Phosphoric acid esters are present in biological materials of all kinds, and it is of interest to know how solvation affects their thermodynamic stabilities. Successive removal of alkyl groups from trialkyl phosphate esters results in even greater increases in hydrophilic character than in carboxylic acid esters, amounting to four to five orders of magnitude for each substitution. This is more than sufficient to account for the favorable standard free energy of hydrolysis of phosphate esters in water. It follows that equilibrium would favor spontane-

> Fig. 5. Electron shifts associated with hydrogen bonding to solvent tend to reinforce each other in imidazole (upper left), and in *p*-nitrophenol (upper right). In pyrazine (below), hydrogen bonding interactions at each nitrogen are weaker than they would be if the other nitrogen were not present.

> Fig. 6. Water-to-vapor distribution coefficients of cyclic compounds; the data are from (4) and (13)

ous formation of phosphate esters, from phosphoric acids and alcohols, in the vapor phase at equilibrium (14).

Group transfer from anhydrides of phosphoric acid occurs commonly in biosynthetic and biophysical phenomena involving adenosine triphosphate. It would be desirable to know how solvent water, or its removal (in anhydrous environments), affects the positions of equilibria of these important reactions. Because of their extreme polarity, the exact hydrophilic character of these compounds remains to be determined experimentally. On the assumption that observed entropy changes may be a good index of changes in solvation, it has been suggested that reactions of phosphoric acid anhydrides are largely determined by differences in solvation between reactants and products (28). This argument is supported by molecular orbital calculations indicating that solvation effects dominate the thermodynamics of hydrolytic equilibria of phosphoric acid anhydrides (29). Even less stable than phosphoric acid anhydrides are cyclic 3',5'phosphodiesters of nucleosides, commonly involved in biological regulation and signaling phenomena. Thermochemical experiments and molecular orbital calculations indicate that the high group transfer potential of these compounds arises from solvation differences between them and the products of their hydrolysis (30). These special effects, confined to fused ring systems, arise in part from solvent-dependent changes in conformational energy.

Nucleic Acid Structure

In neutral aqueous solution, tautomeric equilibria of nucleic acid bases are very one-sided. These equilibria favor keto over enol tautomers, and amino over imino tautomers, by factors in the neighborhood of 10^4 for cytosine, uracil, and adenine derivatives (31). Errors in base pairing, due to the occurrence of rare tautomers, would be expected to lead to mutational events (32). It is of interest to inquire how the abundance of rare tautomers might be affected by transfer from water to a "dry" environment such as may be present at the active site of an enzyme.

In approaching this question, it may first be noted that aromatic compounds tend to be more hydrophilic than saturated ring systems, presumably because of their greater polarizability and the accessibility of their electron clouds to solvent water (Fig. 6). Water affinities are enhanced as in aliphatic compounds, by the



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2-Pyridone

10-10

introduction of amino and hydroxyl substituents. Of special interest are the very much larger increases that are observed with the introduction of imino or keto substituents. The hydrophilic character of pyridine, for example, is increased by a factor of more than 10^6 when a keto group is introduced. As a result, positions of amino-imino and keto-enol equilibria are very strongly affected by removal of heterocyclic compounds from water to nonpolar surroundings (4, 33). Rare enol tautomers become more abundant, and rare imino tautomers become less abundant in the absence of water, by factors in the neighborhood of 10³ (compare the positions of 2-hydroxypyridine and 2-pyridone in Fig. 6).

These observations imply that nucleic acid bases undergo substantial changes in positions of their tautomeric equilibria when they are removed from water to a medium of low dielectric constant. Infrared studies at high temperature indicate that tautomers that are favored in water continue to be favored in its absence (34). Mutational events that would require imino tautomers are even less likely to occur in the absence of water than in its presence (4). However, mutational events that would depend on the incidence of rare enol tautomers are more likely to occur in nonpolar environments than in water (4).

Structures of Proteins and

Peptides in Solution

It has long been suspected that differences between amino acid residues, in the strength of their solvation by water, may be significant in determining the configurations of proteins in solution (35). It would therefore be of interest to have quantitative information about these water affinities. For the amino acids themselves, prospects of obtaining such information directly are dismal. Even using material of the highest specific radioactivity available, efforts to detect glycine in the vapor phase over its concentrated aqueous solutions have been unavailing. From the behavior of methylamine and acetic acid, it can be estimated that the concentration of glvcine, at equilibrium in the vapor phase over a 1M aqueous solution, can be no higher than $10^{-14}M$. Much lower values are expected for the more polar amino acids (21).

Even if methods more sensitive than those available should make it possible to determine free energies of removal of amino acids from water to the vapor phase, it is questionable whether they would serve as good models (even in a relative sense) for the behavior of the corresponding residues in proteins. In proteins, side chains of internal amino acids are flanked by peptide bonds whose negative free energies of solvation are modest in comparison with the enormous negative free energies of solvation of charged ammonium and carboxylate groups. The solvent-organizing power of the latter groups is very great, and could result in major disturbances of the relative distribution properties of nearby substituents (20, 36). Indeed, when free energies of transfer of amino acids from water to alcohol and other nonpolar solvents are compared with their tendencies to appear at the surface of globular proteins, there is no significant correlation (37). These findings seem to indicate either that solvation properties of individual amino acid side chains are irrelevant to protein conformation or that zwitterionic amino acids are misleading in this regard.



Fig. 7. Distribution coefficients for transfer from neutral aqueous buffer to the vapor phase of hydrogen analogs of amino acid side chains, for example, toluene for phenylalanine, or methanethiol for cysteine.

Seeking to avoid perturbations by zwitterionic functions of the free amino acids, not present at internal positions in protein chains, my colleagues and I examined the behavior of the amino acid side chains by themselves. Distribution coefficients, for transfer to dilute aqueous solution from the dilute vapor phase, were determined for 19 side chains commonly found in proteins in their uncharged states at 25°C. These values were corrected for the fraction ionized at pH 7, in order to calculate a scale of "hydration potentials," or overall free energies of transfer of amino acid side chains from the vapor phase to aqueous solution at pH 7 (20). On the resulting scale, spanning a range of 22 kcal/mole, the side chain of arginine is much more hydrophilic than those of the other amino acids (Fig. 7).

The extreme position in these relationships of arginine, the "Pluto" of amino acids, is of interest in relation to reports that guanidination of primary amino groups in proteins results in increased stability to thermal denaturation (38) and tritium exchange (39). In addition, the ratio of arginine to lysine seems to be positively correlated with thermal stability in redox proteins from several organisms (40). Rates of thermal denaturation and tritium exchange may be limited by processes involving transition states in which arginine residues are withdrawn, at least in part, from aqueous surroundings.

Protein structures have now been solved in sufficient numbers to allow a test of Perutz's generalization (37) that the polar character of amino acid side chains determines their tendency to be found at the surfaces of globular proteins. An algorithm, devised by Lee and Richards (41), provides an index of the solvent exposure of different atoms in a protein, making it possible to measure the number of residues of each kind of amino acid that are accessible to solvent (that is "exposed") in that particular protein (37, 42). When these tendencies toward accessibility were compared with a preliminary scale for 18 amino acid side chains of hydration potentials, a close correlation was observed (43). When methods became sufficiently sensitive to include the side chain of arginine, the most hydrophilic residue and the last to be examined, the relationship was found to be closer still (Fig. 8). The level of confidence of this relation ($P < 10^{-7}$) is some orders of magnitude better than that of any correlation involving single properties of free amino acids that has been examined before (20). While specific short-range interactions are doubtless

operative on individual residues packed in the interior of globular proteins, it is evident that amino acid side chains are statistically ordered in a manner that resembles their relative tendencies to pass from the vapor phase to solvent water. Recent studies have shown that hydration potentials have been conserved during the evolution of homologous regions in the sequences of penicillin-sensitive carboxypeptidases and lactamases (44). Hydration potentials are also very strongly correlated with the tendencies of amino acids to be found in β sheets (45), with the orientation of α helices relative to protein surfaces (46), and with the location of transmembrane sequences in lipoproteins (47).

Measurements of water affinities have also provided information concerning the strength and directional character of solvent interactions with the peptide backbones of proteins. We noted earlier that effects of methyl substitution, on the water-to-vapor distributions of amides, suggest that hydrogen bonds involving carbonyl oxygen may be substantially stronger and more common than those involving the N-H group (3). If this were a general rule, then it would be predicted that, in peptides and proteins, solvent water should tend to be found associated with the carbonyl oxygen atoms of peptide bonds more frequently than with the N-H groups of peptide bonds (3). X-ray diffraction studies bear out this prediction, as reflected by the positions of water oxygen atoms that are sufficiently immobile to appear in the electron density map of the protein rubredoxin. Of the 51 water molecules located within hydrogen bonding distance of peptide bonds in this protein, 38 are associated with carbonyl oxygen and 13 are associated with peptide N-H groups (48). Trypsin (49) and actinidin (50) exhibit a similar bias in favor of carbonyl hydration, also indicated by surveys of hydrogen bonds in crystal structures of amino acids and peptides (51). A "big" carbonyl group, that incorporates bound water as part of its steric requirements, has been found to lead to improved agreement between calculated and observed conformational preferences of model peptides (52).

Enzyme Action

We have seen that changing patterns of solvation exert a strong influence on positions of a number of metabolic and structural equilibria in water. Where water is scarce, as, for example, in the clefts in which the active sites of enzymes often appear to be situated, its



Fig. 8. Amino acid distributions observed in actual protein structures (ordinate) plotted as a function of buffer-to-vapor side chain distributions in Fig. 7 (abscissa).

scarcity may entail major consequences for processes occurring there. During the first event in enzyme action, formation of an enzyme-substrate complex, water presumably tends to be stripped away from the substrate and the enzyme at points where they make contact. Small molecules are sometimes bound by an enzyme with affinities inversely related to their affinities for water, suggesting that the enzyme may recognize, or desolvate, those parts of the small molecules that are mainly responsible for their differences in hydrophilic character. Adenosine deaminase, for example, binds the substrate adenosine, the product inosine, and the inhibitor nebularine (unsubstituted purine ribonucleoside) with widely differing affinities that seem to reflect, almost exactly, differences in the ease with which they can be removed from solvent water (3).

Catalysis by enzymes depends on the existence of an enhanced affinity of their active sites for activated intermediates in substrate transformation, as compared with their affinities for substrates in the ground state. Their enhanced affinity is reflected in the observed affinities of enzymes for transition state analog inhibitors (53). It is then reasonable to ask how mechanisms available for enzyme action could involve, or be affected by, changes in the strength and directional character of substrate interactions with solvent water before and during its transformation to product. In addition to their fundamental interest, answers to this question might provide a rational basis for improvements in the design of powerful enzyme inhibitors designed to resemble activated intermediates in substrate transformation.

Chemists realized long ago that rates of reaction are influenced by the solvent environment in which they take place. New techniques for following reactions in the vapor phase have begun to reveal the startling magnitude of these effects. Hydroxide ion, for example, attacks methyl chloride in the vapor phase about 17 orders of magnitude more rapidly than it does in water (54), in a reaction in which net charge is neither created nor destroyed. These results suggest the kind of rate enhancement that an enzyme might in principle achieve by desolvation alone, if it were able to draw the reactive portions of polar substrates into a nonpolar cavity.

If an enzyme were to enhance the reactivity of a substrate by removing it from a watery environment in which its rate of reaction was retarded, the result would be "catalysis by desolvation" (55). Such an enzyme might be strongly inhibited by a substrate analog that was hydrophobic, reducing the difficulty of stripping away solvent water during the binding event. The reaction catalyzed by pyruvate dehydrogenase proceeds, in the absence of enzyme, very much more rapidly in nonpolar solvents than in water (56). Uncharged analogs of thiamine pyrophosphate serve as very potent inhibitors (57). In reactions of this kind, the enzyme presumably drags polar reactants "by the hair" into an environment that destabilizes them relative to a less polar transition state. Thiamine pyrophosphate appears to be well-equipped with chemical appendages that could serve as the "hair."

It is less easy to account for the many reactions that proceed, in the absence of enzymes, through intermediates that are more polar than the starting materials. Ionic or polar intermediates are stabilized, relative to the starting materials, by watery surroundings, so that these reactions are understood to be subject to "solvent catalysis." Nothing useful would seem to be accomplished by removing such reactants to a waterless cavity. The mechanism of such a reaction might change so that no intermediate more polar than the starting material would be generated during the enzymatic process. B-Galactosidase, for example, seems to follow a mechanism involving a covalent glycosyl-enzyme (58), whereas the acid-catalyzed reaction in solution is believed to proceed through a carbonium ion intermediate. Another possible strategy would be for the active site to be designed to stabilize charges that are generated, even more effectively than does solvent water. Only a well-designed chelating agent could extract an ionic intermediate from aqueous solution in preference to a neutral substrate analogous in structure. Electrostatic contributions to transition state stabilization may in principle be very large in certain cases (59). Some of the more effective transition state analog inhibitors of enzymes, such as 2-phosphoglycollate (60) and benzylsuccinate (61) appear to be tightly bound only in their most fully ionized forms. It is also evident that binding sites of hemoproteins, ionophores, and siderophores have evolved to meet requirements of this kind, binding metal ions very tightly in water.

Finally, it seems possible that an enzyme might change the rate of a reaction indirectly, by desolvating the substrate at a site not identical with the site of reaction. Free energies of hydration of complex molecules are not always an additive function of their constituent groups. In pyrazine, for example, solvation at one end of the molecule seems to interfere with solvation at the other end, an influence that is inherently destabilizing. If a reaction passed through a transition state with similar properties, not shared in the same degree by the substrate in the ground state, the reaction would be solvent-retarded. Removal of the nonreacting end of the substrate from water might then be expected to result in catalysis. In another system, a substrate might resemble imidazole or p-nitrophenol in being subject to interactions with solvent at opposite ends of the molecule, that reinforce each other in stabilizing the ground state. If this special stabilization by solvent were absent in the transition state, then the reaction would be solvent-retarded. As in the previous example, one can imagine selective desolvation by an enzyme of a portion of the substrate not identical with the site of reaction. leading to enhanced reactivity of the part remaining in water. It remains to be determined whether enzymes actually use such indirect mechanisms of catalysis by desolvation.

Analysis of the structural origins and directional preferences of these interactions will require the best efforts of physical, organic, and theoretical chemists, and can be expected to lead to better understanding of equilibria and catalytic phenomena in water.

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