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Conformational changes during enzyme catalysis: Role of water in the transition state

(entropy of activation/catalytic rate enhancement/Hofmeister anions/chaotropic salts/amino acid:tRNA ligases)

ROBERT B. LOFTFIELD, E. ANN EIGNER, ANDRZEJ PASTUSZYN, TIMO NILS ERIK LÖVGREN†, AND HIERONIM JAKUBOWSKI‡

Department of Biochemistry, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131

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ABSTRACT **The entropy of activation for the synthesis of Ile-tRNA is high and positive. The only likely source of a high $\Delta S^{\ddagger}$ is the loss of structured water as the enzyme·substrate complex moves toward the transition state. This requires a change in the orientation or nature of water-organizing residues in the interface between the enzyme·substrate complex and the water. Such changes, which may be some distance from the "active site," are coupled to the active site in such a way that the increased entropy and decreased free energy of the water–enzyme interface is available at the "active site" to reduce the free energy of activation. The effects of Hofmeister anions on K_ms and k_{cat}s are consistent with the entropy data.**

Low and Somero (1, 2) have proposed that enzymic catalysis is accompanied by conformational changes in the enzyme·substrate complex that result in the transfer of amino acid residues through the protein–water interface. These transfers, which reduce the free energy of activation, were manifest in their experiments as salt-sensitive extrinsic volumes of activation, which they attributed to the structuring or unstructuring of water in the transition state. In their proposal, the net transfer of water-structuring residues in the transition state could be in either direction as long as the free energy of the protein–water interaction is lower in the transition state than in the ground state. For many reactions the decreased free energy in the transition state will result from decreased enthalpy (for instance, the formation of new hydrogen bonds). In other cases, the lowered $\Delta G^{\ddagger}$ might be expected to result from a high entropy in the transition state as structured interface water is "melted" or released to the bulk phase. Because it is difficult to imagine any source of a high entropy of activation except that resulting from "melting" of structured water, we sought an enzymic system with a high $\Delta S^{\ddagger}$. In such a system we could be assured that the enzyme–water interface was involved in the transition state and could examine the effects of Hofmeister salts on $\Delta G^{\ddagger}$ and $\Delta V^{\ddagger}$ as Low and Somero did.

The search for enzymic reactions with a positive $\Delta S^{\ddagger}$ is simplified by the knowledge that any enzymic reaction with a Q_{10} [$Q_{10} = k_{cat}(T_1)/k_{cat}(T_1 - 10°)$] greater than 3.0 is likely to have a positive $\Delta S^{\ddagger}$ (3). As an example (assuming that absolute reaction rate theory applies) at 20–30°C for $Q_{10} = 3.0$, $E_a \approx 19.4$ kcal mol^{-1} (1 kcal = 4.184 kJ):

$$\Delta H^{\ddagger} = E_a - RT \approx 18.8 \text{ kcal mol}^{-1}$$

$$\Delta G^{\ddagger} = RT \ln(kT/h) - RT \ln k_{cat} \approx 17.4 - RT \ln k_{cat} = 17.4 \text{ kcal mol}^{-1}$$

(if $k_{cat} = 1.0$ sec^{-1})

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$$

$$17.4 = 18.8 - T\Delta S^{\ddagger};\ \Delta S^{\ddagger} \approx +4.5 \text{ cal mol}^{-1} \text{ K}^{-1}$$

If k_{cat} is greater than 1 sec^{-1} or if Q_{10} is greater than 3.0, the entropies of activation will be correspondingly more positive.

The Q_{10} for the formation of Val-tRNA is about 7.0 (4). Yarus and Berg (5) report that the synthesis of Ile-tRNA at either pH 5.5 or pH 7.0 has a Q_{10} near 4.0. Charlier and Grosjean (6) find that synthesis of both Ile~AMP and Ile-tRNA have Q_{10}s greater than 3.5. Other reports (7, 8) suggest that the Q_{10} for these reactions may be greater than 4.0. These reactions probably have high positive entropies of activation. In order to measure the entropy of activation, we have determined the temperature dependence of the several Michaelis constants and the k_{cat}. In a similar way we have determined K_ms and k_{cat}s as functions of Hofmeister anion concentrations.

MATERIALS AND METHODS

Materials. [^{14}C]Isoleucine was obtained from Amersham/Searle; tRNA^{Ile} was purified according to the method of Gillam (9) from crude tRNA (Schwartz Bioresearch) and isoleucine:tRNA ligase was prepared by the method of Lövgren *et al.* (10), from *Escherichia coli*, then stored at −70°C.

Aminoacylation Assays. Unless otherwise indicated, the reaction mixture contained, in 40 μl total volume: 1.75 μM tRNA^{Ile}, 2.0 mM Mg·ATP, 5 mM $MgCl_2$, 20 μM [^{14}C]isoleucine (330 mCi/mol), 0.1 mM dithioerythritol, 20 μg of bovine albumin, and appropriate amounts of enzyme (1 Ci = 3.7×10^{10} becquerels). Various buffers were used, as shown in the legends, with 0.05 M pH 6.5 sodium cacodylate chosen for the determination of the K_ms because it was found to give the lowest K_m for tRNA (6 nM) and saturation was thus easier to achieve.

In general, the reactions were run at 25°C (except as noted) and initiated by the addition of ATP. Four aliquots of 7 μl were removed at intervals of 30 sec to 4 min, pipetted onto a 2.3-cm Whatman 3 MM filter paper disk, then immediately put into a beaker of cold 5% trichloroacetic acid. In experiments conducted at 0–25°C, aliquots were pipetted into chilled pipettes to avoid the artifact due to brief warming of the reaction mixture (8). After three washings with cold 5% trichloroacetic acid and one with cold ethanol, the disks were dried and radioactivity was measured in toluene/2,5-diphenyloxazole/1,4-bis[2-(5-phenyloxazolyl)]benzene in a Beckman scintillation counter (11). ATP:PP$_i$ exchange assays were done by the method of Eigner and Loftfield (12).

All reaction rates were determined from the best-fitting least squares approximation to the four time points. All K_ms and k_{cat}s

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† Present address: Åbo Akademi, Institutionen for Biokemi och Farmaci, Porthansgatan 3, 20500 Åbo 50, Finland.
‡ Present address: Institute of Biochemistry, Agriculture University, U. Wolynska 35, 60-637 Poznan, Poland.

Table 1. Enzymic parameters for the formation of Ile-tRNA^{Ile} as a function of temperature

Temp., °C	K_m(tRNA^{Ile}), μM	K_m(ATP), μM	K_m(Ile), μM	k_{cat}, sec^{-1}
0	0.3	75	2.0	0.018
5	0.4	73	1.7	0.049
11	0.4	60	1.3	0.127
17	0.5	70	1.7	0.31
25	0.5	56	1.6	0.74
37	1.1	72	3.3	3.2

Reaction conditions: pH 7.0 25 mM Pipes [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)]; 5.0 mM $MgCl_2$; unless otherwise indicated, 1.75 μM tRNA^{Ile}, 2.0 mM Mg·ATP, 20 μM [^{14}C]isoleucine.

were determined from Eadie–Hofstee plots (13). k_{cat} is reported in mol of Ile-tRNA synthesized per mol of enzyme per sec.

RESULTS

At each temperature a catalytic rate constant, k_{cat}, was calculated after first determining the Michaelis–Menten constant, K_m, for each of the substrates. As shown in Table 1, the K_ms for isoleucine, ATP, and tRNA^{Ile} are relatively insensitive to temperature. The determination of k_{cat} was carried out with each substrate in great excess over its K_m in order to minimize errors that might derive from variations in the K_ms.

Fig. 1 presents the Arrhenius plot of the data for k_{cat} from Table 1 as well as for the related half reaction in which the rate of formation of E·(Ile~AMP) is measured. In each case the Arrhenius plot is linear over the range 0–25°C; in each case E_a is greater than 25,000 cal mol^{-1} and $\Delta S^{\ddagger}$ is greater than +25 cal mol^{-1} K^{-1}. Table 2 presents the activation parameters for these reactions.

A high positive entropy of activation can be explained only by there being substantially less solvent structure in the transition state than in the ground state. Hofmeister ions (chaotropic salts) would be expected to disrupt the water structure in the ground state, thus reducing the entropy gained in achieving the transition state; consequently these salts would be inhibitory. Table 3 shows that a variety of Hofmeister anions inhibit the synthesis of Ile-tRNA in a variety of buffers according to their position in the Hofmeister series. To the extent that formation of the enzyme·substrates complex involves changes in the structuring of the aqueous solvent, inhibition could result from changes in the Michaelis constants as well as from changes in

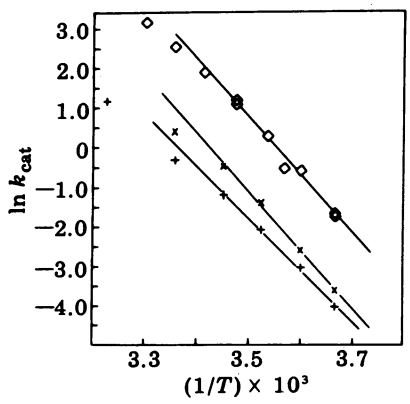

FIG. 1. Arrhenius plot of the k_{cat} data of Table 1 (+), and of similar data for the synthesis of Ile-tRNA^{Ile} in the presence of 100 μM spermine (×, 1.0 mM Mg·ATP, no excess Mg^{2+}) (14), and for the synthesis of E·(Ile~AMP) as determined from ATP:PP_i exchange (◇). The drawn lines are least squares best fitting for all points below 20°C.

Table 2. Reaction parameters at 0°C

Reaction	$\Delta G^{\ddagger}$, kcal mol^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, cal mol^{-1} K^{-1}
Ile-tRNA formation	+18.1	+25.6	+27.5
Ile-tRNA formation (with spermine)	+17.8	+28.8	+40.0
E·(Ile~AMP) formation	+16.8	+28.5	+42.7

Calculated from Fig. 1.

the catalytic rate constant. Table 4 shows that the binding of isoleucine and ATP to the enzyme is progressively facilitated by increasing concentrations of chloride or perchlorate. (K_ms for isoleucine and ATP decrease similarly for other Hofmeister anions.) Contrariwise, the Michaelis constants for the association of enzyme with tRNA^{Ile} increase rapidly with the addition of chloride or perchlorate. The dependence is described accurately by Eqs. 1 (which are the equations that describe competitive inhibition by cooperatively bound inhibitor),

$$\frac{K_m'}{K_m} = 1 + \left(\frac{[I]}{K_I}\right)^n \quad \text{or}$$

$$\log\left(\frac{K_m'}{K_m} - 1\right) = n \log[I] - n \log K_I \qquad [1]$$

in which [I] is the concentration of inhibitory anion, K_m' the Michaelis constant in the presence of the inhibitory anion, K_I the dissociation constant for the enzyme-inhibitor complex, and n the minimum number of inhibitor anions bound to the enzyme in the enzyme-inhibitor complex. Fig. 2 presents a plot of $\log[(K_m'/K_m) - 1]$ vs. $\log[I]$ for three Hofmeister anions. The linearity of the relationship permits accurate estimation of K_ms for intermediate concentrations of anion.

At constant concentrations of substrates, increasing concentrations of dinitrophenoxide, perchlorate, or chloride slow the synthesis of Ile-tRNA as shown by the open symbols of Fig. 3. Part of this inhibition is due to the increasing K_m'(tRNA)s, whose values can be accurately estimated from Fig. 2. From the observed rate and the K_m'(tRNA) it is possible to calculate k_{cat}, which is presented by the filled symbols of Fig. 3. The decreases in k_{cat} reflect noncompetitive inhibition.

Table 3. Relative rates of formation of Ile-tRNA as inhibited by Hofmeister anions

Na salt	Relative rate, % Tris*	Barbital†	Cacodylate‡
None	100	100	100
Acetate	90	111	90
Chloride	61	83	68
Bromide	46	77	57
Nitrate		61	56
Sulfate	19	25	53
Iodide	17	30	40
Thiocyanate	9	21	21
Perchlorate	5	11	11
p-Nitrophenoxide			3

Except for sulfate, the anions are arranged in order of increasing "chaotropic" activity.

* Tris [tris(hydroxymethyl)aminomethane] at 50 mM, adjusted to pH 7.5 with 1.0 M hydrochloric acid, with 30 mM added Hofmeister salt.

† Sodium diethylbarbiturate at 30 mM, adjusted to pH 7.5 with 1.0 M hydrochloric acid, with 25 mM added Hofmeister salt.

‡ Sodium cacodylate buffer at 50 mM, adjusted to pH 7.5 with 1.0 M hydrochloric acid, with 25 mM added Hofmeister salt.

Table 4. K_m(Ile), K_m(ATP), and K_m(tRNA^{Ile}) for the synthesis of Ile-tRNA^{Ile} as a function of Hofmeister salt concentration

Salt	Conc., mM	K_m(Ile), μM	K_m(Mg·ATP), μM	K_m(tRNA^{Ile}), nM
$NaClO_4$	0.0	2.2	50	6.0
	5.0	1.0	47	
	10.0	0.85	29	31
	15.0	0.77	13	88
	20.0	0.31		160
	25.0			366
NaCl	0	2.2	50	6.0
	50	0.9	32	29
	75	0.6	17.5	150
	100	0.45	4	430

Conditions: 50 mM sodium cacodylate buffer, pH 6.5; 25°C, 5.0 mM $MgCl_2$; unless otherwise indicated, 1.75 μM tRNA^{Ile}, 2.0 mM Mg·ATP, 20 μM [^{14}C]isoleucine.

Noncompetitive inhibition can be described by a Hill plot (15).

$$\frac{k_{cat}}{k'_{cat}} = 1 + \frac{[I]^n}{[K_I]^n} \quad \text{or}$$

$$\log\left[\frac{k_{cat}}{k'_{cat}} - 1\right] = n \log[I] - n \log[K_I]. \qquad [2]$$

In Fig. 4 the k_{cat} data of Fig. 3 are plotted according to Eq. 2. For each anion the Hill coefficient is close to 2.0, from which we may infer that at least two anions cooperate to inhibit the enzyme fully. Table 5 presents the Hill coefficients and the apparent K_Is calculated for both the K_m and k_{cat} data. It is notable that K'_m(tRNA) increases as a function of the cube of the concentration of each of the anions, whereas k_{cat} appears to be a function of the square of the anion concentration.

DISCUSSION

Under nonenzymic conditions a nucleophilic displacement on a carbonyl may have an entropy of activation near zero if the leaving group separates before the new bond forms as in the case of the hydrolysis of acetyl phosphate (16). If the new bond forms before the old bond breaks, as in the general base-catalyzed hydrolysis of alkyl trifluoroacetates (17), the $\Delta S^{\ddagger}$ is in the range of -27 to -50 cal mol^{-1} K^{-1}. The high negative $\Delta S^{\ddagger}$ has been interpreted as indicating that several highly ordered water molecules are part of the transition state structure (18).

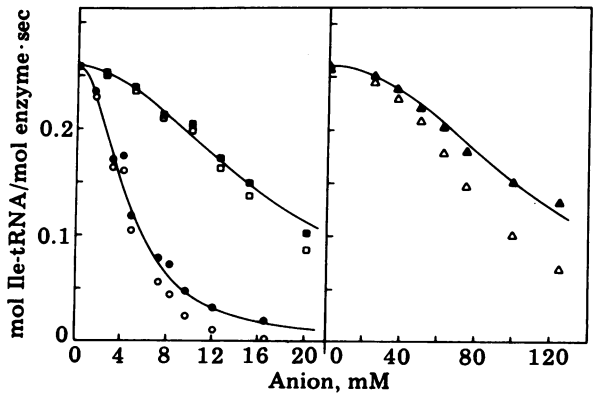

FIG. 3. Rate of Ile-tRNA synthesis as a function of Hofmeister salt concentration. Conditions as in Table 4. Open symbols show observed rates; closed symbols show k_{cat}s—i.e., rates corrected for variations in K_m due to Hofmeister salt; drawn curves are calculated curves based on K_ms and k_{cat}s calculated from the parameters of Table 5. ○ and ●, dinitrophenoxide (0.5 or 0.6 μM tRNA); □ and ■, perchlorate (1.0 μM tRNA); △ and ▲, chloride (0.6 μM tRNA).

The synthesis of Ile-tRNA involves a general base-catalyzed (19) displacement of a tRNA hydroxyl on carbonyl (20) in which the rate-limiting step is formation of the acyl-tRNA bond (8). By analogy we would expect the $\Delta S^{\ddagger}$ of the nonenzymic reaction to be very negative. Instead we observe $\Delta S^{\ddagger}$ to be more positive than +25 cal mol^{-1} K^{-1} for the enzyme-catalyzed reaction.

Among nonenzymatic chemical reactions, those that have high positive $\Delta S^{\ddagger}$ are those in which a highly solvated triply charged cation reacts with a solvated anion to form a transition state complex that is less charged and, therefore, less solvated. Examples include the reactions of Fe^{3+} or Co^{3+} with the anions of H_2O_2 or hydroquinone (21), all of which have $\Delta S^{\ddagger}$ in the range of +50 cal mol^{-1} K^{-1}, exactly the entropy gain anticipated for this type of charge cancellation in water.

In the reaction under consideration there is no such charge cancellation and no reasonable scheme of active site chemistry that would yield a high positive $\Delta S^{\ddagger}$. If the chemistry at the active site is tightly coupled to relatively remote conformational movements of the protein and substrate, one can conceive of several changes that would increase the entropy. On the basis of the temperature coefficients of solubility for glycine, valine, and leucine in 20.3% and 92.6% (wt/wt) ethanol/water mix-

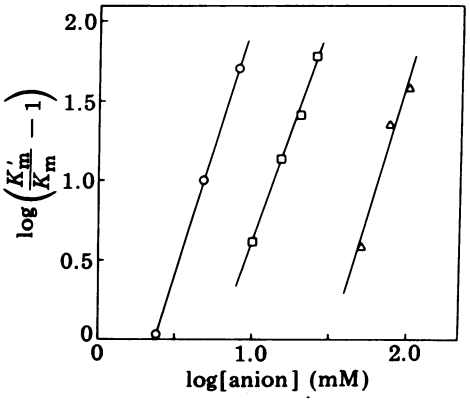

FIG. 2. Michaelis constants for tRNA^{Ile} as a function of Hofmeister salt concentration. Data partly from Table 4. ○, Dinitrophenoxide; □, perchlorate; △, chloride. The best-fitting straight lines correspond to $\log[(K'_m/K_m) - 1]$: = −1.18 + 3.19 log[DNP$^-$]; = −2.21 + 2.83 log[ClO_4^-]; = −5.09 + 3.37 log[Cl^-] (concentrations in mM).

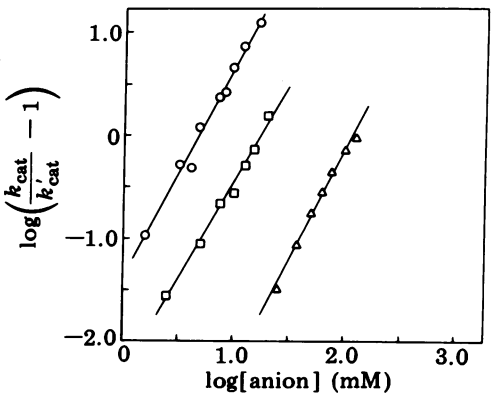

FIG. 4. k_{cat} for the synthesis of Ile-tRNA as a function of Hofmeister salt concentration. Rates from Fig. 3 corrected for K_ms according to Fig. 2. ○, Dinitrophenoxide; □, perchlorate; △, chloride. The best-fitting straight lines correspond to $\log[(k_{cat}/k'_{cat}) - 1]$: = −1.40 + 2.06 log[DNP$^-$]; = −2.35 + 1.89 log[$ClO_4^-$]; = −4.43 + 2.14 log[$Cl^-$] (concentrations in mM).

Table 5. Apparent inhibitor binding constants and Hill coefficients for effects on tRNA binding and on catalytic constants

Anion	K_m(tRNA)*		k_{cat}†	
	K_I, mM	n	K_I, mM	n
Dinitrophenoxide	2.3	3.2	4.8	2.1
Perchlorate	6.0	2.8	17.5	1.9
Chloride	32.6	3.4	117.5	2.1

* K_I and n are defined in Eq 1; K_I equals concentration of inhibitor that doubles the value of K_m(tRNA).

† K_I and n are defined in Eq. 2; K_I equals the concentration of inhibitor that reduces k_{cat} by half.

tures (ref. 22, summarized in ref. 23), one can estimate that the transfer of a valine or leucine side chain from water to a nonaqueous medium is accompanied by about 8 cal mol^{-1} K^{-1} more entropy than the transfer of glycine. Similar estimates based on distribution coefficients (water vs. chloroform or butyl ether) of acetylamino acid ethyl esters (24) suggest that the transfer of norvaline or norleucine from water has about 15 cal mol^{-1} K^{-1} more positive entropy than the transfer of alanine. Thus a conformational movement in which a valine or leucine in the water interface is replaced by a glycine or alanine from within the protein might show an entropy increase of 8–15 cal mol^{-1} K^{-1}. Of course a complete exchange is unlikely, but one can imagine a small conformational movement of many residues that, in aggregate, yields a substantial entropy gain. Another attractive source of positive entropy would be a conformational movement that permitted two oppositely charged surface residues to approach each other. Much of the water structured around each charge would be released as two charges cancelled each other, with an entropy gain of 15–30 cal mol^{-1} K^{-1} (25).

Regardless of the actual mechanism, it appears that the binding of substrates to enzymes and their subsequent reaction lead to decreases and increases in entropy that are most easily interpreted as due to conformational changes that affect water structure. For instance, acetylcholinesterase binds acetylcholine and three less reactive homologous substrates with the entropies shown in Table 6 (26). Because all four substrates have, at pH 7.0, identical charge distributions and similar geometries, it may be presumed that all the initial enzyme-substrate complexes have similar structures and ΔS_us (0–10 cal mol^{-1} K^{-1}). Of these, only the complex containing acetylcholine undergoes a drastic conformational change with the loss of some 15–30 cal mol^{-1} K^{-1}, undoubtedly largely due to structuring of water. It is notable that the entropies of the four transition states relative to the free enzyme and free substrate (i.e., $\Delta S_u + \Delta S^{\ddagger}$) are approximately equal. This suggests that each of the four transition states has a similar extent of order, including the structured solvent.

Table 6. Michaelis constants, catalytic rate constants, and corresponding entropies of association and activation for acetylcholinesterase

Substrate	K_m,* mM	ΔS_u(ass), cal K^{-1} mol^{-1}	k_{ca} (25°C), sec^{-1}	$\Delta S^{\ddagger}$, cal K^{-1} mol^{-1}	($\Delta S_u + \Delta S^{\ddagger}$), cal K^{-1} mol^{-1}
Acetylcholine	0.4–0.9	−(14–29)	$2–3 \times 10^6$	+(16–34)	ca. +2
DMAE acetate	1.2	+3	3×10^5	−(7–11)	−6
MAE acetate	10	+4	1×10^5	−9	−5
AE acetate	16	+8	9×10^3	−9	−1

Taken from Wilson and Cabib (26). DMAE, dimethylaminoethyl; MAE, methylaminoethyl; AE, aminoethyl.

* Considered by Wilson and Cabib (26) to be the dissociation constant of the enzyme-substrate complex.

The thermodynamics of binding of tRNAs to the amino acid:tRNA ligases reveals a pattern parallel to that of acetylcholinesterase. The isoleucine enzyme binds cognate $tRNA^{Ile}$ with 20 cal mol^{-1} K^{-1} lower entropy than it binds noncognate $tRNA^{Val}$ (27), whereas the phenylalanine enzyme binds cognate $tRNA^{Phe}$ with 55 cal mol^{-1} K^{-1} lower entropy than the noncognate $tRNA^{Tyr}$ (28). In the latter case, rapid kinetic techniques have established that both $tRNA^{Phe}$ and $tRNA^{Tyr}$ bind rapidly to the enzyme with similar rates of association and dissociation, but that only $tRNA^{Phe}$ effects the change in conformation that leads to a low-entropy enzyme·tRNA complex (29).

Both the amino acid:tRNA ligases and acetylcholinesterase can be described by the scheme of Fig. 5, the only difference being the absolute charge on substrate and enzyme. Correct and incorrect substrates bind to the enzyme. In all cases the substrate

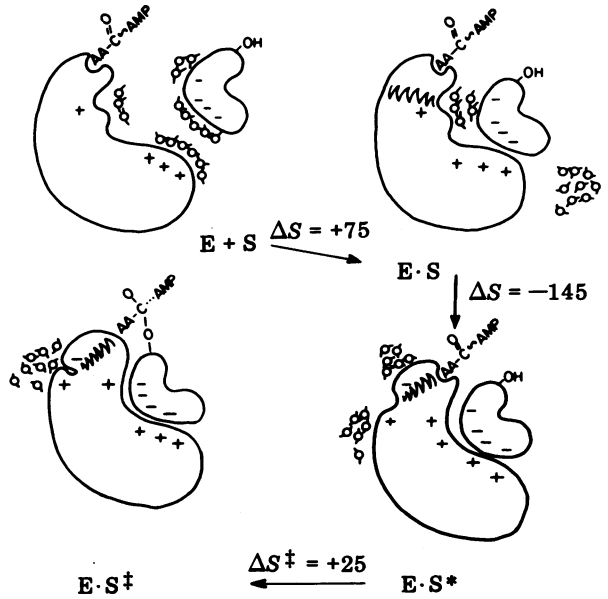

FIG. 5. Schematic representation of enzymic rate enhancement by participation of the enzyme-water interface. The nonspecific association of enzyme and tRNA to form E-S is largely ionic and results in the release of structured water and a high positive entropy. Only part of the substrate has bound to the enzyme. The binding of the remainder of the substrate is exothermic, but forces a conformational change of E-S to E-S* in which about 145 cal mol^{-1} K^{-1} is lost. [Crude estimates of the thermodynamic parameters can be derived from Maass (28, 29) and are useful only to give an appreciation of the direction and possible magnitude of these effects: K_d (E^{Phe}·$tRNA^{Tyr}$) = 2 μM; K_d (E^{Phe}·$tRNA^{Phe}$) = 1.25 μM; K_2 ([E-S*]/[E-S]) = 0.6; ΔS_{ass} (E^{Phe}·$tRNA^{Tyr}$) = 75 cal mol^{-1} K^{-1}, ΔS_{ass} (E^{Phe}·$tRNA^{Phe}$) = 20 cal mol^{-1} K^{-1}.] Because the equilibrium between E-S and E-S* is near unity, there must be about 40 kcal decrease in enthalpy resulting from the formation of new hydrogen bonds, ionic bonds, van der Waals bonds, etc. We propose that the conformational change causes the surface emergence of water-organizing charges that account for the entropy loss. The movement from E-S* to E-S‡ involves surface changes that neutralize the water-organizing foci; this change (high entropy) is coupled to movements at the active site that drive the substrates into a high enthalpy transition state. A similar scheme can be applied to catalysis by acetylcholinesterase. Poor substrates (dimethylaminoethyl acetate, methylaminoethyl acetate, aminoethyl acetate) bind to the enzyme, but predominantly to form high-entropy E-S. Movement to the E-S‡ is therefore entropically negative and catalysis is slow. Acetylcholine forms a complex that is largely in the E-S* conformation from which a positive entropy change facilitates reaching E-S‡.

is known to be ionic and, therefore, solvated; in every case it is known that the enzyme carries countercharges that are also solvated. The high entropy of initial association results largely from the release of bound water as the E·S complex forms. Then, driven by the negative enthalpy (formation of hydrogen bonds, perfect van der Waals fits, etc.) a conformational change occurs only with the correct substrate, the new E·S* complex possessing extraordinarily low entropy. As E·S* moves towards $E{\cdot}S^{\ddagger}$, the foci that organized water in E·S* release the water, permitting the increase in entropy that reduces the free energy of $E{\cdot}S^{\ddagger}$, thereby increasing k_{cat} for the correct substrates. When an incorrect substrate binds to the enzyme, it is either completely unable to proceed to E·S* and $E{\cdot}S^{\ddagger}$ or it must move to $E{\cdot}S^{\ddagger}$ all the way from the ground state of E·S, thereby denying itself the entropic push available to a correct substrate. Jencks (30) has proposed a "Circe effect" in which not all the binding energy is utilized in forming the E·S complex. The full binding energy is expressed in forming an E·S (which need not represent a significant part of the ground state complex) in which full utilization of binding energy has destabilized the complex relative to the transition state. Our E·S* may correspond to Jencks' E·S* or there might be another E·S** between our E·S* and $E{\cdot}S^{\ddagger}$ in which additional water has been lost. In the latter case the high positive $\Delta S^{\ddagger}$ might correspond to $E{\cdot}S^{*} \rightleftharpoons E{\cdot}S^{**}$ rather than to $E{\cdot}S^{*} \rightleftarrows E{\cdot}S^{\ddagger}$. Atkinson (31) has also argued that excess binding energy can be used, not merely to concentrate substrates from dilute solution, but to force enzyme and substrate into an "energetically loaded" or strained conformation that makes the ground state energy closer to that of the transition state.

In the case of the amino acid:tRNA ligases each of the complexes E·S* and $E{\cdot}S^{\ddagger}$ appears to have high entropy relative to free enzyme and free substrates. To the extent that high entropy relfects release of structured water, it would be expected that Hofmeister anions, which destructure water, would reduce the gains in entropy and would interfere with both the formation of E·S complexes and with the achievement of the transition state. The effects we observe (Tables 3 and 4; Figs. 2, 3, and 4) are qualitatively consistent with expectation. Quantitatively, the effects occur at much lower salt concentrations than those required to show nonspecific salting-in effects. It is likely that there are anion-binding sites capable of preventing formation of the E·S complex and other anion-binding sites that are involved in the catalytic process. Fridovich (32) has shown that acetoacetate decarboxylase is noncompetitively inhibited by the binding of a single Hofmeister anion from dilute solution. Whether the anion is binding directly to enzyme or destructuring water associated with enzyme, it is probable that some of the effects are away from the "catalytic sites," because steric considerations make it unlikely that two to five perchlorate or dinitrophenoxide ions can all be bound simultaneously at the catalytic site.

Taken together, the Hofmeister salt effects and the high entropies of activation constitute evidence that the transition state is stabilized by conformational changes from the enzyme·substrate ground state. These changes are relatively distant from the site of chemical reaction and include energy-releasing movements of the water surrounding the enzyme. Although this concept is based on reactions with high positive $\Delta S^{\ddagger}$, entirely comparable rate enhancement can be provided by conformational changes that decrease the $\Delta H^{\ddagger}$ of the enzyme·substrate and of its interaction with water.

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