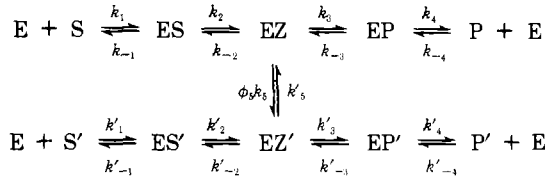
Free-Energy Profile for the Reaction Catalyzed by Triosephosphate Isomerase†

W. John Albery‡ and Jeremy R. Knowles*

ABSTRACT: The experimental results on the interconversion of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate catalyzed by triosephosphate isomerase that are presented in the previous five papers are here collected and analyzed according to the theory presented in the first paper (Albery, W. J., and Knowles, J. R. (1976), *Biochemistry 15,* the first of eight papers in a series in this issue). The rate constants and fractionation factors so derived allow the construction of the Gibbs free-energy profile for this enzyme-catalyzed reaction.

The first paper in this series (Albery and Knowles, 1976a) presented the theory for 16 different experiments involving deuterium or tritium, which may be carried out on the system shown in Scheme I, where E is the enzyme, ES and EP are the

Scheme I



enzyme-bound complexes of the substrates S and P, and EZ is an intermediate that can exchange hydrogen isotopes with the solvent. The primes indicate isotopic substitution by ^2H or by ^3H. For triosephosphate isomerase, S is dihydroxyacetone phosphate and P is D-glyceraldehyde 3-phosphate.

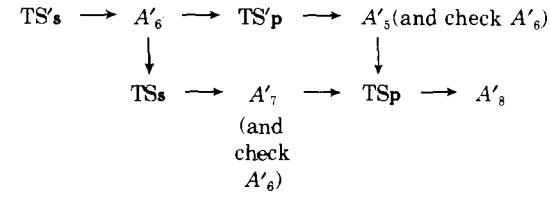
In the next five papers in the series (Herlihy et al., 1976; Maister et al., 1976; Fletcher et al., 1976; Leadlay et al., 1976; Fisher et al., 1976) are presented the results for 11 of the 16 experiments on the triosephosphate isomerase reaction, together with results for the two substrate fractionation factors, Φ_S and Φ_P. From these experimental results, the Gibbs free-energy profile for the reaction is constructed in two stages. First, the results are plotted according to the equations given in Table III of Albery and Knowles (1976a) to find values of the parameters A_n and B_n. These parameters are functions of the different rate constants and fractionation factors. In the second stage of the analysis, the values of A_n and B_n are combined together to allow the derivation of the individual rate constants and fractionation factors. Since there are more pieces of experimental information than unknown parameters, the consistency of the data and the success of the kinetic model for the reaction may be checked.

The first stage of the analysis—the calculation of values for A_n and B_n—has been carried out in the previous five papers (Herlihy et al., 1976; Maister et al., 1976; Fletcher et al., 1976; Leadlay et al., 1976; Fisher et al., 1976). In the discussion sections of these papers, qualitative descriptions of the results have also been presented, and a physical picture of the meaning of the data has been given. (The reader is referred back to these discussions in order to be aware of which experiments define which aspects of the catalyzed reaction.) The present paper describes the second stage of the quantitative analysis, and the construction of the Gibbs free-energy profile.

Experiments Involving Tritium

In Herlihy et al. (1976) and in Maister et al. (1976) we have followed the route suggested in Albery and Knowles (1976a) for the evaluation of A_5', A_6', A_7', and A_8', as shown in Scheme II. Herlihy et al. (1976) report the results from TS's and TS'**p**,

Scheme II



and Maister et al. (1976) those from TS**s** and TS**p**. The derived values of A_n' are collected in Table I. Furthermore, the plots from the TS'**p** and TS's experiments gave straight lines, thereby confirming the value of A_6' derived from the TS's experiment.

Because of the practical difficulties of preparing P' (D-[2-^{3}H]glyceraldehyde 3-phosphate) of high specific radioactivity, we have not carried out the TP'**p** and TP's experiments, and so cannot follow the analogous route to the B_n' parameters. However, the TP**p** and TP**s** experiments are described in Fletcher et al. (1976), and the first of these gives the following relationship between B_7' and B_6':

$$B_7' = (0.33 \pm 0.03) + 0.19(B_6' - 1) \qquad (1.1)$$

This relation is simply derived from the data in Table IV of Fletcher et al. (1976). The second experiment (TP**s**) of Fletcher et al. (1976) yields the value of B_8' in Table I. Also included in this table are the values for the two substrate fractionation factors, Φ_S and Φ_P. The greater practical difficulties associated with the analysis of the tritium content of the small amount (4%) of D-glyceraldehyde 3-phosphate present at equilibrium (compared with the determination of the specific radioactivity of the 96% of dihydroxyacetone phosphate) means that Φ_P has a larger experimental error than has Φ_S. Both fractionation factors are, however, close to unity, which is expected for deuterium or tritium bound to an sp^3 hybridized carbon atom (Schowen, 1972). Furthermore, the

† From the Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138. *Received March 4, 1976.* This work was done partly in Oxford, supported by the Science Research Council, and partly in Cambridge, supported by the National Science Foundation.

‡ Physical Chemistry Laboratory, University of Oxford, Oxford OX1 3QZ, United Kingdom.

TABLE I: Values of Parameters from ^3H Experiments.

$A_5' = 0.058 \pm 0.004$	(Herlihy et al., 1976)
$A_6' = 0.50 \pm 0.03$	(Herlihy et al., 1976)
$A_7' = 0.27 \pm 0.025$	(Maister et al., 1976)
$A_8' = 0.78 \pm 0.01$	(Maister et al., 1976)
$B_5' \simeq 0.02$	(Fletcher et al., 1976)
$B_8' = 0.13 \pm 0.01$	(Fletcher et al., 1976)
$\Phi_S = 1.03 \pm 0.04$	(Fletcher et al., 1976)
$\Phi_P = 1.0 \pm 0.2$	(Fletcher et al., 1976)

sites in dihydroxyacetone phosphate (on C-1) and in D-glyceraldehyde 3-phosphate (on C-2) are very similar, and one would expect them to have nearly identical fractionation factors. Hence, despite the experimental uncertainty of Φ_P, we can be confident that it is within a few percent of unity.

According to Albery and Knowles (1976a), the tritium experiments should provide four pieces of kinetic information [$\Phi_{1,2}$ from A_6' and A_7'; $\Phi_{3,4}$ from A_8'; θ from A_6' and A_7'; and a ratio involving k_5 in A_5'; see Table IV of Albery and Knowles, 1976a] and four checks on the internal consistency of the data. Because two out of the eight possible tritium experiments have not been done, we obtain the four pieces of kinetic information, with two checks.

Equations 7.1 and 7.2 of Albery and Knowles (1976a) state that:

$$\frac{B_5'}{A_5'} = \frac{\Phi_S}{\Phi_P}\frac{A_6'}{B_6'} = \frac{B_8'}{B_7'}\frac{(1 - B_5')}{(1 - A_5'B_5')} = \frac{A_7'(1 - A_5'B_5')}{A_8'(1 - A_5')} \tag{1.2}$$

and

$$\frac{A_7'}{\Phi_S A_6'(1 - A_5')} + \frac{B_7'}{\Phi_P B_6'(1 - B_5')} = 1 \tag{1.3}$$

Using the values of A_5', A_7', and A_8' (from Table I), we can calculate that:

$$B_5' = 0.02 \tag{1.4}$$

Hence the term $A_5'B_5'$ is negligible with respect to unity, and substitution from eq 1.2 into eq 1.3 gives:

$$\frac{A_7'}{1 - A_5'} + B_8' = \Phi_S A_6' \tag{1.5}$$

We can check this equality from the values in Table I, and we obtain

$$\frac{A_7'}{1 - A_5'} + B_8' = 0.42 \pm 0.03$$

and

$$\Phi_S A_6' = 0.51 \pm 0.04$$

Reasonable agreement is found. Giving some weight to the necessary equality of eq 1.5, we obtain the values of the parameters listed in Table II.

From eq 1.2, we find

$$B_7' = 0.34 \pm 0.03 \tag{1.6}$$

and

$$B_6' = 1.14 \pm 0.13 \tag{1.7}$$

We may now examine the second check, by comparing these values of B_6' and B_7' with the relationship of eq 1.1 that was derived from the completely separate experiment, TP**p**. Figure 1 compares eq 1.1, 1.6, and 1.7. For a perfect check, the three full lines should intersect. The broken lines show an intersection

TABLE II: Values of Parameters after Including Equations 1.5 and 1.1.

From eq 1.5	
$A_6' = 0.47$	$B_8' = 0.14$
$A_7' = 0.30$	$\Phi_S = 0.99$
From eq 1.1	
$B_6' = 1.12$	$B_7' = 0.35$
Unchanged (from Table I)	
$A_5' = 0.06$	$\Phi_P = 1.0$
$A_8' = 0.78$	

TABLE III: Values of $\Phi_{1,2}$, $\Phi_{3,4}$, and θ.[a]

$\Phi_{1,2} = 0.14 \pm 0.01$	$\theta = 2.18 \pm 0.3$ (from A_n' data)
$\Phi_{3,4} = 0.83 \pm 0.01$	$\theta = 2.14 \pm 0.3$ (from B_n' data)
	$\theta = 2.2 \pm 0.2$ (mean value)

[a] Calculated from the values in Table I and eq 7.5 of Albery and Knowles (1976a).

that is one-fifth of a standard deviation from each of the lines. This check is therefore extremely successful, and we take the values of B_6' and B_7' given in Table II. Finally, we may now check the values of the parameters in Table II against the identities of eq 1.2 and 1.3:

$$\frac{A_7'}{A_8'(1 - A_5')} = 0.41; \frac{B_8'(1 - B_5')}{B_7'} = 0.40; \frac{\Phi_S A_6'}{\Phi_P B_6'} = 0.42;$$

and:

$$\frac{A_7'}{\Phi_S A_6'(1 - A_5')} + \frac{B_7'}{\Phi_P B_6'(1 - B_5')} = 1.00$$

Having obtained the values of the parameters listed in Table II and demonstrated their internal consistency, we may now calculate the mixed fractionation factors ($\Phi_{1,2}$ and $\Phi_{3,4}$) and θ, from eq 7.3 to 7.5 of Albery and Knowles (1976a). These values are given in Table III.

The parameter θ describes how the intermediate EZ partitions between S and P, and from eq 1.7 and Table IV of Albery and Knowles (1976a):

$$\theta = \left(\frac{k_{-2}}{1 + (k_2/k_{-1})}\right)\left(\frac{1 + (k_{-3}/k_4)}{k_3}\right)$$

The value of θ is about 2 (Table III), which means that in the all-hydrogen system, EZ forms S about twice as frequently as it is converted to P. The low value of $\Phi_{1,2}$ (0.14) shows that, in the pair of steps represented by S → ES → EZ, the process involving proton transfer (ES → EZ: this represents the abstraction of the 1-*pro-R* proton of dihydroxyacetone phosphate by a base at the active site) is significantly rate limiting, and that the binding step (S → ES) is relatively fast. On the other hand, the value of $\Phi_{3,4}$ (0.83) shows that, in the pair of steps represented by EZ → EP → P, the main rate-limiting process does not involve proton transfer and is EP → P (the loss of glyceraldehyde 3-phosphate from the enzyme). The value for $\Phi_{3,4}$ is, however, significantly smaller than unity, and smaller than the value of 1.0 that we expect for the unmixed fractionation factor Φ_4. (This expectation arises from the likelihood that $\Phi_4 = \Phi_P$ since the act of binding the enzyme to the substrate P should not effect the fractionation factor Φ_P.) The experimental error in $\Phi_{3,4}$ is small (see Table III) and we may conclude that this fractionation factor does include a contribution from the lower fractionation factor Φ_3 which relates to

TABLE IV: Values of Parameters from ^1H and ^2H Experiments.

From Putman et al. (1972)
$A_1 = 2.25 \pm 0.08 \times 10^{-6}$ Ms
$A_2 = 2.32 \pm 0.1 \times 10^{-3}$ s
$B_1 = 0.110 \pm 0.006 \times 10^{-6}$ Ms
$B_2 = 0.235 \pm 0.03 \times 10^{-3}$ s

From Leadlay et al. (1976)
$A_3/A_1 = 2.9 \pm 0.25$ $A_4/A_2 = 3.0 \pm 0.5$
$B_3/B_1 = 1.15 \pm 0.14$ $B_4/B_2 = 1.01 \pm 0.25$

From Fisher et al. (1976)
$A_5 = 0.059 \pm 0.005$

TABLE V: Evaluation of Thermodynamic Parameters.[a]

	Eq No.[b]
$A_{10} = 1.9 \pm 0.5$	(9.5)
$B_{10} = 0.0 \pm 0.4$	(9.6)
$A_2 + B_2 - A_{10} - B_{10} = 0.6 \pm 0.6$	
$k_1/k_{-1} = 0.8 \pm 0.2 \times 10^3$ M^{-1}	(9.7)
$k_2/k_{-2} = 0.3 \pm 0.3$	(9.8)
$k_3/k_{-3} = (0.0 \pm 0.4)/(0.6 \pm 0.6)$	(9.9)
$k_4/k_{-4} \geq 0.3 \times 10^{-3}$ M	(9.10)

[a] Values relating to the free carbonyl forms: corrected for the equilibrium proportion of unhydrated form of the substrates, using K_S and K_P (see text). [b] From Albery and Knowles (1976a).

the proton transfer step EZ → EP and will have a value of about 0.1–0.2 (see below). This conclusion is reinforced by considering Figure 1. If Φ_P were 0.83, then the value of B_6' calculated from the A_n' data would be 1.37. The line drawn in Figure 1 corresponding to this value fits the data much less well than the line corresponding to $\Phi_P = 1$.

Experiments Involving Deuterium. In Table IV are collected the values for A_n and B_n obtained by Putman et al. (1972) and Leadlay et al. (1976). Using the value of θ from Table III and eq 7.12 and 7.13 from Albery and Knowles (1976a), we can obtain values for the mixed fractionation factors for deuterium: $\phi_{1,2} = 0.31 \pm 0.03$ and $\phi_{3,4} = 0.92 \pm 0.11$. These values are consistent with the values of $\Phi_{1,2}$ and $\Phi_{3,4}$ in Table III. The value for $\phi_{1,2}$ does deviate from the Swain–Schaad relationship, but the deviation is not significant nor is it known precisely enough to make use of our earlier discussion (Albery and Knowles, 1976a) of the possible breakdown of the Swain–Schaad relationship (Swain et al., 1958). On the basis that this relationship holds, "best" values of $\phi_{1,2} = 0.28$ and $\Phi_{1,2} = 0.16$ are taken.

Next, we see that the values of A_5 and A_5' in Tables II and IV are nearly equal (i.e., there is very little difference between the fraction of ^2H and of ^3H that is transferred from [1(*R*)-^{2}H]- and [1(*R*)-^{3}H]-labeled dihydroxyacetone phosphate to the product 3-phosphoglycerate (Fisher et al., 1975). Using eq 8.3 and 8.4 of Albery and Knowles (1976a), and $\Phi_{3,4} = 0.83$, we conclude that $\phi_5 = 0.94 \pm 0.17$. The fact that ϕ_5 is close to unity means that the rate-determining step for proton exchange between the conjugate acid of the enzyme's catalytic base and the solvent does not involve the transfer of the isotopically labeled proton. The implications of this finding are discussed elsewhere (Fisher et al., 1976). Finally, we require a value for $B_5\theta^{-1}$, and this is obtained from eq 7.10 of Albery and Knowles (1976a):

$$B_5\theta^{-1} = \frac{A_5\phi_{1,2}(1 + B_5/\theta')}{\phi_{3,4}(1 + A_5\theta')} = 0.02$$

TABLE VI: Evaluation of Kinetic Parameters.

	$\alpha = 0.0$	$\alpha = 0.30$	Eq No.[a]
k_1/M^{-1} μs^{-1}	∞	6	(10.3)
k_2/ms^{-1}	1.7	2.2	(10.4)
ϕ_2	0.28	0.23	(10.1)
	$\phi_3 = 0.30$	$\phi_3 = 0.20$	Eq No.[a]
γ[b]	15	28	(10.2)
k_{-4}/M^{-1} μs^{-1}	14	14	(10.6)
$\gamma k_5/k_3$[b]	14	15	(8.4)

[a] From Albery and Knowles (1976a). [b] $\gamma = k_{-3}/k_4$.

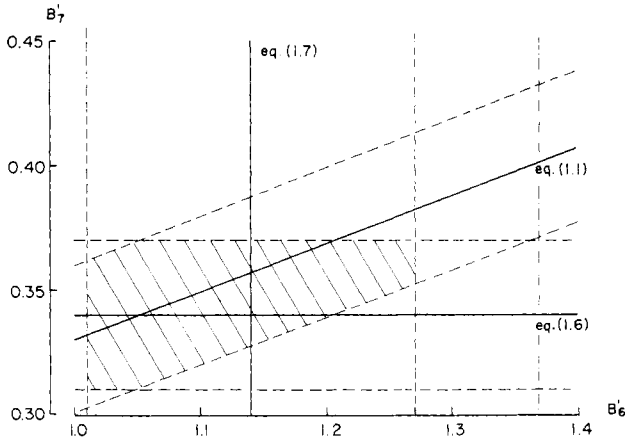


FIGURE 1: Check on the consistency of the results from eq 1.1, 1.6, and 1.7. The full lines represent plots based on the experimental results. The broken lines represent one standard deviation from these values. The hatched area shows the range of values of B_6' and B_7' within one standard deviation of the values in the three equations. The full circle gives the "best" values reported in Table II. The fine - - - - is the value of B_6' with $\Phi_P = 0.83$ rather than $\Phi_P = 1.00$.

Evaluation of the Thermodynamic Parameters. We may now derive the thermodynamics of the free-energy profile (i.e., the free energies of the intermediates), and these results are given in Table V. Within experimental error, both ($A_2 + B_2 - A_{10} - B_{10}$) and B_{10} could equal zero, which (as discussed in Albery and Knowles, 1976a; eq 9.12 and 9.13) means that it is possible that both EZ and EP are kinetically insignificant. There is no doubt, however, that the most stable intermediate is ES.

Evaluation of the Kinetic Parameters. We start with eq 10.7 of Albery and Knowles (1976a):

$$\frac{A_{10}}{1 + \alpha} - \frac{B_{10}}{1 + \gamma} = A_2 - B_2\theta$$

The B_{10} term is negligible with respect to the other terms, and from this equation, we determine $\alpha = 0.0_5 \pm 0.2_5$. It is thus possible that α is close to zero and that transition state 1 is kinetically insignificant. Fortunately, however, we can set some limits on transition state 1. The maximum permissible value of α is 0.3, which results in a value for k_1 (see Table VI) of approximately 6×10^6 M^{-1} s^{-1}. But since k_1 represents the rate of association of dihydroxyacetone phosphate with the enzyme, its value cannot be greater than the diffusion limit, which is likely to be between 10^8 and 10^9 M^{-1} s^{-1} for these molecules (pace Kuo-chen and Shou-Ping, 1974). In Table VI we report values for the rate constants and ϕ_2 for $\alpha = 0.0$ and $\alpha = 0.30$; either value of ϕ_2 is a reasonable one for a proton in

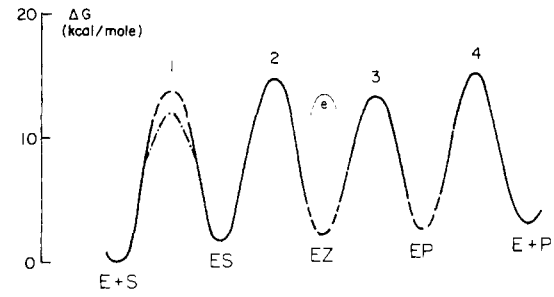


FIGURE 2: The free-energy profile for the reaction catalyzed by triosephosphate isomerase. The standard state is 40 μM [which is the known concentration of triosephosphates in vivo (Williamson, 1965)]. Broken lines indicate limits on those species that may be kinetically insignificant. The line - - - - - for transition state 1 is the diffusion-controlled limit (assuming $k_1 = k_{-4}$). The barrier labeled "e" represents that for the exchange of the proton from the conjugate acid of the enzyme's catalytic base (in the enzyme-enediol intermediate) with a proton from the medium.

TABLE VII: Data for the Free-Energy Profile.

Value	ΔG (kcal mol^{-1})
$\frac{k_1k_2k_3k_4}{k_{-1}k_{-2}k_{-3}k_{-4}} = 0.0031$	3.5
$k_1/k_{-1}^a = 1.3 \pm 0.4 \times 10^3$ M^{-1}	1.8[c]
$k_2/k_{-2} = 0.3 \pm 0.3$	>0.3
$k_4/k_{-4}^a > 1.1 \times 10^{-5}$ M	<0.8[c]
$k_1^a > 10^7$ M^{-1} s^{-1}	14.1[c]
$k_2 = 1.7 \pm 0.5 \times 10^3$ s^{-1}	13.3
$k_{-3}/k_4 = 21 \pm 6$	−1.8
$k_{-4}^a = 3.7 \times 10^8$ M^{-1} s^{-1}	12.0
$\frac{k_5k_{-3}}{k_3k_4} = 14 \pm 3$	−1.6

[a] Values relating to the unhydrated substrate forms: see the text. [b] Calculated from $(k_1k_2k_3k_4)/(k_{-1}k_{-2}k_{-3}k_{-4}) = [B_1(1 + K_S^{-1})]/[A_1(1 + K_P^{-1})]$. [c] Calculated for a standard state of 40 μM: see the text.

flight from carbon. The value of k_2 is relatively insensitive to the value of α.

To determine the other rate constants we must depend on an assumed value of ϕ_3. We know ϕ_2 to be close to 0.28, and we may assume that ϕ_3 (the fractionation factor for a proton abstraction very similar to that represented by ϕ_2) is approximately equal to ϕ_2. In Table VI we report values for $\phi_3 = 0.20$ and $\phi_3 = 0.30$. Since γ is large, the value of k_{-4} is quite insensitive to this. The free energy of the third transition state is related to that of the fourth, through γ, and so we can determine the free energies of transition states 2, 3, 4, and 5, with reasonable certainty, and only transition state 1 is in any doubt.

Before constructing the free-energy profile we allow for the fact that both dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate exist in aqueous solution in equilibrium with their hydrates. The enzyme triosephosphate isomerase only handles the free carbonyl forms of each substrate (Reynolds et al., 1971; Trentham et al., 1969) and the gem-diols (at least, that of dihydroxyacetone phosphate) bind to the enzyme weakly if at all (M. R. Webb, P. E. Johnson, and D. Standring, unpublished work). The equilibrium constants for the dehydration equilibria (>C(OH)$_2$ ⇌ >C=O) for dihydroxyacetone phosphate and glyceraldehyde 3-phosphate are $K_S = 1.44$ and $K_P = 0.039$, respectively (by interpolating between measured values in the literature at 20 and 37 °C: Reynolds et al., 1971; Trentham et al., 1969; Gray and Barker, 1970). The rate constants k_1 and k_{-4} are corrected for the hydration equilibria using:

$$(k_n)_{\text{unhyd}} = (k_n)_{\text{obsd}}(1 + K_N^{-1})$$

where k_n is k_1 or k_{-4} and K_N is K_S or K_P, respectively. The values of the rate constants and thermodynamic parameters used to construct the free-energy profile are collected in Table VII, and the profile itself is illustrated in Figure 2.

Features of the Free-Energy Profile. To present the results in Table VII in pictorial form as a Gibbs free-energy profile, we need to choose a standard state. Thus the position of E + S and E + P with respect to all the intermediates and transition states involved in unimolecular processes (that is, transition states 1, 2, 3, and 4, and ES, EZ, and EP) depends upon the choice of standard state. Rather than use 1 M, we have taken 40 μM since this is the actual concentration of triosephosphate in vivo (Williamson, 1965) and, therefore, provides a better description of the relative stabilities of the reaction components.

As noted in earlier sections, the three areas where the profile is ill-defined are transition state 1 and EZ and EP. These three states are, or are close to being, kinetically insignificant, and the only restrictions that may be set upon these states are (a) that transition state 1 must be no higher than is shown in Figure 2 and no lower than would cause k_1 to be at the diffusion limit, and (b) that intermediate EZ may be no lower than is shown in Figure 2 and no higher than would cause its half-life to be too short to accommodate the observed (>90%) exchange of protons with the medium during the course of the catalyzed reaction. [This half-life depends on the intrinsic pK_a of the conjugate acid of the enzyme's catalytic base. Although the steady-state catalytic activity of the enzyme depends on an ionization of *apparent* pK_a of 6.0 (Plaut and Knowles, 1972), the intrinsic pK_a of the active-site glutamic acid is probably 3.9 (Hartman et al., 1975).]

Overall, we see from Figure 2 that all the intermediates (ES, EZ, and EP) are less stable than the lower enzyme + substrate state (E + S), *and* that the transition states 1, 2, and 3 are more stable than transition state 4, which represents—in the right to left direction—the rate of binding of P to the enzyme. The rate constant, k_{-4}, for this process is 3.7×10^8 M^{-1} s^{-1}, which is close to what is expected for a diffusion-limited process. This value is at the upper end of the range of measured "on" rates for protein–ligand interactions (Hammes and Schimmel, 1970) and is a reasonable estimate from consideration of the size of an enzyme subunit (of molecular weight 25 000: Putman et al., 1972) and a triose phosphate, using Smoluchowski's equation (Smoluchowski, 1917). If there is a significant electrostatic interaction between substrate and enzyme, and if the suggestions of Kuo-chen and Shou-Ping (1974) are correct, however, then the diffusion limit may be somewhat higher than the above figure. On the basis that the highest energy barrier for the interconversion is at least close to the diffusion limit, it is inescapable that this enzyme has reached the end of its evolutionary development. Further erosion of the barriers represented by transition states 1, 2, and 3, or further destabilization of the intermediates ES, EZ, and EP, would have little or no effect on the fluxes S → P or P → S.

Finally, we may note that if we compare the energetics of the enzyme-catalyzed reaction (Figure 2) with the uncatalyzed reaction (Hall and Knowles, 1975), the *difference* between the rates of collapse of the common enediol intermediate to dihydroxyacetone phosphate and to glyceraldehyde 3-phosphate

is very similar. The transition states for each of the enolization processes have been stabilized to the same extent.

The evolutionary state of triosephosphate isomerase as a catalyst and the factors leading to the acceleration by the enzyme of the enolization of dihydroxyacetone phosphate by more than 10^9 will be discussed in more detail in the following paper (Albery and Knowles, 1976b).

References

Albery, W. J., and Knowles, J. R. (1976a), *Biochemistry 15,* first of eight papers in a series in this issue.

Albery, W. J., and Knowles, J. R. (1976b), *Biochemistry 15,* eighth of eight papers in a series in this issue.

Fisher, L. M., Albery, W. J., and Knowles, J. R. (1975), *Symp. Faraday Soc. 10,* 154–159.

Fisher, L. M., Albery, W. J., and Knowles, J. R. (1976), *Biochemistry 15,* sixth of eight papers in a series in this issue.

Fletcher, S. J., Herlihy, J. M., Albery, W. J., and Knowles, J. R. (1976), *Biochemistry 15,* fourth of eight papers in a series in this issue.

Gray, G. R., and Barker, R. (1970), *Biochemistry 9,* 2454–2462.

Hall, A., and Knowles, J. R. (1975), *Biochemistry 14,* 4348–4352.

Hammes, G. G., and Schimmel, P. R. (1970), *Enzymes, 3rd Ed. 2,* 67–114.

Hartman, F. C., LaMuraglia, G. M., Tomozawa, Y., and Wolfenden, R. (1975), *Biochemistry 14,* 5274–5279.

Herlihy, J. M., Maister, S. G., Albery, W. J., and Knowles, J. R. (1976), *Biochemistry 15,* second of eight papers in a series in this issue.

Kuo-chen, C., and Shou-Ping, J. (1974), *Sci. Sin. 17,* 664–680.

Leadlay, P. F., Albery, W. J., and Knowles, J. R. (1976), *Biochemistry 15,* fifth of eight papers in a series in this issue.

Maister, S. G., Pett, C. P., Albery, W. J., and Knowles, J. R. (1976), *Biochemistry 15,* third of eight papers in a series in this issue.

Plaut, B., and Knowles, J. R. (1972), *Biochem. J. 129,* 311–320.

Putman, S. J., Coulson, A. F. W., Farley, I. R. T., Riddleston, B., and Knowles, J. R. (1972), *Biochem. J. 129,* 301–310.

Reynolds, S. J., Yates, D. W., and Pogson, C. I. (1971), *Biochem. J. 122,* 285–297.

Schowen, R. L. (1972), *Prog. Phys. Org. Chem. 9,* 275–332.

Smoluchowski, M. v. (1917), *Z. Phys. Chem. 92,* 129–168.

Swain, C. G., Stivers, E. C., Reuwer, J. F., and Schaad, L. J. (1958), *J. Am. Chem. Soc. 80,* 5885–5893.

Trentham, D. R., McMurray, C. H., and Pogson, C. I. (1969), *Biochem. J. 114,* 19–24.

Williamson, J. R. (1965), *J. Biol. Chem. 240,* 2308–2321.

Evolution of Enzyme Function and the Development of Catalytic Efficiency†

W. John Albery and Jeremy R. Knowles*

ABSTRACT: An efficiency function is proposed that describes the effectiveness of a catalyst in accelerating a chemical reaction. This function depends on the rate constants for the reaction and has a limiting value of unity when the rate of the reaction is controlled by diffusive steps. The evolution of enzymes toward catalytic perfection can be quantified by this function. For the interconversion of dihydroxyacetone phosphate and D-glyceraldehyde phosphate, the efficiency function has values of 2.5×10^{-11} for a simple carboxylate catalyst and 0.6 for the glycolytic enzyme triosephosphate isomerase. Thus the enzyme is almost a perfect catalyst. The improvement in the catalytic efficiency of enzymes, compared with simple organic molecules, is separated into three broad types of alteration to the Gibbs free-energy profile. In order of increasing difficulty these are "uniform binding," "differential binding," and "catalysis of elementary steps." For changes in "uniform binding," the free energies of the bound states remain the same relative to each other but are altered with respect to those of unbound states. Changes in the "differential binding" are more subtle and require the enzyme to discriminate between different bound intermediates. Finally, the most sophisticated improvement involves "catalysis of an elementary step," where an enzyme must discriminate between the transition state and the ground state of the kinetically significant step. These concepts are discussed for enzyme catalysis generally and are applied in particular to the reaction catalyzed by triosephosphate isomerase.

In the previous paper (Albery and Knowles, 1976b) the Gibbs free-energy profile for the reaction catalyzed by triosephosphate isomerase was derived. Qualitatively, it appears from the profile that this enzyme has reached the end of its evolutionary development. Moreover, a comparison of the rates of enolization of dihydroxyacetone phosphate in the presence and absence of the enzyme (Hall and Knowles, 1975) shows that the enzymic reaction is about 10^9 times faster. In this paper we first develop a quantitative measure of the catalytic effi-

† From the Physical Chemistry Laboratory, University of Oxford, Oxford, OX1 3QZ, United Kingdom. (W.J.A.), and the Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138 (J.R.K.). *Received March 4, 1976.* This work was supported by the National Science Foundation.