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Computer Analysis of the Two-Substrate Reaction Catalyzed by Yeast and Bovine Transaldolase†

Ekkehard Kuhn and Karl Brand*

ABSTRACT: Kinetic studies with transaldolase (EC 2.2.1.2) from *Candida utilis* and bovine mammary gland were carried out in order to obtain further information about the different reaction mechanisms of the yeast and the animal enzyme. The data were fitted to different rate equations with a digital computer using a general parameter estimation program. By this method for each enzyme a reaction sequence could be established which is consistent with the data obtained from chemical and kinetic studies. The kinetics of yeast transaldolase indicate a Ping Pong mechanism. No satisfactory fit can be obtained if an ordered ternary complex mechanism is assumed since systematic deviations are always observed between the models and the data. Thus the catalysis of yeast transaldolase proceeds mainly with formation of a binary complex which can be attributed to the dihydroxyacetone enzyme complex formed during incubation of the enzyme with its substrate D-fructose 6-phosphate. The reaction therefore occurs in a stepwise rather than in a concerted manner. On the other hand, the kinetic data obtained with bovine transaldolase could not be explained by a linear mechanism, but the data were in best agreement with a model in which a Random Bi Bi mechanism was assumed. Thus aldol cleavage reaction seems not to involve formation of a binary complex, but to proceed *via* a ternary enzyme–substrate intermediate. No covalent label could be found to be attached to the enzyme protein by the borohydride technique in previously published experiments. This fact suggests that Schiff base formation between enzyme and substrate is not required for the catalytic mechanism in the case of the bovine enzyme. The kinetics of the reaction indicate the formation of a ternary complex by random order combination of substrates with the enzyme protein.

In a previous paper (Kuhn and Brand, 1972) a purification procedure for transaldolase (D-sedoheptulose 7-phosphate: D-glyceraldehyde dihydroxyacetone transferase, EC 2.2.1.2) from bovine mammary gland and some physical properties of this enzyme as well as its modification with sodium borohydride have been described. Using sodium borohydride reduction, Horecker *et al.* (1963a) have shown that yeast transaldolase incubated with uniformly labeled [^{14}C]fructose 6-phosphate forms a stable inactive enzyme–dihydroxyacetone intermediate. Chemical degradation of this intermediate yielded N^6-β-glyceryllysine, indicating the structure of the enzyme–substrate complex being a Schiff base containing dihydroxyacetone linked to the ϵ-amino group of a lysine residue at the active site of the enzyme. In analogous experiments carried out with bovine transaldolase no labeled amino acid derivative could be detected and no label could covalently be incorporated into the enzyme after borohydride treatment (Kuhn and Brand, 1972). From these results it has been concluded that in the case of the animal enzyme the aldol cleavage reaction proceeds *via* a mechanism different from the Schiff base mechanism of yeast transaldolase.

In the present work the kinetics of yeast as well as bovine mammary gland transaldolase are described in detail. The results are based on a computer analysis of initial velocity data. Using a general Fortran program designed to estimate the parameters of nonlinear mathematical models the coefficients of simple as well as more complex rate equations could be determined. Thus the adequacy of different branching and nonbranching two-substrate reaction mechanisms could be proved and for each enzyme a reaction sequence could be established which is consistent with the kinetic data and the data obtained from chemical experiments.

Materials and Methods

D-Fructose 6-phosphate, D-erythrose 4-phosphate, NADH, α-glycerophosphate dehydrogenase, triosephosphate isom-

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erase, Tris, and EDTA were purchased from Boehringer Mannheim, Germany. Yeast transaldolase, Type III, was purified and crystallized from *Candida utilis* according to the procedure of Tsolas and Horecker (1970). Bovine transaldolase was purified as described in a previous publication (Kuhn and Brand, 1972). The reaction velocity was followed by the decrease in absorbance at 334 nm in a coupled optical test system (Tchola and Horecker, 1966). 1 ml of the reaction mixture contained: 0–6.62 μmol of D-fructose 6-phosphate, 0–0.43 μmol of D-erythrose 4-phosphate, 0.15 μmol of NADH, 3.5 IU of α-glycerophosphate dehydrogenase, 30 IU of triosephosphate isomerase, and 0.02 IU of transaldolase. In order to exclude a limitation effect on the reaction velocity minimum, concentrations of α-glycerophosphate dehydrogenase and triosephosphate isomerase were taken from a calibration curve. All measurements were carried out at pH 8.2, 25° with a recording Eppendorf spectrophotometer. Initial rates were obtained graphically from the linear part of the progress curves. Two series of initial rate measurements were made with each enzyme using a constant initial concentration of D-fructose 6-phosphate and different concentrations of D-erythrose 4-phosphate and *vice versa*.

A set of Fortran programs including SPLOT (Ashler and Gellert, 1968) and NLPE (Bard, 1967) was used to process the data. NLPE is designed to estimate unknown parameters in nonlinear mathematical models and to solve nonlinear programming problems. The use of this program and the mathematical ideas on which it is based are explained in the program manual. The programs were run on an IBM system 360/44 with a Fortran IV H compiler. Core storage requirements did not exceed 59255 bites. The running time depended largely on the particular model and the structure of the data. It was usually less than 15 min exclusive of loading.

Theory

Transaldolase (EC 2.2.1.2) catalyzes the following reversible reaction: D-fructose 6-phosphate + D-erythrose 4-phosphate ⇌ D-sedoheptulose 7-phosphate + D-glyceraldehyde 3-phosphate. The reaction is a group transfer reaction of the general type

$$GX + Y \rightleftharpoons X + GY \qquad (1)$$

whereby a chemical group G is transferred from a donor molecule X to an acceptor Y. Since eq 1 represents the overall process, it summarizes a variety of two-substrate reaction mechanisms. If the substrate GX is bound to the enzyme in the first reaction step and the product X leaves the enzyme prior to reaction of the second substrate Y the mechanism can be represented by the reaction sequence in eq 2.

$$E \overset{GX}{\rightleftharpoons} EGX \underset{X}{\rightleftharpoons} EG \overset{Y}{\rightleftharpoons} EGY \underset{GY}{\rightleftharpoons} E \qquad (2)$$

The rate equation has the form

$$v_0 = v^* \frac{s_1 s_2}{c_1 s_1 + c_2 s_2 + c_3 s_1 s_2} \qquad (3)$$

(Cleland, 1963), where v_0 is the initial velocity and s_1 and s_2 are the concentrations of D-fructose 6-phosphate and D-erythrose-4-phosphate, respectively. v^* is a measure of the enzyme concentration e. It is the reaction velocity determined at high concentrations of both substrates (2.65 mM D-fructose 6-P, 0.19 mM D-erythrose 4-P). It was made sure that under these conditions v^* was proportional to e. Each c_i (i = 1, 2, 3) is a function of the rate constants of the mechanism.

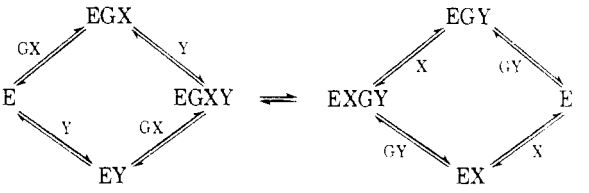
A different mechanism is obtained if formation of a ternary complex is assumed. If GX is still the first combining substrate, the sequence is

$$E \overset{GX}{\rightleftharpoons} EGX \overset{Y}{\rightleftharpoons} EGXY \rightleftharpoons EXGY \underset{X}{\rightleftharpoons} EGY \underset{GY}{\rightleftharpoons} E \qquad (4)$$

The appropriate rate expression has the form

$$v_0 = v^* \frac{s_1 s_2}{c_1 + c_2 s_1 + c_3 s_2 + c_4 s_1 s_2} \qquad (5)$$

(Cleland, 1963). If alternate reaction sequences exist, the mechanism becomes more complex (eq 6). The rate equation

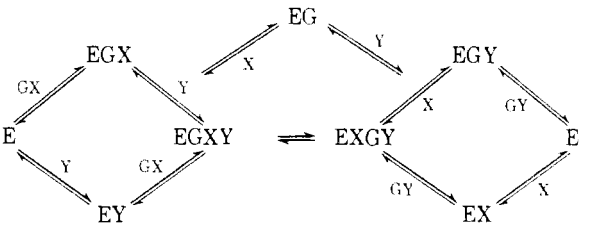


(6)

was derived by Dalziel (1958) (eq 7). By Cleland's definitions

$$v_0 = v^* \times \frac{c_1 s_1 s_2 + c_2 s_1^2 s_2 + c_3 s_1 s_2^2}{1 + c_4 s_1 + c_5 s_1^2 + c_6 s_1 s_2 + c_7 s_1^2 s_2 + c_8 s_1 s_2^2 + c_9 s_2 + c_{10} s_2^2} \qquad (7)$$

the mechanisms discussed so far were called "Ping Pong Bi Bi" (eq 2), "Ordered Bi Bi" (eq 4), and "Random Bi Bi" (eq 6). They can be integrated in a single comprehensive scheme (Wong and Hanes, 1962) (eq 8). Since this scheme is of wide



(8)

generality, we will assume that the transaldolase reaction can correctly be described by one of its partial sequences.

Some of these sequences are homeomorphous, *i.e.*, the appropriate rate expressions are identical in structure and therefore cannot be distinguished by steady-state methods. In the case of nonhomeomorphous mechanisms, however, a distinction is possible by investigating the consistency of different rate equations with a suitable set of experimental data. As will be discussed below, the rate expressions belonging to (8) fall into two different groups. Provided a differentiation between these groups is possible, the identification of the proper reaction sequence is greatly simplified, since the number of possible mechanisms is remarkably reduced. Dalziel (1957) found that the initial rate expressions of all linear mechanisms follow eq 5. This equation holds even if the substrates combine in random order, provided that isomeriza-

tion of the ternary enzyme–substrate complex is the only slow reaction step (Rapid Equilibrium Random Bi Bi mechanism, Cleland (1963)). Equation 5 can be rearranged as

$$\frac{v^*}{v_0} = \left[\frac{c_1}{s_2} + c_3\right]\frac{1}{s_1} + \left[\frac{c_2}{s_2} + c_4\right] \qquad (9)$$

If s_2 is held constant, (9) will be linear with respect to v^*/v_0 and $1/s_1$ and, in consequence of symmetry, the same is true if s_2 is replaced by s_1. Therefore, if a plot of v^*/v_0 *vs.* $1/s_{1,2}$ is linear (group 1 mechanisms), the mechanism will be branched only if simplifying conditions hold. This is the case if all steps in a Random Bi Bi mechanism are very rapid except the interconversion of the two ternary complexes. Otherwise the mechanism will be linear. If double reciprocal plots are nonlinear (group 2 mechanisms), the mechanism will be branched but will not be of the type Rapid Equilibrium Random Bi Bi.

Departure from linearity can be taken into account by adding a quadratic term to the following linear relationship

$$\frac{v^*}{v_0} = B_1 + B_2(1/s_i) \qquad (i = 1, 2) \qquad (10)$$

(eq 10) where B_1 and B_2 are constants. Then (10) takes the form

$$v^*/v_0 = B_1 + B_2/s_i + B_3/s_i^2 \qquad (11)$$

In this equation the size of B_3 determines the departure of the regression line from linearity. If B_3 equals zero, the graph of eq 11 will be linear. It will be curved upwards if $B_3 > 0$ and it will be curved downwards if $B_3 < 0$. However, if the deviation from linearity is small and if the accuracy of the parameter values is not known, it might be difficult to decide whether or not B_3 is different from zero. A practical though not an absolute criterion is obtained, if the standard deviation of the parameters is known. Let σ_3 be the standard deviation of B_3; then the regression line (eq 11) will be linear if $B_3 \leqq \sigma_3$ and it will be nonlinear if $B_3 > \sigma_3$. The method outlined above was applied to the kinetics of yeast and bovine transaldolase, differentiation between linear and nonlinear reciprocal plots becoming more reliable than by graphical analysis alone. A similar method was proposed by Gardiner and Ottaway (1969).

Differentiation between eq 3 and 5 is possible in a similar way. If c_1 is set equal to zero eq 5 takes the same form as eq 3. c_1 practically equals zero if its value is less than its standard deviation σ_1. Consequently, if in eq 5 $c_1 \leqq \sigma_1$, the data can be fitted to eq 3 as well. Then the operative mechanism of the reaction is Ping Pong Bi Bi. If $c_1 > \sigma_1$, the mechanism will be Ordered Bi Bi or Rapid Equilibrium Random Bi Bi.

The discrimination of two substrate mechanisms discussed so far was based on the assumption that the problem of finding the parameters of any of the rate equations used can be solved. For a long time the parameter estimation of enzyme kinetic models, *i.e.*, the functions employed to fit enzyme kinetic data, has been attempted by graphical means. Graphical methods, however, are restricted to simple cases and often do not provide sufficient accuracy. Statistical methods to determine the parameters of the Michaelis–Menten equation were described by Wilkinson (1961) and Cleland (1967). If provisional estimates of the parameters are known, the two latter methods can be extended to more complex rate expressions, since reduction to a linear regression problem is possible by replacing the rate equation by the linear part of a Taylor series expansion. This is done by the classical least-squares method. The method can be used in an iterative way, too. It was pointed out, however, that the iteration can be expected to converge solely if initial parameter estimates are sufficiently good (Pennington, 1965). For example, if Cleland's program is used, provisional estimates of K_m should be within the range of 0.1–1.5 times the optimum value (Gardiner and Ottaway, 1969). In more complicated cases graphical methods are not available or, if the problem is tackled numerically, a set of difficult nonlinear equations is obtained. The finding of useful estimates of the parameters is then not easier than the solution of the original problem of finding their optimum values. Hence current optimization techniques start from a set of more or less arbitrary initial parameter values and proceed improving these by iteration until some best fit criterion is fulfilled and the computation is terminated. A survey on these methods was given by Swann (1969). The methods of Davidon (1959) and Fletcher and Powell (1963) and an alternative Gauss–Newton method are applied by NLPE ("Nonlinear Parameter Estimation and Programming"). This is a general parameter estimation program designed to estimate the parameters of nonlinear mathematical models and to solve nonlinear programming problems (Bard, 1967). NLPE was used to find optimum values of the parameters of all of the models employed to fit the kinetics of yeast and bovine transaldolase.

The variance of v_0 was assumed to be constant. Therefore data were not supplied with weights. In the case of eq 11, however, this leads to a distortion of the Gaussian criterion, since the function minimized is

$$\sum_j \left(\frac{v^*}{v_{0j}} - g(s_j, B_1, B_2, B_3)\right)^2$$

instead of

$$\sum_j (v_{0j} - f(v^*, s_j, B_1, B_2, B_3))^2$$

NLPE is not bound to linear functions. Thus this difficulty can be bypassed, if for the purpose of parameter estimation the following form of eq 11 is used. Then B_1, B_2, and B_3 can be

$$v_0 = \frac{v^*}{B_1 + B_2/s_i + B_3/s_i^2} \qquad (12)$$

calculated by unweighted analysis. Some minor programs were developed by the authors mainly to produce graphical representations of how the models fit the data. In their present forms the programs serve two functions. (1) Using eq 11 and 12, they allow one to decide whether or not reciprocal plots depart from linearity. (2) Data can be fitted to models (3), (5), or (7) and the quality of fit can be compared by graphical and statistical criteria.

Results

1. Yeast Transaldolase. The initial velocity of D-glyceraldehyde phosphate formation was followed by measuring a series of substrate saturation curves. Each set of data was fitted to eq 12. Results are summarized in Table I. As was mentioned in the theory section, plots of v^*/v_0 *vs.* $1/s_i$ can be regarded as linear, if the size of B_3 in eq 11 and 12 is less than the standard deviation of this parameter. It can be seen from Table I that five of the curves show no departure from linearity. For seven curves, however, the condition $B_3 \leqq \sigma_3$ is not fulfilled. The

TABLE I[a]

No.	(S) (mM)	v^* (nmol/(min ml))	B_1	σ_1	B_2 (mM)	σ_2 (mM)	B_3 (mM)^2	σ_3 (mM)^2	Non-linear
				Fixed Substrate: D-Erythrose 4-Phosphate					
1	0.107E-1	0.191E 2	0.276E 1	0.105E 0	0.170E 0	0.431E-1	0.477E-2	0.272E-2	+
2	0.171E-1	0.202E 2	0.196E 1	0.330E-1	0.204E 0	0.144E-1	0.719E-5	0.141E-4	−
3	0.371E-1	0.197E 2	0.102E 1	0.155E-1	0.331E 0	0.124E-1	0.818E-5	0.136E-4	−
4	0.463E-1	0.181E 2	0.960E 0	0.911E-2	0.343E 0	0.134E-1	0.490E-8	0.930E-3	−
5	0.705E-1	0.193E 2	0.795E 0	0.382E-2	0.340E 0	0.682E-2	0.346E-8	0.105E-2	−
6	0.893E-1	0.174E 2	0.642E 0	0.150E-1	0.269E 0	0.186E-1	0.117E-8	0.511E-3	−
				Fixed Substrate: D-Fructose 6-Phosphate					
7	0.513E 0	0.161E 2	0.996E 0	0.401E-1	0.171E-1	0.231E-2	0.455E-4	0.207E-4	+
8	0.776E 0	0.176E 2	0.936E 0	0.298E-1	0.137E-1	0.268E-2	0.629E-4	0.305E-4	+
9	0.131E 1	0.176E 2	0.773E 0	0.301E-1	0.116E-1	0.324E-2	0.144E-3	0.544E-4	+
10	0.148E 1	0.185E 2	0.657E 0	0.207E-1	0.157E-1	0.232E-2	0.979E-4	0.457E-4	+
11	0.210E 1	0.181E 2	0.685E 0	0.184E-1	0.718E-3	0.296E-3	0.481E-3	0.803E-4	+
12	0.261E 1	0.172E 2	0.593E 0	0.194E-1	0.105E-1	0.321E-2	0.189E-3	0.858E-4	+

[a] Kinetic coefficients of yeast transaldolase; optimum values (B_i) and standard deviations (σ_i) of parameters of eq 11 and 12; (S), concentration of fixed substrate; v^*, reaction velocity under saturating conditions; departure from linearity is marked by a plus sign; decimal exponent, 0.107E-1 reads 0.107×10^{-1}.

deviations being fairly small, they are not detected in direct v *vs.* s plots of experimental points.

Without regard to the curvature uncovered by the linearity test the mechanism of yeast transaldolase fell into group 1 of the two groups defined above, *i.e.*, the sequences were non-branching or rapid equilibrium Random Bi Bi. The corresponding models of group 1 mechanisms are eq 3 and 5. As stated earlier, (5) takes the form of eq 3, if c_1 in eq 5 equals zero. Thus (5) cannot account for the data, if the calculation of c_1 yields a value that is not significantly different from zero. From Table II it can be seen that $c_1 < \sigma_1$. Thus (5) cannot be expected to fit the data closer than (3). To verify this conclusion, data were fitted to eq 3 as well. The optimization procedure converged again to the values given in Table II. Substituting $c_1 - c_3$ in eq 3 for the appropriate numerical values (Table II) we obtain a formula that describes the kinetic behavior of yeast transaldolase at pH 8.2, 25° in the range of $0 < s_1 < 6.62$ mM, $0 < s_2 < 0.43$ mM. Figure 1 shows the resulting fit. Data correspond to Table I, No. 3.

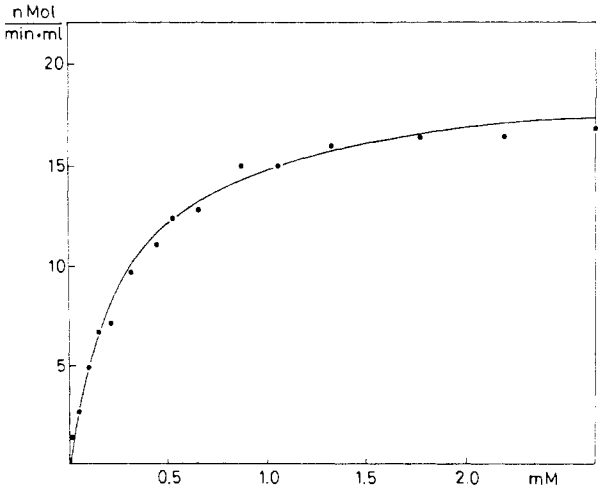


FIGURE 1: Substrate saturation curve of yeast transaldolase, computed by eq 3; abscissa, D-fructose 6-phosphate, mM; ordinate, initial reaction velocity, nmol/(min ml); fixed substrate D-erythrose 4-phosphate; data corresponding to No. 3, Table I.

Equation 3 fits the data well. Moreover, since c_1 is smaller than its standard deviation, an ordered ternary complex mechanism is made unlikely. This suggests that the main pathway of the mechanism of yeast transaldolase is Ping Pong Bi Bi. Equation 3 cannot account, however, for the slight curvature of more than half of the double reciprocal plots. In terms of (8) this fact requires a branching point in the linear Ping Pong reaction sequence.

2. Bovine Transaldolase. Preliminary graphical analysis of the kinetics of bovine transaldolase suggested that many of the substrate saturation curves were faintly sigmoid. Therefore the linearity of double reciprocal plots was tested as described in the Theory section. The results are summarized in Table III. Figure 2 gives a graphical example. This curve corresponds to the broken line in Figure 3. Particularly, if erythrose-4-P is the variable substrate, B_3 is positive and consequently the plots are curved upwards. Therefore eq 5 cannot account for the rate behavior and the mechanism must correspond to some branching sequence of (8) with the exception of a Rapid Equilibrium Random Bi Bi mechanism. Since every branching sequence of (8) contains a ternary enzyme–substrate complex, such a complex must be regarded as an obligatory intermediate of the reaction. On the other hand, if the sequence $E \rightleftharpoons EGX \rightleftharpoons EG \rightleftharpoons EY \rightleftharpoons E$ plays no role in the catalytic

TABLE II[a]

Parameter	Optimum Value	Standard Deviation
c_1	0.141×10^{-7}	0.638×10^{-3}
c_2	0.219×10^{-1}	0.382×10^{-3}
c_3	0.312×10^{0}	0.171×10^{-1}
c_4	0.428×10^{0}	0.110×10^{-1}

[a] Kinetic coefficients of yeast transaldolase; optimum values and standard deviations of parameters of eq 5.

TABLE III[a]

No.	(S) (mM)	v^* (nmol/(min ml))	B_1	σ_1	B_2 (mM)	σ_2 (mM)	B_3 (mM)^2	σ_3 (mM)^2	Non-linear
				Fixed Substrate: D-Erythrose 4-Phosphate					
1	0.665E-2	0.199E 2	0.763E 1	0.254E 0	0.111E-7	0.145E-1	0.795E-3	0.144E-3	+
2	0.164E-1	0.195E 2	0.206E 1	0.901E-1	0.117E 0	0.152E-1	0.823E-3	0.418E-3	+
3	0.306E-1	0.139E 2	0.145E 1	0.466E-1	0.690E-1	0.160E-1	0.219E-2	0.869E-3	+
4	0.313E-1	0.183E 2	0.114E 1	0.467E-1	0.138E 0	0.221E-1	0.663E-3	0.118E-2	−
5	0.156E 0	0.171E 2	0.949E 0	0.266E-1	0.230E 0	0.123E-1	0.286E-8	0.859E-3	−
				Fixed Substrate: D-Fructose 6-Phosphate					
6	0.496E-1	0.210E 2	0.448E 1	0.196E 0	0.186E-8	0.364E-2	0.163E-3	0.906E-5	+
7	0.105E 0	0.222E 2	0.314E 1	0.984E-1	0.931E-9	0.358E-2	0.160E-3	0.296E-4	+
8	0.149E 0	0.277E 2	0.188E 1	0.589E-1	0.347E-3	0.431E-2	0.311E-3	0.558E-4	+
9	0.254E 0	0.195E 2	0.109E 1	0.709E-1	0.218E-9	0.432E-2	0.154E-3	0.490E-4	+
10	0.503E 0	0.244E 2	0.138E 1	0.337E-1	0.128E-2	0.239E-2	0.187E-3	0.294E-4	+
11	0.262E 1	0.171E 2	0.829E 0	0.296E-1	0.724E-8	0.295E-2	0.215E-3	0.470E-4	+
12	0.264E 1	0.262E 1	0.838E 0	0.192E-1	0.650E-2	0.111E-2	0.712E-5	0.788E-5	−

[a] Kinetic coefficients of bovine transaldolase; optimum values (B_i) and standard deviations (σ_i) of parameters of eq 11 and 12; (S), concentration of fixed substrate; v^*, reaction velocity under saturating conditions; departure from linearity is marked by a plus sign; decimal exponent: 0.665E-2 reads 0.665×10^{-2}.

overall process, the mechanism will follow (6) and eq 7 will be the correct model. In spite of its high degree of complexity the parameter estimation procedure converged to provide the values given in Table IV. Using these values and eq 7 we obtain a formula describing the kinetic behavior of bovine transaldolase at pH 8.2, 25° in the range of $0 < s_1 < 2.65$ mM, $0 < s_2 < 0.27$ mM. For practical use of this formula the term with c_7 can be put to zero, since the value of c_7 is much smaller than its standard deviation. However, c_7 being a function of several rate constants of the random mechanism, this leads not to a simplification of the underlying reaction scheme. Figure 3 gives an example of the fit. The deviations of experimental points from the model lie within experimental error. Therefore the kinetics of bovine transaldolase can be described satisfactorily on the basis of a Random Bi Bi mechanism, characterized by the formation of a ternary enzyme–substrate complex. Formation of a binary complex cannot be detected.

Michaelis Constants. K_m values of yeast transaldolase can be calculated from Table II. For D-fructose-6-P, $K_m = c_3/c_4 = 7.28 \times 10^{-4}$ M; for D-erythrose-4-P, $K_m = c_2/c_4 = 5.11 \times 10^{-5}$ M (pH 8.2, 25°). K_m values of bovine transaldolase were determined in a previous work by simple graphical analysis (Kuhn and Brand, 1972). The values obtained were 2×10^{-4} M for D-fructose-6-P and 7×10^{-6} M for D-erythrose-4-P (pH 8.0, 25°). Michaelis constants are not defined for random mechanisms. On this account at least the K_m's of the bovine enzyme are without kinetic meaning.

Discussion

In the case of transaldolase from *C. utilis* the formation of a transaldolase dihydroxyacetone complex was established by several workers (Horecker *et al.*, 1961, 1963b; Venkataraman

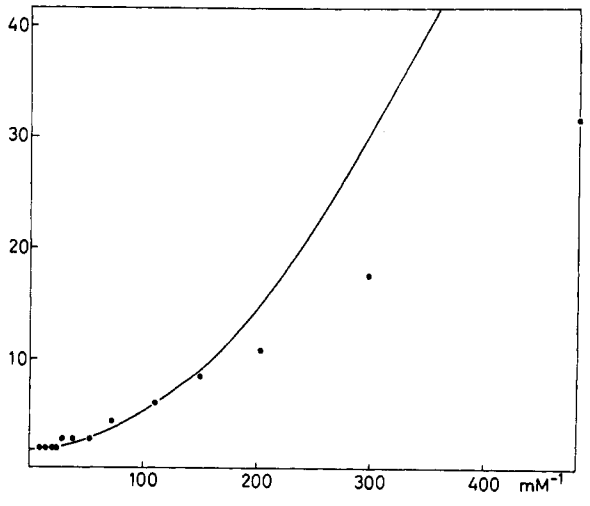


FIGURE 2: Substrate saturation curve of bovine transaldolase, computed from eq 11; double reciprocal form; abscissa, reciprocal concentration of D-erythrose 4-phosphate, mM^{-1}; ordinate, v^*/v_0; data corresponding to No. 8, Table III.

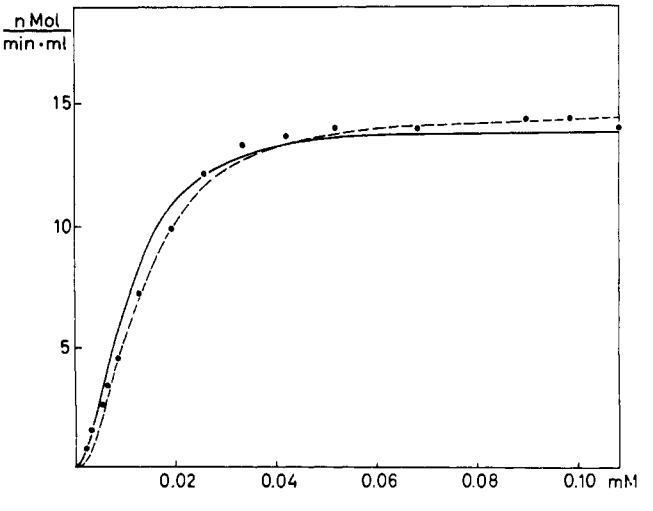


FIGURE 3: Substrate saturation curves of bovine transaldolase, computed from eq 7 (—) and 12 (- - - -); abscissa, D-erythrose 4-phosphate, mM; ordinate, initial reaction velocity, nmol/(min/ml); fixed substrate D-fructose 6-phosphate; data corresponding to No. 8, Table III.

TABLE IV[a]

Parameter	Optimum Value	Standard Deviation
c_1	0.511×10^3	0.26×10^1
c_2	0.255×10^5	0.52×10^1
c_3	0.128×10^7	0.16×10^3
c_4	0.321×10^3	0.19×10^0
c_5	0.615×10^3	0.18×10^0
c_6	0.387×10^2	0.27×10^0
c_7	0.123×10^{-5}	0.22×10^0
c_8	0.137×10^7	0.19×10^3
c_9	0.174×10^1	0.11×10^0
c_{10}	0.192×10^6	0.55×10^2

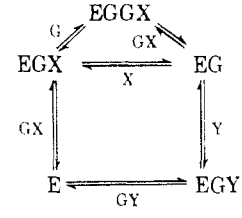
[a] Kinetic coefficients of bovine transaldolase; optimum values and standard deviations of parameters of eq 7.

and Racker, 1961). This complex accumulates when the enzyme is incubated with an excess of D-fructose 6-phosphate in absence of the second substrate D-erythrose 4-phosphate. Presence of the acceptor D-erythrose 4-phosphate in the reaction mixture promotes the reaction beyond the dihydroxyacetone–enzyme complex stage. These findings indicate a Ping Pong mechanism. However, since detection of the Schiff base complex occurs only in absence of the second substrate, it is not necessarily a step in the overall catalytic reaction. The possibility remains therefore that the aldol cleavage reaction proceeds in a concerted rather than in a stepwise manner. In the case of fructosediphosphate aldolase a mechanism involving concerted fructose diphosphate Schiff base formation and dealdolization was discussed in the literature (Morse and Horecker, 1968). On condition that a concerted aldol cleavage mechanism applied to yeast transaldolase, a ternary complex would play the role of an obligatory intermediate of the reaction sequence. The rate data presented here cannot be explained completely in terms of a Ping Pong mechanism. Therefore some consideration must be given to this possibility. On the basis of (8) a more general approach is possible if the linear Ping Pong sequence EGX $\rightleftharpoons$ EG $\rightleftharpoons$ EGY is assumed to be shunted by the parallel pathway EGX $\rightleftharpoons$ EGXY $\rightleftharpoons$ EGY. The branched mechanism obtained is of first degree in GX and of second degree in Y, *i.e.*, the appropriate rate expression contains squared terms of s_2. In principle this fact can account for departure from linearity arising particularly if D-erythrose 4-phosphate is the variable substrate. On this account and for reasons discussed below the rate expression of a mechanism including a binary as well as a ternary complex is likely to provide a perfect fit of the data of the yeast enzyme.

As has been reported in a previous paper (Kuhn and Brand, 1972), no covalently linked dihydroxyacetone could be detected in the case of bovine mammary gland transaldolase by using radioactive labeling techniques. Formation of a Schiff base intermediate therefore seems unlikely. The steady-state analysis presented here gives further evidence of a different reaction mechanism of the bovine enzyme. Since data could not be fitted to eq 5, the kinetics of bovine transaldolase cannot be explained by a binary or by a nonbranching ternary complex mechanism. Since the consistency of (6) with the data could be demonstrated, a ternary complex mechanism seems very likely. Thus, in contrast to the yeast enzyme aldol cleavage reaction and formation of the new carbon–carbon bond should proceed *via* isomerization of a ternary central complex. Consequently covalent catalysis would be of no account for the catalytic power in the case of the animal enzyme. It seems reasonable to suggest that the reaction is promoted mainly by other factors, *e.g.*, acid–base catalysis and proximity or orientation effects.

As can be seen from (8), a ternary as well as a binary complex are expected to form during the reaction and therefore further branching reaction sequences are obtained. The appropriate rate expressions have not been published in the literature. However, computer methods are available by which the rate equations can be derived in algebraic form (Heckmann *et al.*, 1969). Since they can be expected to contain at least the same number of parameters as eq 7, it seems conceivable that they would fit the data as well. In other words, the uniqueness of model 6 is not yet proved. Nondefinite models can provide similar fits with different parameter sets. This becomes more likely as the models are of increasing complexity, since the maximalization procedure tends more frequently to converge to a local maximum of the objective function. Lasch (1969) proposed to take the standard deviation of parameters as a measure of uniqueness. The higher the deviations, the more suspect is the model. The standard deviation of most parameters of model 7 is very low (Table IV). In addition, the numerical values given were obtained in different computer runs with different sets of initial parameter values. Both factors indicate uniqueness of the model. However, the possibility that a binary complex is involved at least to some degree in the reaction cannot be excluded definitely.

As was stated in the theory section, (8) was regarded as the basis of the transaldolase reaction. However, further mechanisms can be written down. Horecker *et al.* (1963a) demonstrated that in the absence of D-erythrose 4-phosphate there is a slow turnover of yeast transaldolase with fructose 6-phosphate as the single substrate. The reaction is described as



Due to the species EGGX which does not appear in (8), this mechanism cannot be integrated in the general two-substrate scheme. The mechanism is of second degree in GX and of first degree in Y. Thus the rate expression contains quadratic terms of s_1 but only linear terms of s_2. Therefore it can account for nonlinear double reciprocal plots only if fructose 6-phosphate is the variable substrate.

Moreover (8) could not be expected to apply if multiple forms or different conformational stages of the enzyme were present, since then each enzymatic species can act as the starting point of different reaction sequences. Then the kinetics of the enzyme system arise from a superposition of its individual components. The assumption would hold neither if there was a single enzymatic species containing two or more active sites per molecule, provided that these are kinetically different and/or coupled by subunit interaction. Yeast transaldolase was reported to consist of two subunits (Tsolas and Horecker, 1970; Tsolas *et al.*, 1970). After borohydride reduction in the presence of ^{14}C-labeled fructose 6-phosphate only 1 equiv of dihydroxyacetone could be found to be incorporated per dimer (Horecker *et al.*, 1963a,b; Brand and Horecker, 1968) and only one critical histidine could be

detected (Brand *et al.*, 1969). Recently, however, evidence for catalytically active monomers of yeast transaldolase could be obtained from studies with matrix-bound subunits of yeast transaldolase (Chan *et al.*, 1973). If each of the monomers followed a Ping Pong mechanism, the deviations from eq 3 established by the linearity test could be evoked by coupling of the subunits of the dimeric molecule.

The purpose of the present investigation was to obtain detailed kinetic data in order to correlate the results with those of chemical studies. The rate behavior of the yeast enzyme is congruent with the formation of a covalent enzyme–substrate intermediate identical with the dihydroxyacetone complex formed by incubation of the enzyme with D-fructose 6-phosphate. Small systematic deviations from a Ping Pong mechanism indicate that concerted dealdolization may be involved to some extent in the catalytic overall process. In the case of the bovine enzyme, the results obtained by radioactive labeling experiments are confirmed, a binary complex mechanism being inconsistent with the rate behavior. From the kinetic data a ternary complex mechanism is proposed, characterized by the aldol cleavage reaction taking place during isomerization of a ternary complex formed by random order combination of substrates with the enzyme protein.

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