Kinetic Studies on Chorismate Mutase–Prephenate Dehydrogenase from *Escherichia coli*: Models for the Feedback Inhibition of Prephenate Dehydrogenase by L-Tyrosine

Joanne Turnbull,[‡,§] John F. Morrison,[*,‡] and W. W. Cleland[∥]

Division of Biochemistry and Molecular Biology, John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia, and the Institute for Enzyme Research, 1710 University Avenue, Madison, Wisconsin 53705

Received December 28, 1990; Revised Manuscript Received April 16, 1991

ABSTRACT: Kinetic studies have been undertaken to elucidate the mechanism of the allosteric inhibition by tyrosine of the prephenate dehydrogenase activity of the bifunctional dimeric enzyme chorismate mutase–prephenate dehydrogenase. The effect of tyrosine on the initial velocity of the reactions in the presence of both prephenate and the alternative substrate, 1-carboxy-4-hydroxy-2-cyclohexene-1-propanoate, have been determined. In addition, investigations have been made of the effect of tyrosine on the inhibition of the reaction by the inhibitory analogues of prephenate, (4-hydroxyphenyl)pyruvate, and (carboxyethyl)-1,4-dihydrobenzoate. The results of the double inhibition experiments indicate clearly that the enzyme possesses a distinct allosteric site for the binding of tyrosine. The initial velocity data obtaind with both substrates have been fitted to the rate equations that describe a wide range of models. From a comparison of the results obtained from studies with the two substrates, and with a knowledge of the value for the dissociation constant of the tyrosine-enzyme complex, definitive conclusions have been reached about the mechanism of the allosteric inhibition. It is concluded that tyrosine combines twice at allosteric sites and in an antisynergistic fashion, while prephenate reacts at both active sites of the dimeric enzyme as well as weakly at one of the allosteric sites. It appears that the latter is simple competition reaction that affects neither the binding of prephenate at the active site nor the rate of product formation. The model also predicts the formation of an active tyrosine–enzyme–prephenate complex that yields product at a much slower rate than does the enzyme–prephenate complex. The same model can account for the kinetic data obtained with 1-carboxy-4-hydroxy-2-cyclohexene-1-propanoate except that this alternative substrate does not react appreciably at the allosteric site on chorismate mutase–prephenate dehydrogenase.

Chorismate mutase–prephenate dehydrogenase from *Escherichia coli* is a dimeric enzyme with a molecular weight of 78 000–88 000 (SampathKumar & Morrison, 1982; Hudson et al., 1984; Turnbull et al., 1990) that catalyzes two consecutive reactions along the pathway for the biosynthesis of L-tyrosine. Chorismate mutase (EC 5.4.99.5) catalyzes the rearrangement of chorismate to prephenate, while prephenate dehydrogenase (EC 1.3.1.12) catalyzes the oxidative decarboxylation of prephenate to (4-hydroxyphenyl)pyruvate in the presence of NAD. Recent studies indicate that the two reactions occur at distinct active sites that exhibit structural similarities (Turnbull & Morrison, 1990).

Steady-state kinetic investigations of the dehydrogenase reaction, over a range of lower prephenate concentrations, have shown that the interaction of the substrate at the active sites of the dimeric enzyme exhibits positive cooperativity if NAD is not saturating (Christopherson, 1985; Turnbull et al., 1990). Further, it has been demonstrated that tyrosine enhances the cooperativity (Hudson et al., 1983; Christopherson, 1985; Christopherson & Morrison, 1985). Thus prephenate dehydrogenase is an allosteric enzyme that is subject to feedback inhibition by tyrosine, which is the end product of one branch of the aromatic biosynthetic pathway. The mechanism of the allosteric inhibition has received only limited attention, but two models have been proposed to account for the observed kinetic effects. Hudson et al. (1983) have proposed that the enzyme exists in solution as an equilibrium mixture of active (dimeric) and inactive (tetrameric) forms with tyrosine (and NAD) binding preferentially to the tetramer. By contrast, Christopherson (1985) concluded that tyrosine acts as an inhibitory analogue of prephenate and, like prephenate, undergoes cooperative interaction at the two active sites of the dimeric dehydrogenase molecule.

The aim of the present investigation was to elucidate the kinetic mechanism of the allosteric inhibition of prephenate dehydrogenase by tyrosine. For this purpose, kinetic studies were undertaken with both prephenate and the alternative substrate, 1-carboxy-4-hydroxy-2-cyclohexene-1-propanoate (CHCP, deoxodihydroprephenate).[1] The results are consistent with the existence on the dehydrogenase of a distinct allosteric binding site for tyrosine at which prephenate may also combine.

Experimental Procedures

Materials

Chorismate and prephenate (Figure 1) were prepared as described previously (Gibson, 1968; Dudzinski & Morrison, 1976). (4-Hydroxyphenyl)pyruvate (HPP) was obtained from Sigma Chemical Co. and was purified according to the procedure of Lindbland et al. (1977). The concentration of HPP was estimated spectrophotometrically at 276 nm by using an

[‡] Australian National University.
[§] Present address: Department of Molecular and Cell Biology, Wendell Stanley Hall, University of California, Berkeley, California 94720.
[∥] Institute for Enzyme Research.

[1] Abbreviations: CEDB, (carboxyethyl)-1,4-dihydrobenzoate; CHCP, 1-carboxy-4-hydroxy-2-cyclohexene-1-propanoate (deoxodihydroprephenate); HPP, (4-hydroxyphenyl)pyruvate; MES, 2-(*N*-morpholino)-ethanesulfonic acid; NAD, oxidized form of nicotinamide adenine dinucleotide.

0006-2960/91/0430-7783$02.50/0

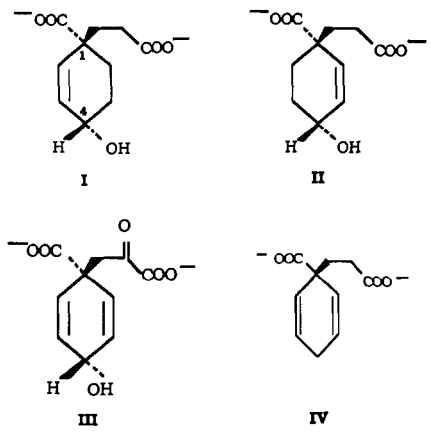

FIGURE 1: Structure of substrates and a substrate analogue for prephenate dehydrogenase: I and II, isomers of 1-carboxy-4-hydroxy-2-cyclohexene-1-propanoate (CHCP); III, prephenate; IV, (carboxyethyl)-1,4-dihydrobenzoate (CEDB).

extinction coefficient of 2.0 mM^{-1} cm^{-1} at pH 7.7 (Knox & Pitt, 1956). L-Tyrosine, supplied by Merck, was prepared as a saturated stock solution in a buffer of 0.1 M (*N*-morpholino)ethanesulfonic acid (MES)/0.051 M *N*-ethylmorpholine/0.051 M diethanolamine (pH 7.2). This method was used to avoid pH adjustment, which leads to the formation of salts that enhance the effectiveness of tyrosine as an inhibitor of prephenate dehydrogenase (unpublished observations of J.T.). CHCP (deoxodihydroprephenate) was prepared as described previously (Turnbull et al., 1990). The preparation contained two isomers of CHCP whose structures are given in Figure 1. The V/K value for one isomer is at least 10-fold greater than that for the other isomer so the kinetic data obtained with CHCP are for the faster reacting isomer. Chorismate mutase–prephenate dehydrogenase was isolated from *E. coli* JFM30 as described by Turnbull et al. (1990) and purified to a specific activity of 54 for the dehydrogenase and 65 for the mutase. (Carboxyethyl)-1,4-dihydrobenzoate (CEDB, Figure 1) was a gift from Professor Glenn Berchtold of the Massachusetts Institute of Technology. All other chemicals were of the highest purity available commercially.

Methods

Determination of Enzyme Activities and Protein Concentration. Prephenate dehydrogenase activity was measured at 30 °C by following the formation of NADH from NAD at 340 nm with a Varian 219 spectrophotometer (Heyde & Morrison, 1978). Assays contained 0.1M MES/0.051 M *N*-ethylmorpholine/0.051 M diethanolamine (pH 7.2), 1 mM DTT, and 1 mM EDTA. Extinction coefficients of 6400 and 6200 were used to calculate initial velocities when the substrates were prephenate and CHCP, respectively. Maximum velocity (V) is expressed in units of reciprocal seconds. Protein concentrations were determined by the method of Bradford (1976).

Analysis of Kinetic Data. Velocity data, which were obtained by varying the inhibitor concentration (I) at a fixed substrate concentration (A) and which gave rise to a curvilinear plot, were fitted to eq 1. In this equation, V represents the uninhibited reaction velocity while b, c, and d are empirical constants.

$$v = \frac{V(1 + dI)}{1 + bI + cI^2} \tag{1}$$

Steady-state velocity data obtained by varying the concentration of two inhibitors (I and J) at a fixed substrate concentration (A) were fitted to eq 2 in log form.

$$v = \frac{V\left[1 + \frac{2K_{ia}}{A}\left(1 + \frac{J}{2K_j}\right)\right]}{\left[1 + \frac{2K_{ia}}{A}\left(1 + \frac{J}{2K_j}\right)\right]^2 + \frac{4K_{ia}^2}{A^2}\left(\frac{I}{K_i}\right)\left(1 + \frac{I}{K_I} + \frac{J}{K_{Ij}}\right)} \tag{2}$$

Values were obtained for the maximum velocity (V) and for the dissociation constants (K_i, K_j, K_I and K_{Ij}) of the various enzyme–inhibitor complexes. Velocity data for the inhibition of the dehydrogenase by tyrosine were fitted to the log forms of eqs 3–10.

$$v = \frac{V\left(1 + \frac{V_3K_aI}{V\alpha K_iA}\right)}{1 + \frac{K_a}{A} + \frac{K_aI}{\alpha K_iA} + \frac{K_{ia}K_aI}{K_iA^2} + \frac{K_{ia}K_aI^2}{K_IK_iA^2}} \tag{3}$$

$$v = V\left[1 + \frac{2K_{ia}}{A}\right] \Big/ \left(1 + \frac{2K_{ia}}{A}\right)^2 + \left(\frac{4K_{ia}}{K_{Ia}}\right)\frac{I}{A} + \frac{4K_{ia}^2}{K_i}\left(1 + \frac{I}{K_i}\right)\frac{I}{A^2} + (\text{coef})\frac{I^2}{A} \tag{4}$$

$$v = V\left[1 + \frac{2K_a}{A} + (\text{coef})\frac{1}{A}\right] \Big/ \left(1 + \frac{2K_{ia}}{A}\right)^2 + (\text{coef})\frac{I}{A} + (\text{coef})\frac{I}{A^2} + (\text{coef})\frac{I^2}{A^2} + (\text{coef})\frac{I^2}{A} \tag{5}$$

$$v = V\left[\left(1 + \frac{2K_{ia}}{A}\right)\left(1 + \frac{K_{aa}}{A}\right) + (\text{coef})\frac{I}{A^2}\right] \Big/ \left(1 + \frac{2K_{ia}}{A}\right)^2\left(1 + \frac{K_{aa}}{A}\right) + (\text{coef})\frac{I}{A^3} + (\text{coef})\frac{I}{A^2} + (\text{coef})\frac{I^2}{A^3} \tag{6}$$

$$v = V\left(1 + \frac{2K_{ia}}{A}\right)\left(1 + \frac{K_{aa}}{A}\right)^2 \Big/ \left(1 + \frac{2K_{ia}}{A}\right)^2\left(1 + \frac{K_{aa}}{A}\right)^2 + (\text{coef})\frac{I}{A^4} + (\text{coef})\frac{I}{A^3} + (\text{coef})\frac{I^2}{A^4} \tag{7}$$

$$v = \frac{V\left(1 + \frac{2K_{ia}}{A}\right)\left(1 + \frac{K_{aa}}{A}\right)\left(1 + \frac{I}{K_i}\right)}{\left(1 + \frac{2K_{ia}}{A}\right)^2\left(1 + \frac{K_{aa}}{A}\right)\left(1 + \frac{I}{K_i}\right) + (\text{coef})\frac{I^2}{A^3}} \tag{8}$$

$$v = \frac{V\left(1 + \frac{2K_{ia}}{A}\right)\left(1 + \frac{I}{K_i}\right)}{\left(1 + \frac{2K_{ia}}{A}\right)^2\left(1 + \frac{I}{K_i}\right) + (\text{coef})\frac{I^2}{A^2}} \tag{9}$$

$$v = V\left[\left(1 + \frac{2K_{ia}}{A}\right) + (\text{coef})\frac{I}{A} + (\text{coef})I + (\text{coef})I^2\right] \Big/ \left[\left(1 + \frac{2K_{ia}}{A}\right)^2 + (\text{coef})\frac{I}{A} + (\text{coef})\frac{I}{A^2} + (\text{coef})\frac{I^2}{A^2} + (\text{coef})\frac{I^2}{A} + (\text{coef})I + (\text{coef})I^2\right] \tag{10}$$

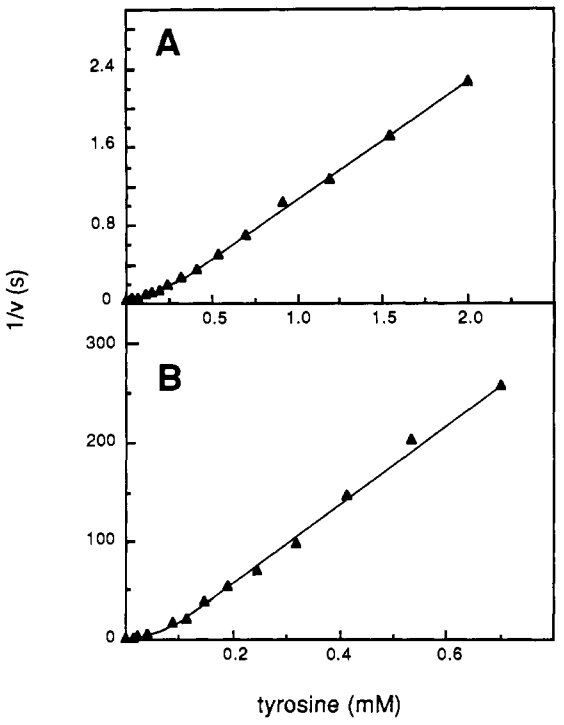

FIGURE 2: Inhibition of prephenate dehydrogenase by tyrosine. (A) NAD and prephenate were held at fixed concentrations of 2 mM and 125 μM, respectively. Initial velocity data were fitted to eq 1 to yield the parameter values of $V = 29 \pm 1\ s^{-1}$, $b = 12 \pm 1\ \mu M^{-1}$, $c = 77 \pm 14\ \mu M^{-2}$, and $d = 1.9 \pm 0.5\ \mu M^{-1}$. (B) NAD and CHCP were held at fixed concentrations of 2 mM and 100 μM, respectively. Initial velocity data were fitted to eq 1 to yield the parameter values of $V = 0.82 \pm 0.06\ s^{-1}$, $b = 27 \pm 13\ \mu M^{-1}$, $c = 2700 \pm 500\ \mu M^{-2}$, and $d = 7.5 \pm 2.0\ \mu M^{-1}$. The parameter values were used to draw the curves.

In the above equations, the terms in I (or J) and A denote inhibitor and substrate concentrations, respectively; V is the maximum velocity of the reaction. The coefficients terms include complex combinations of dissociation constants (K_i, K_I, K_{ia}, K_{IA}, K_{aa}) that are associated with the various enzyme complexes that are assumed to form. Weighted fits of the velocity data were made to the log form of the equations. The weighting factor was $\sqrt{v}$, which assumes that the error increases with increasing velocity although not in a proportional manner. Velocity data were fitted to the appropriate equations by use of a VAX 8700 computer.

Results

Inhibition of Prephenate Dehydrogenase by Tyrosine. The inhibition of the dehydrogenase reaction was studied over a wide range of tyrosine concentrations to determine the nature of the inhibition. Reaction mixtures contained a saturating concentration of NAD and fixed nonsaturating concentrations of either prephenate or the fast isomer of CHCP (cf. Materials). With both substrates, the data yielded curvilinear plots of $1/v$ against tyrosine (Figure 2A,B) and could be fitted to eq 1. This equation is a 2/1 function (Cleland, 1963) that describes the positive cooperativity that is observed in Figure 2. Thus, the data can be interpreted to indicate that the presence of a tyrosine molecule at one allosteric binding site on one subunit of the dimeric enzyme influences the binding of a second tyrosine molecule at the allosteric binding site on the other subunit. The data exclude the possibility that the presence of tyrosine on the enzyme simply makes the binding of substrate at the active site more difficult. Under these circumstances, the inhibition data would be described by a 2/2 function with I^2 terms in both the numerator and denominator of the rate equation. The results obtained with prephenate are in agreement with those reported by Christopherson (1985).

Effect of Tyrosine on Binding of Substrate Analogues. To establish if prephenate dehydrogenase possesses a distinct allosteric binding site for tyrosine, double inhibition experiments were performed with (4-hydroxyphenyl)pyruvate (HPP) and (carboxyethyl)-1,4-dihydrobenzoate (CEDB), which act as linear competitive inhibitors with respect to prephenate (SampathKumar & Morrison, 1982; Turnbull & Morrison, 1990). The double inhibition experiments with CEDB and tyrosine, as well as with HPP and tyrosine, yielded the unusual plots that are illustrated in Figure 3. The data for each plot could be fitted to eq 2, which describes a model (Figure 4) that allows for substrate (A) or inhibitory analogue (J) to combine twice at the active sites of the dimeric enzyme, for tyrosine (I) to combine twice at a specific allosteric site on each subunit and for both tyrosine and the inhibitory substrate analogue to be present on the enzyme at the same time. Although tyrosine and prephenate exhibit structural similarities, the model does not allow for any interaction of tyrosine at the active site of the enzyme. This is because arogenate, which is the transaminated amino acid corresponding to prephenate and which is also related structurally to tyrosine, competes poorly with prephenate for binding at the active site of prephenate dehydrogenase. The results of the analysis (Table I) establish the existence on the enzyme of an allosteric site for tyrosine binding since a finite value was obtained for K_{Ij}. They also indicate that the dissociation constant for the interaction of the first molecule of tyrosine with the enzyme (K_i) is very much lower than the dissociation constant for the binding of the second molecule of tyrosine (K_I). The K_I/K_i

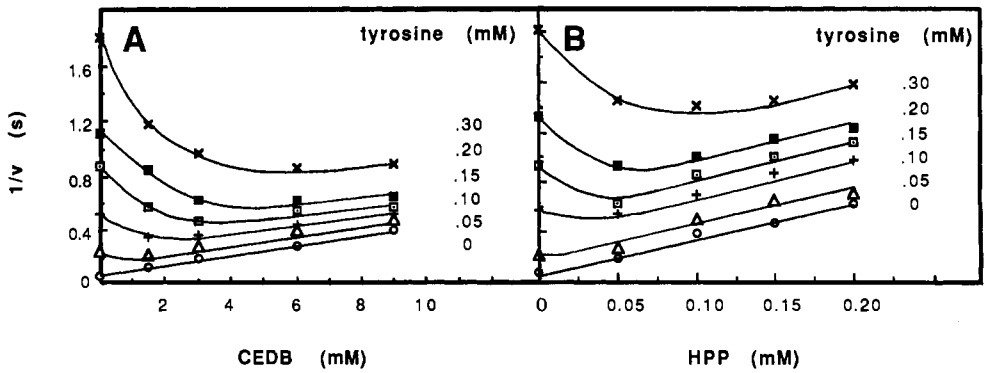

FIGURE 3: Double inhibition of prephenate dehydrogenase by (A) tyrosine and (carboxyethyl)-1,4-dihydrobenzoate (CEDB); (B) tyrosine and (4-hydroxyphenyl)pyruvate (HPP). Assays were performed with prephenate and NAD held constant at concentrations of 40 μM and 2 mM, respectively. Initial velocity data were fitted to eq 2.

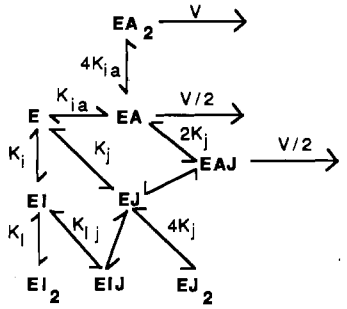

FIGURE 4: Kinetic scheme for the interaction with the prephenate dehydrogenase–NAD complex of tyrosine (J) and the inhibitory substrate analogues (I) (carboxyethyl)-1,4-dihydrobenzoate and (4-hydroxyphenyl)pyruvate. A denotes prephenate.

Table I: Values of Dissociation Constants Obtained for Interaction of Tyrosine and (4-Hydroxyphenyl)pyruvate (HPP) or Tyrosine and Carboxyethyldihydrobenzoate (CEDB) with Prephenate Dehydrogenase

parameter	reaction[a]	parameter values[b] HPP	CEDB
V (s^{-1})		29 ± 2	29 ± 2
K_j (μM)	EJ ⇌ E + J	5.5 ± 0.3	420 ± 10
K_i (μM)	EI ⇌ E + I	4.7 ± 0.7	4.7 ± 0.4
K_I (μM)	EI_2 ⇌ EI + I	240 ± 70	340 ± 60
K_{Ij} (μM)	EIJ ⇌ EI + J	26 ± 4	10300 ± 2600
$K_i K_I$ (μM^2)	EI_2 ⇌ E + 2I	1130 ± 200	1600 ± 200
$K_i K_{Ij}$ (μM^2)	EIJ ⇌ E + I + J	130 ± 10	47 ± 10

[a] I, J, and E denote tyrosine, HPP or CEDB, and enzyme–NAD, respectively. [b] The Michaelis constant for prephenate (K) was determined by fitting initial velocity data obtained by varying the concentration of prephenate (A) to the equation $v = VA/(K + A)$. Parameter values were determined by using the relationships $K = 2K_{ia}$ and $2K_{ia}/A = 0.925$. The fixed concentration of prephenate (A) used in the assay was 40 μM.

ratio of 40–70 far exceeds the expected value of 4 for the independent binding of two tyrosine molecules. Thus the presence of one tyrosine molecule on the enzyme hinders the binding of a second molecule by a factor of 10–18. It should also be noted that, if the presence of tyrosine on the enzyme did not affect the formation of an enzyme–NAD–HPP or an enzyme–NAD–CEDB complex, the K_{Ij}/K_j ratio would be 2. The ratio of 4.6 observed with HPP suggests that tyrosine may affect slightly the binding of this substrate analogue. By contrast, it can be concluded from the K_{Ij}/K_j ratio of 24 obtained with CEDB that tyrosine reduces the binding of this molecule by a factor of 12.

Mechanism of Inhibition of Dehydrogenase by Tyrosine. A kinetic approach has been made to elucidate the effects of tyrosine on the interaction of the non-nucleotide substrate with prephenate dehydrogenase under conditions where NAD was present at a saturating concentration. It is under these conditions that double-reciprocal plots of velocity as a function of substrate concentration are linear (Turnbull et al., 1990). Initial velocity data were obtained by varying the concentration of tyrosine from 0 to 2 mM in the presence of different fixed concentrations of prephenate or CHCP. The fixed concentrations of prephenate were 25, 125, and 500 μM, while the fixed concentrations of CHCP were 50, 100, and 1000 μM. The total number of data points was 42 with prephenate and 30 with CHCP. The initial velocity data were fitted to equations that describe models which could account for the allosteric inhibition of the dehydrogenase reaction. These models were derived from the most general scheme that might apply and that is illustrated in Figure 5. The scheme allows for (a) the presence of one active site and one allosteric site on each subunit of the dimeric enzyme; (b) the binding of two

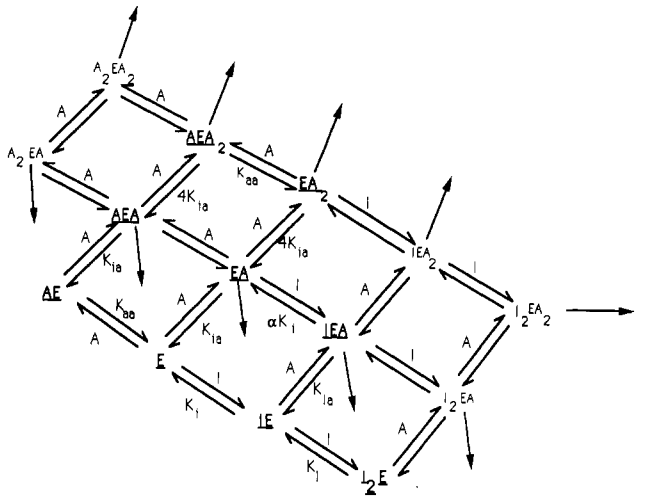

FIGURE 5: General scheme for the inhibition of prephenate dehydrogenase by tyrosine: A, substrate; I, tyrosine. The binding of substrate at the active site of the enzyme is denoted by writing A to the right of the symbol for the enzyme–NAD complex, E. The combination of tyrosine (or substrate) at an allosteric site is denoted by writing I (or A) to the left of E. Single arrows indicate the enzyme complexes that could give rise to product. The pathway presented in the underlined larger boldface type represents that for the inhibition by tyrosine in the presence of prephenate.

substrate molecules at the active sites and two tyrosine molecules at the allosteric sites; the reaction of tyrosine at the active site is not considered because the structurally related arogenate binds so poorly at the active site; (c) the simultaneous presence of both substrate and tyrosine on the enzyme so that tyrosine may or may not influence the rate of product formation; and (d) the binding of substrate at one or both allosteric sites, so as to prevent tyrosine binding, without having any effect on substrate binding at the active site or on the rate or product formation.

The general scheme is far too complex for data analysis. Thus the initial velocity data, as obtained with prephenate and CHCP, were fitted to rate equations that describe a set of simpler kinetic models. These models, which form part of the complete scheme (Figure 5), are summarized in Table II, which also lists the rate equations to which the data were fitted. It should be noted that the models have been divided into two groups according to whether substrate does or does not bind at the allosteric site. The models whose initial velocity equations yielded fits to the velocity data with each substrate are listed in Table III, together with values for the appropriate kinetic parameters.

Discussion

The present investigation emphasizes the complexities associated with the elucidation of the kinetic mechanism for a reaction catalyzed by an enzyme that exhibits allosteric properties. It also draws attention to the importance of performing the kinetic studies with alternative substrates and of having thermodynamic data for the binding of the allosteric inhibitor. The two substrates used for the studies on prephenate dehydrogenase were the natural substrate, prephenate, and the chemically synthesized analogue, CHCP.

It will be assumed now and justified later that the initial velocity data obtained with prephenate as substrate can best be described by model 14. This model, which is summarized in Table II and outlined in the underlined boldface type of Figure 4, proposes that (a) all the reactions occur under rapid equilibrium conditions; (b) prephenate (A) binds at one or other of the two equivalent active sites on the dimeric de-

Table II: Enzyme Complexes for Models To Describe the Allosteric Inhibition of Prephenate Dehydrogenase by Tyrosine[a]

model no.	EA[b,c]	EA_2[b]	IE[c]	I_2E	IEA[c]	I_2EA[c]	IEA_2	I_2EA_2	AEA[d]	AEA_2[d]	A_2EA[d]	A_2EA_2[d]	eq[e]
Group A: Substrate Binds at Active Site Only													
1	+	+	+	+									4(i)
2	+	+	+	+	+0								4(ii)
3	+	+	+	+	++								5(ii)
4	+	+	+	+	+0	+0							4
5	+	+	+	+	++	+0							5
6	+	+	+	+		+0							4(iii)
7	+	+	+−	+	+0								5(iv)
8	+0	+	+	+	++								3(v)
9	+0	+	+	+									3(vi)
10	+	+	+−	+									9
11	+	+	+	+	++		++	++					10
Group B: Substrate Binds at Both Active and Allosteric Sites													
12	+	+	+	+					++	++			6(vii)
13	+	+	+	+	+0				++	++			6(viii)
14	+	+	+	+	++				++	++			6
15	+	+	+−	+	+0				++	++			6(ix)
16	+	+	+−	+	++				++	++			6(xi)
17	+	+	+	+					++	++	++		7(xi)
18	+	+	+−	+	+0				++	++	++		7(xii)
19	+	+	+−	+					++	++			8

[a] Complexes involving substrate (A) and the allosteric inhibitor, tyrosine (I), that can form with dimeric prephenate dehydrogenase (E) are illustrated in Figure 4. The placing of symbols to the left and to the right of E denotes binding at the allosteric and active sites, respectively; a statistical factor is associated with EA as A can bind at each of two sites on the dimeric enzyme. [b] (+) complex forms and gives rise to products; (+0) complex forms but does not yield product. [c] (+) complex forms; (++) complex forms and yields product but at a slower rate than from EA; (+0) complex forms but is inactive; (+−) presence of I on enzyme does not influence binding of A. [d] Combination of substrate at the allosteric site is considered to have no effect on the rate of product release. The dissociation constant for this interaction is denoted by K_{aa}. [e] (i) modified form of equation without terms in I/A (IEA) and I^2/A (I_2EA); (ii) no I^2/A (I_2EA) term; (iii) no I/A (IEA) term in numerator; (iv) no I/A (IEA) term in numerator; no I/A^2 (EI) or I^2/A (I_2EA) in denominator; (v) αK_i represents dissociation constant for loss of I from IEA; (vi) α = infinity; (vii) no I/A^2 (IEA) term in numerator or denominator; (viii) no (coef) I/A^2 term as IEA is inactive; (ix) no I/A^2 term in numerator as IEA is inactive; no I/A^3 term in denominator as EI is equivalent to E; (x) no I/A^3 term as EI is equivalent to E; (xi) no I/A^3 term as IEA is not formed; (xii) no I/A^4 term as EI is equivalent to E.

Table III: Dissociation Constants for Complexes Involving Enzyme–Tyrosine–Substrate in the Prephenate Dehydrogenase Reaction

	values of dissociation constants[a] obtained with							
	prephenate				CHCP			
model no.	K_i	K_iK_I	K_iK_{Ia}	σ	K_i	K_iK_I	K_iK_{Ia}	σ
1	3.9 ± 0.4	3200 ± 200	–	0.0863	1.5 ± 0.3	1300 ± 600	–	0.157
2	3.5 ± 0.3	3300 ± 200	3090 ± 250	0.0735	–	–	–	–
6	3.7 ± 0.4	3300 ± 300	–	0.087	–		–	–
10	66 ± 9	200 ± 30	–	0.121	46 ± 8	35 ± 10	–	0.165
12	–	–	–	–	2.0 ± 0.4	112 ± 20	–	0.093
13	3.9 ± 0.4	2600 ± 200	950 ± 150	0.068	–	–	–	–
14	3.6 ± 0.4	1400 ± 300	690 ± 180	0.066	–	–	–	–
15	–	1200 ± 200	86 ± 20	0.123		27 ± 9	92 ± 10	0.070
16	–	45 ± 9	30 ± 10	0.094	–	–		
17	–	–		–	4.2 ± 0.5	81 ± 10	–	0.079
18	–	930 ± 200	88 ± 15	0.168	–	64 ± 10	550 ± 150	0.066
19	–	–	–	–	830 ± 320	52 ± 8	–	0.077

[a] K_i (μM), K_iK_I, (μM^2), and K_iK_{Ia} (μM^2) denote dissociation constants for the reactions of EI to E + I, EI_2 to E + 2I, and EAI to E + I + A, respectively. Where a dash is shown, one or more coefficients in the fitted equation were negative or a convergent fit was not obtained.

hydrogenase (E) to form two EA complexes for which the dissociation constant is K_{ia} (It should be noted that E represents E-NAD as NAD was present always at a saturating concentration); (c) a second molecule of prephenate combines at each vacant active site on the enzyme to form an EA_2 complex whose dissociation constant is $4K_{ia}$ and which yields product at twice the rate of the EA complex; (d) tyrosine (I) binds at a specific allosteric site on each of the two subunits in the same random manner as does prephenate to yield IE and I_2E complexes with dissociation constants of K_i and K_I, respectively. (Note that reaction of a ligand at the allosteric binding sites of the enzyme is indicated by writing the symbol for the ligand to the left of the enzyme-NAD symbol, E.); (e) I_2E is a dead-end complex, while IE can react with A to form an IEA complex that yields product at a reduced rate compared with EA; (f) prephenate can react at one allosteric site on the enzyme to form AEA and AEA_2 complexes with a dissociation constant of K_{aa}; it is assumed that substrate simply competes with tyrosine for the allosteric binding sites and does not have any effect on the binding of substrate at the active site or on the rate of product formation; and (g) there is no interaction of tyrosine at the active site; arogenate is such a poor inhibitor of the enzyme that any binding of tyrosine at the active site should be negligible.

The proposed model includes an IEA complex, which is necessary for plots of $1/v$ against tyrosine to be described by a 2/1 function as observed (Figure 2). It also includes a dead-end I_2E complex. Such a complex must form to account for the finding that the velocity of the reaction varies with the tyrosine concentration in the presence of a saturating concentration of prephenate. Thus the mechanism of the allosteric inhibition of prephenate dehydrogenase by tyrosine differs from the simple K_m type of allosteric inhibition observed with aspartate transcarbamylase (Gerhart & Pardee, 1962; Gerhart

& Schachman, 1965). It was necessary to allow for the reaction of prephenate at the allosteric site as, in the absence of such interaction, one of the parameter values was negative.

The form of eq 6, which describes the aforementioned mechanism, is both qualitatively and quantitatively in accord with the experimental data obtained with prephenate (Table III). The equation predicts that plots of $1/v$ against tyrosine or $1/v$ against 1/[prephenate] in the presence of tyrosine will be curvilinear (Figure 2) and that in the absence of tyrosine, double-reciprocal plots of velocity as function of prephenate concentration will be linear (Christopherson & Morrison, 1985; Turnbull et al., 1990). The fit of the data to this equation, which gives the lowest σ value (Table III), shows that there is antisynergistic binding of tyrosine at the allosteric site. Indeed, the binding of the second molecule is about two orders of magnitude weaker than the binding of the first molecule. The K_I value of 290 μM is comparable to those of 240 and 340 μM that were determined from the double inhibition experiments with tyrosine and HPP (or CEDB) (Table I). Since these experiments yielded correct values for the dissociation constants of the enzyme–HPP and enzyme–CEDB complexes, the data lend support to model 14. The value of 3.5 μM for the dissociation constant of the tyrosine–enzyme complex agrees with the value of 4.7 μM determined from the double inhibition experiments (Table I), and this agreement again offers support for the proposed model. The value compares well with that of 2.0 μM as reported by Hudson et al. (1983) from binding studies. The latter study also indicated that there appeared to be a weaker interaction with the enzyme of a second molecule of tyrosine. The presence of tyrosine on the enzyme has a marked effect on the rate of product formation as the tyrosine–enzyme–prephenate complex yields product at only 6% of the rate obtained with the enzyme–prephenate complex.

Apart from showing that the experimental data with prephenate are in accord with model 14, there are grounds for positive discrimination against the other models listed in Table II. Models 1, 2, 6, 10, 15, 16, and 18 are described by functions to which the data could be fitted, although the resulting σ values are higher than that obtained with model 14. Models 12, 15, 16, and 18 can be eliminated because they do not yield a value for the dissociation constant (K_i) of the tyrosine–enzyme complex, which is known to form (Hudson et al., 1983). The value of 66 μM for the dissociation constant of this complex, as determined by fitting the data to the equation that describes model 10, is very much greater than the thermodynamic value of 2 μM. Thus the model must be eliminated. While models 1, 2, and 6 yield correct values for K_i, the values determined for the product K_iK_I (cf. Table II) are not in agreement with any value determined for the same product with CHCP as substrate. It is axiomatic that this value must be independent of the substrate used to determine it. On this basis, models 1, 2, and 6 must be eliminated from consideration. It is noteworthy that model 13 does not yield the correct value for K_iK_I.

The data obtained for the allosteric inhibition of prephenate dehydrogenase by tyrosine with CHCP as substrate could be fitted to the equations that describe seven models (Table II). But when the arguments elaborated above in relation to the prephenate data are taken into account, several models cannot apply. Models 10 and 19 do not yield the correct value of the dissociation constant (K_i) for the tyrosine–enzyme complex, while models 12 and 17 do not give rise to a value of about 1400 μM^2 as obtained with model 14 for the prephenate data. The only remaining model is model 1. This model must apply even though the fitting of the CHCP data to the equation that describes this model gives the second highest σ value. Model 1 differs from model 14 only in that there is no formation of a tyrosine–enzyme–substrate complex and no combination of substrate at the allosteric sites. If it is considered that these complexes are not detected because CHCP binds more weakly to the tyrosine–enzyme complex and at the allosteric site than does prephenate, then the difference between the two models is only one of degree.

The conclusions reached about the kinetic mechanism for the allosteric inhibition of prephenate dehydrogenase by tyrosine differ from those of earlier investigators. Christopherson (1985) proposed that the inhibtion data obtained with tyrosine could be explained on the basis that both tyrosine and prephenate bind at the active site of the enzyme. Hudson et al. (1983) postulated that a dynamic equilibrium exists between an active dimeric form and an inactive tetrameric form of the enzyme, that tyrosine and NAD bind more tightly to the tetramer than to the dimer, and that tyrosine both facilitates the polymerization reaction and competes with prephenate for the active site of the enzyme. Apart from being inconsistent with the present findings, it has not been possible to demonstrate in this laboratory that tyrosine is involved with or induces the polymerization of chorismate mustase–prephenate dehydrogenase (Christopherson & Morrison, 1985).

ACKNOWLEDGMENTS

We thank Mrs. Irene Davies for skilled technical assistance.

REFERENCES

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.
Christopherson, R. I. (1985) *Arch. Biochem. Biophys. 240*, 646–654.
Christopherson, R. I., & Morrison, J. F. (1985) *Biochemistry 23*, 1116–1120.
Cleland, W. W. (1963) *Biochim. Biophys. Acta 67*, 104–137.
Dudzinski, P. K., & Morrison, J. F. (1976) *Prep. Biochem. 6*, 113–121.
Gerhart, J. C., & Pardee, A. B. (1962) *J. Biol. Chem. 237*, 891–896.
Gerhart, J. C., & Schachman, H. K. (1965) *Biochemistry 4*, 1054–1062.
Gibson, F. (1968) *Biochem. Prep. 12*, 94–98.
Harada, K., & Wolfe, R. G. (1968) *J. Biol. Chem. 243*, 4131–4137.
Heyde, E., & Morrison, J. F. (1978) *Biochemistry 17*, 1573–1580.
Hudson, G. S., Howlett, G. J., & Davidson, B. E. (1983) *J. Biol. Chem. 258*, 3114–3120.
Hudson, G. S., Wong, V., & Davidson, B. E. (1984) *Biochemistry 23*, 6240–6249.
Knox, W. E., & Pitt, B. M. (1957) *J. Biol. Chem. 225*, 675–688.
Lindbland, B., Lindstedt, G., Lindstedt, S., & Rundgren, M. (1977) *J. Biol. Chem. 252*, 5073–5084.
Monod, J., Wyman, J., & Changeaux, J. P. (1965) *J. Mol. Biol. 12*, 88–118.
SampathKumar, P., & Morrison, J. F. (1982) *Biochim. Biophys. Acta 702*, 204–211.
Turnbull, J., & Morrison, J. F. (1990) *Biochemistry 29*, 10255–10261.
Turnbull, J., Cleland, W. W., & Morrison, J. F. (1990) *Biochemistry 29*, 10245–10254.