strate and not free to move in the active site. Addition of the hydroxyl radical to the *re* face of the carbon radical results in formation of (*R*)-carnitine. This topographical arrangement predicts both the observed retention of configuration and the stereospecificity of hydrogen atom abstraction, while allowing for the intermediacy of a carbon radical.

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Chorismate Mutase–Prephenate Dehydrogenase from *Escherichia coli*: Positive Cooperativity with Substrates and Inhibitors

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ABSTRACT: Investigations have been made at pH 6.0 of the effect of chorismate and adamantane derivatives on the mutase and dehydrogenase activities of hydroxyphenylpyruvate synthase from *Escherichia coli*. When used over a wide range of concentrations, chorismate 5,6-epoxide, chorismate 5,6-diol, adamantane-1,3-diacetate, adamantane-1-acetate, adamantane-1-carboxylate, and adamantane-1-phosphonate give rise to nonlinear plots of the reciprocal of the initial velocity of each reaction as a function of the inhibitor concentration. The inhibitors do not induce the enzyme to undergo polymerization and have only a small effect on the $s_{20,w}$ value of the enzyme as determined by using sucrose density gradient centrifugation. At low substrate concentration, low concentrations of adamantane-1-acetate cause activation of both the mutase and dehydrogenase activities while at higher concentrations this compound functions as an inhibitor. When chorismate and prephenate are varied over a wide range of concentrations, double-reciprocal plots of the data indicate that the reactions exhibit positive cooperativity. The addition of albumin eliminates the cooperative interactions associated with substrates but has little effect on those associated with inhibitors.

The immediate precursor for the biosynthesis of tyrosine in the Enterobacteriacae is 4-hydroxyphenylpyruvate whose formation from chorismate (eq 1) is catalyzed by a bifunctional enzyme that possesses chorismate mutase (EC 5.4.99.5) and prephenate dehydrogenase (EC 1.3.1.12) activities. The enzyme, which has also been referred to as hydroxyphenylpyruvate synthase (Christopherson et al., 1983), is a dimeric

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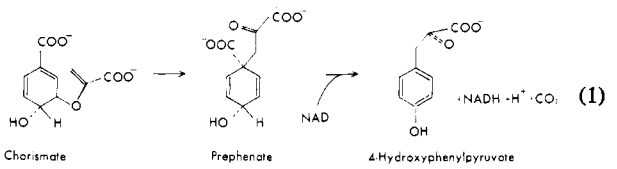

(1)

protein that consists of two identical subunits and has a molecular weight of 88 000 (SampathKumar & Morrison, 1982a). Since the product of the first reaction of eq 1 is a substrate for the second reaction, considerable interest has centered on the relationship between the sites at which the two reactions occur. There have been several indications that the mutase and dehydrogenase reactions do not take place at separate sites (Koch et al., 1972; Heyde & Morrison, 1978; Hudson & Davidson, 1981; Rood et al., 1982). More recent data suggest that chorismate and prephenate are bound at separate active sites for the mutase and dehydrogenase reactions and that there is a close spatial relationship between the two sites (Christopherson et al., 1983).

From previous studies on hydroxyphenylpyruvate synthase, it appeared that both reactions conform to Michaelis–Menten kinetics except in the presence of tyrosine which acts as an allosteric end-product inhibitor of the dehydrogenase reaction (Koch et al., 1971). The present investigations show that, at concentrations of chorismate or prephenate which are lower than those used previously, the reactions do not conform to Michaelis–Menten kinetics. Further, it has been demonstrated that analogues of chorismate act like tyrosine in that they cause inhibitions of the mutase and dehydrogenase reactions that are not of the classical type.

Experimental Procedures

Materials

Procedures for the purification of hydroxyphenylpyruvate synthase and the synthesis of chorismate derivatives have been described by Christopherson et al. (1983). Bovine serum albumin, bovine hemoglobin, horse liver alcohol dehydrogenase, and lactate dehydrogenase from bovine muscle were purchased from Sigma Chemical Co. Adamantane-1-phosphonate was kindly supplied by Professor Glenn Berchtold. All other compounds were obtained as described previously (Christopherson et al., 1983).

Methods

Determination of Enzyme Activity. Mutase and dehydrogenase activities were measured at pH 6.0 in the presence of 50 mM 2-(*N*-morpholino)ethanesulfonic acid, 25 mM *N*-ethylmorpholine, and 25 mM diethanolamine. This three-component buffer system has been used in all studies on the enzyme as it can be used over a wide range of pHs without changes in ionic strength (Ellis & Morrison, 1982). A pH value of 6.0 was chosen because the substrate analogues under investigation are more inhibitory at pH 6.0 than at neutral pH. The conversion of chorismate to prephenate was followed at 290 nm while the oxidative decarboxylation of prephenate in the presence of NAD was followed at 340 nm. Both reactions were monitored by using a Cary 118 spectrophotometer which was set at the maximum scale expansion of 0–0.02.

Sucrose Gradient Ultracentrifugation. Linear sucrose gradients from 10% to 40% (w/v) were prepared by stepwise addition of 2.9 mL of 10%, 20%, 30%, and 40% (w/v) sucrose solutions to the bottom of centrifuge tubes which were then left for 90 min at room temperature to allow formation of a linear gradient by diffusion. The sucrose gradients also contained 20 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 7.5), 1 mM dithiothreitol, 1 mM ethylenediaminetetraacetic acid (EDTA), and other components as indicated in the text. Samples (200 μL) containing 61 μg of pure hydroxyphenylpyruvate synthase, three marker proteins (1 mg of hemoglobin, 1 mg of alcohol dehydrogenase, and 150 μg of lactate dehydrogenase), 20 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, 1 mM EDTA, and other components indicated in the text were equilibrated at 0 °C for 1 h prior to being layered onto the appropriate gradient (of similar composition). Sedimentation profiles for the synthase were developed by centrifugation at 41 000 rpm in a Beckman SW 41 Ti rotor for 65 h at 5 °C using a Beckman L8-70 ultracentrifuge. Five drop (164 μL) fractions of each gradient were collected by puncturing the bottom of the tube. Prephenate dehydrogenase was assayed as described above and the marker proteins as outlined by Traut & Jones (1977). The sedimentation coefficient of the synthase in the presence of various effectors was determined from a calibration curve of the three standard proteins for each gradient using published *s* values: hemoglobin, 4.3 S; alcohol dehydrogenase, 5.1 S; and lactate dehydrogenase, 7.0 S (Smith, 1973).

Analysis of Data. Data that yielded curvilinear double-reciprocal plots of velocity as a function of substrate concentration were fitted to eq 2 by using weighting factors of $1/v^2$

$$v = \frac{V(A^2 + dA)}{A^2 + bA + c} \qquad (2)$$

and the computer program of Cleland (1979). Data obtained with compounds that could both activate and inhibit enzyme activity were fitted to the same equation but with $1/I$ replacing A. Analyses were performed by using the modification of the STEPIT program described by Duggleby & Morrison (1979). The STEPIT program was obtained from the Quantum Chemistry Program Exchange at Indiana University as part of the STEPT package (QCPE 307).

Results

Inhibition of Hydroxyphenylpyruvate Synthase by Derivatives of Chorismate and Adamantane. Previous studies on the inhibition of hydroxyphenylpyruvate synthase by chorismate and adamantane derivatives showed that these compounds are linear competitive inhibitors with respect to chorismate in the mutase reaction and with respect to prephenate in the dehydrogenase reaction (Christopherson et al., 1983). Such studies were performed by using a range of substrate and inhibitor concentrations over which the reactions could be considered as conforming to Michaelis–Menten kinetics. However, this relationship does not hold when lower concentrations of the substrates and/or higher concentrations of the inhibitors are used to study the mutase and dehydrogenase reactions.

The data of Figure 1a,b show that Dixon plots of the reciprocal of the initial velocity of both the mutase and dehydrogenase reactions against the concentration of the substrate analogues, chorismate 5,6-epoxide and chorismate 5,6-diol, are markedly curved at higher concentrations of the inhibitors. The curvature is less pronounced with the dehydrogenase than with the mutase reaction. This may well be due to the presence of NAD as the enzyme was saturated to about the same degree with each of the nonnucleotide substrates. The similar behavior of the epoxide and diol as inhibitors of the two reactions contrasts with that of 5,6-dihydrochorismate. However, the difference may simply be a consequence of the fact that 5,6-dihydrochorismate is a relatively poor inhibitor. The limited amount of material available

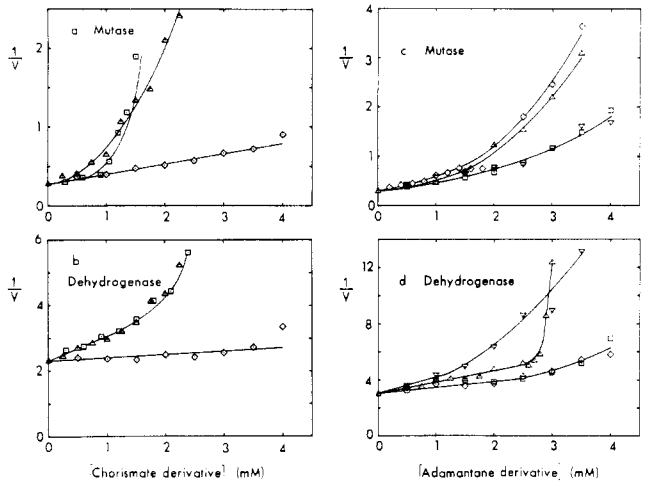

FIGURE 1: Dixon plots for the inhibition of mutase and dehydrogenase activities of hydroxyphenylpyruvate synthase by derivatives of chorismate and adamantane. (a and b) Chorismate 5,6-epoxide (△); chorismate 5,6-diol (□); 5,6-dihydrochorismate (◇). (c and d) Adamantane-1-acetate (△); adamantane-1,3-diacetate (▽); adamantane-1-phosphonate (◇); adamantane-1-carboxylate (□). Initial reaction velocities were determined by using 0.21 μg/mL synthase and 200 μM chorismate for the mutase assay and 0.07 μg of the synthase and 200 μM prephenate plus 100 μM NAD for the dehydrogenase assay. Reaction velocities are expressed in micromolar per minute.

precluded the use of a wider range of concentrations.

Nonlinear Dixon plots are also observed for the inhibition of the reactions by a series of adamantane derivatives (Figure 1c,d) which have been considered as analogues of the transition-state complex for the conversion of chorismate to prephenate (Andrews et al., 1977) and which can act as inhibitory analogues of both chorismate and prephenate (Christopherson et al., 1983). For the mutase reaction, the effectiveness of the inhibitors decreases in the order adamantane-1-phosphonate > adamantane-1-acetate > adamantane-1-carboxylate ≃ adamantane-1,3-diacetate. The same order is not maintained with the dehydrogenase reaction for which the order of effectiveness can be considered as adamantane-1,3-diacetate > adamantane-1-acetate > adamantane-1-carboxylate ≃ adamantane-1-phosphonate. However, it should be noted that there is a marked increase in the curvature of the plot with adamantane-1-acetate (Figure 1d) when its concentration exceeds 2.5 mM. When the concentration of NAD for the prephenate dehydrogenase reaction was increased from 0.1 to 1.0 mM, higher concentrations of the inhibitors were required to demonstrate curvature in the plots. In this connection, it is of interest that the presence of NAD on the enzyme has been shown to hinder the binding of all chorismate analogues to the enzyme (Christopherson et al., 1983).

Kinetics of the Reactions in the Absence of Inhibitors. The nonlinearity of Dixon plots in the presence of inhibitory analogues of chorismate (Figure 1) suggested that the binding of an inhibitor molecule to one subunit of the enzyme may cause an interaction between the two subunits which enhances the binding of a second inhibitor molecule. On this basis, it might be expected that similar interactions would be associated with substrate binding. To test this hypothesis, initial velocities for the mutase and dehydrogenase reactions were determined over a much larger range of substrate concentrations than had been used previously (Christopherson et al., 1983; Sampath-Kumar & Morrison, 1982; Heyde & Morrison, 1978). The results (Figure 2) indicate that positive cooperativity occurs with both reactions as judged by the nonlinearity of the double-reciprocal plots of initial velocity as a function of chorismate or prephenate concentration. It should be noted that nonlinearity is not apparent until the concentrations of substrates fall below 10 μM. The data of Figure 2 were fitted to eq 2 to yield parameter values for the mutase reaction of $b = 14 \pm 5$ μM, $c = 0.8 \pm 26$ μM², $d = -3.4 \pm 0.1$ μM, and $V = 1.7 \pm 0.2$ μM min⁻¹ and for the dehydrogenase reaction of $b = 18 \pm 4$ μM, $c = 21 \pm 19$ μM², $d = -1.5 \pm 0.2$ μM, and $V = 0.76 \pm 0.06$ μM min⁻¹. Since the c values are ill-defined and the d values are negative, it can be concluded that the data are not described by eq 2. The poor fit of the data to eq 2 was not due to enzyme inactivation. Progress curves obtained for the mutase and dehydrogenase reactions at low substrate concentrations, where cooperativity is observed, and a range of enzyme concentrations were superimposable when plotted with time multiplied by enzyme concentration as the independent variable (Selwyn, 1965). Positive cooperativity for the mutase and dehydrogenase activities was also observed at pH 7.5. The curvature of double-reciprocal plots at this pH was more marked than those obtained at pH 6.0 (Figure 2).

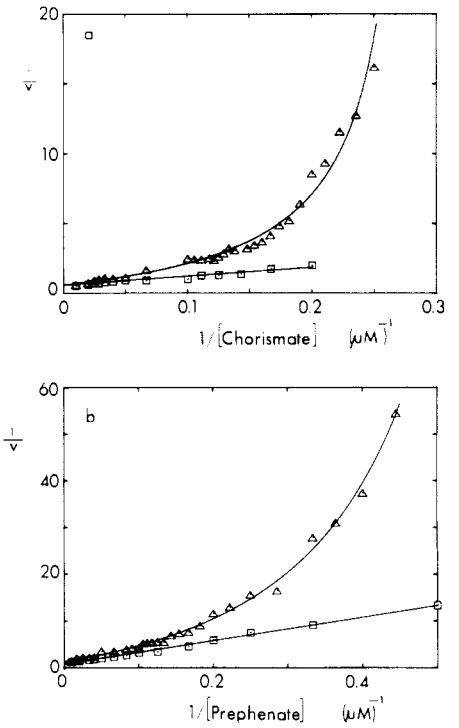

FIGURE 2: Lineweaver–Burk plots for the mutase (a) and dehydrogenase (b) activities of hydroxyphenylpyruvate synthase. Assays were performed in the absence (△) and the presence (□) of 100 μg/mL albumin using 0.21 μg/mL synthase and 40 μM NAD for the dehydrogenase assay. The curves through the data points for mutase and dehydrogenase were generated from eq 2 by using parameter values given in the text. Reaction velocities are expressed in micromolar per minute.

The Hill coefficients calculated from the data of Figure 2 were 3.5 for the mutase reaction and 2.7 for the dehydrogenase reaction. Hill coefficients were also determined for the data of Figure 1 by plotting log $(v_i/v_0 - v_i)$ against log I where v_0 and v_i are initial velocities in the absence and presence of inhibitor, respectively. The respective values obtained for the mutase and dehydrogenase were 2.1 and 2.0 with chorismate 5,6-epoxide (Figure 1a,b) and 1.4 and 2.0 with adamantane-1-acetate (Figure 1c,d). At high concentrations of the adamantane derivatives, more extreme upward curvature was observed in the Dixon plots. Such low reaction velocities (e.g., adamantane-1-acetate of Figure 1d) could not be accurately determined and were not included in the Hill plots.

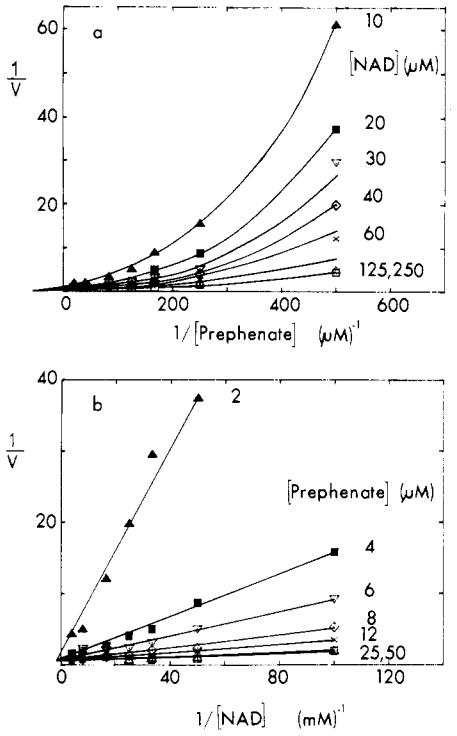

FIGURE 3: Effect of substrate concentration on the velocity of the dehydrogenase reaction as plotted with prephenate (a) or NAD (b) as the variable substrate. The amount of enzyme used was 1.1 μg/mL, and velocities are expressed in micromolar per minute.

A more detailed kinetic analysis of the dehydrogenase reaction is shown in Figure 3. Positive cooperativity with respect to prephenate is observed over a range of NAD concentrations from 10 to 250 μM. It should be noted that at the lowest prephenate concentration used of 2 μM, 10% conversion of prephenate to 4-hydroxyphenylpyruvate represents a difference of 6 scale units out of 100 with the Carey 118 spectrophotometer set at the maximum scale expansion of 0–0.02. A replot of the data of Figure 3a with NAD as the variable substrate (Figure 3b) gave rise to a linear double-reciprocal plot. Thus, it may be concluded that there are no cooperative interactions for the binding of NAD with the enzyme. The finding that the same set of data can be plotted to yield either curvilinear or linear double-reciprocal plots (Figures 3a,b) attests to the accuracy of the initial velocity data.

Effect of Albumin on Reaction Kinetics. Previous investigations have shown that bovine serum albumin forms a relatively stable 1:1 complex with hydroxyphenylpyruvate synthase and acts as an activator of the dehydrogenase through a lowering of the dissociation constants for the binary enzyme complexes (SampathKumar & Morrison, 1982b). The inclusion of albumin (100 μg/mL) in assay mixtures for the mutase and dehydrogenase abolished the nonlinearity of double-reciprocal plots but had little effect upon the apparent maximum velocities of the reactions (Figure 2). More detailed investigations of the effect of albumin on the values for the kinetic parameters associated with the mutase and dehydrogenase reactions yielded the results that are listed in Table I. These studies were performed over a range of substrate concentrations which yielded linear double-reciprocal plots in the absence of albumin. In accord with the data of Figure 2 and in contrast to the results obtained at pH 7.7 by SampathKumar & Morrison (1982b), albumin has little activating effect on the dehydrogenase over the higher range of prephenate concentrations. On the other hand, albumin does cause activation of the mutase through the lowering of the K_m value for chorismate. However, the marked effect of albumin on each enzyme activity is observed only at relatively low concentrations of chorismate or prephenate. Albumin (100 μg/mL) had little effect upon the inhibition of the mutase and dehydrogenase activities of the synthase by adamantane-1-acetate and adamantane-1-phosphonate when the experiments were performed as described for Figure 1c,d. The reduction in the inhibition of the dehydrogenase activity by these compounds at a concentration of 4 mM was less than 20%. The inhibition by adamantane-1-phosphonate in the presence of albumin was also studied over the range of chorismate and prephenate concentrations for which Michaelis–Menten kinetics appear to be obeyed. The resulting values of the dissociation constants for the combination of the inhibitor with different forms of enzyme were somewhat different from those reported previously for reactions in the absence of albumin (Christopherson et al., 1983). The dissociation constants for the enzyme–inhibitor complex in the presence (and absence) of albumin were 360 ± 50 μM (200 ± 20 μM) and 420 ± 200 μM (140 ± 20 μM) as determined from the mutase and dehydrogenase reactions, respectively, while those for the enzyme–NAD–inhibitor complex were 410 ± 70 μM (720 ± 60 μM).

Table I: Values of Kinetic Parameters for Hydroxyphenylpyruvate Synthase at pH 6.0 in the Absence and Presence of Albumin[a]

		albumin concn (μg/mL)	
reaction	kinetic parameter[b]	0	100
mutase	K_a	43 ± 5	14 ± 2
dehydrogenase	K_{ib}	14 ± 5	22 ± 5
	K_b	38 ± 10	22 ± 4
	K_{ic}	59 ± 20	162 ± 42
	K_c	163 ± 43	167 ± 22

[a] Chorismate and prephenate concentrations were varied from 20 to 100 μM. All values are given in micromolar and were calculated on the assumption that the reaction conforms to a rapid equilibrium, random mechanism at pH 6.0 as it does at pH 7.7 (SampathKumar & Morrison, 1982b). [b] K_a, K_b, and K_c represent Michaelis constants for chorismate, prephenate, and NAD, respectively, while K_{ib} and K_{ic} denote dissoication constants for the interaction with the free enzyme of prephenate and NAD, respectively.

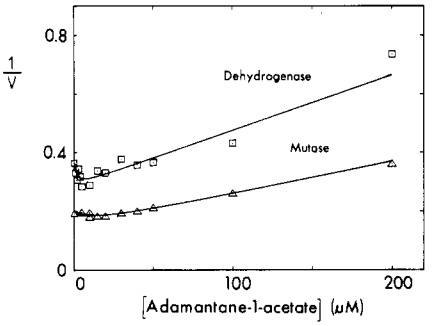

FIGURE 4: Activation of the mutase and dehydrogenase activities of hydroxyphenylpyruvate synthase by adamantane-1-acetate. The fit of the data to eq 2 with $A = 1/I$ yielded parameter values for the mutase reaction of $b = 0.059$ μM, $c = 0.00042$ μM^2, $d = 0.074$ μM, and $V = 5.0$ μM min^{-1} and for the dehydrogenase reaction of $b = 0.66$ μM, $c = 0.0043$ μM^2, $d = 0.84$ μM, and $V = 2.8$ μM min^{-1}. The values were used to draw the curves. Assays were performed with 2.1 μg/mL synthase and 10 μM chorismate for the mutase reaction or 1.1 μg/mL synthase, 6 μM prephenate, and 50 μM NAD for the dehydrogenase reaction.

Activation and Inhibition of the Enzyme by Adamantane-1-acetate. The effects of adamantane-1-acetate on the mutase and dehydrogenase reactions in the presence of low concentrations of chorismate (10 μM) or prephenate (8 μM) are illustrated in Figure 4. The fit of the data of Figure 4 to eq 2 with A equal to $1/I$ yielded values for each of the parameters

Table II: Sedimentation Coefficients for Hydroxyphenylpyruvate Synthase in the Presence of Different Ligands[a]

condition	$s_{20,w}$[b] (S)
control	4.73 ± 0.04
1.0 mM NAD	4.75
4.0 mM adamantane-1-acetate	4.86
4.0 mM adamantane-1-acetate, 1.0 mM NAD	4.92
1.0 mM chorismate 5,6-epoxide	4.85
1.0 mM chorismate 5,6-epoxide, 1.0 mM NAD	4.88
2.0 mM L-tyrosine	4.97
2.0 mM L-tyrosine, 1.0 mM NAD	5.14

[a] A 200-μL sample containing hydroxyphenylpyruvate synthase (61 μg) was applied to a linear 10–40% (w/v) sucrose gradient of 11.5 mL containing 20 mM Tris-HCl, pH 7.5, 1 mM dithiothreitol, 1 mM EDTA, and the indicated ligands. [b] Obtained by reference to three internal standard proteins sedimented in each gradient as described under Experimental Procedures.

that were used to draw the curves. However, the errors associated with these parameters were high because of the difficulty of obtaining well-determined initial velocity values at low substrate concentration. There is an initial activation which reaches a maximum value of 7% with the mutase and 18% with the dehydrogenase. Although the activation is small, it was reproducible. At higher concentrations of adamantane-1-acetate, both reactions are inhibited. Results similar to those of Figure 4 have been observed with aspartate transcarbamylase (Gerhart & Pardee, 1963; Collins & Stark, 1971). In the presence of a low concentration of aspartate, both dicarboxylic acids and *N*-phosphonacetyl-L-aspartate cause initial activation of the enzyme followed by the expected inhibition at higher inhibitor concentrations.

Sedimentation Coefficients of Enzyme–Ligand Complexes. Sucrose density gradient centrifugation of hydroxyphenylpyruvate synthase in the absence and presence of different ligands suggested that there may be a small variation in the value of the sedimentation coefficient of the enzyme in the presence of substrate analogues, but not in the presence of NAD (Table II). By contrast, there is a distinct, although small, change in the value when tyrosine alone or tyrosine and NAD are added. This finding differs from the results of Hudson et al. (1983), who reported that, in the presence of NAD and tyrosine, the enzyme exists as a tetrameric species. Sedimentation profiles for the free enzyme as well as for the enzyme–chorismate 5,6-epoxide and enzyme–NAD–tyrosine complexes are illustrated in Figure 5. The inclusion of three internal protein standards in each tube allowed determination of $s_{20,w}$ values with standard deviations of less than 3%.

Discussion

Previous kinetic investigations on the reactions catalyzed by hydroxyphenylpyruvate synthase indicated that, in the absence of tyrosine, both the mutase and dehydrogenase reactions conform to Michaelis–Menten kinetics (Heyde & Morrison, 1978; SampathKumar & Morrison, 1982a,b). Thus, the enzyme appeared to differ in its kinetic properties from most other allosteric enzymes. The present findings establish that this is not the case and that the dimeric hydroxyphenylpyruvate synthase does exhibit the kinetic properties which characterize many allosteric enzymes. The first indication that the synthase cannot be considered as a simple classical enzyme in the absence of tyrosine came from studies in which chorismate and adamantane derivatives were utilized as inhibitory analogues of chorismate and prephenate (Figure 1). The results clearly show that there are interactions associated with the binding to the enzyme of multiple molecules of inhibitor. Such interactions are not due simply to the

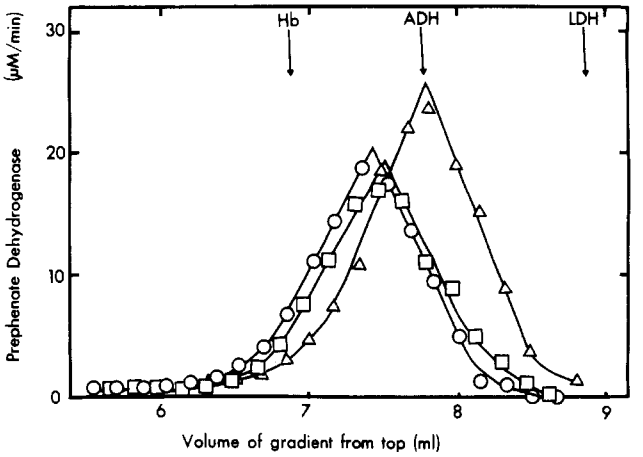

FIGURE 5: Sucrose gradient sedimentation of hydroxyphenylpyruvate synthase in control buffer (○), 4.0 mM chorismate 5,6-epoxide (□), or 2.0 mM L-tyrosine plus 1.0 mM NAD (△). Protein standards used were hemoglobin (Hb), alcohol dehydrogenase (ADH), and lactate dehydrogenase (LDH). Other details are described in Table II and under Experimental Procedures.

combination of two molecules of a dead-end inhibitor at the same site on each of two independent subunits as the data did not fit to an equation which describes a parabola. It seemed possible that the results (Figure 1) could be accounted for simply on the basis that the binding of an inhibitor molecule to one subunit influences the binding of a second inhibitor molecule to the other subunit. However, quantitative evidence to support this hypothesis was not obtained as the data did not fit well to equations that were the ratios of quadratic polynomials.

The values determined for Hill coefficients from data for the mutase and dehydrogenase reactions suggest that a minimum of three to four molecules of inhibitor undergo interaction with the enzyme. The idea that two molecules of inhibitor react at each of two subunits with interaction between the subunits is in accord with the conclusion that the binding of chorismate and prephenate occurs at distinct active sites (Christopherson et al., 1983). Further, there is the possibility that chorismate analogues can also act as tyrosine analogues. The combination of inhibitor at the active and allosteric sites on each of two subunits would allow for the marked degree of interaction as well as the binding of the required number of inhibitor molecules. In this connection, it must be mentioned that, as yet, there is no evidence for the presence on each subunit of a specific binding site for tyrosine. The possibility that nonlinear Dixon plots (Figure 1) arise as a consequence of enzyme polymerization in the presence of inhibitors can be eliminated. The results of density gradient centrifugation (Figure 5) and unpublished cross-linking experiments with dimethyl suberimidate show that neither tyrosine nor inhibitory analogues of chorismate cause enzyme polymerization. However, inhibitors may induce small changes in enzyme conformation (Table II) that could be responsible for the cooperative interaction of multiple inhibitor molecules.

The data obtained as a result of undertaking extensive kinetic investigations on the mutase and dehydrogenase reactions over wide concentration ranges of the nonnucleotide substrates confirm the allosteric nature of hydroxyphenylpyruvate synthase (Figure 2). The kinetic data could not be described by a 2:1 function (eq 2), and thus the kinetics of neither the mutase nor the dehydrogenase reactions can be explained in terms of the interactive combination of two molecules of chorismate or prephenate with catalysis being the rate-limiting step(s) in the reaction sequence. Such a result is not surprising

as there are few allosteric enzymes for which the kinetic data can be described in quantitative terms [cf. Barritt & Morrison (1972), Schramm & Morrison (1969), and Morrison (1969)]. It might be noted that, over a range of higher prephenate concentrations, the dehydrogenase conforms to a random mechanism for which the catalytic step is rate limiting (SampathKumar & Morrison, 1982b). The data of Figure 2 were not fitted to an equation which is the ratio of cubic polynomials and which would describe the interaction of two molecules of substrate with the enzyme under steady-state conditions (Morrison, 1969). Studies in this laboratory have shown that, for the fitting of data to such a function, the random error must be less than 2%.

The ability of adamantane-1-acetate to activate both the mutase and dehydrogenase reactions at low concentrations of the nonnucleotide substrate draws attention to the interrelationship between substrate and inhibitor binding (Figure 4). It can be envisaged that an initial combination of either substrate or inhibitor with one subunit of the free enzyme facilitates subsequent binding of substrate to the second subunit. Further increases in inhibitor concentration would lead to its competition with substrate for the substrate binding site of the second subunit. A similar activation phenomenon has been observed with inhibitors of aspartate transcarbamylase (Collins & Stark, 1971). It is also of particular interest that the cooperative interaction for substrate and inhibitor binding to the enzyme is observed with both the mutase and dehydrogenase reactions. Such findings lend additional support to earlier postulates that there is a close spatial relationship between the sites at which the two reactions occur.

The present results point once again to the similarity of the properties of the mutase and dehydrogenase sites on hydroxyphenylpyruvate synthase. Not only nonlinear kinetics are observed with each activity at low substrate concentrations but also the addition of albumin causes activation of both enzymic activities and a change in kinetic behavior to the Michaelis–Menten type. The effect of albumin is not specific as several enzymes, including lysozyme, creatine kinase, arginine kinase, urease, and aspartate aminotransferase, act in the same manner (SampathKumar, 1978). However, it remains to be demonstrated if these other proteins give a 1:1 complex with the synthase. These findings also raise the question of the intracellular control of tyrosine biosynthesis through specific protein interaction involving the enzyme. Certainly the aromatic amino acid aminotransferase of *Escherichia coli*, which catalyzes the conversion of hydroxyphenylpyruvate to tyrosine, undergoes interaction with the synthase (Powell & Morrison, 1979). It is yet to be established if such an interaction has kinetic effects and if other proteins from *E. coli* function as activators of hydroxyphenylpyruvate synthase.

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Registry No. NAD, 53-84-9; chorismic acid, 617-12-9; prephenic acid, 126-49-8; chorismate 5,6-epoxide, 61414-76-4; chorismate 5,6-diol, 84520-94-5; adamantane-1,3-diacetate, 17768-28-4; adamantane-1-acetate, 4942-47-6; adamantane-1-carboxylate, 828-51-3; adamantane-1-phosphonate, 23906-88-9; chorismate mutase, 9068-30-8; prephenate dehydrogenase, 9044-92-2; hydroxyphenylpyruvate synthase, 94859-19-5.

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