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Studies of the Reactions of Lamb Liver Serine Hydroxymethylase with L-Phenylalanine: Kinetic Isotope Effects upon Quinonoid Intermediate Formation†

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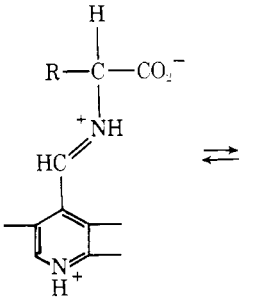
ABSTRACT: Serine hydroxymethylase (EC 2.1.2.1) reacts with L-phenylalanine in the absence of tetrahydrofolic acid to form an enzyme-bound amino acid–pyridoxal 5′-phosphate quinonoid intermediate (EQ_{503}) which is associated with the appearance of a new absorbance band at 503 nm. The enzyme also catalyzes the labilization of tritium from L-[α-^{3}H]phenylalanine. From the dependence upon the concentration of L-phenylalanine of the kinetic parameters for the approach to equilibrium and of the absorbance of the equilibrium concentration of EQ_{503}, the rate and/or equilibrium constants for the following minimal kinetic scheme were evaluated: E + S $\rightleftharpoons$ ES $\rightleftharpoons$ EQ_{503}, where ES is the enzyme-bound amino acid–pyridoxal 5′-phosphate Schiff base. The molar absorptivity of EQ_{503} at pH 8.00 is 4.1×10^4 M^{-1} cm^{-1} (per pyridoxal 5′-phosphate). Experiments with L-[α-^{1}H]- and L-[α-^{2}H]phenylalanine in H_2O and D_2O have established that there are kinetic deuterium isotope effects of 5–6-fold on the rate constant for the formation of EQ_{503} from ES and on the rate constant for the reverse reaction. These results are direct evidence that a hydrogen (proton) is transferred in the transition state of the ES $\rightleftharpoons$ EQ_{503} interconversion and, thus, for a general base catalytic contribution by the enzyme. The observed stereochemistry in which reactivity of serine hydroxymethylase is with L- and not D-phenylalanine is unexpected in terms of current formulations regarding pyridoxal 5′-phosphate mediated reactions.

Resonance stabilized quinonoid intermediates (II, eq 1) have been postulated to occur after the loss of the leaving group (α-hydrogen, CO_2, >C=O, etc.) from the Schiff bases formed from amino acids and pyridoxal 5′-phosphate (PLP)[1] during racemization, transamination, β elimination, decarboxylation, and dealdolization reactions catalyzed by PLP enzymes (Braunstein, 1947; Metzler et al., 1954; Morino and Snell, 1967; Jencks, 1969). In studies of several PLP model systems (Thanassi and Fruton, 1962; Matsumoto and Matsushima, 1972; Schirch and Slotter, 1966; Maley and Bruice, 1968) and enzymes (Morino and Snell, 1967; Jenkins, 1961; Schirch and Jenkins, 1964; Fonda and Johnson, 1970; Martinez-Carrion et al., 1970; Ulevitch and Kallen, 1977a) including serine hydroxymethylase (EC 2.1.2.1), an absorbance band at about 500 nm has been observed and assigned as a quinonoid intermediate.



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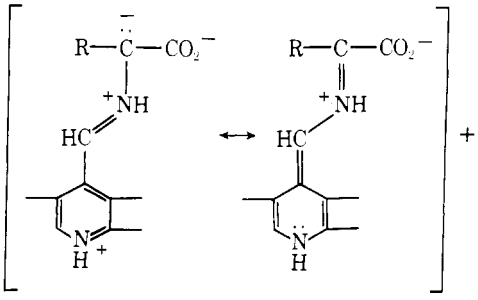

II (1)

During studies of the mechanism of the serine hydroxymethylase catalyzed cleavage of β-phenylserine to glycine and benzaldehyde (Ulevitch and Kallen, 1977a), the reaction of L-phenylalanine with the enzyme was investigated. A new

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[1] Abbreviations: buffer A, 0.05 M Hepes, 0.001 M EDTA, 0.025 M Na_2SO_4, pH 8.00; EDTA, ethylenediaminetetraacetic acid; E_t, total tetrameric enzyme (unless noted otherwise); Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; PLP, pyridoxal-5′-phosphate; THF, tetrahydrofolic acid.

absorbance peak appears at 503 nm when L-phenylalanine is mixed with serine hydroxymethylase and remains unchanged for at least 24 h. The present study provides direct evidence that the absorbance band at 503 nm results from the formation of a quinonoid intermediate (II'). This paper describes the experiments which led to the formulation of a minimal kinetic scheme that describes the formation of EQ_{503} from L-phenylalanine and serine hydroxymethylase (eq 2)

$$E \underset{k_{-1}}{\overset{k_1[S]}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EQ_{503} \qquad (2)$$
$$(I') \qquad (II')$$

$$K_1 = [E][S]/[ES] \qquad K_2 = [ES]/[EQ_{503}]$$

where ES and EQ_{503} are the enzyme bound amino acid in Schiff base linkage with PLP and the conjugate base thereof, respectively.

Experimental Section

Materials. Serine hydroxymethylase of specific activity 2.3 U/mg was purified and stored as described elsewhere (Ulevitch and Kallen, 1977b), D,L- and L-[α-^{3}H]phenylalanine (Calbiochem, A Grade), Hepes, D,L-[α-^{2}H]phenylalanine (gift of Dr. D. Porter), D_2O, 99.8 atom % deuterium (International Chemical and Nuclear Corp.), L-[2,3-^{3}H]phenylalanine (Amersham/Searle), D,L-α-methyl-*m*-methoxyphenylalanine (Nutritional Biochemicals Corp.) and other materials of reagent grade were utilized without further purification. Solutions were made with deionized water of greater than 5×10^5 ohms cm specific resistance and contained 10^{-3} M EDTA.

Methods. Kinetic measurements and spectra were recorded on a Gilford 2000 and a Cary 14 (0–0.1 absorbance slide wire) with cell compartments thermostated at 25 ± 0.1 °C. Pseudo-first-order rate constants were calculated from change in absorbance at 503 nm as described elsewhere (Kallen and Jencks, 1966; Kallen, 1971). Kinetic experiments were performed at pH or pD values of 8.00: 0.05 M Hepes, 0.001 M EDTA, ionic strength adjusted to 0.1 M with Na_2SO_4 (buffer A). The pD values of solutions in D_2O were determined according to the following equation (Salomaa et al., 1964):

$$pD = pH_{\text{meter reading}} + 0.4$$

The pH or pD values at the conclusion of each kinetic run were within ±0.03 unit of the expected value.

Tritium Exchange Experiments. Purified serine hydroxymethylase (Ulevitch and Kallen, 1977b) (110 μM active sites) was incubated with 0.075 M L-phenylalanine, pH 8.00, containing L-[^{3}H]phenylalanine (1.1×10^6 cpm), total volume of 0.5 mL. An identical solution without enzyme served as control. At 3, 17, 23.5, and 35 h after the addition of enzyme, a 0.1-mL aliquot was removed and frozen, and the solvent was collected by sublimation under reduced pressure. Samples of solvent (40 μL) were counted in 10 mL of counting fluid (3 L of dioxane, 3 g of naphthalene, 12 g of 2,5-diphenyloxazole) with an Intertechnique scintillation counter. The estimated error limits for equilibrium and kinetic constants in this study are ±10%.

Results and Discussion

Spectral Characteristics of the Reaction of L-Phenylalanine with Serine Hydroxymethylase. After mixing serine hydroxymethylase with L-phenylalanine, visible absorbance changes (~425 nm) are observed within 1 min (Figure 1) and are too rapid for the time course to be observed by conventional spectrophotometric techniques. The relatively slower appearance of a new absorbance band at about 500 nm[2] (Figure 1) can, however, be followed by ordinary spectrophotometric methods and once formed this spectrum is unchanged for >24 h. There are no spectral changes in the region of 500 nm observed when either 0.1 M D-phenylalanine or 0.1 M D, L-α-methyl-*m*-methoxyphenylalanine is mixed with the enzyme. These results are consistent with eq 2 in which the rapid formation of the enzyme-bound L-phenylalanine–PLP Schiff base (I'), characterized by spectral changes in the region of 430 nm (Martell, 1963), is followed by the slow appearance of the enzyme-bound quinonoid intermediate characterized by the absorbance band at 503 nm (Figure 1).

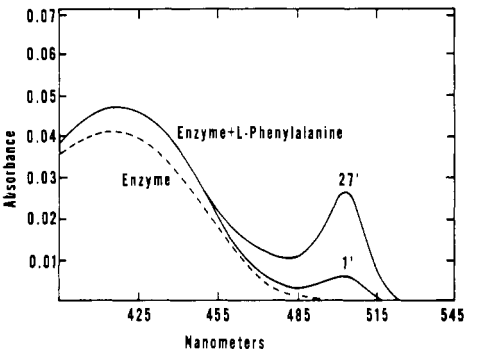

FIGURE 1: Visible absorbance spectrum of serine hydroxymethylase before (- - -) and after (—) mixing with L-phenylalanine in split compartment cuvette (pathlength = 0.88 cm) at 25 °C at pH 7.50, 0.05 M Hepes, 0.001 M EDTA, 0.025 M Na_2SO_4. After mixing: 0.05 M L-phenylalanine and 0.35 mg/mL serine hydroxymethylase (2.3 U/mg).

[^{3}H]Phenylalanine Exchange Reaction Catalyzed by Serine Hydroxymethylase. Serine hydroxymethylase catalyzes the release of counts from [^{3}H]phenylalanine at a rate of 70 cpm per hr per 40-μL aliquot: the rate of release of counts is negligible in the absence of enzyme. The results of the exchange experiments are evidence that serine hydroxymethylase is capable of hydrogen labilization from L-phenylalanine and that the base on serine hydroxymethylase involved in proton activation exchanges with solvent. Furthermore, the spectral and tritium exchange data together indicate that, while the system comes to chemical equilibrium within about an hour, isotopic equilibrium is not reached until a much greater time has elapsed. We have chosen to refer to the circumstance of the attainment of a chemical equilibrium *without* isotopic equilibrium as an equilibrium state.

Kinetics of the Reaction of L-[α-^{1}H]Phenylalanine with Serine Hydroxymethylase. There is a rectangular hyperbolic dependence of the initial rate of EQ_{503} formation (v_i) upon the concentration of L-phenylalanine, [S] (eq 3), where [E_t] is the enzyme subunit concentration and K_1 and k_2 are defined by eq 2. The values of $k_2 = 0.049$ min^{-1} and $K_1 = 0.046$ M are calculated from the ordinate and abscissa intercepts, respectively, of a double-reciprocal presentation of the data (Figure 2A) and eq 3 (Table I), where $v_i = \Delta A_{503}/\Delta t \epsilon_{503}^{EQ}$, $\Delta A_{503}/\Delta t$ is the initial slope of an A_{503} vs. time plot, and ϵ_{503}^{EQ} is the molar absorptivity (per site) of EQ_{503} at 503 nm.

$$v_i/[E_t] = k_2/(K_1/[S] + 1) \qquad (3)$$

The rate constant k_{-2} may be evaluated by a "chase" experiment with the addition of L-serine (0.01 M final concentration) to a reaction mixture of serine hydroxymethylase and L-phenylalanine at equilibrium with respect to the formation of EQ_{503}. The value of the apparent inhibition constant, K_i, is 0.0007 M for competitive inhibition by L-serine of the serine hydroxymethylase catalyzed cleavage of D,L-*erythro*-β-phenylserine (Ulevitch and Kallen, 1977b). The L-serine at

[2] There are associated circular dichroism changes at 503 nm as well.

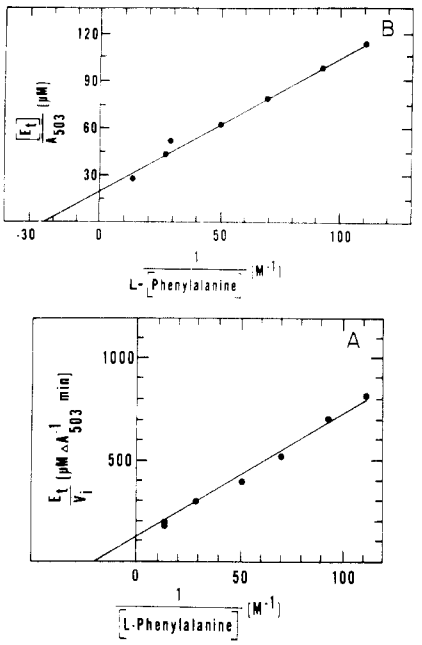

FIGURE 2: (A) Plot of $[E_t]/v_i$ vs. 1/[L-phenylalanine], pH 8.00, 0.05 M Hepes, 0.001 M EDTA, ionic strength = 0.1 M with Na_2SO_4, 25 °C, 503 nm, $[E_t]$ = 272 μM (per PLP). The solid line is calculated from eq 3 and the constants contained in Table I. (B) Plot of $[E_t]/A_{503}$ vs. 1/[L-phenylalanine], pH 8.00, 0.05 M Hepes, 0.001 M EDTA, ionic strength = 0.1 M with Na_2SO_4, 25 °C, 503 nm. $[E_t]$ = 4.4 μM (per site). The solid line is calculated from eq 6 and the constants contained in Table I.

TABLE I: Rate and Dissociation Constants for the Reaction of L-[α-^{1}H]- and L-[α-^{2}H]Phenylalanine with Serine Hydroxymethylase in H_2O and D_2O[a] at pH or pD 8.00, 25 °C, Ionic Strength, 0.1 M.

Constant	H_2O	D_2O
K_1 (M)	0.046, 0.055	0.050
K_2^H	3.5, 3.2	0.38
K_2^D	22.0	2.1
k_2^H (min^{-1})	0.043, 0.049	0.076
k_2^D (min^{-1})	0.007	0.014
k_{-2}^H (min^{-1})	0.154, 0.15	0.029
k_{-2}^D (min^{-1})	0.154[b]	0.029[b]
k_{obsd}^H (min^{-1})	0.182[c]	0.064[d]
k_{obsd}^D (min^{-1})	0.157[c]	0.033[d]
ϵ_{503} (M^{-1} cm^{-1})[e]	4.1×10^4	4.1×10^4

[a] For definition of constants see eq 2. [b] Assumed to be same as k_{-2}^H (see text). [c] 0.041 M L-[α-^{1}H]phenylalanine and L-[α-^{2}H]phenylalanine. [d] 0.043 M L-[α-^{1}H]phenylalanine and L-[α-^{2}H]phenylalanine. [e] Per PLP site.

a concentration of 0.01 M reacts "instantaneously" to bind free enzyme and additional free enzyme molecules as they are regenerated by the breakdown of EQ_{503} (the reversal of eq 2). The enzyme-serine complexes do not absorb at 503 nm and hence the rate of disappearance of EQ_{503} is a first-order decay, k_{-2} = 0.154 min^{-1} (not shown). Thus, the value of $K_2 = k_{-2}/k_2$ = 0.154/0.049 = 3.15.

There is an exponential increase in absorbance at 503 nm (EQ_{503}) upon mixing L-phenylalanine and serine hydroxymethylase (not shown), the time constant for which is independent of both the enzyme concentration or the presence of D-phenylalanine. The equation for the dependence of the rate constant for the appearance of EQ_{503}, k_{obsd}, upon the L-phenylalanine ([S]) concentration (based upon eq 2 and a rapid equilibrium assumption for the formation of I′ from E and S)

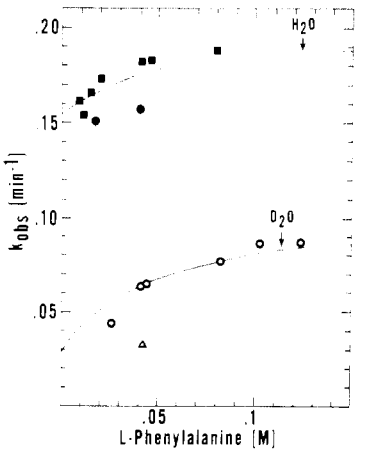

FIGURE 3: Dependence of the pseudo-first-order rate constant (k_{obsd}) for EQ_{503} formation upon L-phenylalanine concentration at pH 8.00 (buffer A), 25 °C (filled symbols and unfilled symbols, respectively), in H_2O and D_2O ionic strength 0.1 M. (■, ○) L-[α-^{1}H] Phenylalanine; (●, △) L-[α-^{2}H]phenylalanine. The solid lines are calculated from eq 4 and the constants contained in Table I. Serine hydroxymethylase: 10-20 μM active site concentration.

describes a rectangular hyperbola with a finite ordinate intercept, k_{-2} = 0.15 min^{-1} (eq 4), and is in accord with the observed data (Figure 3), although the hyperbola is quite shallow for the data obtained in light water. The solid line in Figure 3 is calculated from eq 4 (Ulevitch, 1971) and the constants K_1, k_{-2}, and k_2 in Table I determined in the initial velocity and "chase" experiments previously described. The value of K_1 is not well determined from this type of experiment in this circumstance.

$$k_{obsd} = k_2\{[S]/([S] + K_1)\} + k_{-2} \quad (4)$$

At saturating substrate concentrations, $[S] \gg K_1$, the k_{obsd} values become asymptotic to the line determined by the maximum k_{obsd} value (eq 5):

$$\lim_{[S]\to\infty} k_{obsd_{max}} = k_2 + k_{-2} \quad (5)$$

From eq 5 and the values of $k_{obsd_{max}}$ and k_{-2}, k_2 = 0.043 min^{-1} and $K_2 = k_{-2}/k_2$ = 0.15/0.043 = 3.5.

Although the pseudo-first-order rate constants for the appearance of EQ_{503} demonstrate only a small dependence on L-phenylalanine concentration in water (Figure 3), a marked substrate concentration dependence of the equilibrium absorbance of EQ_{503} is observed (Figure 2B) and is described by eq 6 for the mechanism of eq 2 (Ulevitch, 1971):

$$\frac{1}{A_{503}} = \frac{K_1K_2}{[E_t]\epsilon_{503}^{EQ}}\frac{1}{[S]} + \frac{K_2 + 1}{\epsilon_{503}^{EQ}[E_t]} \quad (6)$$

where A_{503} and ϵ_{503}^{EQ} are the absorbance at 503 nm and the molar absorptivity (per PLP) for EQ_{503} at 503 nm, respectively, and $[E_t]$ is the enzyme subunit concentration (cf. Jenkins, 1970). The solid line in Figure 2B is calculated from eq 6 employing an estimate of the molar absorptivity of the EQ_{503} species and the kinetic constants contained in Table I. The agreement between the observed data and the calculated line provides a further corroboration of these values of the constants K_1 and K_2. The value of the molar absorptivity of EQ_{503} of 4.1×10^4 M^{-1} cm^{-1} (per site) at pH 8.00 may be compared with the apparent molar absorptivity at 492 nm for the sum of all binary complexes including the quinonoid in-

termediate formed from *erythro*-β-hydroxy-D,L-aspartate and aspartate aminotransferase of 2.2×10^4 M^{-1} cm^{-1} (per site) at pH 8.25 (Fonda and Johnson, 1970; Jenkins, 1964) and the value of about 4×10^4 M^{-1} cm^{-1} at pH 7.5 for the quinonoid intermediate formed from glycine and serine hydroxymethylase (Ulevitch and Kallen, 1977a). In the former case, as frequently obtains, the distribution function of the enzyme among binary complexes is unknown, and the molar absorptivity values are inevitably apparent values.

Kinetics of the Reaction of D,L-[α-^{2}H]Phenylalanine with Serine Hydroxymethylase. The exponential increase in absorbance at 503 nm observed when D,L-[α-^{2}H]phenylalanine is mixed with serine hydroxymethylase (Figure 4) is characterized by a pseudo-first-order rate constant that is approximately 15% less than that observed for [α-^{1}H]phenylalanine at the same amino acid concentration, 0.04 M (Figure 3). In contrast to this small effect of substitution of deuterium for hydrogen at C_α of phenylalanine on the rate constant for the appearance of EQ_{503} both the initial rate of formation and the equilibrium absorbance of EQ_{503} are decreased approximately sixfold by the presence of deuterium at C_α (Figure 4). These experimental observations are consistent with a kinetic isotope effect, produced by the presence of deuterium at $C_{\alpha'}$ almost entirely on k_2. The initial rates of EQ_{503} formation clearly reflect the kinetic deuterium isotope effect upon k_2 (dashed lines, Figure 4). From the rectangular hyperbolic dependence of the initial rates of EQ_{503} formation upon the concentration of L-[^{2}H]phenylalanine (not shown), the values of K_1 and k_2 are 0.055 M and 0.007 min^{-1}, respectively (eq 3). This gives a direct estimate of the kinetic deuterium isotope effect upon k_2 of about 6 (Table I). The same conclusion regarding the magnitude of the deuterium isotope effect upon k_2 can be arrived at somewhat less directly from the equilibrium absorbance measurements in the following manner. The labilization of counts to solvent during equilibration of serine hydroxymethylase with [^{3}H]phenylalanine indicates that a hydrogen (proton) of phenylalanine is activated by the enzyme and further that the basic group involved in hydrogen (proton) removal can exchange with solvent. This solvent exchange is evidence that the reprotonation of EQ_{503} to form ES involves a solvent derived protium regardless of whether the L-phenylalanine initially contained protium or deuterium or tritium at the C_α position, (i.e., $k_{-2}^H = k_{-2}^D$). With this equality of k_{-2} values and from the relationships expressed in eq 7

$$\frac{K_2^D}{K_2^H} = \frac{k_{-2}^D/k_2^D}{k_{-2}^H/k_2^H} = \frac{k_2^H}{k_2^D} \simeq 6 \qquad (7)$$

for protio and deuterio substrates, a kinetic deuterium isotope effect of about 6 is apparent for k_2 based upon the K_2^D value of 22.0. With the value of K_2^D (see above), the values of K_1 and the molar absorptivity of EQ_{503} are calculated to be 0.055 M and 4.1×10^4 M^{-1} cm^{-1} (per site) at pH 8.00 from the abscissa and ordinate intercepts (eq 6), respectively, of plots of $1/A_{503}$ against $1/[S]$ (not shown). The agreement of rate and dissociation constants determined by the several methods for the protio and deuterio substrates and the satisfactory accounting for the experimental observations as indicated by the calculated curves in Figures 2–4 are strong evidence in support of eq 2 and provide direct evidence that hydrogen is transferred in the transition state during the formation of EQ_{503} (Jencks, 1969) for the L-phenylalanine reaction. Furthermore, since k_{-2} is the dominant term in eq 4 (Table I), the sixfold isotope effect yields only a 15% decrease in k_{obsd}, Table I, as seen in Figure 3.

The values of the molar absorptivity of EQ_{503} and K_1 are reasonably insensitive to the presence of deuterium at the C_α position (Table I).

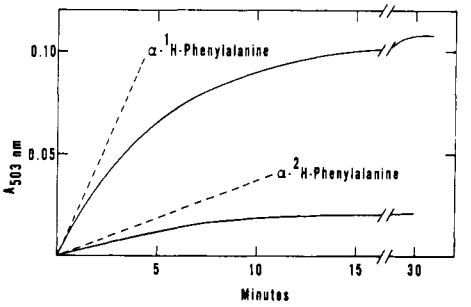

FIGURE 4: The effect of substitution of deuterium for hydrogen at the α carbon of L-phenylalanine on the time course of EQ_{503} formation at pH 8.00, 25 °C, ionic strength 0.1 M. Upper curve: L-[α-^{1}H]phenylalanine, final absorbance at 503 nm, 0.12. Lower curve: L-[α-^{2}H] phenylalanine, final absorbance at 503 nm, 0.02. Substrate concentration, 0.041 M, serine hydroxymethylase, 6.8 μM. The dashed lines are calculated from eq 3 and constants contained in Table I.

Kinetics of EQ_{503} Formation from [α-^{1}H]- and [α-^{2}H]-Phenylalanine in D_2O. From the types of measurements previously described (eq 3, 4, and 6) for EQ_{503} appearance, values for K_1, k_2, and k_{-2} in D_2O have been evaluated for the reactions of L-[α-^{1}H]- and L-[α-^{2}H]phenylalanine with serine hydroxymethylase (pD = 8.00).

The value for k_{-2} determined from a "chase" experiment with L-serine indicates that there is a 5.3-fold solvent kinetic deuterium isotope effect upon k_{-2} and that k_{-2} in D_2O is insensitive to the initial presence of deuterium at C_α since $k_{-2}^H = k_{-2}^D = 0.029$ for L-[α-^{1}H]- and L-[α-^{2}H]phenylalanine, respectively, in D_2O. These measurements of k_{-2} indicate that the base which removes the light or heavy hydrogen (proton) from C_α rapidly exchanges with solvent and are consistent with the aforementioned tritium exchange data.

The rate constant, k_{obsd}, at a given concentration of L-phenylalanine shows a greater sensitivity to the presence of deuterium at C_α in D_2O than was observed in H_2O (Figure 3) largely as a consequence of the diminished contribution of k_{-2} to k_{obsd} in eq 4. For example, at a L-phenylalanine concentration of 0.043 M, the value of k_{obsd} for the exponential appearance of EQ_{503} for the [α-^{2}H] substrate is about one-half the k_{obsd} value for [α-^{2}H]phenylalanine in D_2O. These data may be compared with the 15% decrease in k_{obsd} values for [α-^{2}H]- as opposed to [α-^{1}H]phenylalanine in H_2O. In addition, equilibrium absorbance at 503 nm decreases by 5.4-fold when deuterium is substituted for protium at C_α in D_2O. From eq 4 and the substrate dependence of the rate of appearance of EQ_{503} (k_{obsd}), the values for constants K_1 and k_2 are 0.05 M and 0.076 min^{-1} in D_2O (Table I). The calculated ratio of absorbance at 503 nm in D_2O with respect to that in H_2O based on the constants of Table I at 0.043 M L-phenylalanine is about 3 and may be compared with a measured value of 2.5. The dissociation and rate constants and the molar absorptivity values utilized in calculating the curves which adequately describe all of the experimental observation in terms of eq 2 are summarized in Table I.

The Mechanism of the Reaction of L-Phenylalanine with Hydroxymethylase. Evidence from tritium exchange experiments, kinetic studies of the rate of formation of EQ_{503}, absorbance measurements of equilibrium concentration of EQ_{503}, and the initial rate of EQ_{503} appearance provide support for the mechanism of eq 2.

The measurement of about a 5–6-fold kinetic deuterium isotope effect upon k_2 is direct evidence that hydrogen is

transferred in the transition state during the formation of EQ_{503}. Although the rate constant for proton removal is slow, 0.043 min^{-1} at pH 8.00, evidence discussed elsewhere (Kallen and Blanck, 1973; Schirch and Jenkins, 1964; Chen and Schirch, 1973) indicates that the rate of proton removal from amino acid substrates catalyzed by serine hydroxymethylase may be quite slow especially when tetrahydrofolic acid (THF) derivatives are not present. The rate of proton removal in the absence of THF from glycine at pH 7.5 is about 14 min^{-1} (Ulevitch and Kallen, 1977a) and from D-alanine at pH 8.7 is estimated to be approximately 1–2 min^{-1} (Schirch and Jenkins, 1964).

Although the present experiments were conducted at only a single pH (pD) value (cf. Jencks, 1969), it is of interest to compare the values of k_2, the rate constant for proton removal, in H_2O and D_2O (Table I). Although the molar absorptivity of EQ_{503} and the K_1 values are essentially unchanged in D_2O compared with H_2O, the small change in k_2 values may reflect small alterations in the protein conformation and/or small shifts in the pK of the general base on serine hydroxymethylase which is involved in the proton removal in D_2O (Bunton and Shiner, 1961; Bell and Kuhn, 1963; Jencks, 1969).

One important question concerning the reaction of L-phenylalanine with serine hydroxymethylase presently remains unanswered. According to Jordon and Akhtar (1970) and Besmer and Arigoni (1968), the stereochemistry of proton removal from glycine catalyzed by serine hydroxymethylase in the presence of THF is *pro-S*. The report of Schirch and Jenkins (1964) of the formation of a quinonoid intermediate when D-alanine is added to serine hydroxymethylase is consistent with the removal of the *pro-S* hydrogen of glycine since the α proton of the D-amino acid is in a stereochemically equivalent position to the *pro-S* hydrogen of glycine. Further, the report of Schirch is consistent with the proposals of Dunathan (1966, 1971) regarding the stereochemical control of PLP enzymes: a PLP-dependent aldolase, serine hydroxymethylase, utilizing L-(R) isomers as substrates might be able to remove the α hydrogen of the appropriate D-(S)-amino acid isomer.

The results obtained with L-phenylalanine are not expected based upon both the proposals of Dunathan (1966, 1971) and studies of the stereochemistry of proton removal from glycine (Jordan and Akhtar, 1970; Besmer and Arigoni, 1968; Ulevitch and Kallen, 1977b). With L-phenylalanine there is exchange of the α proton that is stereochemically equivalent to the nonexchanging *pro-R* hydrogen of glycine. Hence, despite the slower cleavage of the α-C–H bond of L-phenylalanine than the α-C–H bonds of glycine and D-alanine, in view of the unexpected stereochemistry of the L-phenylalanine reaction, it seems that some modification is required of the current views regarding the stereochemical control of PLP enzymes.

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