PH dependence of the absorbance and31P NMR spectra of O-acetylserine sulfhydrylase in the absence and presence of O-acetyl-L-serine

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pH Dependence of the Absorbance and ^{31}P NMR Spectra of O-Acetylserine Sulfhydrylase in the Absence and Presence of O-Acetyl-L-serine[†]

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ABSTRACT: O-Acetylserine sulfhydrylase (OASS) is a pyridoxal 5′-phosphate (PLP)-dependent enzyme which catalyzes the final step in the biosynthesis of L-cysteine in *Salmonella*, viz., the conversion of O-acetyl-L-serine (OAS) and sulfide to L-cysteine and acetate. UV–visible spectra of OASS exhibit absorbance maxima at 280 and 412 nm with pH-independent extinction coefficients over the range 5.5–10.8. Addition of OAS to enzyme results in a shift in the absorbance maximum from 412 to 470 nm, indicating the formation of an α-aminoacrylate Schiff base intermediate [Cook, P. F., & Wedding, R. T. (1976) *J. Biol. Chem. 251*, 2023]. The spectrum of the intermediate is also pH independent from 5.5 to 9.2. The observed changes in absorbance at 470 nm at different concentrations of OAS were used to calculate a K_d of 3 μM for OAS at pH 6.9. As the pH decreases, the K_d increases an order of magnitude per pH unit. The ^{31}P NMR signal of the bound PLP has a pH-independent chemical shift of 5.2 ppm in the presence and absence of OAS. These results indicate that the phosphate group is present as the dianion possibly salt-bridged to positively charged groups of the protein. In agreement with this, the resonance at 5.2 ppm has a line width of 20.5 Hz, suggesting that the cofactor is tightly bound to the protein. The sulfhydrylase was also shown to catalyze an OAS deacetylase activity in which OAS is degraded to pyruvate, ammonia, and acetate. The activity was detected by a time-dependent disappearance of the 470-nm absorbance reflecting the α-aminoacrylate intermediate. The rate of disappearance of the intermediate was measured at pH values from 7 to 9.5 using equal concentrations of OAS and OASS. The rate constant for disappearance of the intermediate decreases below a pK of 8.1 ± 0.1, reflecting the deprotonation of the active-site lysine that originally formed the Schiff base with PLP in free enzyme. A possible mechanism for the deacetylase activity is presented where the lysine displaces α-aminoacrylate which decomposes to pyruvate and ammonia.

Cysteine biosynthesis in the enteric bacterium *Salmonella typhimurium* is catalyzed by two enzymes. The first, serine transacetylase, catalyzes the formation of O-acetyl-L-serine (OAS)[1] from acetyl-CoA and L-serine, while the second, O-acetylserine sulfhydrylase, catalyzes the formation of L-cysteine from sulfide and OAS. The latter enzyme is dimeric with a subunit molecular weight of 34 450 (Levy & Danchin, 1988) and has 1 mol of PLP tightly bound per subunit (Becker et al., 1969).

Cook and Wedding (1976) have shown that the enzyme has a ping-pong mechanism. This mechanism requires that acetate

[1] Abbreviations: OAS, O-acetyl-L-serine; OASS, O-acetylserine sulfhydrylase; PLP, pyridoxal 5′-phosphate; F, α-aminoacrylate intermediate; Tris, tris(hydroxymethyl)aminomethane; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; Mes, 2-(N-morpholino)ethanesulfonic acid; Hepes, N-(2-hydroxyethyl)piperazine-N′-2-ethanesulfonic acid; Taps, 3-[[tris(hydroxymethyl)methyl]amino]-propanesulfonic acid; Ches, 2-(N-cyclohexylamino)ethanesulfonic acid.

is β-eliminated from OAS in the first half-reaction to generate α-aminoacrylate in Schiff base with the active-site PLP. The production of α-aminoacrylate is accompanied by increases in absorbance at 470 and 320 nm and a concomitant decrease in the absorbance at 420 nm (Becker et al., 1969). The second half-reaction then consists of nucleophilic attack by sulfide on α-aminoacrylate to produce L-cysteine. There are two dead-end complexes allowed, E·sulfide and F·OAS (where F is α-aminoacrylate in Schiff base with the active-site PLP), stemming from the observation of double-competitive substrate inhibition by both OAS and sulfide (Cook & Wedding, 1976).

The stereochemistry at C-3 of cystine for its formation has been determined by Floss et al. (1976). These authors prepared (2S,3R)- and (2S,3S)-[³H]-L-serine, converted the serines to O-acetyl-L-serine, and used the labeled substrates to prepare L-cysteine via the O-acetylserine sulfhydrylase reaction. The cysteine produced retained configuration in both cases consistent with a ping-pong kinetic mechanism in which sulfide adds to the same face of the α-aminoacrylate intermediate as that from which the acetyl group leaves.

Although the kinetic mechanism is reasonably well-defined, little is known concerning the protonation state of cofactor, substrate, and enzyme functional groups in the active site. In this study, the pH dependence of the UV–visible and ³¹P NMR spectra of free enzyme and the α-aminoacrylate intermediate and the dissociation constant for OAS were obtained. These data and others were used to deduce the protonation state of specific active-site groups. In addition, an OASS deacetylase side reaction catalyzed by the sulfhydrylase was identified and characterized.

MATERIALS AND METHODS

Chemicals and Enzymes. O-Acetyl-L-serine, pyruvate, NADH, lactate and alanine dehydrogenases, and all buffers were purchased from Sigma. O-Acetylserine sulfhydrylase was purified according to Hara et al. (1990). The resultant enzyme was pure by the criterion of SDS–PAGE and had a final specific activity of 800 units/mg using a computer-assisted sulfide ion selective electrode assay (Hara et al., 1990). A unit is defined as the amount of enzyme required to convert 1 μmol of sulfide to cysteine in 1 min at pH 7.6 and 25 °C.

UV–Vis Spectral Studies. Absorbance spectra were measured on a Cary 15 dual-beam recording spectrophotometer set at 0.5 full-scale recording at 5 nm/min and 10 nm/cm. In all cases, the blank consisted of all components minus enzyme. Additions made to the experimental cuvette were also made to the blank. Spectra were also obtained using a Hewlett-Packard Model 8452A photodiode array spectrophotometer, recording over the same wavelength range. Spectra were obtained with 1 mg/mL OASS as a function of pH with the following buffers at 100 mM final concentration: Mes, 5.5–6.5; Hepes, 6.5–8.0; Taps, 8.5–9.0; K₂CO₃, 10.0–11.0. To determine the pH dependence of the spectrum of the α-aminoacrylate intermediate, the above experiments were repeated in the presence of 10 mM OAS.

Calculation of the K_d for O-Acetyl-L-serine. Absorbance spectra were recorded in the absence of added OAS and in the presence of increasing amounts of OAS (added to both sample and blank). The increase in absorbance at 470 nm allows calculation of the concentration of the intermediate using a calculated extinction coefficient of 9760 M⁻¹ cm⁻¹. The amounts of free OAS and enzyme were then calculated, allowing calculation of the K_d according to [OAS][OASS]/[intermediate]. These calculations were carried out for each of the OAS concentrations used, and the final K_d was an average of all of the calculated values. Titrations were carried

out as a function of pH. As the pH approached 7, absorbance readings at 470 nm decreased with time. Thus, readings were restricted to 470 nm only at pH values of 6.5 and above. Absorbance readings were generally stable for 30 s after addition of OAS. Subsequent additions of OAS were not made until the absorbance decreased to zero at pH values greater than 7, or separate cuvettes containing the same amount of OASS were used for each OAS concentration (both gave identical results). Data were also collected with β-chloro-L-alanine above pH 7; β-chloro-L-alanine is a substrate for OASS (Cook & Wedding, 1977).

³¹P NMR Spectra. All ³¹P NMR spectra were recorded with a Bruker WH-180 wide-bore spectrometer at 72.86 MHz. Spectra were collected making use of 20-mm quartz NMR tubes, and samples were titrated to the new pH value using 0.1 N KOH or by dialysis against buffer containing 2 mM EDTA titrated to the appropriate pH. Each sample tube contained a concentric 5-mm NMR tube with D₂O which served as the field/frequency lock. The pH and OASS activity were determined before and after all measurements with no change observed. All spectra were collected with a 3-s acquisition time, a 90° pulse, and broad-band proton decoupling (2.5–3.5 W) against a reference of 85% H₃PO₄. In all cases, about 20 000–30 000 scans were accumulated. An exponential line broadening of 10 Hz was applied prior to Fourier transformation. Positive chemical shifts are downfield from the reference 85% H₃PO₄.

Data Processing. Data for the pH dependence of the first-order disappearance of the 470-nm intermediate were fitted using eq 1 and a FORTRAN program developed by Cleland

$$\log y = \log [C/(1 + H/K_1)] \tag{1}$$

(1979). In eq 1, y is the value of the rate constant at any pH, C is the pH-independent value of the rate constant, H is the hydrogen ion concentration, and K_1 is the acid dissociation for a group on enzyme.

RESULTS

pH Dependence of the Ultraviolet–Visible Spectrum of O-Acetylserine Sulfhydrylase. The absorbance spectrum of native enzyme was obtained over the wavelength range 260–480 nm. This was carried out as a function of pH from 5.5 to 10.85. Examples of spectra at several pH values are shown in Figure 1. Additon of OAS to enzyme results in changes in the absorbance spectrum as shown in Figure 2 (top). A difference spectrum calculated from these data is shown in Figure 2 (bottom).

Examples of spectra measured in the presence of OAS at several pH values are shown in Figure 3. The spectrum observed for the intermediate is pH independent from 5.5 to 9 over the wavelength range 300–540 nm. At low pH, however (e.g., pH 5.5), the absorbance in the UV region (240–290 nm) is increased compared to that obtained at pH 7 and above.

Dissociation Constant for O-Acetyl-L-serine. Using the observed changes in absorbance, the enzyme can be titrated with OAS (Becker et al., 1969). The observed spectral changes represent the equilibrium [E][OAS]/[F][acetate] where E is free enzyme and F is the species absorbing at 470 nm. However, since the dissociation constant for acetate is high (Cook & Wedding, 1976), the expression reduces to [E]-[OAS]/[F]. The dissociation constant for OAS was calculated as discussed under Materials and Methods using data similar to those depicted in Figure 2, with the exception that the OAS concentration was varied. The K_d was determined over the pH range 5.5–7. Above pH 7, the degradation of OAS and the 470-nm intermediate precludes accurate determination of
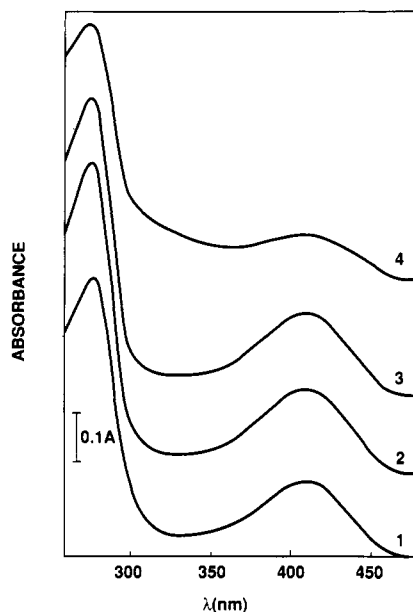
FIGURE 1: pH dependence of the UV–visible spectrum of *O*-acetylserine sulfhydrylase. Absorbance spectra were measured on a Cary 15 dual-beam recording spectrophotometer as described under Materials and Methods. Spectra were obtained at the pH values specified below. Spectrum 1, pH 10.85; 2, 8.34; 3, pH 6.54; 4, pH 5.50 (at pH 5.50, OASS concentration is 0.5 mg/mL while at all other pH values OASS concentration is 1 mg/mL).
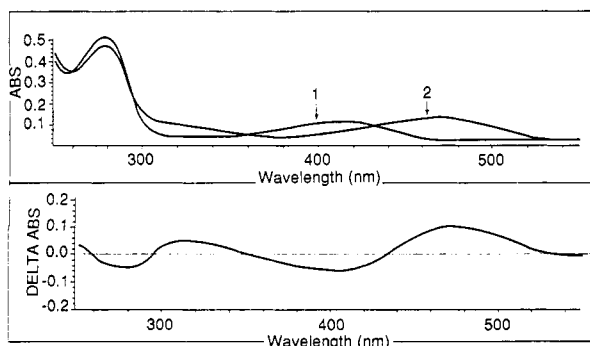

FIGURE 2: Spectra of OASS obtained in the presence and absence of OAS at pH 7 in 100 mM Hepes. Spectra were obtained using a Hewlett-Packard Model 8452A photodiode array spectrophotometer. Top: spectrum 1 was recorded using 0.47 mg/mL OASS, and spectrum 2 was obtained after the addition of 1 mM OAS. Bottom: difference spectrum obtained by subtracting spectrum 1 from spectrum 2. The difference spectrum shows an increase in absorbance at 470 and 320 nm and a decrease at 412 and 280 nm after adding OAS to the native enzyme.

the K_d. The K_d for OAS is 3 μM at pH 6.9 and increases an order of magnitude per pH unit as the pH decreases.

pH Titration of the Enzyme-Bound 5'-Phosphate of PLP via ^{31}P NMR. The ^{31}P NMR spectrum of free OASS at pH 7.75 is shown in Figure 4. The calculated chemical shift is pH independent from 6.5 to 8.4 with an average value of 5.24 ± 0.06. Similar titrations were carried out in the presence of 10 mM OAS. The calculated chemical shift is again pH independent from 6.5 to 7.55 with an average value of 5.2 ± 0.03. A line width of 20.5 Hz was calculated once corrected for the exponential line broadening of 10 Hz.

OAS Deacetylase Activity. While the above UV–visible spectra were being collected, a time-dependent decrease in the absorbance at 470 nm (α-aminoacrylate intermediate) was observed (data not shown). A plot of the log of the absorbance vs time indicated that the process was first order in intermediate (data not shown). The rate of disappearance increases
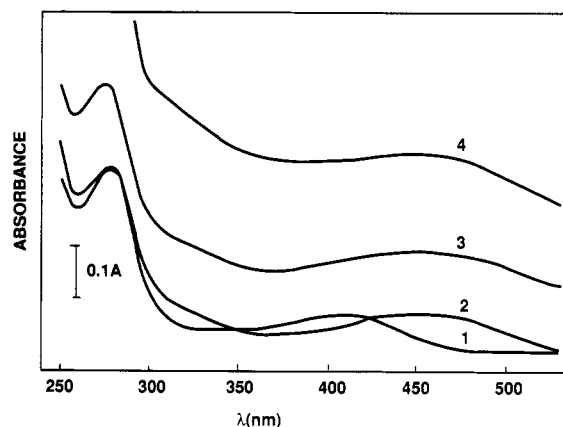

FIGURE 3: pH dependence of the UV–visible spectrum of OASS in the presence of OAS. Spectra were obtained as described in Figure 1 except that these spectra (2–4) were recorded in the presence of 10 mM OAS. Spectra were obtained at different pH values as specified below. Spectrum 1, native enzyme (no OAS), pH 9.02; 2, pH 9.02; 3, pH 7.76; 4, pH 6.06.
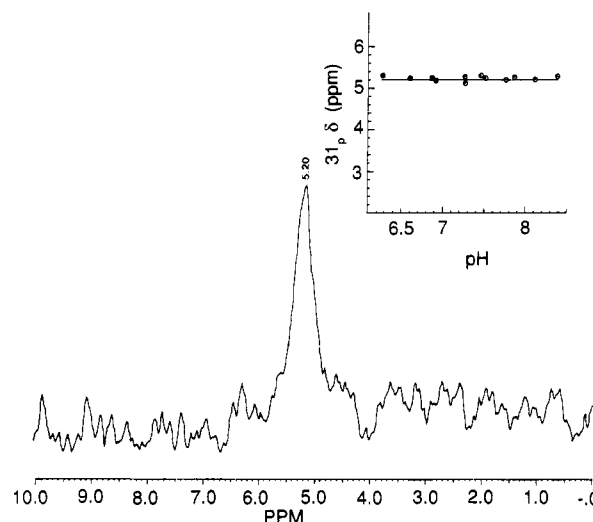

FIGURE 4: pH dependence of the ^{31}P chemical shift of PLP bound to OASS. Spectra were measured as described under Materials and Methods. The chemical shift is pH independent from 6.5 to 8.4 with an average value of 5.24 ± 0.06. In the presence of 10 mM OAS, the chemical shift is pH independent from 6.5 to 7.55 with an average value of 5.20 ± 0.03.

as the pH is increased. To characterize this reaction, experiments were carried out with the concentration of OAS (0.085 mM) equal to the concentration of OASS (0.085 mM) at pH values from 7 to 9.5 using the buffers cited under Materials and Methods. The pH dependence of the first-order rate constant is shown in Figure 5. The rate constant decreases below a pK of 8.1 ± 0.1 and has a pH-independent value of 0.10 ± 0.01 s^{-1}.

The products of the reaction of OAS with enzyme were determined enzymatically using alanine dehydrogenase. The enzyme alanine dehydrogenase catalyzes the oxidative deamination of either alanine or serine to pyruvate or hydroxy-pyruvate and ammonia (Grimshaw et al., 1981). No measurable production of serine or alanine was detected as measured by the alanine dehydrogenase reaction at pH 9.5 in the presence of saturating NAD$^+$ (5 mM). Ammonia was produced, however, as demonstrated by the reductive amination of pyruvate catalyzed by alanine dehydrogenase. Ammonia reactions were carried out at pH 9 in 100 mM Taps with 0.2 mM NADH, 10 mM pyruvate, and 20 units of alanine dehydrogenase with OAS and OASS concentrations maintained
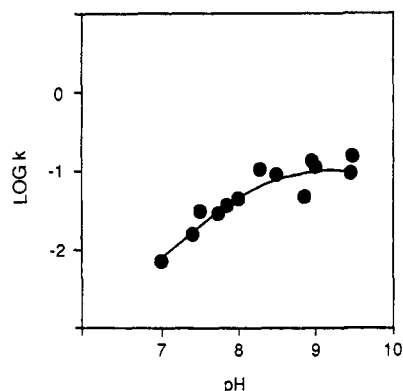
FIGURE 5: pH dependence of the disappearance of absorbance of the 470-nm intermediate. Experiments were carried out as described under Materials and Methods. Data were fitted using eq 1 giving a pK of 8.1 ± 0.1. The solid line is theoretical from the fit, and the points are experimental values. OAS forms an external Schiff base (II) with PLP, with concomitant release of the active-site lysine from the internal Schiff base (I). The α-proton is abstracted from II via a general base, initiating the elimination of the acetate group and the formation of the α-aminoacrylate intermediate (III), which has absorption maxima at 470 nm. As the pH increases, the ϵ-amino group of the active-site lysine becomes deprotonated (IV) and in a transaldimination reaction generates from enzyme and free α-aminoacrylate (V). The latter tautomerizes to iminopyruvate (VI) and is hydrolyzed nonenzymically to pyruvate and ammonia (VII). (VII), the iminopyruvate, is dissociated into pyruvate and ammonia.

at 0.2 mM and 1 mg/mL, respectively. There was also pyruvate produced as monitored by the lactate dehydrogenase reaction. Pyruvate reactions were monitored at pH 7 in 100 mM Hepes, 0.2 mM NADH, and 20 units of lactate dehydrogenase with OAS and OASS concentrations maintained at 0.2 mM and 1 mg/mL, respectively. In all cases, the reaction was initiated by the addition of OASS. A stoichiometric amount of pyruvate and ammonia were produced (measured pyruvate/ammonia = 1.1). The calculated turnover number for the deacetylase reaction is 0.07 s^{-1}, while the turnover number for the OASS reaction with sulfide as a substrate is 430 s^{-1} (Hara et al., 1990).

DISCUSSION

The λ_{max} at 412 nm represents a hydrogen-bonded species in which the proton donor is the protonated form of the Schiff base between the PLP and the ϵ-amino group of an enzyme lysine and the proton acceptor is the ionized phenolic oxygen at C-3 of PLP (Kallen et al., 1985). The spectrum of OASS is pH independent from pH 5.5 to 10.85 over the wavelength range 300–500 nm, suggesting that the imine of the internal Schiff base does not titrate over this pH range. The extinction coefficient of native enzyme at 412 nm is 7600 M^{-1} cm^{-1} (Becker et al., 1969), and as can be seen, the extinction coefficient of the intermediate at 470 nm is slightly higher than this value (ϵ_{470} = 9760 M^{-1} cm^{-1}). The λ_{max} at 470 nm obtained in the presence of OAS (Figure 2) represents a hydrogen-bonded species in which the proton donor is the protonated form of the Schiff base between the PLP and the α-amino of aminoacrylate and the proton acceptor is the ionized phenolic oxygen at C-3 of PLP (Cook & Wedding, 1976; Schnackerz et al., 1979a). Assignment of the intermediate as the α-aminoacrylate Schiff base is made on the basis of the following criteria: (1) the ping-pong nature of the kinetic mechanism in which isotope exchange between acetate and OAS is observed; (2) the similarity between the absorbance spectrum of the species generated by mixing OAS and OASS and the spectrum of an analogue of α-aminoacrylate in Schiff base with PLP prepared by Schnackerz et

Scheme I: Proposed Mechanism for the Hydroxylase Activity of *O*-Acetylserine Sulfhydrylase[a]
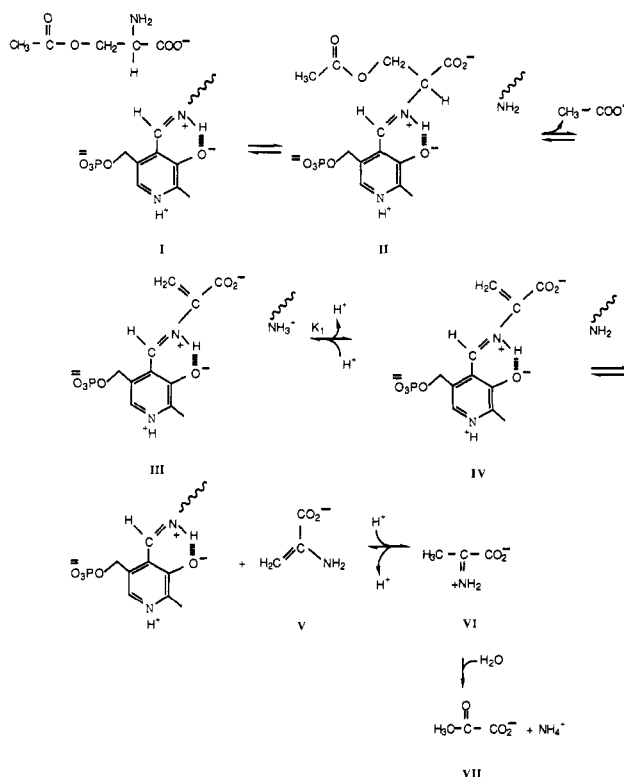
[a] OAS forms an external Schiff base (II) with PLP, with concomitant release of the active-site lysine from the internal Schiff base (I). The α-proton is abstracted from II via a general base, initiating the elimination of the acetate group and the formation of the α-aminoacrylate intermediate (III), which has absorption maxima at 470 nm. As the pH increases, the ϵ-amino group of the active-site lysine becomes deprotonated (IV) and in a transaldimination reaction generates free enzyme and free α-aminoacrylate (V). The latter tautomerizes to iminopyruvate (VI) and is hydrolyzed nonenzymically to pyruvate and ammonia (VII). (VII), the iminopyruvate, is dissociated into pyruvate and ammonia.

al. (1979a). The intermediate spectrum is pH independent from pH 5.5 to 9 over the wavelength range 290–540 nm, suggesting that the imine of the α-aminoacrylate Schiff base also does not titrate over this pH range. However, at pH values below 6, the absorbance in the wavelength range 240–290 nm increases with time when the sulfhydrylase is placed in Mes or Pipes buffer. The difference spectrum obtained by subtracting the spectrum obtained at zero time from that obtained after incubation for several minutes yields an increase centered at 280 nm. Thus, changes in protein structure consistent with denaturation are suggested. In agreement with this, the protein loses activity and begins to precipitate after 30 min at pH 5.5. The enzyme in phosphate buffer at these low pH values experiences none of these changes. Preincubation of the enzyme with 50 mM phosphate, pH 5.6, followed by the addition of Mes to a final concentration of 100 mM also results in no detectable changes in the spectrum or activity of OASS. Phosphate appears to stabilize the active form of the enzyme, suggesting the presence of an anion binding site on the OASS.

The pH independence of the spectral bands associated with the PLP imine in the internal Schiff base (I of Scheme I) and the α-aminoacrylate Schiff base intermediate (III of Scheme I) suggests either that OAS must selectively bind with its α-amino unprotonated or that if it is protonated must be deprotonated by an enzymic general base other than the imine nitrogen. In the former case, the Schiff base lysine once released could act as the general base to abstract the α-proton

in the β-elimination reaction, while in the latter it could be either the lysine or the general base that originally accepted a proton from the α-amino of OAS once a proton was transferred to the lysine. The same residues that served a role as general base in the first half-reaction would presumably play a role as general acid in the second half-reaction. These questions will have to await further studies that are presently in progress.

The lack of a pH-dependent change in the absorbance of the internal Schiff base is a common occurrence among PLP-dependent enzyme. Threonine dehydratase from *Escherichia coli* exhibits a pH-independent absorbance at 415 nm from pH 6 to 9 in the absence and presence of the allosteric effector AMP (Shizuta, 1969). Similar results have been reported for the tyrosine phenol lyase for the Schiff base absorbance at 430 nm (Kumagai et al., 1979), although in the presence of NH_4^+ or K^+, the absorbance does increase. The absorption maximum is 415 nm for the D-serine dehydratase, and its absorbance is pH independent over the pH range 5.5–9.5 (Schnackerz et al., 1979a). Nagasawa et al. (1982) have reported that the 418-nm absorption of the 3-chloro-D-alanine chloride-lyase Schiff base is also pH independent from 6 to 9.

Aspartate aminotransferase exhibits a pH-dependent change in the internal Schiff base absorbance (Braunstein, 1973), giving a pK of about 6 reflecting the acid dissociation constant of the imine nitrogen hydrogen-bonded to the phenolic oxygen of the cofactor. The low pK of the aminotransferase is likely a result of a perturbation of the pK of the imine from a high value above 10 to the observed value around 6 by the proximity of two positively charged arginines that ion-pair the α- and β-carboxyls of aspartate. In agreement with this, the pK of the imine increases to 8 when α-ketoglutarate binds to the E–PLP form of the enzyme, neutralizing the arginine that binds the α-carboxyl group (Kiick & Cook, 1983; Velick & Vavra, 1962; Jenkins & D'Ari, 1966; Haddad et al., 1977; Eichele et al., 1978).

pH Titration of the Enzyme-Bound 5'-Phosphate of PLP via ^{31}P NMR. ^{31}P NMR has been shown to be a powerful tool to obtain direct evidence on the environment of the phosphate group of PLP bound to enzymes (Schnackerz, 1984, 1986). In addition, the pH dependence of the ^{31}P NMR spectrum provides information concerning the ionization state of enzyme-bound PLP. A pH-independent chemical shift of the phosphorus resonance of OASS was observed in the presence and absence of OAS in the pH range of 6.5–8.4. The value of the chemical shift suggests that the 5'-phosphate exists as the dianion over the entire pH range for the E–PLP and α-aminoacrylate Schiff base forms of the enzyme. The value of 5.2 ppm for the chemical shift of the phosphate group of PLP in OASS is high when compared to other PLP-dependent enzymes. The chemical shift of PLP in the β₂ subunits of tryptophan synthase is 4.5 ppm (Schnackerz & Bartholmes, 1983). Upon reduction of the β₂ subunit by borohydride, a further downfield shift to 5.4 ppm is observed. Qualitatively similar results were obtained for D-serine dehydratase (α,β-elimination) in the presence of isoserine. A pH-independent chemical shift of 4.2 ppm is observed for bound PLP from pH 6.3 to 7.7. The above observations for tryptophan synthase β₂ and the D-serine dehydratase cofactor–inhibitor complex (external Schiff base) were attributed to PLP complexes that are excluded from interaction with water and fixed in their dianionic form via a rigid salt bridge (Schnackerz et al., 1979b). In the absence of the inhibitor, the ^{31}P NMR chemical shift of PLP in D-serine dehydratase is pH dependent with a

pK of 6.4. The chemical shift of 5.2 ppm for PLP bound to OASS likely indicates a similar strong salt bridge excluded from solvent.

Another indication of the strong interaction of the cofactor phosphate group with OASS is the line width of 20.5 Hz. For a protein of 68 900 molecular weight, a line width of this magnitude indicates that the PLP is tightly bound and tumbling with the protein (Schnackerz et al., 1979b). The line width for PLP rigidly bound to OASS can be calculated using the line width (55–60 Hz) and molecular weight (200K) of phosphorylase (a protein known to bind PLP rigidly; Schnackerz et al., 1979b) and the molecular weight (68.9K) of OASS. Calculated values of the line width are 18–21 Hz in agreement with the proposed rigid binding of the cofactor to OASS.

OASS Deacetylase Activity. A new side reaction catalyzed by OASS was identified and characterized in which the α-aminoacrylate intermediate (470-nm species) decomposes to pyruvate and ammonia, regenerating the active form of the enzyme. A possible mechanism for this reaction is shown in Scheme I. The protonated internal Schiff base (I) of PLP and the ε-amino group of a lysine residue of OASS undergo in the presence of OAS a transaldimination reaction to generate the external Schiff base (II) of OAS and PLP. α-Aminoacrylate in Schiff base with the active-site PLP (III) is produced from the external Schiff base, and acetate is released to complete the first half of the reaction (Cook & Wedding, 1976). The lysine that originally formed the Schiff base with the active-site PLP in free enzyme is likely protonated at the end of the elimination reaction. The unprotonated form of the active-site lysine (IV) undergoes a transaldimination reaction to generate free α-aminoacrylate (V) and regenerate E–PLP. The α-aminoacrylate then nonenzymically tautomerizes to iminopyruvate (VI), which is spontaneously hydrolyzed to pyruvate and ammonia (VII). The pH dependence of the α-aminoacrylate Schiff base disappearance gives a pK of 8.1 ± 0.1, reflecting the deprotonation of the active-site lysine (pK_1 in Scheme I). A similar pK value (8.2) is observed for the pH dependence of the V/K for 5-thio-2-nitrobenzoate (TNB, an alternative substrate for sulfide), and presumably also with sulfide as a substrate (Nalabolu et al., 1991). [The kinetic parameter V/K for the second half-reaction reflects free TNB (or sulfide dependent on the substrate used) and free α-aminoacrylate Schiff base (III).]

The β₂ subunit of tryptophan synthase catalyzes an α,β-elimination reaction of L-serine, forming pyruvate and ammonia (Crawford & Ito, 1964). Excitation of the β₂–L-serine complex at 405 nm generates a strong fluorescence at 500 nm, termed the "aqua band" (Goldberg et al., 1968). The fluorescence emission is likely a result of formation of the external Schiff base which precedes formation of the α-aminoacrylate intermediate. The highly fluorescent 420-nm-absorbing β₂–L-serine species undergoes a very slow decay to yield pyruvate and ammonia. The rate of this decay is subject to a primary kinetic isotope effect when L-[α-^{2}H]serine is substituted for L-[α-^{1}H]serine (Miles & McPhie, 1974; Drewe & Dunn, 1985). The dehydratase activity of the β₂ protein has a pH optimum in the range of 7.8–8.2 (Crawford & Ito, 1964), similar to that obtained for O-acetylserine sulfhydrylase.

The α-aminoacrylate intermediate appears only transiently when D-serine reacts with D-serine dehydratase to yield pyruvate and ammonia (Schnackerz et al., 1979a). The pH optimum for this reaction is around 7.8 and highly dependent on monovalent cations like K^+ and NH_4^+ (Dupourque et al., 1966). Tyrosine phenol-lyase and tryptophan indole-lyase both

catalyze the same type of reaction, elimination of phenol from tyrosine and indole from tryptophan, with the subsequent formation of pyruvate and ammonia (Kiick & Phillips, 1988a,b). Once phenol and indole are removed, the α-aminoacrylate intermediate is formed which can then undergo transaldimination and hydrolysis as described above. For OASS which primarily catalyzes a β-replacement reaction, replacing acetate with sulfide, the α-aminoacrylate Schiff base is a stable intermediate which can easily be prepared and studied. However, as discussed above, the enzyme catalyzes a side reaction in which the intermediate decomposes to pyruvate and ammonia, the same products of the β-elimination reactions described above.

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