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Resolution of Pyridoxal 5′-Phosphate from *O*-Acetylserine Sulfhydrylase from *Salmonella typhimurium* and Reconstitution of Apoenzyme with Cofactor and Cofactor Analogues as a Probe of the Cofactor Binding Site

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A procedure has been developed to prepare the apoenzyme of *O*-acetylserine sulfhydrylase (apoOASS) by first converting the native enzyme to the α-aminoacrylate intermediate and dialyzing against 5 M guanidinium chloride. Aposulfhydrylase is stable for at least a month in buffers containing phosphate or phosphate analogues. Reconstitution of aposulfhydrylase with pyridoxal 5′-phosphate (PLP), 2′-methyl PLP (2′-MePLP), and pyridoxal 5′-deoxymethylenephosphonate (PDMP) results in enzymatically competent proteins. Pyridoxal in the absence and presence of phosphate and pyridoxal 5′-phosphate monomethyl ester are unable to form a Schiff base with apoOASS. The reconstitution of apoOASS with PLP is highly cooperative judged by the initial rate of activity regained and shows no evidence of saturation with PLP. The reconstituted enzymes have been studied using ^{31}P NMR spectroscopy. The ^{31}P NMR of the aposulfhydrylase reconstituted with PLP exhibits a chemical shift of 5.2 ppm, identical to that of native enzyme. The latter has been interpreted in terms of a strong ionic interaction between enzyme and the 5′-phosphate of PLP (P. F. Cook, S. Hara, S. Nalabolu, and K. D. Schnackerz, 1992, *Biochemistry* 31, 2298–2303). Reconstitution with 2′-MePLP gives a lower chemical shift of 4.95 ppm, suggesting a weaker ionic interaction at the 5′-phosphate when compared to native enzyme. The PDMP-reconstituted enzyme gives a chemical shift of 23.7 ppm, consistent with the monoanionic form of the bound phosphonate. All of the chemical shifts are pH independent. The apoenzyme has also been reconstituted with pyridoxal 5′-sulfate. Although the resulting enzyme is not active in the overall reaction, it forms the external Schiff base. The PDMP- and 2′-MePLP-reconstituted enzymes have also been studied in the presence of amino acid reactants and analogues, and results are discussed in terms of the mechanism of OASS.

The biosynthesis of L-cysteine in *Salmonella typhimurium* is initiated by the acetylation of L-serine catalyzed by serine transacetylase. In a second reaction, *O*-acetyl-L-serine (OAS)[2] is converted to L-cysteine in the presence of sulfide by *O*-acetylserine sulfhydrylase (OASS; EC 4.2.99.8) Two OASS isozymes, A and B, have been described in *S. typhimurium* (1). The A and B isozymes are thought to be required for aerobic and anaerobic growth, respectively, and OASS-B will utilize thiosulfate as a substrate in addition to sulfide (2). The A and B isozymes are both dimeric with molecular masses of 68.9 (3) and about 64 kDa (4), respectively. Both proteins consist of two identical subunits each containing 1 mol PLP covalently bound in a Schiff base linkage to an enzyme lysine (5). The lysine that forms a Schiff base linkage has been identified as Lys-42 (unpublished work from this laboratory).

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[2] Abbreviations used: PLP, pyridoxal 5′-phosphate; 2′-MePLP, 2′-ethyl-3-hydroxy-4′-formyl-5′-hydroxymethylpyridine 5′-phosphate; PLS, pyridoxal 5′-sulfate; OASS, *O*-acetylserine sulfhydrylase; OAS, *O*-acetyl-L-serine; TNB, 5-thio-2-nitrobenzoate; DTE, dithioerythritol; cAAT, cytosolic aspartate aminotransferase; PDMP, pyridoxal 5′-deoxymethylenephosphonate; Mes, (2-[*N*-morpholino]ethanesulfonic acid; Ches, 2-(*N*-cyclohexylamino)ethanesulfonic acid; apoOASS, *O*-acetylserine aposulfhydrylase.

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O-Acetylserine sulfhydrylase catalyzes its reaction via a BiBi ping pong kinetic mechanism (4, 6). In the first-half reaction OAS is converted to α-aminoacrylate in Schiff base with the active site PLP, and this intermediate absorbs maximally at 330 and 470 nm, while in the second-half reaction the α-aminoacrylate intermediate is converted to cysteine (6, 7). Recently, the pH dependence of the kinetic parameters has been used to propose an overall chemical mechanism for the sulfhydrylase (8). *O*-Acetylserine binds with its α-amine unprotonated and an enzyme group is required protonated to assist in the elimination of acetate. In the second-half reaction the enzyme group must be unprotonated and the Schiff base lysine must be protonated to donate a proton upon formation of the cysteine external aldimine.

Resolution of the PLP cofactor and reconstitution with cofactor analogues has been used successfully to study the cofactor binding site of a number of PLP-dependent enzymes (9–13). Resolution of the cofactor from PLP-dependent enzymes has been achieved using several different methods including: (a) incubation of holoenzyme with hydroxylamine at neutral pH (tryptophan synthase) (14), (b) formation of the pyridoxamine phosphate form of an aminotransferase by incubation with the amino acid substrate followed by displacement of the cofactor phosphate with high concentrations of inorganic phosphate (aspartate aminotransferase) (15), and (c) incubation of the protein with L-cysteine forming the thiazolidine derivative with PLP, followed by release of the cofactor, facilitated by the deforming buffer "imidazole-citrate" (D-serine dehydratase) (16). None of these procedures are able to resolve PLP from OASS.

In this paper we describe a method which allows the preparation of aposulfhydrylase by dialyzing the α-aminoacrylate intermediate of OASS in the presence of 5 M guanidinium chloride. The properties of the apoenzyme have been established. In addition, we have tested several PLP analogues for their ability to bind and to form a catalytically active enzyme with *O*-acetylserine aposulfhydrylase. This study indicates that the sulfhydrylase functions optimally only when the cofactor phosphate group is present as the dianion.

MATERIALS AND METHODS

Chemicals. Pyridoxal 5′-deoxymethylenephosphonate was kindly donated by Dr. O. Saiko (Merck, Darmstadt). Pyridoxal 5′-phosphate monomethyl ester, prepared by the method of Pfeuffer *et al.* (17), was a gift of Dr. J. Ehrlich. Pyridoxal 5′-sulfate was prepared by the method of Kuroda (18). 2′-Methylpyridoxal 5′-phosphate was a gift of Dr. K. Feldmann. Guanidinium chloride and *O*-acetyl-L-serine were obtained from Sigma. All other chemicals were of the highest quality available from commercial sources.

Enzymes. A rapid purification procedure has been developed for OASS-A making use of gradient elution from Q-Sepharose Fast Flow and phenyl–Sepharose columns. The procedure gives homogeneous enzyme with 50% yield in 3 days (19). A plasmid pRSM40 has been obtained from Dr. N. M. Kredich at Duke University. This plasmid includes a 1484 bp *Cla*I–*Dra*I fragment containing the cysK coding region and promoter. From the overproducing strain 25 mg of pure OASS-A is isolated from 10 g of wet cell paste (overnight growth), compared to 75 mg isolated from 300 g of wild-type wet cell paste. The latter has now been successfully scaled up to growing 10 liters in a fermenter giving about 50–60 g of wet cell paste that yields 900 mg of pure OASS-A. Recently the above purification procedure has been adapted to the HPLC (4).

Enzyme assay. The enzymatic activity of OASS was monitored as described by Tai *et al.* (4) using a spectrophotometric assay monitoring the disappearance of TNB [13,600 M^{-1} cm^{-1} (20)].

Preparation of O-acetylserine aposulfhydrylase. OASS-A (2–3 mg/ml) was incubated with 10 mM OAS to form the protonated Schiff base between PLP and α-aminoacrylate as indicated by the absorbance changes of the holoenzyme from 412 to 470 nm (6, 7). The incubation mixture is transferred to a dialysis bag and dialyzed against 50 ml resolving buffer containing 100 mM Mes, pH 6.5, 10 mM freshly prepared OAS, 0.1 mM DTE, and 5 M guanidinium chloride for 24 h. Finally the resolved sulfhydrylase is dialyzed against two changes of 500 ml 0.1 M potassium phosphate, pH 7.0, containing 0.1 mM DTE. All other resolving systems used successfully for other PLP-dependent enzymes as stated in the introduction were unproductive in the case of OASS-A. It appears that the cofactor binding site has to be at least partially unfolded for the release of the α-aminoacrylate intermediate.

Reconstitution kinetics. Kinetic studies of reconstitution of aposulfhydrylase with PLP were carried out by monitoring the appearance of activity with time using the TNB assay. One hundred microliters of a 5.17 mg/ml solution of apoOASS was incubated with variable amounts of PLP (0.25 to 1.67 mM) in 100 mM potassium phosphate, pH 7, to a final volume of 120 μl. A small background rate of the apoOASS was observed and subtracted from all subsequent activity measurements. At different times, aliquots of 20 μl were assayed for regain of activity, until the activity was constant. Independent of the PLP concentration, the final activity was constant and gave an average value of 0.035 $\pm$ 0.001 μM/min, approximately 95% of the activity of native OASS-A.

NMR measurements. ^{31}P NMR spectra were collected at 121.4 MHz on a Bruker AM300 SWB superconducting spectrometer using a 10-mm multinulear probe head. The protein sample (2 ml) and D_2O (0.2 ml) in a 10-mm NMR tube was kept at 20 $\pm$ 0.1°C using a thermostatted continuous air flow. A spectral width of 2000 Hz was acquired in 8K datapoints with a pulse angle of 60°. Spectra reported are not proton-decoupled. Proton decoupling gives a 50% decrease in the linewidth of the phosphonate resonance, but has no effect on the phosphate resonance. The pH was measured before and after the NMR experiments with no change. An exponential line broadening of 10 Hz was applied prior to Fourier transformation. Positive chemical shifts in ppm are downfield changes with respect to 85% H_3PO_4.

RESULTS AND DISCUSSION

Characterization of aposulfhydrylase. The absorption spectrum of aposulfhydrylase in 0.1 M potassium phosphate, pH 7.0, 0.1 mM DTE shows a single absorption maximum at 280 nm. The typical absorbance of the internal PLP Schiff base of holosulfhydrylase at 412 nm is absent, indicating that the cofactor has successfully been removed. Apoenzyme preparations were typically found to have 3–5% holoenzyme present. However, the holoenzyme activity can be eliminated by

reduction with borohydride without harm to the apoenzyme. Aposulfhydrylase is stable at 4°C for at least a month in buffers containing 100 mM phosphate or phosphate analogues, such as arsenate, molybdate, or borate. Dialysis against other buffers results in irreversible denaturation. apoOASS does not bind inorganic phosphate very tightly. Removal of phosphate from apoOASS by dialysis followed by ^{31}P NMR does not show a signal for bound inorganic phosphate. In contrast, aspartate apoaminotransferase has been shown by ^{31}P NMR spectroscopy to bind one inorganic phosphate per subunit in the cofactor binding site after removal of phosphate by exhaustive dialysis (21–23).

Reconstitution of aposulfhydrylase with PLP or PLP analogues. The viability of apoenzyme preparations is routinely checked by incubating apoenzyme (2 μM) in 0.1 M potassium phosphate, pH 7.0, with 2 μM PLP and monitoring the enzymatic activity at various time intervals. The appearance of reconstituted enzyme is first order as shown by a first-order plot of the data in Fig. 1, indicating that the slow step is not the initial combination of enzyme and PLP. *O*-Acetylserine sulfhydrylase is fully reconstituted in about 3 h, as demonstrated in Fig. 1. The initial velocity of reconstitution of aposulfhydrylase with PLP to give holoenzyme was obtained by repeating the experiment shown in Fig. 1 as a function of PLP concentration. A plot of the initial rate against PLP is shown in the inset of Fig. 1. The reconstitution process is highly cooperative as shown by the near-zero rate at low PLP concentrations prior to a linear increase of the rate at higher PLP concentrations.

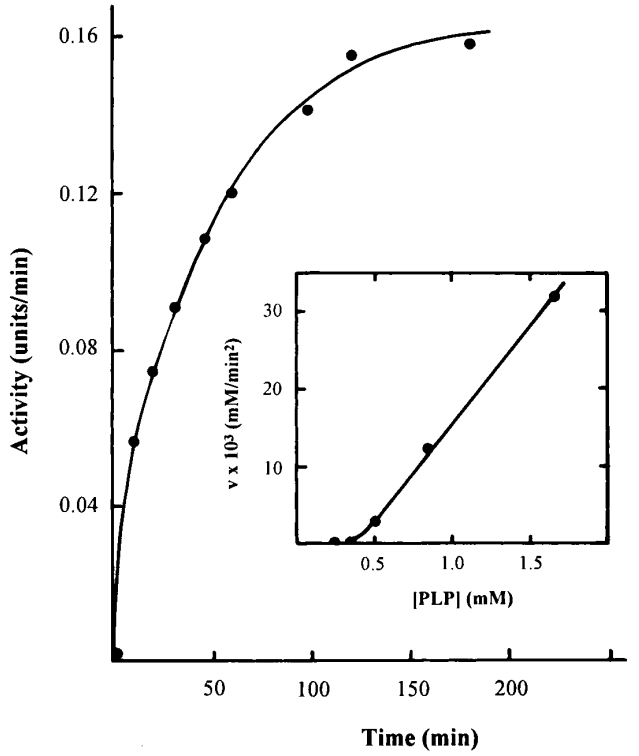

FIG. 1. Kinetics of the reconstitution of *O*-acetylserine aposulfhydrylase with PLP. Aposulfhydrylase was incubated with 2 μM PLP in 0.1 M Hepes, pH 7.0. The enzymatic activity was monitored at various times by using the TNB method (see Materials and Methods). (Inset) Plot of the initial rate of reconstitution vs [PLP]. Velocity is given as activity of OASS in mM/min regained per min.

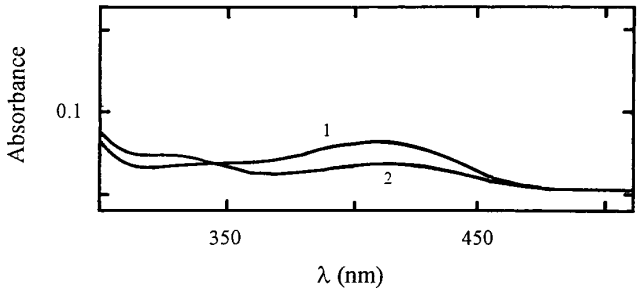

FIG. 2. The uv–visible spectra of PLS–OASS in the absence and presence of amino acids. Spectrum 1 is with 14.5 μM PLS–OASS, in 100 mM Hepes, pH 7, 25°C. Spectrum 2 represents the addition of 4 mM L-cysteine, pH 9.

Pyridoxal 5′-phosphate analogues such as PDMP, 2′-MePLP, and pyridoxal 5′-sulfate form an internal Schiff base with aposulfhydrylase to produce protein species absorbing at 418, 412, and 415 nm, respectively. The first two enzyme species exhibit enzymatic activity. Similar reactivation capacity for these two PLP analogues has been found for D-serine dehydratase (16). The pyridoxal 5′-sulfate-reconstituted sulfhydrylase, however, is enzymatically inactive. Arginine apodecarboxylase from *Escherichia coli* and apotryptophanase, by contrast, show some activity when reconstituted with pyridoxal 5′-sulfate (9). Cofactor analogues, like pyridoxal and PLP monomethyl ester, are unable to form a Schiff base with apoOASS and therefore cannot initiate enzymatic activity. In contrast, aposerine hydroxylmethyltransferase can be completely reactivated with PLP monomethyl ester (11).

Characterization of pyridoxal 5′-sulfate O-acetylserine sulfhydrylase. Aposulfhydrylase reconstituted with PLS gives the spectrum of a typical PLP-enzyme with λ_{max} values at 278, 336, and 415 nm, and a ratio of the absorbance at 278 to that at 415 of 3.1 (Fig. 2). The absorption spectra can be compared to that of native OASS which has λ_{max} values of 278 and 412 nm and a ratio of about 3.5 for A_{278}/A_{412} (7). Addition of OAS results in a decrease in the absorbance at 415 nm concomitant with a slight increase in the absorbance at 480–485 and 320–330 nm (data not shown). Compared to the value of 412 nm for the native enzyme (7), the λ_{max} of the protonated internal Schiff base of PLS–OASS is 415 nm, slightly red-shifted. In addition, only a very

small amount of either a quinonoid or α-aminoacrylate intermediate is formed. If it is the α-aminoacrylate intermediate that is formed, it would be expected to absorb at 470–478 nm by analogy to all other PLP analogs tested, e.g., 2′-MePLP and PDMP (unpublished results). The absorbance at 480–485 suggests the formation of the more highly conjugated quinonoid intermediate. The spectrum in the presence of OAS did not change after 3 min or upon addition of twice the concentration of OAS, and the PLS–OASS has for all intents and purposes no activity. With native enzyme, L-cysteine forms an external Schiff base intermediate that absorbs at 418 nm, while L-serine gives a mixture of external Schiff base species absorbing maximally at 320 and 418 nm. The 320-nm band is interpreted as a tautomeric form of the external Schiff base with serine (24). Addition of 40 mM L-serine to PLS–OASS at pH 9 results in a slight red shift to 417 nm (data not shown), while 4 mM L-cysteine results in a decrease in the absorbance at 415 nm, and appearance of new bands at 320 and 417 nm (Fig. 2). Thus, changes observed with L-cysteine and L-serine are opposite to those observed with native enzyme. Taken together, data suggest a different orientation of the PLS cofactor compared to PLP in the native enzyme. The lack of appreciable activity of PLS–OASS must result from the apparent inability of the enzyme to eliminate acetate and form the α-aminoacrylate intermediate. The inability to catalyze the elimination of acetate is likely a result of a change in the orientation of the cofactor in relation to Lys-42, the general base that abstracts the α-proton, and another enzyme group that interacts with the side chain of OAS maintaining acetate out of the C2–C3 plane (8).

^{31}P NMR spectra of aposulfhydrylase reconstituted with PLP and PLP analogues. The ^{31}P NMR spectra of native OASS-A exhibit a single pH-independent resonance at 5.2 ppm, indicating a dianionic cofactor phosphate in a strong salt bridge linkage to group(s) on the enzyme (Fig. 3) (7). A similar resonance was found for the PLP-reconstituted aposulfhydrylase (Fig. 3). The additional resonances observed at about 3.6 and 2 ppm represent a small amount of contaminating PLP and inorganic phosphate, respectively. Upon reduction of the Schiff base linkage with sodium borohydride the ^{31}P signal of reduced OASS shifts upfield to 4.8 ppm (Fig. 3) concomitant with an increase in linewidth. The decrease in chemical shift upon reduction signals a looser binding of the 5′-phosphate. In all cases the ^{31}P chemical shift is pH independent.

Incubation of aposulfhydrylase with PDMP results in an enzyme species with about 2% activity compared to native enzyme (unpublished results), and a pH-independent resonance at 23.7 ± 0.6 ppm over the pH range 6.0 to 8.5 (Fig. 4). A model Schiff base between PLP and *N*-acetyllysine methyl ester gives a pH-dependent chemical shift which has pH-independent values of 23.6 and 20.5 ppm at low and high pH, respectively (Fig. 4A) (25). It thus appears that the phosphonate derivative binds as the monoanion, and this may account in part for the low activity of the PDMP–OASS. For the phosphonate signal of PDMP–OASS, a linewidth of 40 Hz has been estimated for the non-proton-decoupled spectrum. The proton-decoupled spectrum, however, gives a linewidth of 20 Hz identical to that of native enzyme. The value of 20 Hz estimated for wild-type enzyme is the maximum linewidth predicted for a rigidly bound 5′-phosphate tumbling with the protein of a molecular mass of 68.9 kDa (7).

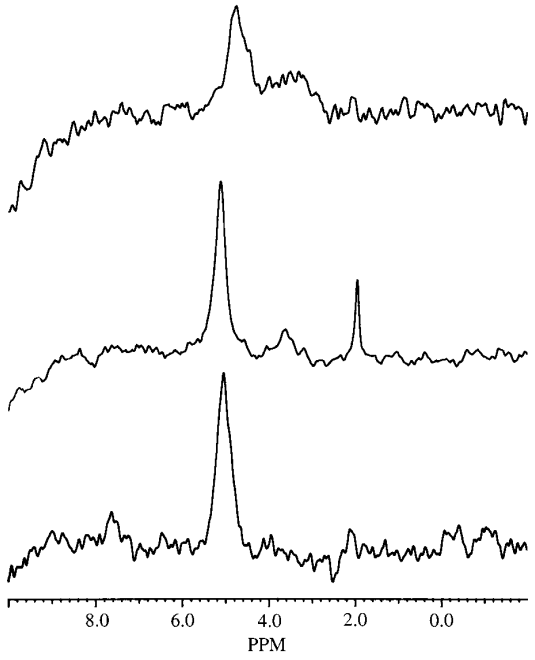

FIG. 3. ^{31}P NMR spectra of native (32,768 scans; bottom), reconstituted (33,564 scans; middle), and sodium borohydride-reduced *O*-acetylserine sulfhydrylase (21,452 scans; top) at pH 7.0. Spectra were measured as described under Materials and Methods.

The ^{31}P chemical shift of the cofactor analogue Schiff base in PDMP–D-serine dehydratase (35% activity when compared with native enzyme) is pH dependent with limiting chemical shift values of 18.5 to 19.5 ppm and a p*K* value of 7.4. Since the same p*K* is measured for a model Schiff base between PLP and N-acetyllysine methylester the former data suggest the exposure of the phosphonate group to solvent (Fig. 4A) (25). In the presence of 25 mM isoserine the apparent p*K* of the isoserine-cofactor analogue Schiff base is lowered from 7.4 to 6.35, suggesting that addition of the substrate analogue causes an additional interaction with the phosphonate or a change to a more hydrophobic environment of the phosphonate (26). Substitution of PLP with PDMP in cytosolic aspartate aminotransferase

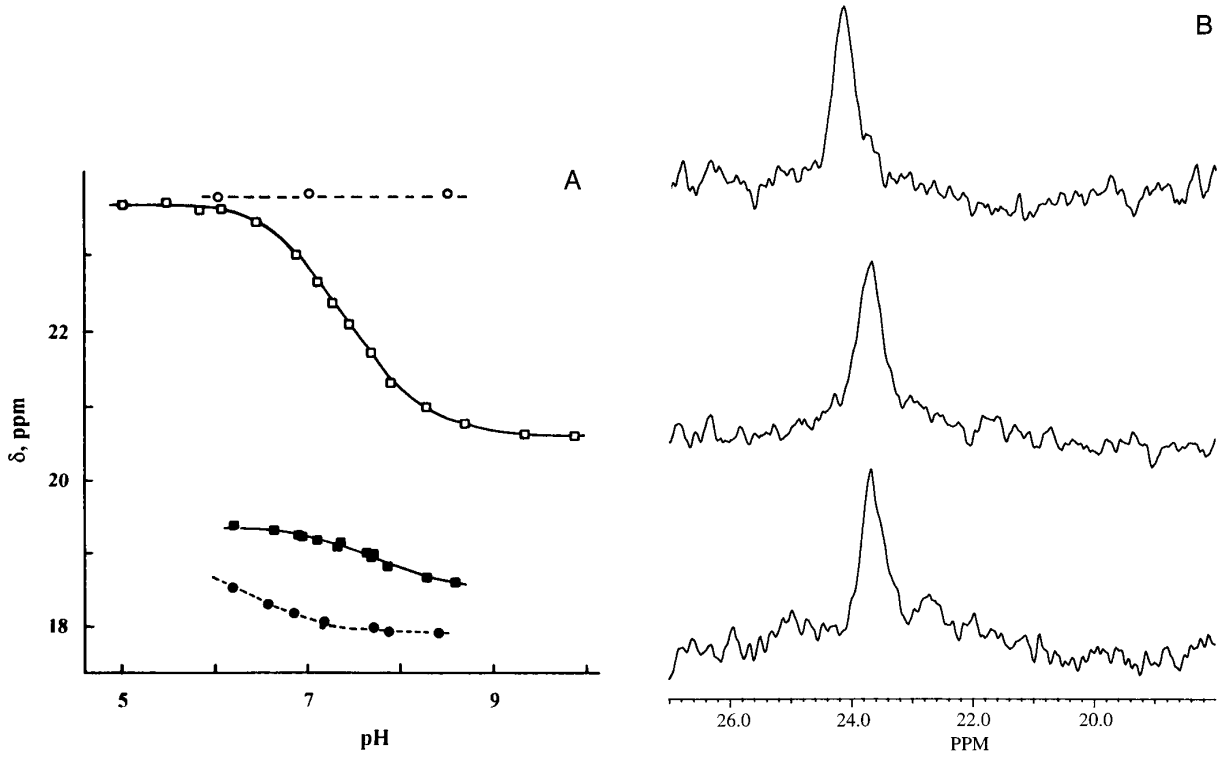

FIG. 4. ^{31}P NMR spectra of PDMP-reconstituted OASS and the pH dependence of ^{31}P chemical shifts of PDMP bound as Schiff base to OASS and D-serine dehydratase. (A) The pH dependence of ^{31}P chemical shifts of the PDMP Schiff base with *N*-acetyllysine methyl ester (□), bound to OASS (○), and bound to D-serine dehydratase in the absence (■) and presence (●) of 25 mM isoserine (Schnackerz and Feldmann, 1980). (B) The ^{31}P NMR spectrum of PDMP-reconstituted OASS is shown alone, in 100 mM Hepes, pH 7 (23,728 scans; bottom); plus 1 mM OAS, in 100 mM Mes, pH 6.5 (23,344 scans; top), and plus 4 mM L-cysteine, in 100 mM Ches, pH 9 (27,068 scans; middle). Experiments were performed in 50 mM Hepes or Mes, 1 mM DTE, and 1 mM EDTA. For experimental details, see Materials and Methods. Transitions were obtained by iterative computer analysis.

(cAAT) gives an enzyme species with 5% activity. A phosphonate ^{31}P signal at 24.9 ppm that is pH independent over the pH range 5.5 to 8.5 is observed for the PDMP–cAAT. At pH values lower than 5, the signal of cAAT-bound PDMP shifts upfield. A very low pK of 3.7 reported for the protonated aldimine in the PDMP derivative has been estimated (27).

Replacement of PLP with 2′-MePLP in OASS-A yields a resonance at 4.95 ppm (Fig. 5). Alterations in ^{31}P chemical shift can result from configurational changes around the cofactor 5′-phosphate side chain, leading to a structure with a near-eclipsed pair of bonds. Such a stressed configuration produces slight alterations in bond angles around the phosphorus atom which in turn cause the observed change in ^{31}P chemical shift. Additionally, changes in the strength of ion pairing to the 5′-phosphate can lead to changes in chemical shift, or changes in the hydrophobicity around the 5′-phosphate side chain. The slight upfield shift from 5.2 ppm for native enzyme to 4.95 ppm for 2′-MePLP–OASS suggests that either the bonds around the ^{31}P nucleus are less eclipsed or that a weaker ionic interaction with the cofactor phosphate exists. A linewidth of 22 Hz is estimated for the resonance at 4.95 ppm, indicating a cofactor that is rigidly bound to the protein, similar to native enzyme (7). The ^{31}P signal is identical from pH 7 to 8. Attempts to collect data at pH 6.3 resulted in precipitation of the enzyme.

^{31}P NMR spectra of PDMP- and 2′-MePLP–OASS in the presence of amino acids. The addition of OAS to PDMP–OASS results in the formation of the α-aminoacrylate intermediate, while cysteine gives a mixture of α-aminoacrylate intermediate and external Schiff base (24). The ^{31}P NMR spectrum for PDMP–OASS in the presence of 1 mM OAS exhibits a chemical shift of 24.2 ppm, downfield from the signal at 23.6 ppm for enzyme alone (Fig. 4B). The downfield shift indicates a looser binding of the 5′-phosphonate in the presence of OAS compared to the enzyme alone. Addition of L-cysteine produces no change in the phosphonate chemical shift (Fig. 4B). The linewidth estimated for enzyme in the presence of OAS is 40 Hz for the non-proton-decoupled spectra, while it increases to 49 Hz in the presence of cysteine. The increase in linewidth from 40 to 49 Hz likely reflects the mixture of α-aminoacrylate intermediate and external Schiff base.

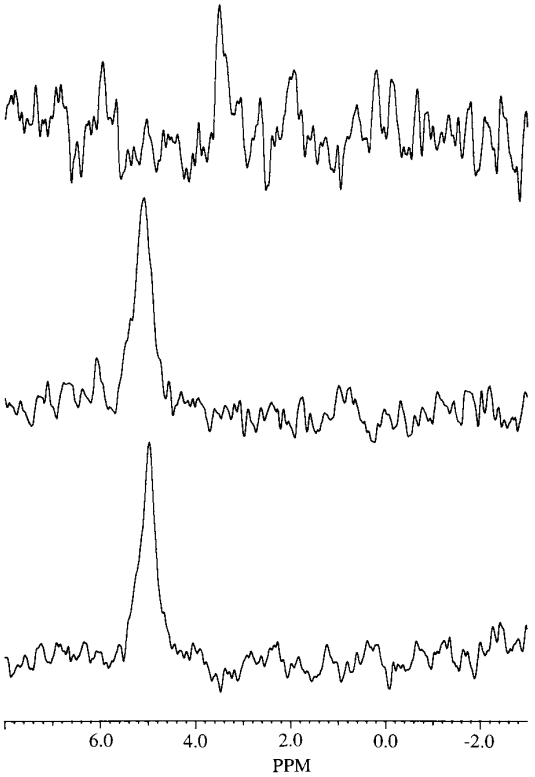

FIG. 5. ^{31}P NMR spectrum of 2′-MePLP-reconstituted OASS. The ^{31}P NMR spectrum of 2′-MePLP-reconstituted OASS is shown alone, in 100 mM Hepes, pH 8, at 20°C (26,372 scans; bottom); plus 53 mM OAS, in 100 mM Hepes, pH 7.6, at 4°C (6148 scans; top), and plus 10 mM L-cysteine, in 100 mM Ches, pH 9, at 20°C (27,192 scans, middle). For experimental details, see Materials and Methods.

Addition of OAS to 2′-MePLP–OASS results in formation of the α-aminoacrylate intermediate, while addition of L-cysteine produces the external Schiff base (unpublished results). The ^{31}P NMR spectrum for 2′-MePLP–OASS in the presence of 10 mM cysteine at pH 9 shows a downfield shift of the resonance by 0.14 ppm, similar to that observed for wild-type enzyme (Fig. 5). Addition of 53 mM OAS at pH 7.6 and 4°C exhibits a resonance at 3.6 ppm (Fig. 5). Addition of 53 mM OAS at pH 7.6 and 4°C exhibits a resonance at 3.6 ppm (Fig. 5). The experiment had to be carried out at 4°C because of the precipitation observed at 20°C. Furthermore, the number of transients collected was limited due to the fact that OAS is unstable at pH values above 6.5. The significant shift[3] observed upon addition of L-cysteine suggests a tighter binding of the 5′-phosphate in the external Schiff base compared to internal Schiff base or a more hydrophobic environment. The linewidth increases from 20 to 38 Hz as the external Schiff base is formed. The value of 20 Hz estimated for wild-type enzyme is the maximum linewidth predicted for a rigidly bound 5′-phosphate tumbling with the protein of a molecular mass of 68.9 kDa (7). The linewidth of 38 Hz for the external Schiff base is likely to reflect a mixture of species. In contrast, the large upfield shift observed in the presence of OAS suggests a significant weakening of the interactions between the 5′-phosphate of the cofactor and protein. The weaker interactions are accompanied by formation of the α-aminoacrylate intermediate which requires a change in hybridization from sp^3 to sp^2 at C2 and C3 of the substrate. Thus, the presence of the 2′-methyl group, probably as a result of steric considerations to allow changes in geometry, requires a movement of the 5′-phosphate. The linewidth estimated in the presence of OAS is 20 Hz, identical to that of free enzyme. Despite the movement of the 5′-phosphate, it remains rigidly bound.

[3] Although the downfield shift is slight (0.14 ppm) it has been observed in 10 different experiments comparing enzyme plus and minus L-cysteine.

Conclusions. A method to effectively resolve the cofactor PLP from *O*-acetylserine sulfhydrylase has been described. Changes in the 5′-phosphate sidechain are not tolerated; the sidechain has to have a double negative charge to produce full enzymatic activity. The properties of the aposulfhydrylase have been established. Apoenzyme can be fully reconstituted with PLP.

ACKNOWLEDGMENTS

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