The Reaction of L-Serine *O*-Sulfate with Aspartate Aminotransferase*

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ABSTRACT: In the presence of the aldimine form of aspartate aminotransferase, L-serine *O*-sulfate undergoes a β-elimination reaction leading to the formation of equimolar amounts of pyruvate, protons, ammonium, and sulfate ions. The reaction was followed by measuring either proton release in a pH-Stat or pyruvate production by a coupled reduced nicotinamide–adenine dinucleotide dependent reducing system. While the initial rates determined by the two methods are identical, the progress curves, though both biphasic, are quite different. This difference is explained by a slow transamination between L-serine *O*-sulfate and the aldimine enzyme to give the aminic enzyme, which is unable to catalyze the β-elimination reaction. Pyruvate and other keto acids, by transaminating with the aminic enzyme, regenerate the aldimine enzyme and thus maintain the rate of β elimination at a higher level.

Spectrofluorimetric evidence indicates that transamination does occur between L-serine *O*-sulfate and pyridoxal phosphate in the presence of aspartate aminotransferase. In addition, an irreversible reaction takes place between this substrate and aspartate aminotransferase resulting in complete inactivation of the enzyme. Understanding of the various events occurring in the system has allowed the determination of the steady-state kinetic parameters for the β-elimination reaction. At pH 6.5 and 25°, K_m was 7×10^{-2} M and $k_{cat} = 12$ sec^{-1}. Transamination proceeds much more slowly. These findings show that aspartate aminotransferase is capable of catalyzing, on the same substrate, two simultaneous reactions; the progress of one of these reactions prevents the other from occurring. The irreversible inactivation of the enzyme by a substrate analog provides a way of specifically labeling the active site.

Cytoplasmic pig heart aspartate aminotransferase (AAT)[1] (EC 2.6.1.1.) has been shown to be capable of combining with several amino acids other than aspartate and glutamate. Novogrodsky and Meister (1964) have shown that the aldimine form of AAT transaminates with L-methionine, L-serine, L-arginine, and L-phenylalanine. Spectral and chemical evidence shows that α-methylaspartic acid binds to the enzyme, forming an enzyme–substrate Schiff base which, however, is not further transformed (Fasella *et al.*, 1966). Erythro β-hydroxyaspartic acid undergoes slow transamination; in this case the reaction is characterized by the rapid formation of a complex with an absorption maximum at 490 mμ attributed to a quinoid intermediate formed after elimination of the α hydrogen from the enzyme-bound substrate (Jenkins, 1961). It has also recently been observed that AAT catalyzes an α,β-elimination reaction with β-chloroglutamate producing ammonium ions, chloride, and 2-oxoglutaric acid (Manning *et al.*, 1968). It was concluded that in this case the electronegativity of the chlorine substituent was responsible for the prevalence of α,β elimination over transamination.

Molecular models show that L-serine *O*-sulfate and the normal substrate L-glutamate possess similar stereochemical arrangements of functional groups; for this reason the behavior of L-serine *O*-sulfate with AAT was investigated. It had been reported in a preliminary communication (John *et al.*, 1968) that AAT catalyzes the production of pyruvate, protons, sulfate, and ammonium ions in equimolar amounts from L-serine *O*-sulfate, and that the enzyme is inactivated in the process. This paper describes a more detailed investigation of the reaction.

Experimental Procedure

Materials

L-Serine *O*-sulfate was synthesized as described by Tudball (1962). AAT was prepared according to the modification described by Martinez-Carrion *et al.* (1967) of the method of Jenkins *et al.* (1959). Malic dehydrogenase, lactic dehydrogenase, and NADH were bought from Boehringer.

Methods

Assay of Transaminase Activity. Transaminase activity was assayed in the presence of 5×10^{-4} M 2-oxoglutarate, 3.1×10^{-3} M L-aspartate, and pH 7.5 Tris-HCl buffer (0.1 M in Tris) at 25°. Production of oxaloacetate was followed by two methods, the choice of which depended upon the sensitivity required. Either the increase in optical density at 260 mμ due to production of enol oxaloacetate, or the decrease in optical density at 340 mμ due to the oxidation of NADH (1×10^{-4} M) in the presence of malic dehydrogenase (7 μg/ml) was followed, the latter method being about six times more sensitive than the former.

Assay of the β Elimination of L-Serine O-Sulfate by AAT. The progress of the β-elimination reaction was followed con-

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[1] Abbreviations used are: AAT, L-aspartate 2-oxoglutarate aminotransferase; PLP, pyridoxal 5-phosphate; PMP, pyridoxamine 5-phosphate; NEM, *N*-ethylmaleimide.

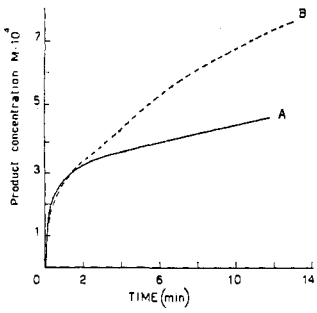


FIGURE 1: Progress of the β elimination of L-serine *O*-sulfate catalyzed by AAT. (A) Followed spectrophotometrically. (B) Followed with the pH-Stat. Initial conditions: L-serine *O*-sulfate, 0.04 M; NaCl, 0.01 M; AAT, 0.1 mg/ml; pH 6.5; 25°.

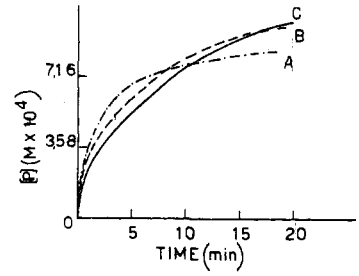


FIGURE 2: Effect of pyruvate on the progress of the β elimination of L-serine *O*-sulfate catalyzed by AAT and followed with the pH-Stat. (A) 4×10^{-3} M pyruvate; (B) 8×10^{-4} M pyruvate; (C) no added pyruvate. Other experimental conditions as in Figure 1.

tinuously by two methods. Hydrogen ion production was recorded using a pH-Stat (Titrator–Titrigraph combination, Radiometer, Copenhagen), the pH being maintained constant by the addition of small amounts of dilute NaOH prepared from standard solutions. Pyruvate production was measured spectrophotometrically by following the decrease in optical density at 340 mμ due to the oxidation of NADH in the presence of lactic dehydrogenase (1 μg/ml). The spectrophotometric assay was conducted in the absence of buffer in order to simulate conditions in the pH-Stat assay where buffer cannot be included. It was found that the pH of the assay mixture did not change during the course of the reaction, when LDH and NADH were included in the system.

In some spectrophotometric experiments cacodylate buffer (pH 6.5, 0.1 M) was included in the reaction mixture. The shapes of the progress curves thus obtained were very similar to those obtained at the same pH in the absence of buffer. The absolute rates were approximately 50% lower.

Spectral Determinations. Absorption spectra and other optical density measurements were made using a Beckman DK-2A recording spectrophotometer. Fluorescence spectra and other fluorimetric measurements were made using a series 115 Farrand recording spectrofluorimeter.

Results

β-Elimination Reaction of L-Serine O-Sulfate in the Presence of AAT. Using the conditions and procedures described by Thomas and Tudball, (1967), pyruvate, sulfate and ammonium ions were formed in equimolar amounts after L-serine *O*-sulfate was incubated for 1 hr with AAT (3.35 μmoles of products/mg of enzyme). As is the case with *threo*-β-chloro-L-glutamic acid (Manning *et al.*, 1968) it is the aldimine form rather than the aminic form of AAT that catalyzes β elimination from L-serine *O*-sulfate.

Progress of the β Elimination from L-Serine O-Sulfate Catalyzed by AAT. Preliminary experiments indicated that the progress curve of the β-elimination reaction was not linear and that the nature of the progress curve depended both upon experimental conditions and on the method chosen to follow the reaction.

Under the conditions shown in Figure 1B, the progress curve obtained using the pH-Stat assay was found to have two distinct phases. The rate of the reaction decreased rapidly at first, then more slowly. Eventually (portion of the progress curve not shown in the figure) it fell to zero before 5% of the initial concentration of substrate had been used. When a new aliquot of the enzyme was added to the same solution, the reaction started again, following a course similar to the previous one. The addition of a new aliquot of substrate did not result in further reaction. The spectrophotometric assay system also showed a biphasic progress curve (Figure 1A); but in this case the rate during the second phase was only 25% of that determined by the pH-Stat method and did not appear to decrease during the time of the experiment.

Neither sulfate nor ammonium ions were found to affect the course of the reaction when added initially at concentrations similar to those which would result from their formation as products of the reaction. However, when 1×10^{-3} M pyruvate was present in the initial mixture, the reaction proceeded more rapidly at first, then slowed down, coming to a halt much sooner than in those experiments in which no pyruvate was added. The effect of pyruvate was concentration dependent, reaching a maximum at 1×10^{-4} M. A series of progress curves obtained at different initial pyruvate concentrations is shown in Figure 2.

The probability that the difference between the progress curves determined by the two methods could be attributed to the removal of pyruvate in the spectrophotometric system, coupled with the observation that the initial inclusion of pyruvate in the pH-Stat system produced changes in the form of the progress curve, led us to investigate the effect of another keto acid, namely, 2-oxoglutaric acid, on the reaction. We found that when 3.5×10^{-3} M 2-oxoglutarate was included in the reaction mixture the progress curves determined by either method became very similar, as shown in Figure 3.

In the presence of added or produced keto acids β elimination from L-serine *O*-sulfate stopped completely when only a small fraction of the initial substrate had been transformed. Since the reaction resumed upon addition of fresh enzyme and did not upon addition of fresh substrate, it seemed probable that the enzyme had been inactivated, at least as far as β elimination from L-serine *O*-sulfate was concerned. Experiments were carried out to establish whether the enzyme had also lost its capacity to catalyze transamination between its normal substrates. Taking advantage of the fact that transaminase activity can be followed at AAT concentrations 100 times lower than those required to follow the β-elimination reaction, samples were taken from the reaction mixture described in Figure 3 at various times, rapidly diluted by a factor

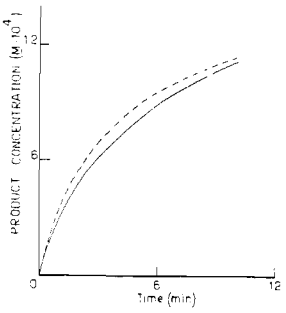


FIGURE 3: Comparison of the progress curves for β elimination of L-serine *O*-sulfate catalyzed by AAT determined spectrophotometrically (—) and with the pH-Stat (– – –) in the presence of 2-oxoglutarate (4×10^{-3} M).

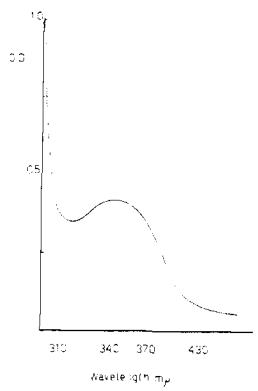


FIGURE 4: Spectrum of inactivated AAT after dialysis against sodium pyrophosphate–HCl (pH 8.0) and 0.1 M in sodium pyrophosphate. Protein concentration = 10 mg/ml.

of at least 50 times[2] in pH 7.5 Tris-HCl buffer (0.1 M in Tris) and then assayed for aspartate transaminase activity as described under Methods. It was found that all through the reaction loss of transaminase activity paralleled the decrease in the β-elimination rate.

In order to establish whether the inactivation was reversible or irreversible, a preparation of enzyme (16 mg/ml), inactivated in the presence of pyruvate (0.002 M) and of L-serine *O*-sulfate (0.04 M) to a residual activity of less than 1% of the original, was separated from the small molecules present in the system by chromatography on a 60 × 1.4 cm column of Bio-Gel P-10. The protein fraction of the effluent was concentrated to 2 ml by ultrafiltration. The spectrum of the enzyme was identical before and after removal of the small molecules; its absorption maximum was at a wavelength 15 mμ shorter than that of the native aldimine enzyme (Figure 4). Moreover, after removal of L-serine *O*-sulfate and of other small molecules, the transaminase activity, assayed under standard conditions, remained less than 1% of the original. This loss of 99% of the initial activity could be attributed to complete inactivation of 99% of the enzyme molecules or to a fall by a factor of 100 in the catalytic activity of all the molecules. It is possible to distinguish between these alternatives by an all or none test. With AAT, such a test can be carried out by examining either the transamination between the aldimine form of the enzyme and one of its amino acid substrates, or the reverse reaction. Both reactions can be conveniently studied by following the spectral shift which occurs when the aldimine and amine forms of the enzyme are interconverted. The spectrum of the inactivated enzyme (10 mg of protein/ml) in pH 8.0 pyrophosphate-HCl buffer (0.08 M in pyrophosphate) was not affected by the addition of either 2-oxoglutarate (final concentration 4×10^{-3} M) or L-aspartate (final concentration 7×10^{-2} M) or both together.

Moreover an aliquot of the above solution containing the inactivated enzyme and L-aspartate was made 3×10^{-4} M in NADH and the absorption at 340 mμ was measured before and after the addition of malate dehydrogenase (final concentration 5 μg/ml). Again, no spectral change was observed: this proves that oxaloacetate was not present in detectable amounts.

[2] Preliminary controls had shown that this large dilution reduced the rate of both β elimination and inactivation to a negligible value.

These results show that, most probably, 99% of the enzyme molecules are completely inactivated.

Another preparation of the inactivated enzyme was submitted to the procedure normally used for resolution of the coenzyme (Scardi *et al.*, 1963); pyridoxal phosphate was then added at various concentrations and the transaminase activity of the resulting preparation was measured after 3 min of incubation at room temperature. Only 1.7% of the activity could be recovered after incubation with excess pyridoxal phosphate (10^{-5} M), under conditions which allow full reactivation of the native apoenzyme (Martinez-Carrion *et al.*, 1967).

Progress of the Inactivation of AAT in the Presence of L-*Serine O-Sulfate.* Preliminary experiments had indicated that the rate of inactivation of aspartate aminotransferase in the presence of L-serine *O*-sulfate increased with enzyme concentration more than would be expected if it varied linearly with enzyme concentration. This phenomenon was clearly demonstrated by an experiment conducted in the presence of 3.5×10^{-3} M 2-oxoglutarate at two enzyme concentrations, *viz.*, 1×10^{-5} and 1×10^{-6} M, using the spectrophotometric assay system to follow β elimination. For the lower enzyme concentration 10-mm path-length cuvets and 1.8×10^{-4} M NADH were used, whereas for the higher concentration 1-mm cuvets and 1.8×10^{-3} M NADH were used. A direct comparison of the rate of change of absorbance at 340 mμ is shown in Figure 5.

The progress curve of enzyme inactivation can be conveniently studied in the presence of excess pyruvate; the small amount of pyruvate formed by β elimination is then negligible relative to the amount initially present and inactivation is monophasic (*cf.* Figure 6). Under these conditions, a plot of the logarithm of enzyme activity *vs.* time is not linear, while a plot of the reciprocal of remaining transaminase activity *vs.* time is (Figure 7). The linearity of this plot may be a coincidence since inactivation results from a complex phenomenon involving various simultaneous processes such as the enzyme's action on L-serine *O*-sulfate and the regeneration of the PLP form of the enzyme by transamination.

Transamination of L-*Serine O-Sulfate.* The markedly biphasic nature of the progress curve of both β elimination and inactivation under conditions where pyruvate is continuously removed can be explained by assuming that L-serine *O*-sulfate

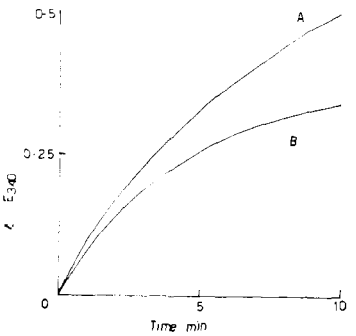


FIGURE 5: Dependence of the progress of AAT-catalyzed β elimination of L-serine *O*-sulfate on the AAT concentration. (A) AAT = 0.5 mg/ml; NADH = 1.8×10^{-3} M; 1-mm cuvet. (B) AAT = 0.05 mg/ml; NADH = 1.8×10^{-4} M; 10-mm cuvet. Initial conditions: L-serine *O*-sulfate, 0.04 M; NaCl, 0.01 M; 2-oxoglutarate, 0.004 M; pH 6.5; 25°.

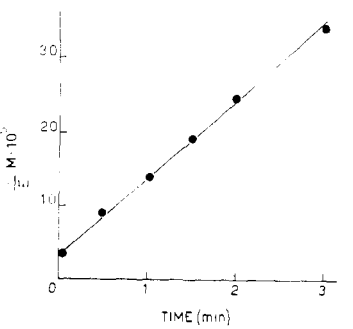


FIGURE 7: Reciprocal plot of AAT inactivation by L-serine *O*-sulfate. Initial conditions: L-serine *O*-sulfate, 0.04 M; NaCl, 0.01 M; pyruvate, 0.004 M; AAT, 0.11 mg/ml; pH 6.5; 25°. Samples were diluted and assayed for transaminase activity as described in Figure 6.

slowly transaminates with the aldimine enzyme. The amine enzyme thus formed would not be able to react with L-serine *O*-sulfate to catalyze β elimination or to undergo inactivation.

The second, slow, phase in the β elimination and in the inactivation progress curves would occur when the slow transamination between PLP- and PMP-enzymes, L-serine *O*-sulfate, and hydroxypyruvate sulfate, has reached equilibrium. The approach to this equilibrium must necessarily be slow to explain the biphasic nature of the progress curve. If transamination occurred at about the same rate as the initial β elimination (*e.g.*, 5×10^{-4} M/min in the presence of 1×10^{-6} M enzyme) equilibrium would be reached so rapidly that the progress curve would not appear to be biphasic. Detection of such a slow transamination is quite difficult because high enzyme concentrations cannot be used owing to the very rapid inactivation under the latter conditions. In order to support the hypothesis that transamination occurs between the coenzyme of AAT and L-serine *O*-sulfate, a method depending upon the strong fluorescence of free PMP was used. It is known (Turano *et al.*, 1964) that in the presence of NEM the affinity of the AAT protein for its coenzyme decreases by

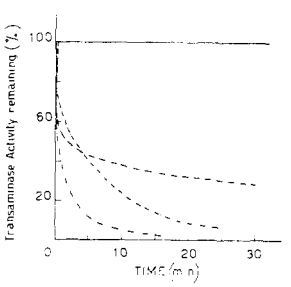


FIGURE 6: Effect of pyruvate on the rate of inactivation of AAT by L-serine *O*-sulfate. (A) No pyruvate added (–·–·–). (B) Pyruvate (0.002 M) present initially (–··–··–). (C) Pyruvate continuously removed by the inclusion of LDH and NADH (– – –). (D) 0.002 M pyruvate in the absence of L-serine *O*-sulfate (—). Initial conditions: L-serine *O*-sulfate, 0.04 M; AAT, 0.05 mg/ml; NaCl, 0.01 M; pH 6.5; 25°. Samples taken at various times from the above reaction mixture were rapidly diluted at least 50 times in 0.1 M Tris-HCl buffer (pH 7.5) and assayed for transaminase activity.

many orders of magnitude, so that AAT is transformed into a PLP-amino acid transaminase. Therefore, any transamination between L-serine *O*-sulfate and PLP in the presence of NEM-treated AAT should lead to the formation of free PMP, which can be detected fluorimetrically. A solution containing 0.2 mg/ml of the aldimine form of aspartate aminotransferase, 1.8×10^{-4} M PLP, Tris-HCl (0.1 M in Tris) (pH 7.8), 0.03 M L-serine *O*-sulfate, and 0.048 M NEM in a 2 × 2 mm fluorimeter cuvet was monitored for fluorescence emitted at 400 mμ using an exciting light of 350 mμ. These wavelengths were chosen because they were found to provide optimal conditions for the detection of PMP fluorescence in solutions containing NEM and PLP. An increase in emission was observed over the course of about 40 min. Both the emission and excitation spectra of the product were identical with those of PMP and the increase of fluorescence did not occur in the absence of L serine *O*-sulfate. The progressive increase in fluorescence was converted into PMP equivalents using a calibration curve constructed by the inclusion of authentic PMP in an identical system and is illustrated graphically in Figure 8.

Determination of Steady-State Parameters. Knowledge of the factors influencing the various reactions occurring between L-serine *O*-sulfate and AAT permitted the study of the β-elimination reaction under conditions when the effects of inactivation and transamination were negligible. The reaction was carried out at low enzyme concentration to minimize inactivation and in the presence of 2-oxoglutarate to maintain the enzyme in the aldehyde form.

The reaction mixture contained 6×10^{-8} M AAT and 1.8×10^{-3} M 2-oxoglutarate in 0.01 M NaCl. The spectrophotometric assay was used with NADH (1.8×10^{-4} M) and lactic dehydrogenase (10 μg/ml) in 10-mm cuvets at 25°. The pH was adjusted to 6.5 with NaOH. Under these conditions, the progress was linear over several minutes and good estimates of initial rates could be made. A double-reciprocal plot of initial velocities *vs.* initial L-serine *O*-sulfate concentrations is shown in Figure 9. From these data the K_m was determined to be 7×10^{-2} M and V_{max} 5×10^{-5} M min^{-1}.

Discussion

The results suggest that after the initial binding of L-serine *O*-sulfate to AAT, one of three pathways may be followed lead-

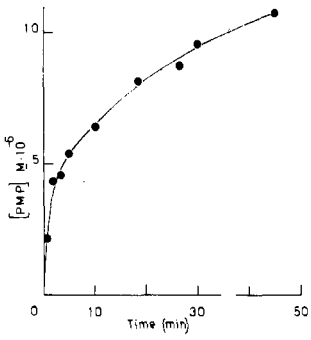


FIGURE 8: Formation of PMP from PLP in the presence of L-serine *O*-sulfate, AAT, and NEM. For experimental details, see text.

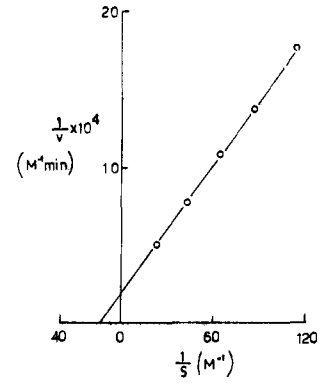
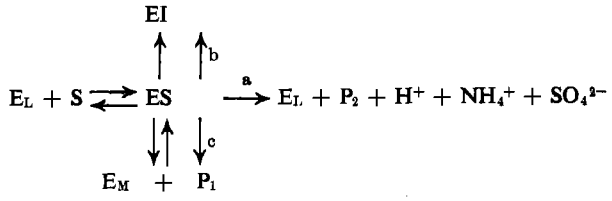


FIGURE 9: Double-reciprocal plot for AAT-catalyzed β elimination of L-serine *O*-sulfate. For experimental details, see text.

ing respectively to β elimination (a), inactivation (b), or transamination (c)



where E_L = aldimine enzyme, E_M = amine enzyme, ES = enzyme–substrate complex, EI = inactivated enzyme, P_1 = hydroxypyruvate sulfate, P_2 = pyruvate, and S = serine *O*-sulfate.

In the experiment of Figure 1A, where pyruvate is continuously removed, both β elimination and inactivation occur rapidly at first; with the progress of the reaction, however, the rates for both these reactions decrease because a slow transamination with L-serine *O*-sulfate converts some of the aldimine enzyme into the amine enzyme which is unable to catalyze β elimination or to undergo inactivation. This slow transamination continues until an equilibrium is established between the aldimine and the amine forms of the enzyme L-serine *O*-sulfate and hydroxypyruvate sulfate; during the establishment of this equilibrium, the rate of β elimination falls as a result of both inactivation and formation of amine enzyme. When equilibrium in the transaminating system has been reached, β elimination continues at a rate which is reduced only by inactivation. This phase corresponds to the latter part of the curve shown in Figure 1A.

Under the conditions of Figure 1B, the pyruvate produced by β elimination is allowed to accumulate and to transaminate with the amine enzyme regenerating the aldimine enzyme which is capable of catalyzing β elimination and of undergoing inactivation. Both the latter events proceed therefore at a higher rate when pyruvate is allowed to accumulate (Figure 1B) than when it is removed (Figure 1A).

When pyruvate, or another keto acid, is added initially, the transamination equilibrium is so strongly in favor of the aldimine form of the enzyme, that the formation of amine enzyme becomes negligible and the progress curves for β elimination and for inactivation no longer have the biphasic form attributed to the slow transamination between aldimine enzyme and L-serine *O*-sulfate.

That both β elimination and inactivation occur seems well established, but the evidence for transamination is not quite so strong. Assuming that transamination is responsible for the biphasic progress curve of Figure 1A, an estimate of the amount of transamination occurring can be made from the data of this figure and of Figure 6C. Such a calculation shows that under the conditions described in these figures less than 40% of the total enzyme present would eventually transaminate to the amine form, *i.e.*, 1 ml of incubation mixture would contain a maximum of 8×10^{-10} mole of transamination products under the conditions of Figure 1A and a maximum of 4×10^{-10} mole under the conditions of Figure 6C. This makes the detection of these products extremely difficult. However, by using special conditions (*cf.* Results), spectrofluorimetric evidence for transamination between L-serine *O*-sulfate and the coenzyme of AAT could be obtained. Moreover, the effect of keto acids on the reaction progress provides strong indirect evidence for transamination. These observations, coupled with the fact that the enzyme's normal function is to catalyze transamination, seem to justify the proposal that the aldimine enzyme slowly transaminates with L-serine *O*-sulfate.

It is known that aspartate aminotransferase can catalyze either β elimination or transamination depending upon the nature of the substituent in the β position of the substrate. This can be readily accounted for in terms of the classical Snell–Braunstein theory on the mechanism of action of pyridoxal enzymes (Metzler *et al.*, 1954; Braunstein, 1964). When the substrate is L-aspartate or L-glutamate, transamination occurs; when the substrate is *threo*-β-chloro-L-glutamate, β elimination occurs (Manning *et al.*, 1968). The substrate L-serine *O*-sulfate provides a new case in that both reactions occur at the same time. This system is remarkable in that the same active site participates in two reactions, one of which is capable of destroying (or regenerating) the coenzyme necessary for the other; a similar case has been described by Novogrodsky *et al.* (1963) for aspartate ω-decarboxylase. As pointed out by these authors, such phenomena could form the basis of metabolic controls systems. In AAT, aspartate decarboxylase and possibly other pyridoxal-dependent enzymes, the interconversion of the forms through which regulation would be mediated does not depend upon conformational changes or on association–dissociation phenomena, but rather on the simple conversion of a functional group in the active site from a state CH=N, which can combine with the substrate for β elimination, to a state CH_2NH_2, which cannot. Further data on this

point have been recently presented by Tate and Meister (1969).

It is worthwhile comparing the kinetic parameters for the β elimination of L-serine *O*-sulfate with those for the reactions catalyzed by the same enzyme on other substrates. An understanding of the various reactions occurring in the present complicated system suggested the experimental conditions in which β elimination could be studied without significant interference from the other reactions. In the presence of small amounts of keto acids the effect of transamination is practically eliminated. The equilibrium for transamination is such that this can be achieved with 2-oxoglutarate concentrations much lower than those which competitively inhibit the enzyme (Jenkins and Sizer, 1959). Moreover, at enzyme concentrations of less than 10^{-7} M inactivation is sufficiently slow to allow an accurate estimate of the initial rate of the β-elimination reaction. Under the conditions shown in Figure 9, the K_m for L-serine *O*-sulfate is 7×10^{-2} M and k_{cat} is 12 sec^{-1} ($k_{cat} = V_{max}$/enzyme concentration). Although values for K_m obtained under different conditions of pH, ionic strength, and type of anions cannot be precisely compared, present data suggest that L-serine *O*-sulfate has an affinity for the aldimine form of AAT a little lower than that for L-glutamate (Jenkins and D'Ari, 1966) and for *threo*-chloro-L-glutamate (Manning *et al.*, 1968) and considerably lower than that for L-aspartate (Jenkins and Taylor, 1965) for α-methylaspartate (Fasella *et al.*, 1966; Hammes and Tancredi, 1967) and for *erythro*-hydroxyaspartate (Jenkins, 1964). The catalytic rate constant for the elimination of L-serine *O*-sulfate is similar to that for *threo*-chloro-L-glutamate (Manning *et al.*, 1968) and more than one order of magnitude lower than that for the rate of the slowest step in the transamination between the aldimine form of AAT and its real substrates L-aspartate and L-glutamate (Fasella and Hammes, 1967; Banks *et al.*, 1968). The rate of the β elimination from L-serine *O*-sulfate catalyzed by AAT is nevertheless 10^4 times greater than the corresponding rate in a nonenzymic model system consisting of $CuCl_2$ and PLP (R. A. John, unpublished results).

Inactivation by substrate analogs has been described for other PLP enzymes, namely, L-aspartate ω-decarboxylase (Miles and Meister, 1967) and threonine dehydrase (Labow and Robinson, 1966; Moss *et al.*, 1965; Boll and Holzer, 1965; McLemore and Metzler, 1968; Nishimura and Greenberg, 1961; Phillips and Wood, 1965). Inactivation of AAT by L-serine *O*-sulfate, however, is novel in that the inactivation is irreversible (John *et al.*, 1968). Using uniformly labeled L-[^{14}C]serine *O*-sulfate it has been shown that inactivation is accompanied by the stable binding of a C-3 residue to the enzyme. After denaturation and partial digestion, the radioactivity remains attached to a peptide fraction (John and Fasella, 1968). The chemical composition of the radioactive peptide and of the L-serine *O*-sulfate–AAT derivative will be reported elsewhere. Chemical considerations suggest that the enzyme might be inactivated by alkylation of a group at or near the active center by an aminoacrylic intermediate formed during the β-elimination reaction. The amino group of this intermediate would interact with the coenzyme causing the observed permanent shift of the absorption maximum from 362 mμ in the native aldimine enzyme to about 345 mμ in the inactive enzyme (*cf.* Figure 4). These findings suggest a general approach to the specific inactivation and labeling of pyridoxal enzymes, through substrate analogs.

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