Reactions of Glutamate 1-Semialdehyde Aminomutase with R- and S-Enantiomers of a Novel, Mechanism-Based Inhibitor, 2,3-Diaminopropyl Sulfate†

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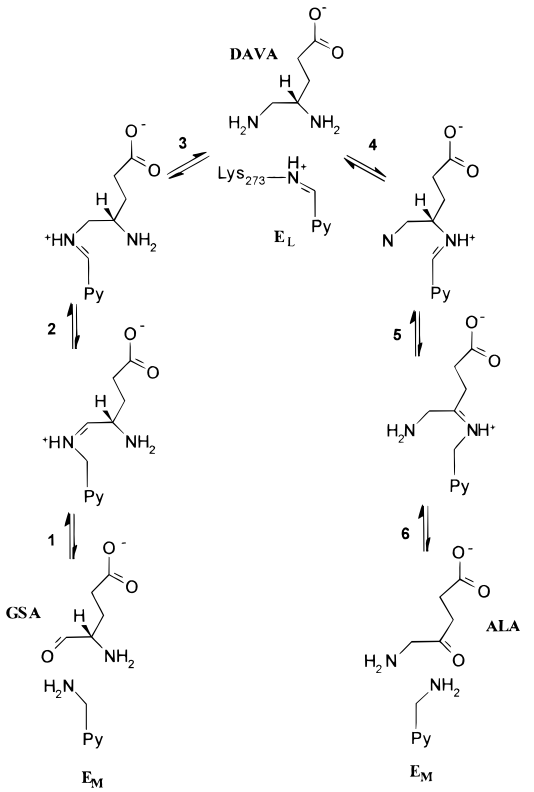
ABSTRACT: Glutamate semialdehyde aminomutase is a recognized target for selective herbicides and antibacterial agents because it provides the aminolevulinate from which tetrapyrroles are synthesized in plants and bacteria but not in animals. The reactions of the enzyme with R- and S-enantiomers of a novel compound, diaminopropyl sulfate, designed as a mechanism-based inhibitor of the enzyme are described. The S-enantiomer undergoes transamination without significantly inactivating the enzyme. The R-enantiomer inactivates the enzyme rapidly. Inactivation is accompanied by the formation of a 520 nm-absorbing chromophore and by the elimination of sulfate. The inactivation is attenuated by simultaneous transamination of the enzyme to its pyridoxamine phosphate form but inclusion of succinic semialdehyde to reverse the transamination leads to complete inactivation. The inactivation is attributed to further reactions arising from generation of an external aldimine between the pyridoxal phosphate cofactor and the 2,3-diaminopropene that results from enzyme-catalyzed β-elimination of sulfate.

In plants, the 5-aminolevulinate from which tetrapyrroles such as heme and chlorophyll are assembled is made from glutamate 1-semialdehyde in a reaction catalyzed by the pyridoxal phosphate-dependent enzyme, glutamate semialdehyde aminomutase (*1*). This enzyme is absent from mammals, which instead synthesize aminolevulinate from glycine and succinyl-CoA in a reaction catalyzed by a different enzyme (*2*). Because no counterpart of glutamate semialdehyde aminomutase exists in animals, the enzyme is a potential target for safe, selective herbicides and antibacterial agents.

Although glutamate semialdehyde aminomutase catalyzes the exchange of amino and oxo functions within the same molecule, it is clear that the enzyme is structurally homologous to the aminotransferases (*3*) and that the catalytic mechanism (Scheme 1) is analogous to classical transamination (*4*, *5*). Successful mechanism-based inhibitors of aminotransferases have been designed by including a good leaving group at the β-carbon of an amino acid, thereby producing a structure which resembles the true substrate sufficiently to bind at the active site and to undergo all of the catalytic steps of the natural reaction up to and including proton abstraction from Cα (*6*). Elimination of the β-substituent at this point in the reaction produces an enamine which is not a normal intermediate and which reacts covalently with the enzyme thereby inactivating it. Sulfate is both a good leaving group and carries a formal negative charge. Thus, serine-*O*-sulfate, a close structural analogue,

Scheme 1: Glutamate Semialdehyde Aminomutase Reaction Mechanism with Natural Substrate[a]



[a] The reaction begins and ends with the enzyme in the E_M form. GSA, glutamate 1-semialdehyde; DAVA, 4,5-diaminovalerate; ALA, 5-aminolevulinate.

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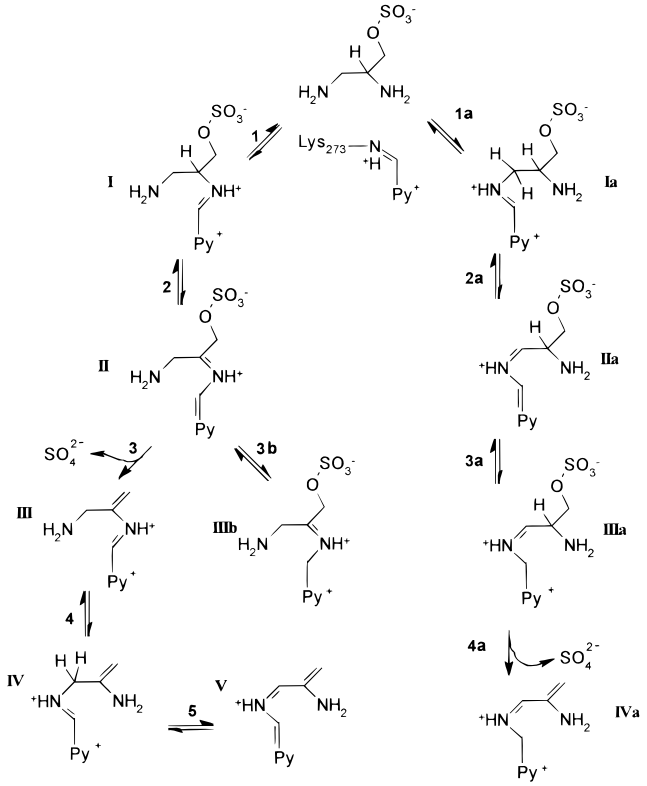
Scheme 2: Mechanisms Proposed for Multiple Reactions of the Enantiomers of 2,3-Diaminopropyl Sulfate with Glutamate Semialdehyde Aminotransferase



of glutamate is an effective mechanism-based inhibitor of aspartate aminotransferase (*7*, *8*). Correspondingly, ethanolamine-*O*-sulfate, an analogue of the inhibitory neurotransmitter 4-aminobutyrate (GABA), selectively inactivates 4-aminobutyrate aminotransferase (*9*).

The glutamate semialdehyde aminomutase-catalyzed reaction (Scheme 1) begins and ends with the enzyme in the pyridoxamine form (E_M)[1], and the initial reaction is with the oxo-group of glutamate semialdehyde (*4*, *5*). However, as in the oxo acid to amino acid half-reaction of classical transamination, the catalytic mechanism requires the formation of a pyridoxaldimine form of the enzyme by net 1,3-prototropic shift from C-4′ of the coenzyme to the substrate (step 5, Scheme 1). The intermediate, 4,5-diaminovalerate, dissociates from this form of the enzyme to some extent (*10*) to give significant concentrations of a form of the enzyme (E_L) in which the cofactor is present as an internal aldimine of pyridoxal phosphate with a lysine residue. Because free diaminovalerate reacts rapidly with E_L (*11*), the structurally analogous 2,3-diaminopropyl sulfate might be expected to react but to undergo β-elimination (Scheme 2, step 3) with possible inactivation of the enzyme by the enamine (Scheme 2, structure III) that is generated.

Earlier work with aminotransferases has shown that multiple reactions occur with artificial substrates of this kind because the mechanism includes partitions at which intermediates can undergo more than one reaction 7. Hence, β-elimination, transamination, and inactivation might be expected to occur simultaneously during the reaction of diaminopropyl sulfate with glutamate semialdehyde aminomutase. Measurement of sulfate production and residual enzyme activity provide convenient methods for measuring β-elimination and inactivation, respectively. Transamination is measurable by changes in absorption spectrum and effects on the other two reactions that are reversed by an appropriate oxo substrate. In this paper, we report the interactions of both R- and S-enantiomers of 2,3-diaminopropyl sulfate with glutamate semialdehyde aminomutase. It should be noted that the R-enantiomer is sterically equivalent to (*S*)-diaminovalerate, the natural form of this dissociable intermediate.

EXPERIMENTAL PROCEDURES

Materials. Details of the synthesis of the enantiomers of 2,3-diaminopropyl sulfate will be reported elsewhere. Before use, both compounds were separated from contaminating inorganic sulfate and diaminopropanol by ion-exchange partition. Each compound (50 mg) dissolved in 0.5 mL of water was loaded on 4 cm × 1 cm column of Dowex-1 (1 × 4−400) equilibrated with 0.1 M NH_4OH. The column was washed with water (20 mL) and eluted with 0.5 M HCl. Fractions containing diaminopropyl sulfate, detected by thin-layer chromatography, were dried. Purity was checked by paper electrophoresis in 70 mM potassium phosphate buffer, pH 7.0. Inorganic sulfate was removed on a Dowex 50W (1 × 4−400) anion-exchange column (1 cm × 4 cm, H^+ form in water), washed with water and eluted with 0.5 M NH_4-OH. Fractions containing diaminopropyl sulfate (identified by thin-layer chromatography) were freeze-dried repeatedly to eliminate residual ammonia.

(*S*)-Glutamate-1-semialdehyde was synthesized by ozonolysis of 12 mg of (*S*)-4-aminohex-5-enoate (a gift from Hoechst Marion Roussel, Cincinnati, OH) dissolved in 1 mL of 0.5 M HCl (*13*, *14*). Ozone was bubbled for 4 min at 0 °C (0.42 mmol min^{-1}) by supplying a Wallace and Tiernan type B ozonator with oxygen at a rate of 25 1 h^{-1}. The purity of the glutamate-1-semialdehyde was confirmed by thin-layer chromatography. All other reagents were from Sigma-Aldrich Co. Ltd., U.K. Wild-type glutamate semialdehyde aminomutase was expressed, purified, and converted to its pyridoxaldimine form as described (*11*).

Measurement of Enzyme Activity, Diaminopropyl Sulfate, and Inorganic Sulfate. Enzyme activity was measured in the presence of 4,5-diaminovalerate as described earlier (*14*). Diaminopropyl sulfate was quantified by its reaction with *o*-phthaldialdehyde. Solutions containing the compound (30 μL) were mixed with an equal volume of 60 mM *o*-phthaldialdehyde containing 230 mM 2-mercaptoethanol in 0.1 M $NaHCO_3$ at pH 10. After 5 min, absorbance at 452 nm was measured, and concentrations were calculated using a value of $\epsilon_{452} = 5800\ M^{-1}\ cm^{-1}$ established in separate experiments. Inorganic sulfate was measured by turbidimetry (*15*).

Kinetic Procedures. Stopped flow experiments were carried out on a SF-1 stoppped-flow spectrophotometer (Hi-Tech, Salisbury, U.K.). Data were acquired and displayed using the IS-2 software suite provided with the instrument. Product formation during the period from 0.2 to 7.0 s was measured by stopping the reaction at appropriate times using a quenched-flow apparatus (*16*). Reactions lasting longer than

[1] Abbreviations: E_M, glutamate semialdehyde aminomutase with the cofactor as pyridoxamine 5′-phosphate; E_L, Glutamate semialdehyde aminomutase with the cofactor as an internal aldimine of pyridoxal 5′-phosphate with a Lys273.

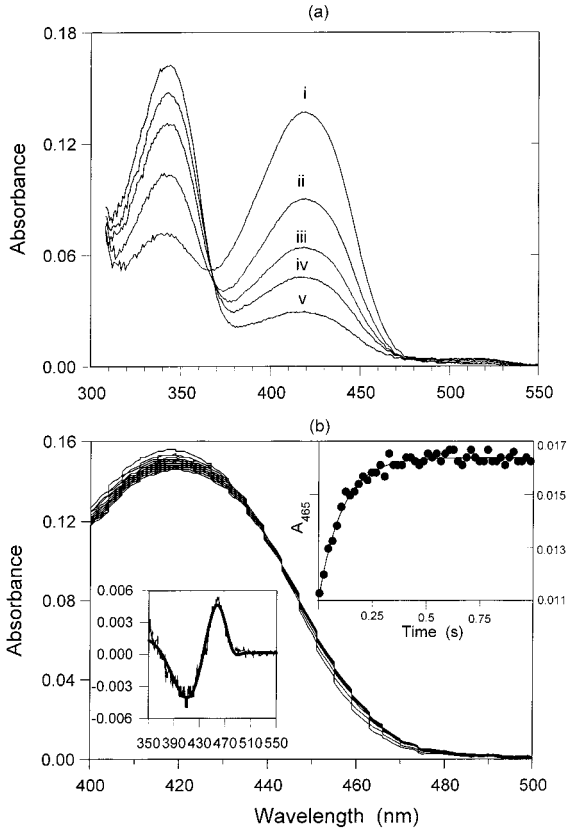


FIGURE 1: Changes in absorption spectrum of glutamate semialdehyde aminomutase during reaction with (*S*)-diaminopropyl sulfate. The enzyme (15 μM) in 0.1 M tricine buffer, pH 7.9, was stopped-flow mixed with (*S*)-diaminopropyl sulfate (3.5 mM) dissolved in the same buffer, and spectra were recorded at intervals. (a) Spectra i−iv recorded at 10 s intervals, spectrum v after 5 min. (b) Changes occurring near 460 nm, spectra recorded at 20 ms intervals. Absorbance increase at 465 nm (●) was fitted to an exponential process. The rate constant for the fit is 9.4 s^{-1}. The difference spectrum shown in the lower inset was calculated by subtracting the first spectrum from that at 1 s. The smooth line is that returned as the best fit of the difference between two log-normal curves.

7.0 s were stopped manually. Simulation of processes containing reversible steps, curve-fitting procedures, and statistical analyses were performed using the data manipulation software of Scientist (Micromath, Salt Lake City). Slow changes in absorption spectra were determined with a Hewlett-Packard model 8452 diode-array spectrophotometer. The following equations were used to fit data:

$$k_{obs} = \frac{k_{obs}}{K_d + [S]} \quad (1)$$

$$P = \frac{k_{cat}k_1}{(k_1 + k_2)^2} E_L^0\{1 - e^{-(k_1+k_2)t}\} + \frac{k_{cat}k_2}{k_1 + k_2} E_L^0 t \quad (2)$$

RESULTS

Changes in Absorption Spectrum and Inactivation. Both R- and S-enantiomers of diaminopropyl sulfate reacted with the enzyme to produce rapid changes in the absorption spectrum of the enzyme-bound cofactor. The spectral changes were distinctly different for each enantiomer.

The major change observed with the S-enantiomer (Figure 1a) was the conversion of the chromophore absorbing maximally at 420 nm due to the E_L form of the enzyme, to one absorbing maximally at 340 nm. This absorbance change followed an exponential course and was characterized by an observed rate constant that increased hyperbolically toward a limiting value as (*S*)-diaminopropyl sulfate concentration was increased. The data fitted well to eq 1 with best-fit values of $k_{max} = 0.055 \pm 0.005$ s^{-1} and $K_d = 5 \pm 1$ mM. Addition of succinic semialdehyde to the 340 nm-absorbing form present at the end of the reaction converted the enzyme back to its initial 420 nm absorbing E_L form. Two additional minor reactions were evident. A small exponential decrease in absorbance at 420 nm ($k = 9.4$ s^{-1}) coincided with a small but clear transient increase at approximately 460 nm (Figure 1b). The difference spectrum for this change (Figure 1b) was calculated by subtracting the initial spectrum from that at 1 s. The components of the difference spectrum were determined by best-fitting the data to the difference between two log-normal curves (*17*). The fit returned λ_{max} values of 418 and 425 nm for the first and last spectrum. During the slow reaction which produced the major transition between 420 and 340 nm species, a small increase at 520 nm also occurred with the same rate constant (0.02 s^{-1}) as the main 420 to 340 nm conversion. Assay of enzyme activity in samples taken from the reaction mixtures, including those to which succinic semialdehyde had been added, showed no detectable loss of enzyme activity at any stage in the reaction. In the presence of 15 μM enzyme and sufficient succinic semialdehyde to reverse the transamination completely, inorganic sulfate was formed at a steady rate of 0.023 μM s^{-1}, allowing the elimination rate constant for the S-enantiomer to be estimated as 0.0016 s^{-1}.

The reaction of E_L with (*R*)-diaminopropyl sulfate produced very different changes in the enzyme absorption spectrum (Figure 2). The major change was a large, initially rapid, increase in absorbance with a maximum at 520 nm. The increase in absorbance was biphasic, the faster phase (Figure 2a) being complete in a few seconds and the slower increase lasting for approximately 1 h (Figure 2b). Thereafter, absorbance decreased over more than 24 h. The increase at 520 nm in the fast phase was accompanied by a smaller increase at 340 nm and by a decrease at 420 nm. The slower increase at 520 nm in the second phase was accompanied by a decrease at 340 nm. The 420 nm chromophore was absent throughout the slow phase. Inclusion of succinic semialdehyde in the reaction mixture produced significant changes in the reaction profile (Figure 3). The amplitude of the increase in A_{520} almost doubled and the reaction became monophasic. Measurement of enzyme activity in samples withdrawn at intervals from identical solutions showed that inactivation paralleled the increase in 520 nm absorbance (Figure 3). (However, note that the inverted percentage scale showing enzyme activity is slightly different for the two sets of data.) This is because the extent of inactivation per unit absorbance increase was larger when succinic semialdehyde was included.

The course of the reaction observed at 520 nm depended systematically on both succinic semialdehyde and (*R*)-diaminopropyl sulfate concentration (Figure 4). Data taken from the first 250 s, before the decrease in A_{520} had made a significant contribution, fitted well to two exponential processes. The complete data set obtained at four (*R*)-diaminopropyl sulfate concentrations was globally fitted to

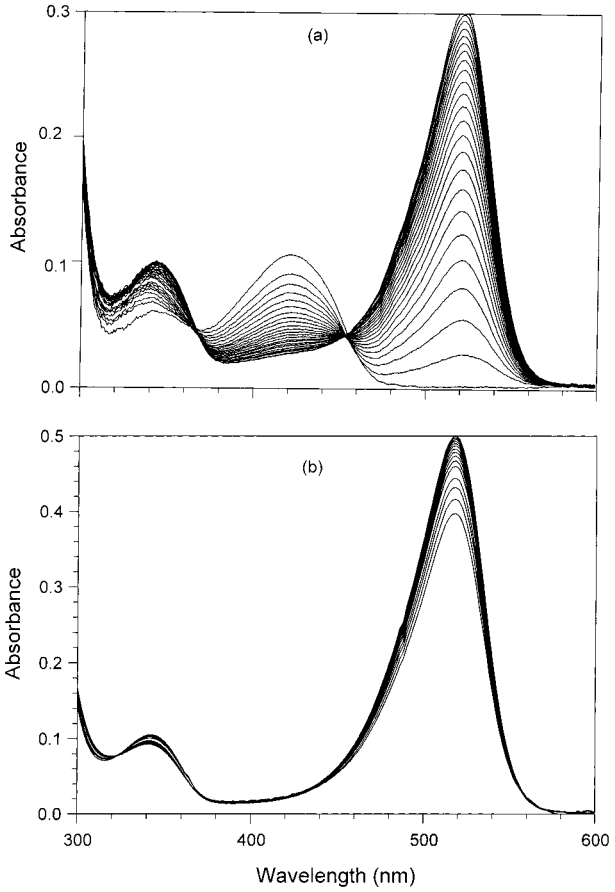


FIGURE 2: Changes in absorption spectrum of glutamate semialdehyde aminomutase during reaction with (*R*)-diaminopropyl sulfate. (a) The enzyme (15 μM) in 0.1 M tricine buffer, pH 7.9, was mixed with (*S*)-diaminopropyl sulfate (10 mM) dissolved in the same buffer. Absorption spectra were recorded at 0.24 s intervals. (b) Spectra recorded at 2 min intervals beginning 2 min after mixing so that the changes associated with the two stages in the reaction are well separated.

Scheme 3 by numerical integration of the relevant differential rate equations. In Scheme 3, *Q* is considered to be the species that absorbs at 520 nm.

The constants of best fit were $K_s = 1.83 \pm 0.01$ mM, $k_1 = 0.077 \pm 0.001$ s^{-1}, $k_2 = 0.0072 \pm 0.0003$ s^{-1}, $k_3 = 0.3037 \pm 0.0002$, and ϵ_{520} for $Q = 32\,900 \pm 170$ M^{-1} cm^{-1}.

After approximately 1 h, A_{520} reached a maximum and then decreased exponentially toward zero with a half-time of 9 h. No 520 nm-absorbing chromophore was present in the spectrum after 48 h, and no further detectable changes occurred. A broad and poorly defined maximum at 330 nm was evident. The enzyme sample was inactive.

Formation of Inorganic Sulfate and Depletion of Substrate. The reaction of both enantiomers with the enzyme resulted in production of inorganic sulfate. In both cases, a burst was followed by a slow, almost constant rate of sulfate production. The rate of depletion of (*R*)-diaminopropyl sulfate was also monitored and found to coincide closely with sulfate production except that a burst of greater amplitude was observed (Figure 5). In the case of the S-enantiomer, both the burst and the steady-state rate of sulfate production were approximately three times greater than in the case of the R-enantiomer. We were unable to measure substrate depletion with the S-enantiomer because of shortage of material.

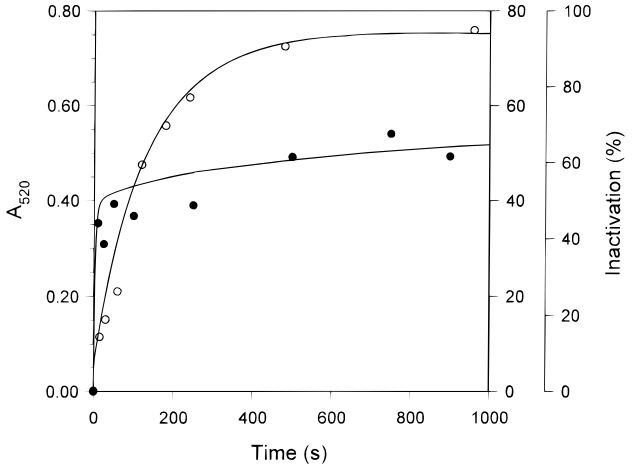


FIGURE 3: Correlation of changes in absorbance at 520 nm with loss of enzyme activity during reaction with (*R*)-diaminopropyl sulfate. Experimental conditions as in Figure 2. The continuous lines are A_{520}. The experimental points show loss of enzyme activity on an inverted scale in the absence of succinic semialdehyde (○) and in the presence of 5 mM succinic semialdehyde (●). Enzyme activity was measured after stopping the inactivation by mixing 20 μL of solution with 280 μL of 4 mM DAVA in 0.1 M tricine/HCl, pH 7.9. Percentage inactivations in brackets are for the experiment with succinic semialdehyde.

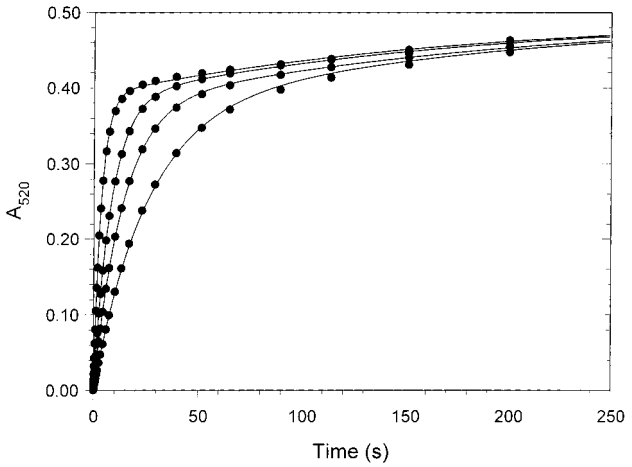
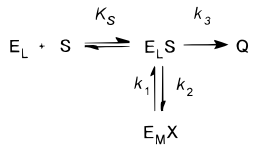


FIGURE 4: Dependence of reaction on (*R*)-diaminopropyl sulfate concentration. The increase in A_{520} was measured for 250 s in reactions conducted at a series of R-diaminopropyl sulfate concentrations. Each data set was fitted to Scheme 3 by numerical integration of the appropriate differential equations. All of the data were used in the fit but, for clarity, only one in 25 of the data points is shown

Scheme 3



Reactivation. The ability of the inactivated enzyme to recover its activity was measured after separating it from diaminopropyl sulfate by gel filtration on Sephadex G-25 (Figure 6). When the separation was carried out after a 1 h reaction with 1 mM (*R*)-diaminopropyl sulfate and 5 mM succinic semialdehyde, i.e., at the point when A_{520} had reached its maximum level, 95% of the activity returned in 5 h in an exponential process governed by a rate constant of 0.54 h^{-1}. The fitting process predicted that 98.3% of the

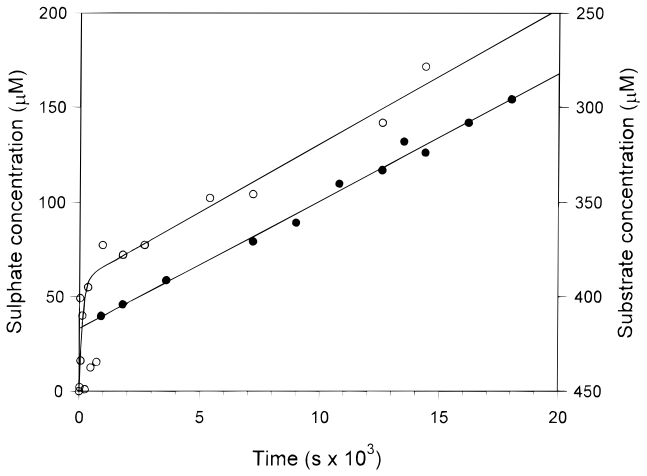


FIGURE 5: Production of sulfate and depletion of substrate. The enzyme (15 μM) was reacted with (*R*)-diaminopropyl sulfate (450 μM). Inorganic sulfate (○) and (*R*)-diaminopropyl sulfate (●) were measured in samples removed at intervals. Experimental conditions as in Figure 1. The continuous line through the points indicating (*R*)-diaminopropyl sulfate concentration were predicted by eq 2 using values of $(k_1 + k_2) = k_{obs} = 0.0075$ s^{-1} (taken from A_{520} increase in the presence of succinic semialdehyde); $k_{cat}k_1/(k_1 + k_2)^2$-$[E_0]$ = burst = 60 μM; $k_{cat}k_2/(k_1 + k_2)[E_0] = 0.006$ μM s^{-1} steady state decrease in diaminopropyl sulfate (from this figure).

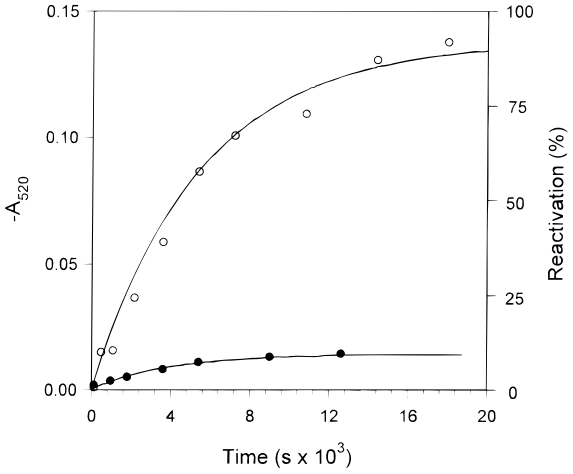


FIGURE 6: Restoration of enzyme activity. The enzyme (15 μM) was reacted with DAPS (450 μM) for 1 h (○) or 71 h (●) at 25 °C before separating on a column of Sephadex G-25 to stop the reaction. Samples (20 μL) were removed at intervals and assayed for enzyme activity. Specific activity was calculated using $\epsilon_{280} =$ 42 800 M^{-1} cm^{-1}.

original activity would return at infinite time. When (*R*)-diaminopropyl sulfate (10 mM) and the enzyme were mixed in the presence of succinic semialdehyde (10 mM) and left for 71 h before gel filtration, much less activity returned (14.5% predicted at infinite time) although the course of reactivation was governed by the same rate constant.

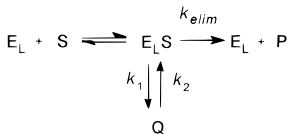
DISCUSSION

The differences between the reactions that occur when the E_L form of glutamate semialdehyde aminomutase is treated with (*S*)- or (*R*)-diaminopropyl sulfate are clearly due to the different stereochemistry at C-2. The main absorbance change observed when the enzyme is reacted with the S-enantiomer is the same as that occurring with the natural intermediate, diaminovalerate, in that the 420 nm absorbance is converted to 340 nm absorbance in a reaction that is reversed by succinic semialdehyde. It seems certain that, as in the case of diaminovalerate, these changes are due to reversible conversion of the enzyme to the pyridoxamine form and we conclude that the S-enantiomer acts predominantly as a transamination substrate. The main absorbance change observed with the R-enantiomer is the very large increase at 520 nm in a process that inactivates the enzyme. Chromophores with absorbance maxima at approximately 500 nm in reactions of pyridoxal-dependent enzymes are generally considered to be due to the extensively delocalized carbanion-quinonoid structures that occur when a proton is abstracted from Cα (*19*). Clearly a reaction, other than transamination, predominates with this enantiomer.

The difference between the enantiomers can be interpreted in terms of Scheme 2. It seems probable that both enantiomers form the aldimines I and Ia through N2′ and N3′, respectively. However, we propose that, because (*R*)-diaminopropyl sulfate is sterically analogous to (*S*)-diaminovalerate, it binds so that its C-2 proton is held orthogonal to the cofactor ring plane such that it is readily abstracted through stabilization of the developing negative charge (*20*). Conversely, the C-2 proton of (*S*)-diaminopropyl sulfate, when bound to the coenzyme through its 2-amino group, would not be expected to be aligned at 90° to the coenzyme ring plane. Thus, C-2 deprotonation would not be favored and the N2′-external aldimine would have no other fate than to dissociate reversibly. It therefore seems likely that the main reactions of the S-enantiomer occur through formation of the N3′-external aldimine (Ia) in a manner analogous to reversal of the first half of the natural reaction. The process is approximately 25 times slower ($k = 0.055$ s^{-1}) than that observed with diaminovalerate itself ($k = 1.6$ s^{-1}, ref *18*). The rapid increase at 465 nm and accompanying fall at 420 nm shows that a detectable transient intermediate occurs. The λ_{max} of one of the components of the difference spectrum (418 nm) is exactly that of the protonated aldimine in the free enzyme. The transient chromophore that is formed in this process has $\lambda_{max} = 425$ nm, indicating that it is also a protonated aldimine. This could be I or Ia or a mixture of both. The small increase above 500 nm occurs simultaneously with the main spectral change from 420 to 340 nm, suggesting that it is due to rate-limiting formation of the quinonoid intermediate, IIa, in the transamination process. Sulfate elimination also occurs when succinic semialdehyde is included to maintain the enzyme in the E_L form so that it can react repeatedly with the (*S*)-diaminopropyl sulfate. Assuming that the C-2 proton is not abstracted from this enantiomer to a significant extent, this observation suggests that the aldimine IIIa can eliminate hydrogen sulfate in a β,γ-fashion (step 4a in Scheme 2). Elimination of SO_4^{2-} from the quinonoid IIa may also make a contribution but the spectra show that very little of this intermediate is present, presumably because it is reprotonated very much faster than it is formed. Estimates of elimination rate constant (0.0016 s^{-1}) and transamination rate constant (0.055 s^{-1}) for the S-enantiomer show that transamination is favored 34-fold.

Assigning a structure to the inactive, 520 nm-absorbing chromophore that is formed in such large amounts with (*R*)-diaminopropyl sulfate is necessarily speculative because of the limited evidence available. However, there seems to be no reason for the quinonoid intermediate (II) in Scheme 2

Scheme 4



to be particularly stable, and in the corresponding reactions with analogous sulfato amino acid analogues and the relevant aminotransferases, no corresponding intermediate is detectable. Elimination of sulfate by step 3 in Scheme 2 results in the intermediate III from which a quinonoid structure could be formed by attack of an enzyme nucleophile at Cβ. Although this possibility cannot be ruled out, there seems to be no reason for this quinonoid structure to be stable. A further quinonoid structure (V) would be possible after transaldimination (Scheme 2, step 4) between the two amino groups on the 2,3-diaminopropene that is formed when sulfate is eliminated. Formation of an external aldimine (IV) with the 3-amino group would allow stereospecific labilization of one of the C-3 protons as has been observed for the elimination of the C-2 proton from N4′-(2″-phosphoethyl)-pyridoxamine 5′-phosphate by glutamate decarboxylase (*21*). This would bring the vinyl group into conjugation in the resulting quinonoid structure (V) possibly accounting for the stability and high λ_{max} of the chromophore.

The biphasic course of the increase in A_{520} over the first 250 s of reaction fits well to Scheme 3 (Figure 4). E_MX in Scheme 3 seems likely to be the ketimine IIIb in Scheme 2. If the 520 nm chromophore Q in Scheme 3 is the structure shown as V in Scheme 2, it is necessary to propose that steps 3 and 4 are fast enough that aldimines III and IV do not accumulate. The abolition of the fast phase by inclusion of succinic semialdehyde supports this interpretation because it demonstrates that this phase is due to reversible conversion of the enzyme to a form that is not directly converted to the inactive 520 nm-absorbing chromophore. However, inclusion of succinic semialdehyde also lowered the initial rate of increase in A_{520} from 0.04 s^{-1} to 0.003 s^{-1}. This may be a secondary effect due to formation of an abortive complex between E_L and succinic semialdehyde.

The rate of (*R*)-diaminopropyl sulfate depletion decreased over several minutes at the beginning of the reaction, resulting in a burst of approximately 60 μM in product formation. The burst cannot be due to transamination because succinic semialdehyde was present in this experiment. Furthermore, the burst in the A_{520} profile attributed to transamination occurs on a much shorter time scale. It seems likely that this burst is due to slowing of the β-elimination reaction as a result of formation of the inactive 520 nm chromophore. The two sets of data can be accommodated by a model (Scheme 4) in which β-elimination slows down greatly as the enzyme converts almost completely but reversibly to the inactive 520 nm chromophore (Q in Scheme 4).

Equation, 2 which describes the process depicted in Scheme 4 assuming that [S] is essentially saturating throughout, provided an adequate fit to both sets of data (Figure 5).

Values for the constants in Scheme 4 can be estimated from the parameters used to fit the data of Figure 5. The β-elimination constant k_{elim} is estimated to be 0.31 s^{-1} but, because the pseudo-equilibrium between dead-end complex Q and E_LS is 75-fold in favor of Q ($k_1 = 0.0074$ s^{-1} and $k_2 = 0.0001$ s^{-1}), after completion of the burst, the actual rate of sulfate formation from (*R*)-diaminopropyl sulfate is three times slower than from the S-enantiomer. Scheme 4 also accounts for the very slow restoration of enzyme activity that occurs when the 520 nm chromophore is separated from (*R*)-diaminopropyl sulfate.

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REFERENCES

1. Kannangara, G., Gough, S. P., Bryant, P., Hoober, J. K., Kahn, A., and von Wettstein, D. (1988) *Trends Biochem. Sci. 13*, 139−143.
2. Jordan, P. M., and Shemin, D. (1972) in *The Enzymes* (Boyer, P. D., Ed.) 3rd ed., Vol. 7, 339−356, Academic Press, New York.
3. Hennig, M., Grimm, B., Contestabile, R., John, R. A., and Jansonius, J. N. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 4866−4871.
4. Smith, M. A., Kannangara, C. G., Grimm, B., and von Wettstein, D. (1991) *Eur J. Biochem. 202*, 749−757.
5. Pugh, C. E., Harwood, J. L., and John, R. A. (1992) *J. Biol. Chem. 267*, 1584−1588.
6. Palfreyman, M. G., Bey, P., Metcalf, B., and Sjoerdsma, A. (1987) *Essays Biochem. 23*, 28−81.
7. John, R. A., and Fasella, P. (1969) *Biochemistry 8*, 4477−4482.
8. Ueno, H., Likos, J. J., and Metzler, D. E. (1982) *Biochemistry 21*, 4387−4393.
9. Fowler, L. J., and John, R. A. (1972) *Biochem. J. 130*, 569−573.
10. Tyacke, R., Harwood, J., and John, R. A. (1993) *Biochem. J. 293*, 697−701.
11. Tyacke, R. J., Contestabile, R., Grimm, B., Harwood, J. L., and John, R. A. (1995) *Biochem. J. 309*, 307−313.
12. Grimm, B., Smith, A. J., Kannangara, C. G., and Smith, M. A. (1991) *J. Biol. Chem. 266*, 12495−12501.
13. Gough, S. P., Kannangara, C. G., and Bock, C. K. (1991) *Carlsberg. Res. Commun. 54*, 99−108.
14. Pugh, C., Nair, S, P. Harwood, J. L., and John, R. A. (1991) *Anal. Biochem. 198*, 43−46.
15. Sorbo, B. (1987) *Methods Enzymol. 143*, 3−6.
16. Eccleston, J. F., Messerschmidt, R. G., and Yates, D. W. (1980) *Anal. Biochem. 106*, 73−77.
17. Johnson, R. J., and Metzler, D. E. (1971) *Methods Enzymol. 18A*, 433−470.
18. Smith, M. A., King, P. J., and Grimm, B. (1998) *Biochemistry 37*, 319−329.
19. Schirch, V., and Slotter, R. A. (1966) *Biochemistry 5*, 3175−3181.
20. Dunathan, H. C. (1966) *Proc Natl. Acad. Sci. U.S.A. 55*, 712−716.
21. Tilley, K., Akhtar, A., and Gani, D. (1994) *J. Chem. Soc., Perkin Trans. 1*, 3079−3087.

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