Mechanisms and Stereochemistry of the Activation of (2*S*)- and (2*R*)-Serine *O*-Sulfate as Suicide Inhibitors for *Escherichia coli* Glutamic Acid Decarboxylase

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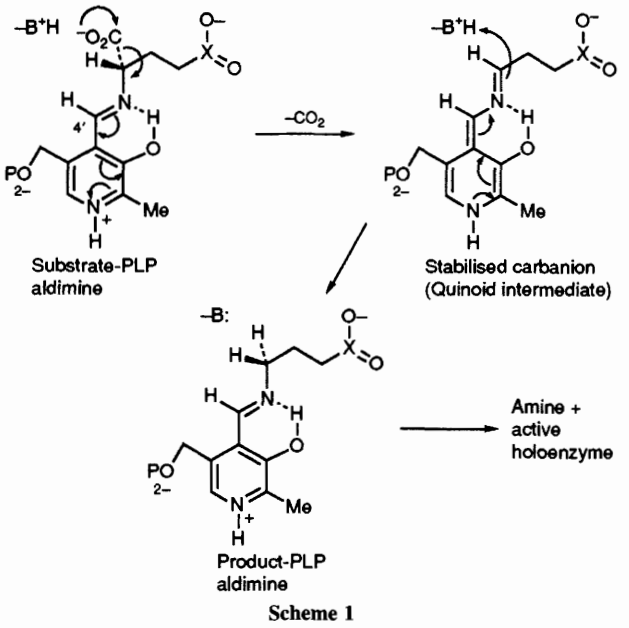
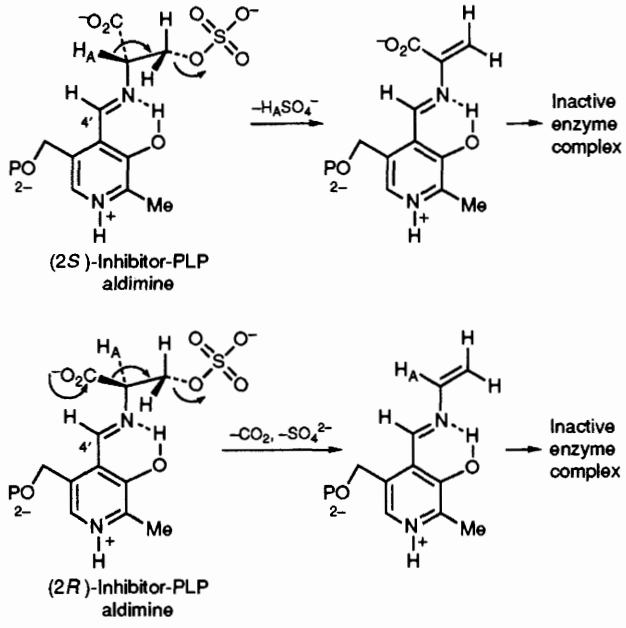
E. coli glutamic acid decarboxylase is inactivated by both enantiomers of the suicide inhibitor serine *O*-sulfate, inactivation by the (2*S*)-enantiomer involving C^{α}–H bond cleavage and inactivation by the (2*R*)-isomer involving C^{α}-decarboxylation; both processes occur on the 4′-*Re*-face of the coenzyme, the opposite face to that utilised in the physiological decarboxylation reaction.

Pyridoxal 5′-phosphate (PLP) dependent glutamic acid decarboxylase (GAD) catalyses the decarboxylation of (2*S*)-glutamic acid **1** to give γ-aminobutyric acid (GABA) and carbon dioxide. The enzyme is completely specific for the (2*S*)-enantiomer of the substrate and the reaction occurs with retention of configuration[1] at C^{α} on the 4′-*Si*-face of the coenzyme.[2] Thus, the distal anionic ω-carboxylate group resides on the 3′-OH side of the coenzyme, Scheme 1, X = C.

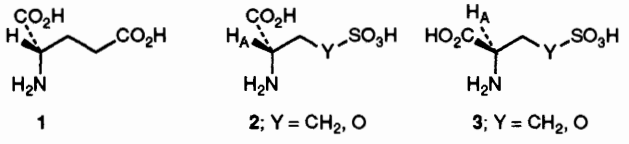
Replacing the ω-carboxylate group in glutamic acid by a sulfonic acid group gives homocysteic acid **2** (Y = CH_2) and, as expected, only the (2*S*)-enantiomer serves as a substrate for the enzyme, Scheme 1, X = SO. However, in contrast to expectations, Metzler and coworkers had shown that the isosteric glutamic acid analogue, (2*S*)-serine *O*-sulfate ester **2** (Y = O) did not undergo decarboxylation during a suicide inhibition process but, instead, underwent α,β-hydrogen



Scheme 1



Scheme 2



sulfate elimination.[3] Given that the carboxy groups of the external aldimines of both compounds, homocysteic acid and serine *O*-sulfate, should be optimally disposed for C^{α}–CO_2 bond cleavage on the 4′-*Si*-face of the coenzyme, Scheme 1, this was a curious result and hitherto could not be explained. Moreover, our finding that (2*R*)-serine *O*-sulfate **3** (Y = O) acts as an irreversible inhibitor for GAD could not be rationalised within the context of the known chemistry of pyridoxal phosphate. Clearly this latter suicide substrate would be expected to eliminate hydrogen sulfate without decarboxylation, given that its α-proton should be correctly disposed for removal by the conjugate base of the active site acid which serves to protonate the stabilized carbanion of GABA following the decarboxylation step in the physiological reaction, Scheme 1.[4]

In order to verify and further probe Metzler's proposed mechanism for suicide inactivation by (2*S*)-serine *O*-sulfate[3] and to gain insight into the mechanism of inactivation by the (2*R*)-enantiomer, α-deuteriated (2*S*)- and (2*R*)-serine were prepared.[5] Samples of the unlabelled and deuteriated serines were then converted to the corresponding *O*-sulfates **2** (Y = O, H_A = H and ^{2}H) and **3** (Y = O, H_A = H and ^{2}H),† using literature methods,[6] and the rates of GAD inactivation were measured‡ for each of the isotopmers at a range of concentrations. Using these data, the deuterium isotope effects were determined for each enantiomer.

For the (2*S*)-enantiomer, the observed isotope effects were normal; ^{D}V (or k_H/k_D) was 1.0 and $^{D}(V/K)$ [or $(k_H/k_D)/(K_H/K_D)$] was 2.3, suggesting that C^{α}–H bond cleavage does, indeed, occur during the inactivation of GAD. The large size of $^{D}(V/K)$ compared to ^{D}V indicates that the reaction commitments to C–H bond cleavage are not large and that C–H bond cleavage is not the most significant transition state in the inactivation process (see Cleland[7] for a review on the interpretation of isotope effects). Thus, the inactivation of GAD by (2*S*)-serine *O*-sulfate involves the removal of a proton from the 4′-*Re*-face of the coenzyme.

For (2*R*)-serine *O*-sulfate, the observed isotope effects for inactivation were inverse at all measured concentrations of the suicide substrate. The averaged apparent values of k_H/k_D ranged from 0.9 at 2 mmol dm^{-3} to 0.34 at 7 mmol dm^{-3} and, while clearly inverse, ^{D}V could not be determined accurately from the narrow range of inhibitor concentrations amenable to kinetic analysis. Nevertheless, the result indicates that C^{α}–H bond cleavage does not occur during the inactivation process and suggests that C^{α}–CO_2^- bond cleavage occurs. The observed values of the isotope effect are too large to represent only the expression of secondary α-isotope effects, where α-H is more stiffly bonded in the transition state than in the external aldimine,[8] but could represent the supression of alternative pathways for substrate processing (upon the introduction of deuterium) which do not cause enzyme inactivation (an induced isotope effect), or a combination of both.

Interestingly, the observed large inverse isotope effect is not easily accommodated by a stepwise mechanism where the loss of carbon dioxide gives a pyridoxal-stabilised carbanion which slowly eliminates sulfate. This is because any such second slow elimination step would dominate the reaction coordinate profile and mask the isotope effect.

Note that the determined value of $^{D}(V/K)$ for the inactivation of GAD by (2*R*)-serine *O*-sulfate was 1.0 indicating that there is a large reaction commitment to the first isotopically sensitive step and that the suicide substrate is extremely sticky,[7] in contrast to the (2*S*)-enantiomer.

In order to verify the unexpected conclusions that (2*S*)- and (2*R*)-serine *O*-sulfate lose a proton and a carboxy group respectively in their inactivations of GAD, samples of (2*S*)-[U-^{14}C]- and (2*RS*)-[U-^{14}C]serine *O*-sulfate were prepared from the appropriately labelled serines. The uniformity of the label in the (2*S*)-[U-^{14}C]serine was checked by chemical decarboxylation[9] and the racemic material was prepared from

† All compounds and intermediates gave the expected spectral and analytical data. The chiral integrity of the C^{α}-centre in samples of serine *O*-sulfate was verified by examination of the *N*-(1*S*,4*R*)-camphanamide derivatives.

‡ Inactivation rates were determined by measuring residual activity in aliquots of the enzyme–inhibitor solution at various times after the addition of the inhibitor, using (2*S*)-[1-^{14}C]glutamic acid as the assay substrate. Plots of $\log_e$(activity) *versus* time gave straight lines of slope $-k_{App}$ for any given concentration of the suicide substrate where k_{cat} (for inactivation) and K_m were determined by fitting values of k_{App} at different substrate concentrations to the Michaelis–Menten equation. Isotope effect errors are less than ±20% of the quoted values.

this sample *via* azlactone racemisation. Each of the ^{14}C-serine *O*-sulfates was incubated with GAD and any liberated CO_2 was collected in barium hydroxide solution. The radioactivity of the incubation solutions and the barium hydroxide solutions were determined by scintillation counting when the inactivations were complete. No $^{14}CO_2$ was released during the inactivation of the enzyme by the (2*S*)-isomer, but the sample of (2*RS*)-[U-^{14}C]-serine *O*-sulfate gave $^{14}CO_2$. These results confirm the conclusion of the isotope effect studies.

In view of the strong evidence that the distal–SO_3^- binding group in each of the external aldimines of the two suicide substrates should occupy the same position,[2] both inactivation events must involve the loss of electrofuges from the 4′-*Re*-face of the coenzyme, Scheme 2. In the light of the fact that the enzyme has evolved to decarboxylate and reprotonate substrates on the 4′-*Si*-face, these appear to be puzzling results. However, the stereochemcial and kinetic data§ reported here are most consistent with concerted processes, an *E*2 type elimination in the case of (2*S*)-serine *O*-sulfate and an early C–OSO_3^- bond ionisation in the case of (2*R*)-serine *O*-sulfate. The torsional conformation of the distal OSO_3^- binding group which leaves during each reaction should then be *anti* to the 4′-*Re*-face electrofuge. In these conformations the activation energies for the concerted processes could be lower than those for the alternative stepwise (stabilised carbanion) 4′-*Si*-face processes which are expected for pyridoxal systems[4] but, which are not observed here, Scheme 2.

We thank the SERC and Merck, Sharp and Dohme Neuroscience Research Centre for a CASE studentship to J. E. R. and Professor D. Arigoni for useful discussion.

Received, 11th September 1992; Com. 2/04893K

§ Note that α-hydrogen exchange with the solvent did not occur during the inactivation reactions mediated by either of the suicide substrates.

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