When several quenching agents which do not interact among themselves are present in solution, the Stern–Volmer equation will be

$$F_0/F = 1 + \sum_i k_q^i \tau_a [Q_i] \quad (1)$$

Since in the racemic mixture $[Q_L] = [Q_D] = {}^1/_2[Q_{DL}]$, it follows that for DL-methionine

$$\frac{F_0}{F} = 1 + (k_q^L + k_q^D)\tau_a \frac{[Q_{DL}]}{2} = 1 + \frac{(k_q^L + k_q^D)}{2}\tau_a[Q_{DL}]$$

Indeed, the observed rate constant for quenching by DL-methionine (5.9×10^7 M^{-1} s^{-1}) is close to the average of the rate constants for quenching by the pure antipodes (5.7×10^7 M^{-1} s^{-1}).

The marked stereoselectivity which exists in the interaction between LADH and methionine is revealed by the ratio of 3 between the rate constants for quenching. It is well established that the specificity of most enzymes toward the stereochemistry of their substrates is very high and in many cases even absolute, so that only one enantiomer participates in the enzymatic reaction.[11] It should be remembered, however, that this high stereospecificity is usually revealed in the interaction between an enzyme and its natural substrate, whose exact fit into the active site is crucial. Moreover, it is the overall reaction which shows the stereospecificity, while no indication is given as to the degree of discrimination between the two antipodes of the substrate in binding to the enzyme (many examples of ligands which bind rapidly and tightly but are inactive are known—i.e., inhibitors for enzymatic reactions; hence the inactivity of an antipode does not necessarily reflect lack of binding).

LADH is a somewhat unusual dehydrogenase in that it shows a broad specificity toward its substrates and oxidizes a wide range of primary and secondary alcohols.[12] This lack of sensitivity for the alkyl group of the alcohol being oxidized is thought to result from the fact that the alkyl binding domain in LADH is a hydrophobic site which combines with a variety of aliphatic groups with comparable affinities. This suggests that LADH may bind and react with both enantiomers of a secondary alcohol. Indeed it was found by Dickinson and Dalziel that LADH oxidizes both D- and L-2-butanol.[13] The oxidation rates of the two enantiomers differed by a factor of about 3, which is similar to the ratio of the rate constants for quenching by methionine reported here. This similarity is, most probably, a coincidence since the diffusion of methionine studied by us was into the adenine binding site of LADH and not into the substrate binding site. Also, as was mentioned above, the rate of enzymatic oxidation of an enantiomer does not necessarily reflect its rate of binding to the enzyme. Indeed, from the large rate constants for enzyme–ligand associations (10^7–10^8 M^{-1} s^{-1}), it is clear that this initial stage of the enzymatic reaction is not the rate-determining step in the oxidation and another process along the catalytic pathway must be responsible for the observed stereospecificity. The results of the present study clearly demonstrate chiral discrimination between two enantiomers of a small molecule interacting with LADH. Although methionine is not a substrate of this enzyme, the results obtained may be of a general significance in understanding the interactions in the first step of the enzymatic reaction—that of the primary association between enzyme and ligand.

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(12) H. Sund and H. Theorell, *Enzymes, 2nd Ed.*, **7**, 25 (1963).
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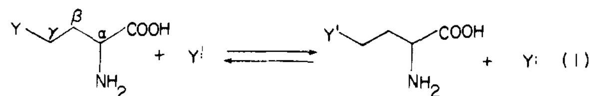
Received July 28, 1980

Stereochemical Course of γ Replacement on an Amino Acid Substrate in a Pyridoxal Phosphate Dependent Enzymatic Reaction

Sir:

Pyridoxal-P (PLP) is an obligate cofactor for a variety of enzyme-catalyzed transformations at the α, β, γ, or ω carbons of amino acid substrates by facilitated formation of stabilized substrate carbanion intermediates in catalysis. The subset of enzymes catalyzing γ replacement of a potentially good leaving group Y by an alternate nucleophile Y′ (eq 1), a formal substi-



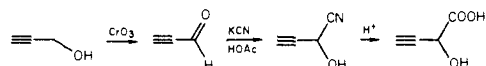
tution at an unactivated carbon, in fact acts via sequential formation of α and then β-substrate–carbanion PLP equivalents, followed by elimination of substituent Y to yield the key intermediate **2**.[1,2] This fully conjugated species is then captured by Y′ to yield a conjugate addition product which is unraveled by chiral α and β protonation to yield the product amino acid.

We report here for the first time the stereochemical outcome of such replacement, at the γ carbon of *O*-succinylhomoserine (**1**), during conversion to cystathionine (**3**), by the *Salmonella* enzyme, cystathionine γ-synthetase,[3,4] in the methionine biosynthetic pathway (Scheme I). We have recently demonstrated that L-vinylglycine (**4**), a natural compound,[5] is an excellent alternate substrate to succinylhomoserine, since **4** is converted to **2** merely by α-H abstraction from the amino acid–PLP aldimine complex.[6] Using specifically deuterated vinylglycines, we have determined first the stereochemistry of the second half-reaction, **2** → **3**, by degradation of cystathionine to homoserine. Then these [4-^{2}H]homoserines were chemically succinylated to **1a** or **1b** and converted enzymatically to **3**, and thereby the stereochemistry of the first half-reaction, **1** → **2**, was uncovered.

(*Z*)-DL-[4-^{2}H]Vinylglycine (**4a**) was prepared by exchange of 2-hydroxy-3-butynoate[7] in $^2H_2O/O^2H^-$ (p^{2}H 9.5) followed by methylation to yield methyl 2-hydroxy-3-[4-^{2}H]butynoate. Partial reduction with hydrogen and Lindlar catalyst yielded *Z*-methyl [4-^{2}H]vinylglycolate. Deuterium enrichment at the 4*Z* position was estimated greater than 98.5% by ^{1}H NMR. This was then converted by standard steps[8] to (*Z*)-DL-[4-^{2}H]vinylglycine (**4a**)[9] (4% total yield from propynal). A parallel sequence of preparation, with omision of deuterium exchange of 2-hydroxy-3-butynoate but with use of deuterium gas in the reduction step, led to the product (*E*)-DL-[3,4-$^2H_2$]vinylglycine (**4b**).[9]

Incubation of **4a** or **4b** with purified[3,4] *Salmonella* cystathionine γ-synthetase and L-cysteine in 0.1 M potassium phosphate buffer (pH 8.4) at 37 °C produced monodeuteriocystathionine samples (102 and 67 mg, respectively), which precipitated out of solution

(1) Davis, L.; Metzler, D. *Enzymes, 3rd Ed.* **1972**, *17*, 42.
(2) Walsh, C. "Enzyme Reaction Mechanisms"; Freeman: San Francisco, CA; 1979.
(3) Guggenheim, S., Flavin, M. *J. Biol. Chem.* **1969**, *244*, 3722.
(4) Guggenheim, S., Flavin, M. *J. Biol. Chem.* **1971**, *246*, 3562 [102 mg (1 mmol) of (*Z*)-DL-[4-^{2}H]vinylglycine and 60 mg (0.5 mmol) of L-cysteine were dissolved in 5 mL of 50 mM potassium pyrophosphate buffer (pH 8.2); 5 units of cystathionine γ-synthetase (20 units/mg) added; incubated under argon atmosphere at 37 °C for 24 h].
(5) Dardenne, G.; Casimar, J.; Marlier, M. *Phytochemistry* **1974**, *13*, 1897.
(6) Johnston, M.; Marcotte, P.; Donovan, J.; Walsh, C. *Biochemistry*, **1979**, *18*, 1729.
(7)



(8) Rando, R. R.; *Biochemistry*, **1974**, *13*, 3859. Marcotte, P.; Walsh, C. *Ibid.* **1976**, *15*, 3070.
(9) (E)-DL-[3,4-^{2}H]Vinylglycine (**4b**): δ 5.42–5.48 (br, s 1 Sh), 4.23–4.25 (s, 1 H). (*Z*)-DL-[4-^{2}H]Vinylglycine (**4a**): δ 5.95 (m, 1 H), 5.53 (d, *J* = 11 Hz, 1 H), 4.22 (d, *J* = 7 Hz, 1 H).

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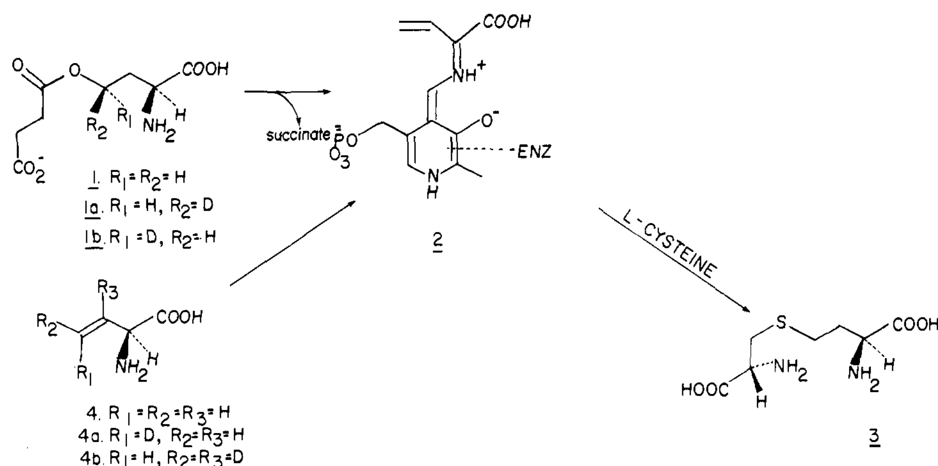
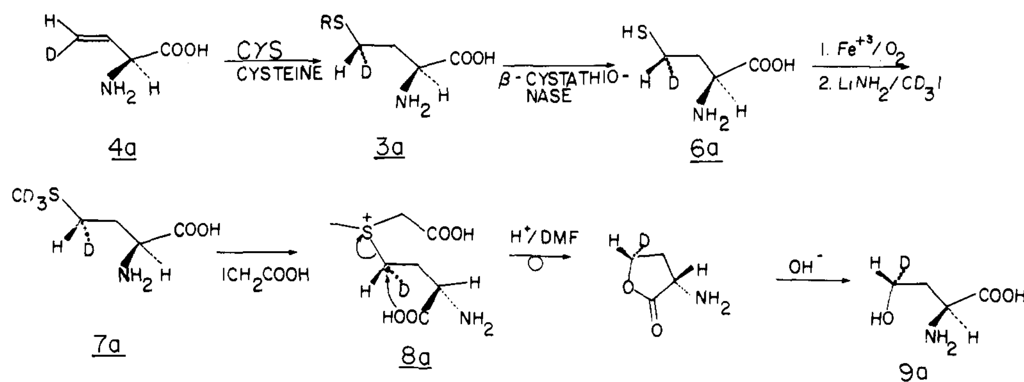
Scheme I



Scheme II



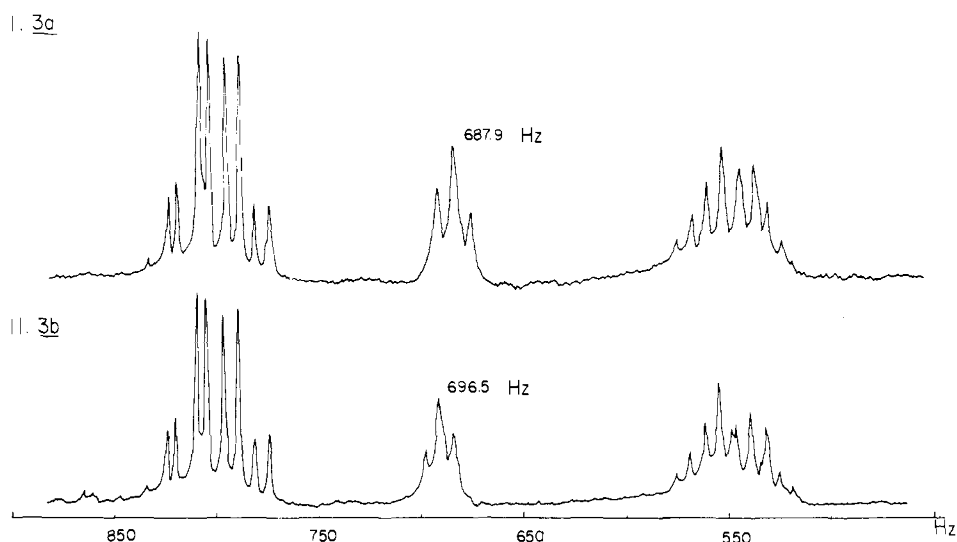


Figure 1. 270-MHz NMR of γ-^{2}H-L-cystathionine samples **3a**,**3b**.

essentially free of L-cysteine and D-vinylglycine. These samples were then subjected to rigorous purification by LC[10] and crystallization. NMR analysis at 270 MHz (Figure 1) of the cystathionine samples **3a**,**b** confirmed deuterium content and opposite diastereomeric location at C4. Deuterium at the C3 location of **4b** was washed out during the incubation.[11] The absolute chirality of C4 of each L-[4 ^{2}H]cystathionine sample was then determined by combined enzymatic and chemical degradation to L-[4-^{2}H]-homoserines (**9a**,**b**), as outlined in Scheme II for **4a** → **9a**. β-Cystathionase[12] action generates homocysteine along with pyruvate as coproduct; the amino acid product was isolated and purified as the homocystine dimer. A dividend of the route is generation of chiral L-[4-^{2}H]methionine samples, e.g., **7a**, along the way. Trideuteriomethyl iodide was utilized to simplify the 270-MHz NMR pattern in **7a** and **7b**. Only the acid-catalyzed lactonization in Scheme I affects configuration at C4, with one inversion. The homoserine samples **9a** and **9b** were purified by preparative TLC[13]

(10) Bio-Rad Amnax-5 cation-exchnge column (0.2 N sodium citrate buffer, pH 3.25 at 50 °C).

(11) Posner, B. I.; Flavin, M. *J. Biol. Chem.* **1972**, *247*, 6412. Flavin and colleagues, studying enzymic exchange at the prochiral β-hydrogens of L-homoserine, noted that *both* hydrogens were exchangeable, albeit at disparate rate, with H_R exchanged some 100-fold faster than H_S. Total washout of deuterium from C3 of vinylglycine suggests product cystathionine may be in exchange equilibrium *before* release from the active site.

(12) β-Cystathionase prepared from E. coli: Dr. J. S. Hong, Brandeis University, unpublished observation.

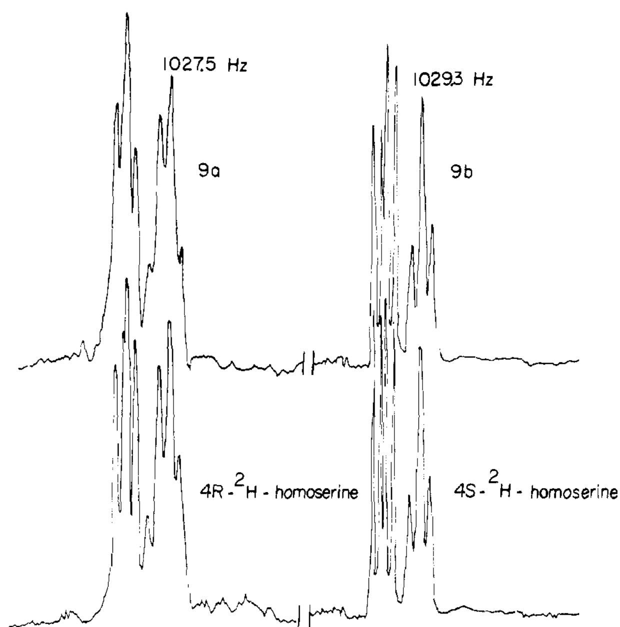
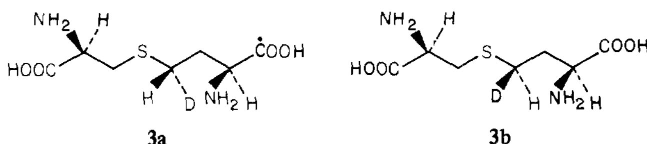


Figure 2. 270-MHz spectra of **9a** and **9b** together with that of authentic (*R*)-L-[4-^{2}H]homoserine and (*S*)-L-[4-^{2}H]homoserine.

and obtained as crystalline samples (5.5 mg of **9a** and 3.2 mg of **9b**, respectively). The 270-MHz spectra are shown in Figure 2 along with that of authentic (*R*)-L-[4-^{2}H]homoserine and (*S*)-L-[4-^{2}H]homoserine (we have recently determined the absolute chirality of such homoserine samples for just this purpose).[14] Clearly **9a** is (*R*)-L-[4-^{2}H]homoserine as shown and the product cystathionine **3a** must be *S*-4-^{2}H as is the methionine sample **7a**. Homoserine **9b** is in turn a *S*-4-^{2}H species and the cystathionine sample **3b** is a *R*-4-^{2}H isomer.

The stereochemical outcome in the first half-reaction could then be determined by chemical succinylation[15] of **9a** → **1a** and **9b** → **1b**, followed by cystathionine γ-synthetase mediated conversion to monodeuteriocystathionines upon incubation with L-cysteine. After purification, cystathionine from **1a** had its γ-H at 696.5 Hz (t, J = 7.0 Hz) and that from **1b** at 687.9 Hz by 270-MHz NMR, confirming that **1a** gives **3b**, (*R*)-L-[4-^{2}H]cystathionine,



given the absolute stereochemistry for monodeuteriocystathionines deduced via Scheme I. **1b** yields the opposite, *S*-4-^{2}H isomer **3a**.

Thus the overall γ-replacement process occurs with *retention of stereochemistry at the γ carbon (C4)* undergoing substitution. Fuganti and co-workers[16] reported in a preliminary way that the H_R proton at the β carbon of *O*-succinylhomoserine is removed; if this result is validated, then the β-H_R,γ-*O*-succinyl elimination would be a syn elimination to a cisoid form of conjugated intermediate **2**, given the sequences observed here: (*R*)-[4-^{2}H]-succinylhomoserine → (*E*)-[4-^{2}H]vinylglycine-PLP α-anion → (*R*)-[4-^{2}H]cystathionine and (*S*)-[4-^{2}H]succinylhomoserine → *Z*-4-^{2}H adduct → (*S*)-[4-^{2}H]cystathionine.

Acknowledgment. We thank Professor J. S. Hong for the generous gift of homogeneous β-cystathionase and Professor A. Redfield for the assistance and the use of his 270-MHz NMR spectrometer.

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Received May 27, 1980

(13) Whatman, No. 1 Paper, 1-butanol-acetic acid-water (12:3:5), R_f = 0.2, located by ninhydrin spray and workup by extraction with hot water.
(14) Chang, M. N.; Walsh, C. *J. Am. Chem. Soc.* **1980**, *102*, 2499.
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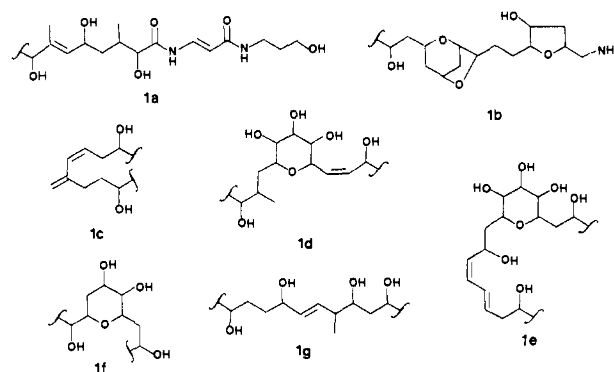
(16) Coggiola, D.; Fuganti, C. *Experientia* **1977**, 33 (7), 847.

Periodate Oxidation of *N*-(*p*-Bromobenzoyl)palytoxin

Sir:

The structure elucidation of palytoxin, an exceedingly poisonous substance from marine soft corals of the genus *Palythoa*,[1,2] presents a formidable challenge to the organic chemist because of its high molecular weight and lack of familiar repeating structural units such as those found in peptides and polysaccharides. Our work on palytoxin from Hawaiian *Palythoa toxica* and a Tahitian *Palythoa* sp. has suggested that the molecular weight is 3300 and that four nitrogens exist in the molecule.[3] Recently the molecular weight of palytoxin from Okinawan *P. tuberculosa* has been determined to be 2681.1 ± 0.35 by ^{252}Cf-plasma desorption mass spectrometry, implying that three nitrogens are present rather than four.[4] Chemical evidence indicates that two nitrogens are present in a β-amidoacrylamide-containing unit (**1a**) located at one terminus of the molecule.[3,5,6] Unit **1a** contains the λ_{263} chromo-



phore.[2] We report here that a third nitrogen, which accounts for the basicity of palytoxin, is present as a primary amino group in a unit (**1b**) situated at the other end of the molecule.

Treatment of palytoxin from Hawaiian or Tahitian *Palythoa* with *p*-bromobenzoic ethylcarbonic anhydride[7] in aqueous acetone at 0 °C leads to *N*-(*p*-bromobenzoyl)palytoxin. Oxidation of the derivatized toxin (25 mg) with $NaIO_4$ (50 mg) in H_2O at 0 °C for 9 min followed by $NaBH_4$ reduction of the resulting aldehydes and subsequent acetylation (Ac_2O/pyridine/N_2) of the alcohols gives a mixture of acetates which are separable by LC (silica gel, EtOAc to 5% EtOH-EtOAc). The degradation product possessing the *N*-*p*-bromobenzoyl group is a crystalline diacetate, mp

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(3) Moore, R. E.; Woolard, F. X.; Sheikh, M. Y.; Scheuer, P. J. *J. Am. Chem. Soc.* **1978**, *100*, 7758.
(4) Macfarlane, R. D.; Uemura, D.; Ueda, K.; Hirata, Y. *J. Am. Chem. Soc.* **1980**, *102*, 875.
(5) Hirata, Y.; Uemura, D.; Ueda, K.; Takano, S. *Pure Appl. Chem.* **1979**, *51*, 1875.
(6) The trisubstituted double bond is *E* since the ^{13}C NMR of palytoxin (Me_2SO-d_6) shows a signal at δ 12.70 for the olefinic methyl carbon.
(7) The mixed anhydride was prepared from triethylammonium *p*-bromobenzoate and ethyl chloroformate in wet acetone at 0 °C.

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