ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS
Vol. 190, No. 2, October, 553-559, 1978

Stereochemistry of the β-Cyanoalanine Synthetase and S-Alkylcysteine Lyase Reactions[1]

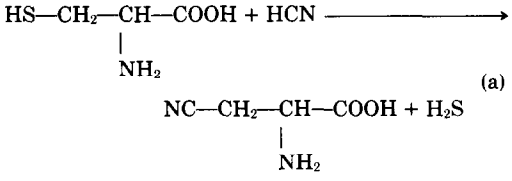
MING-DAW TSAI,* JANET WEAVER,* HEINZ G. FLOSS,* ERIC E. CONN,† RICHARD K. CREVELING,‡ AND MENDEL MAZELIS‡

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, and Department of Biochemistry and Biophysics† and Department of Food Science and Technology,‡ University of California, Davis, California 95616*

Received February 16, 1978; revised April 27, 1978

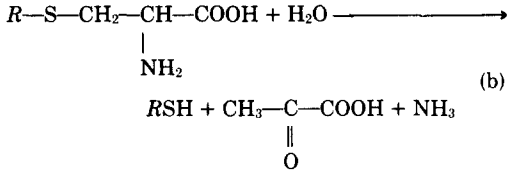
The stereochemistry of the replacement of the SH-group of cysteine by CN catalyzed by β-cyanoalanine synthetase was studied using cysteine stereospecifically tritiated at C-3. Analysis of the resulting β-cyanoalanine by conversion into fumarate via aspartate and malate showed that the reaction had occurred with retention of configuration at C-3. Using cystine stereospecifically labeled at C-3 with tritium or with tritium and deuterium, it was found that the α,β-elimination reaction catalyzed by S-alkylcysteine lyase involves stereospecific replacement of the β-substituent of the substrate by a hydrogen derived from the solvent, D_2O or H_2O, with retention of configuration to give pyruvate containing a chiral methyl group. The results are discussed, particularly in the light of mechanistic proposals by Braunstein and co-workers.

During the past few years, the stereochemical course of a number of β-replacement and α,β-elimination reactions of amino acids catalyzed by pyridoxal phosphate-containing enzymes has been elucidated (1-9). All the enzymes studied were of microbial origin and the reactions were invariably found to proceed with retention of the steric relationships of the ligands at the β-carbon atom, fitting into the concept developed by Dunathan (10, 11) that pyridoxal phosphate-catalyzed reactions all take place on only one side of a planar coenzyme-substrate complex. We wished to determine whether the same stereochemistry is seen with pyridoxal phosphate enzymes from higher plants and chose to examine two enzymes which catalyze rather unique reactions in plant metabolism. These are β-cyanoalanine synthetase (EC 4.4.1.9) (12-14) which catalyzes reaction (a):



(a)

and S-alkyl-L-cysteine lyase (EC 4.4.1.6) (15) which catalyzes reaction (b):



(b)

where R can be an alkyl or the cysteinyl (—S—CH_2—$CH(NH_2)COOH$) residue.

EXPERIMENTAL PROCEDURES

Materials. The chemicals used were of reagent grade or highest purity available commercially. (3*R*)- and (3*S*)-[3-^{3}H]cysteine and (3*S*)-[3-^{14}C,3-^{2}H,3-^{3}H]cysteine were prepared from (3*R*)- and (3*S*)-[3-^{3}H]serine and (3*S*)-[3-^{14}C,3-^{2}H,3-^{3}H]serine, respectively, as described previously (5), and were stored in the form of the corresponding cystines. Ion exchange resins were purchased from Bio-Rad. Lactate dehydrogenase (pig

[1] This work was supported by Grants GM18852 and GM05301 from the National Institute of General Medical Sciences, United States Public Health Service, and by Hatch Funds from the United States Department of Agriculture.

0003-9861/78/1902-0553$02.00/0

heart) was obtained from Sigma. L-Asparaginase (*Escherichia coli*), glutamate-oxalacetate transaminase (pig heart), and malate dehydrogenase (pig heart) were purchased from Boehringer Mannheim. β-Cyanoalanine synthetase was purified through the alumina C_γ step of Akopyan *et al.* (13) and had a specific activity of 3.9 IU/mg protein. *S*-Alkyl-L-cysteine lyase, purified from *Acacia farnesiana* seedlings as described by Mazelis and Creveling (15), had a specific activity of 28 IU/mg protein.

Radioactivity determinations. Radioactivity on chromatograms was located using a Packard model 7201 radiochromatogram scanner. The radioactivity of compounds in solution was determined in a Beckman LS 100 or LS 250 liquid scintillation counter using 2,5-diphenyloxazole (PPO) and 1,4-bis[2-(methyl-5-phenyloxazolyl)]benzene (dimethyl POPOP) in toluene or toluene/ethanol as scintillator solution. Counting efficiencies and the spillover of ^{14}C into the tritium channel were determined by internal standardization with [^{14}C]toluene and [^{3}H]toluene.

S-Alkyl-L-cysteine lyase reaction. The reaction mixture contained: 0.005 M L-cystine, 0.2 ml (0.5 μCi ^{3}H); 0.05 M tricine buffer, pH 7.8, 0.35 ml; 0.5 mM pyridoxal phosphate, 0.05 ml; 0.01 M NADH in 0.01 M tricine buffer, pH 7.8, 0.4 ml; lactate dehydrogenase, 10 IU; and *S*-alkyl-L-cysteine lyase, 10 IU. The incubation was carried out at 37° for 30 min and the reaction was observed spectrophotometrically by following the decrease in the absorption at 340 nm. In the case of the cystine samples containing only tritium, the reaction mixture before addition of the enzymes was lyophilized, twice taken up in 1 ml D_2O, and evaporated and then dissolved in 1 ml D_2O. After incubation, the reaction was stopped by boiling for 2 min and the proteins were removed by dialysis. The lactate was isolated by ether extraction of the acidified reaction mixture and was oxidized to acetate by heating with 12 ml of oxidation mixture (153 mg $K_2Cr_2O_7$; 24 ml concentrated H_2SO_4; made up to 100 ml with water) on a steam bath for 20 min under argon atmosphere (16). The acetate was isolated by steam distillation, mixed with [^{14}C]acetate and analyzed for chirality according to the procedure of Cornforth *et al.* (17) and Lüthy *et al.* (18).

β-Cyanoalanine synthetase reaction. A 0.5 μCi sample of the stereospecifically labeled cystine (10 μCi ^{3}H/μmol) was converted into cysteine by incubation at room temperature with 1 μl mercaptoethanol in 0.2 ml H_2O (flushed with nitrogen) overnight. The solution was then added to 1 μmol L-cysteine and evaporated to dryness, followed by addition of 1 ml isopropanol and evaporation to dryness again. This cysteine sample was then mixed with 0.01 M NaCN, 0.1 ml; 0.2 M Tris buffer, pH 8.5, 0.15 ml; and 0.05 ml β-cyanoalanine synthetase (100 IU/ml). Incubation was carried out at room temperature for 1 h. The incubation mixture was then evaporated to dryness, followed by addition of isopropanol and evaporation to dryness again. The proteins were then removed by dialysis and the crude mixture was passed through a cation exchange column (Dowex 50W, acid form). The material eluted with 1 *N* NH_4OH was collected and evaporated to dryness. The sample from a parallel incubation with L-[U-^{14}C]-cystine was spotted on a silica gel plate (Baker) and chromatographed in $CHCl_3$/EtOH/HOAc/H_2O 50:32:10:8. A ninhydrin-positive spot, coinciding with authentic β-cyanoalanine (R_F 0.23), was observed which corresponded to 40% of the added radioactivity.

Degradation of β-cyanoalanine. The samples obtained from the above incubation were diluted with 2 μmol cold β-cyanoalanine and mixed with 0.5 ml of 0.5 M potassium phosphate buffer (pH 7.8) containing: α-ketoglutarate, 7 μmol; NADH, 5 μmol; asparaginase, 4 IU; glutamate-oxalacetate transaminase, 4 IU; and malate dehydrogenase, 60 IU. The incubations were carried out at 25°C for 80 min and the reactions stopped by boiling for 2 min. The reaction was followed by observing the decrease in the absorption at 340 nm. Proteins were removed by dialysis, and the malate was isolated by passing the reaction mixture through a cation exchange column (Dowex 50W, 200–400 mesh, acid form) and collecting the water effluent. [U-^{14}C]-Malate was then added as reference and the malate was further purified on an anion exchange column (Dowex AG 1X8, 200–400 mesh, formate form). After thorough washing with water, the column was eluted with a formic acid gradient (300 ml 1 *N* formic acid and 300 ml H_2O) and the fractions corresponding to malate were collected. After evaporation to dryness, the malate samples were divided into four fractions, and one of them was counted for its $^{3}H/^{14}C$ ratio. The second fraction was incubated with 10 IU of fumarase in 2.0 ml of 0.04 M sodium phosphate buffer, pH 7.4, for 2 h at room temperature. The progress of the fumarase reaction was followed by observing the increase in the absorption at 240 nm; it was complete in the first few minutes of incubation. After lyophilization, the resulting fumarate was counted to determine its $^{3}H/^{14}C$ ratio. The third fraction was incubated with 120 IU of malate dehydrogenase in 0.5 ml glycine buffer (1 M, pH 9.5) containing 2 μmol 3-acetylpyridine-adenine dinucleotide (APAD) at room temperature for 3 h and the reaction was followed spectroscopically by the increase in the absorption at 365 nm (19). After lyophilization to remove the distillable tritium, the residual oxalacetate was counted to determine its $^{3}H/^{14}C$ ratio. The fourth fraction was used for a control incubation under the same conditions which were used to convert β-cyanoalanine into malate, except that both NAD^+ and NADH were added. Two percent tritium activity was distilled out after such a control incubation with the malate from (3*S*)-[3-^{3}H]-cysteine. This loss could be caused either by the trace amount of fumarase present in the malate dehydrogenase, or by the tritium exchange of oxalacetate which might be present in trace amounts in equilib-

rium with malate. If the latter explanation were true, it would be expected to destroy the chirality of malate to a significant extent. When the malate sample after the control incubation was converted to fumarate, the $^{3}H/^{14}C$ ratio of fumarate was approximately the same as that obtained from malate directly.

RESULTS

The steric course of both enzyme reactions could be studied using samples of cysteine labeled stereospecifically at C-3, which were prepared by a route developed during our previous work on *O*-acetylserine sulfhydrase (5). Three labeled species of cysteine were prepared, two carrying tritium in the *pro*-3*R* and *pro*-3*S* hydrogen, respectively, and the third labeled with deuterium in the *pro*-3*R* and with tritium in the *pro*-3*S* position. In the latter, every tritiated molecule also contained deuterium. For ease of isolation, handling and storage, these cysteine samples were air-oxidized to the corresponding cystines. These served directly as substrates for *S*-alkylcysteine lyase.

For the conversion into β-cyanoalanine, the cystine samples were reduced to cysteine by incubation with mercaptoethanol followed by removal of the reducing agent and incubation with β-cyanoalanine synthetase and cyanide. A control incubation with ^{14}C-cystine indicated only 40% conversion into β-cyanoalanine, the rest of the radioactivity being distributed between unreacted starting material and a new compound, presumably a product of the utilization of residual mercaptoethanol in place of cyanide. Without purification, the β-cyanoalanine formed was converted into L-malate by the reaction sequence outlined in Fig. 1. The malate was purified by ion-exchange chromatography and the tritium distribution between the two heterotopic hydrogens at C-3 was determined using a procedure applied by Rose (20). Tritium from the *pro*-3*R* position was released into water by incubation with fumarase, whereas the total amount of tritium present at C-3 of malate was determined by enzymatic oxidation to oxalacetate and exchange of the latter with water.

Table I summarizes the results of the degradation of the three malate samples derived from the cyanoalanines obtained by using the three cysteine species as substrates in the β-cyanoalanine synthetase reaction. Clearly, the two malate samples derived from cysteine of 3*S* configuration also contained most of the tritium in the *pro*-3*S* position, whereas the sample from (3*R*)-cysteine was labeled predominantly in the *pro*-3*R* position. Therefore, cysteine is converted into β-cyanoalanine with net retention of configuration at carbon atom 3. At least one of the three L-malate samples, the one derived from 3*R* cysteine, shows a rather low degree of apparent chiral purity, and the 3*S* tritiated sample, too shows some scrambling of the tritium into the *pro*-3*R* position. This could (a) reflect a less than complete chiral purity of the starting cysteine samples, (b) could be due to some nonenzymatic enolization at the stage of oxalacetate, a transient intermediate in the conversion of cyanoalanine to malate, or it may (c) indicate a small and varying degree of racemization occurring in the β-cyanoalanine synthetase reaction itself. We con-

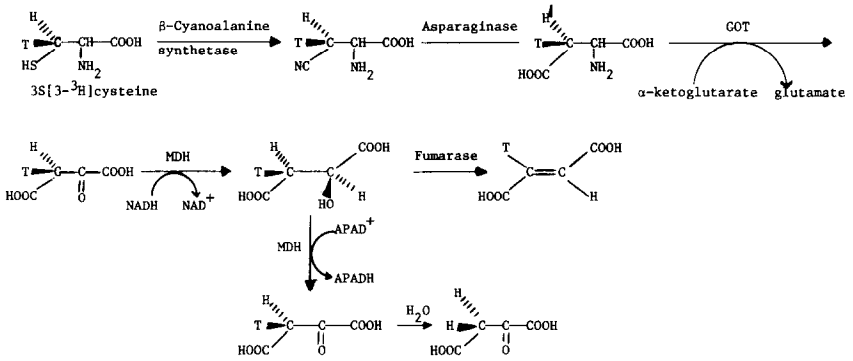


FIG. 1. Determination of the steric course of the β-cyanoalanine synthetase reaction.

TABLE I

CONFIGURATIONAL ANALYSIS OF MALATE SAMPLES DERIVED FROM β-CYANOALANINE SYNTHESIZED ENZYMATICALLY FROM STEREOSPECIFICALLY LABELED CYSTEINE

	(3*S*)-[3-^{3}H]-cysteine	(3*R*)-[3-^{3}H]-cysteine	(3*S*)-[3-^{14}C,3-^{2}H, 3-^{3}H]cysteine
Malate (^{3}H/^{14}C)	1.31[a]	7.85[a]	5.19
Fumarate (^{3}H/^{14}C)	1.10	3.25	4.84
% ^{3}H Retention in fumarase reaction	84.0	41.4	93.2
% ^3H_R in malate	16.0	58.6	6.8
Oxalacetate + APADH (^{3}H/^{14}C)	0.15	0.69	0.50
% ^{3}H Retention in MDH reaction	11.5	8.8	9.6
% (^3H_R + ^3H_S) in malate	88.5	91.2	90.4
$^3H_R/^3H_R$ + ^3H_S in malate (%)	18.1	64.3	7.5

[a] [U-^{14}C]malate was added as reference.

sider (a), possibly with a small contribution from (b), to be the most likely explanation. Firstly, cysteine samples prepared by the same route have also shown varying degrees of apparent chiral purity when analyzed by a different procedure (5), and secondly, as discussed below, the products from the *S*-alkycysteine lyase reaction show different degrees of chiral purity paralleling those seen in the β-cyanoalanine synthetase reaction with the same substrate samples. A control experiment with malate from (3*S*)-[3-^{3}H]cysteine indicated up to 2% tritium exchange. However, this ^{3}H exchange was found not to cause appreciable change in the ^{3}H/^{14}C ratio of fumarate when the malate after control incubation was converted to fumarate. If it were due to enolization of oxalacetate, it would be expected to cause up to 10% racemization based on the theory derived by Sedgwick and Cornforth (21). Thus, enolization at the oxalacetate stage during the conversion of β-cyanolanine to malate may contribute slightly to the scrambling of tritium between the *pro*-3*R* and *pro*-3*S* positions, but it cannot account for all of it in at least two of the experiments.

To determine the steric course of the protonation at C-3 in the *S*-alkylcysteine lyase reaction, the two tritiated cystine samples were incubated with the enzyme in D_2O. The product pyruvate was reduced to lactate *in situ* with lactate dehydrogenase and excess NADH. A third experiment was carried out with stereospecifically tritiated and deuterated cystine in H_2O to provide for the eventuality that the third hydrogen making up the pyruvate methyl groups is not derived from the medium but comes from within the coenzyme-substrate complex. The lactate samples from all three experiments were purified, oxidized to acetate, and then analyzed for the chirality of the labeled methyl group by established procedures (7, 17, 18). The chirality analysis of acetate involves conversion into acetylcoenzyme A and condensation with glyoxylate, catalyzed by malate synthetase, to give malate which is then incubated with fumarase. Due to the deuterium isotope effect in the malate synthetase reaction, samples of chirally labeled acetate will give rise to an uneven tritium distribution between the two hydrogens at C-3 (17, 18). Optically pure *R*-[2-^{2}H,-2-^{3}H]acetate is expected to give malate showing 79.7% tritium retention in the fumarase reaction, the malate from pure *S* isomer should show 20.3% tritium retention upon incubation with fumarase (22).

The results are summarized in Table II. For each experiment, two independent complete analyses were carried out. In each experiment, the methyl group formed was chiral, albeit of low chiral purity. Inasmuch as *R*-[3-^{3}H]cystine in D_2O has yielded chirally labeled pyruvate of *R* configuration, it follows that the third hydrogen has been added stereospecifically at C-3 on the same side at which the β-substituent has been removed, *i.e.*, the reaction proceeds with net retention of configuration at C-3 (Fig. 2). The results also indicate that the hydrogen added at C-3 originates from the solvent, as has been found for tryptopha-

TABLE II

CHIRALITY ANALYSIS OF THE METHYL GROUP OF PYRUVATE (LACTATE) FORMED FROM STEREOSPECIFICALLY LABELED CYSTINE WITH *S*-ALKYLCYSTEINE LYASE

Substrate and condition: $^3H/^{14}C$ of	(3*R*)-[3-^{3}H]cystine in D_2O		(3*S*)-[3-^{3}H]cystine in D_2O		(3*S*)-[3-^{14}C,3-^{2}H,3-^{3}H]cystine in H_2O	
Cysteine	—	—	—	—	4.67	4.67
Lactate	—	—	—	—	1.99	2.14
Acetate	0.771[a]	2.05[a]	2.55[a]	0.994[a]	1.93	—
Malate	0.643	1.46	2.31	0.863	1.75	1.75
Fumarate	0.347	0.81	1.02	0.385	0.975	0.977
% ^{3}H Retention in fumarase reaction[b]	54.0	55.5	44.1	44.6	55.7	55.8
% ^{3}H loss in fumarase reaction[c]	46.6	46.2	57.2	56.0	43.4	42.6

[a] ^{14}C-Acetate was added as reference.

[b] From $^3H/^{14}C$ value.

[c] From tritium activity of the water from the fumarase reaction.

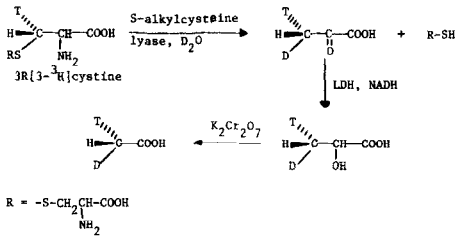


FIG. 2. Steric course of the *S*-alkylcysteine lyase reaction.

nase (7), D-serine dehydratase (6, 8) and tyrosine phenol-lyase (9), and not from within the coenzyme-substrate complex, as has been demonstrated in the case of tryptophan synthetase β_2 protein (23). The low chiral purity of the methyl group in the products may be due to some extent, to incomplete chiral purity of the substrate, but to a larger extent it must be due to racemization accompanying the reaction itself. This has also been observed in the tyrosine phenol-lyase reaction (9) and may either reflect reversibility of the overall reaction or may be due to reversible protonation/deprotonation of the methyl group formed before the release of the pyruvate from the enzyme.

DISCUSSION

The finding that β-cyanoalanine synthetase and *S*-alkylcysteine lyase catalyze the reaction at the C-3 of their substrates with retention of configuration conforms with the stereochemistry deduced for other enzyme reactions of this general type (1-9, 23). Thus, these two unique plant enzymes operate with the same stereochemistry as the microbial pyridoxal phosphate-containing enzymes catalyzing β-replacement and α,β-elimination reactions, which have been studied so far, and they seem to conform to Dunathan's concept (10, 11) that pyridoxal phosphate enzymes operate on only one face of a planar coenzyme-substrate complex.

As we have discussed previously (5), cleavage and formation of a bond at the β carbon atom of the amino acid-pyridoxal phosphate Schiff's base in the β-replacement reactions can be expected to be subject to stereoelectronic control, requiring that the bond to be broken or formed must be aligned perpendicular to the π plane. Because the reaction proceeds with retention of configuration at C-3 of the amino acid, it follows that the leaving group and the incoming substituent must align on the same side of the π plane and, inasmuch as two objects cannot be in the same place at the same time, the reaction must either proceed by a ping-pong mechanism or must involve a conformational change of the enzyme during the catalytic process, which reorients the two groups relative to the π plane. For *O*-acetylserine sulfhydrase,

which also catalyzes β-replacement with retention of configuration (5), ping-pong kinetics have recently been demonstrated (24). In the case of β-cyanoalanine synthetase, Braunstein and co-workers (13) have demonstrated that α-hydrogen exchange in unreacted substrate is slow in the absence of the second substrate and is significantly stimulated by addition of cyanide. Inasmuch as product formation is accompanied by complete exchange of the α-hydrogen, a ternary complex is implicated in the reaction. This argues against a ping-pong mechanism for β-cyanoalanine synthetase and points to the alternative, a conformational reorientation of the enzyme as part of the catalytic process.

Based on their observation that α-hydrogen exchange in unreacted substrate in the absence of cyanide is slower than the overall reaction rate, a finding also made with other β-replacement-specific enzymes (25), and on inhibitor experiments, Braunstein and co-workers (13) have suggested that these reactions involve a direct displacement of the β-substituent by the incoming group (25, 26), rather than the widely assumed elimination-addition mechanism via an α,β-unsaturated Schiff's base intermediate (27). Such a direct displacement is difficult to reconcile with our stereochemical data, because it would presumably involve an S_N2-like process, which should result in inversion of configuration at C-3. To be compatible with the stereochemical results, the direct replacement of the β-substituent would have to involve a double displacement process with participation of an anionic group of the enzyme situated on the "protein side" of the coenzyme-substrate Schiff's base. Although this is certainly possible, there does not seem to be any evidence for such a process in any pyridoxal phosphate-catalyzed β-replacement reaction. It must be kept in mind, however, that the hydrogen exchange data of the Russian workers do not rule out an α,β-unsaturated reaction intermediate. After all, the reaction is accompanied by incorporation of fully one solvent hydrogen into the product, and even if it were not, this could be due to internal recycling of the α-hydrogen, which has been demonstrated for some other pyridoxal phosphate enzymes (7, 10, 23). Therefore, it is quite possible that efficient labilization of the α-hydrogen as well as the overall reaction require a ternary complex and that binding of cyanide brings the enzyme into a conformation which is catalytically competent for cleavage of the C_α-H bond.

Including the present report on *S*-alkylcysteine lyase, five enzymes catalyzing α,β-elimination have now been investigated for the stereochemistry of the replacement of the β-substituent by a hydrogen and, in each case, the reaction proceeds in a retention mode. Three enzymes, tryptophanase (28), tryptophan synthetase β_2 protein (23) and tyrosine phenol-lyase (H. Kumagai, E. Schleicher, H. G. Floss, unpublished results) have been examined further to establish the conformation of the amino acid C_α-C_β bond in the active site, and for each it was shown that the β substituent and H_α are *syn*. Although this has not been shown yet, the same conformation is also likely for *S*-alkylcysteine lyase. In a new classification of pyridoxal phosphate enzymes, Braunstein (26) a few years ago proposed three categories for the enzymes catalyzing β-replacement and/or α,β-elimination reactions: *A2a.* α,β-eliminating lyases (conformation of X^β *trans* to H^α); *A2b.* β-replacing lyases (conformation of X^β *cis* to H^α); *A2c.* mixed function β-lyases (α,β-eliminating and β-replacing; free rotation around C^α-C^β bond). The stereochemical studies carried out since then, however, indicate very strongly that probably all these enzymes operate on only one face of the coenzyme-substrate complex and that, therefore, the β-substituent and H_α are *syn* oriented in all of them. Thus, whereas Braunstein's classification may still be valid based on other criteria, some of the underlying stereochemical and mechanistic assumptions must be abandoned or modified on the basis of more recent data.

REFERENCES

1. SKYE, G. E., POTTS, R., AND FLOSS, H. G. (1974) *J. Amer. Chem. Soc.* **96**, 1593–1595.
2. FUGANTI, C., GHIRINGHELLI, D., GIANGRASSO, D., GRASSELLI, P., AND AMISANO, A. S. (1974) *Chim. e Indust.* **56**, 424.

3. FUGANTI, C., GHIRINGHELLI, D., GIANGRASSO, D., AND GRASSELLI, P. (1974) *J. Chem. Soc. Chem. Commun.* 726–727.
4. SAWADA, S., KUMAGAI, H., YAMADA, H., AND HILL, R. K. (1975) *J. Amer. Chem. Soc.* **97,** 4334–4337.
5. FLOSS, H. G., SCHLEICHER, E., AND POTTS, R. (1976) *J. Biol. Chem.* **251,** 5478–5482.
6. YANG, J. Y., HUANG, Y. Z., AND SNELL, E. E. (1975) *Fed. Proc.* **34,** 496.
7. SCHLEICHER, E., MASCARO, K., POTTS, R., MANN, D. R., AND FLOSS, H. G. (1976) *J. Amer. Chem. Soc.* **98,** 1043–1044.
8. CHEUNG, Y. F., AND WALSH, C. (1976) *J. Amer. Chem. Soc.* **98,** 3397–3398.
9. KUMAGAI, H., YAMADA, H., SAWADA, S., SCHLEICHER, E., MASCARO, K., AND FLOSS, H. G. (1977) *J. Chem. Soc. Chem. Commun.* 85–86.
10. DUNATHAN, H. C. (1971) *Adv. Enzymol.* **35,** 79–134.
11. DUNATHAN, H. C., AND VOET, J. G. (1974) *Proc. Nat. Acad. Sci. USA* **71,** 3888–3891.
12. HENDRICKSON, H. R., AND CONN, E. E. (1969) *J. Biol. Chem.* **244,** 2632–2640.
13. AKOPYAN, T. N., BRAUNSTEIN, A. E., AND GORYACHENKOVA, E. V. (1975) *Proc. Nat. Acad. Sci. USA* **72,** 1617–1621.
14. AKOPYAN, T. N., AND GORYACHENKOVA, E. V. (1976) *Biokhimiya* **41,** 906–914.
15. MAZELIS, M., AND CREVELING, R. K. (1975) *Biochem. J.* **147,** 485–491.
16. SIMON, H., AND FLOSS, H. G. (1967) Bestimmung der Isotopenverteilung in markierten Verbindungen, p. 50, Springer-Verlag, Berlin.
17. CORNFORTH, J. W., REDMOND, J. W., EGGERER, H., BUCKEL, W., AND GUTSCHOW, C. (1970) *Eur. J. Biochem.* **14,** 1–13.
18. LÜTHY, J., RÉTEY, J., AND ARIGONI, D. (1969) *Nature* **221,** 1213–1215.
19. WITT, I. (1974) *in* Methods of Enzymatic Analysis (Bergmeyer, E. U., ed.), Ed. 2, pp. 1593–1596, Academic Press, New York.
20. ROSE, I. A. (1970) *J. Biol. Chem.* **245,** 6052–6056.
21. SEDGWICK, B., AND CORNFORTH, J. W. (1977) *Eur. J. Biochem.* **75,** 465–479.
22. LENZ, H., AND EGGERER, H. (1976) *Eur. J. Biochem.* **65,** 237–246.
23. TSAI, M.-D., SCHLEICHER, E., POTTS, R., SKYE, G. E., AND FLOSS, H. G. (1978) *J. Biol. Chem.* **253,** 5344–5349.
24. COOK, P. F., AND WEDDING, R. T. (1976) *J. Biol. Chem.* **251,** 2023–2029.
25. TOLOSA, E. A., MASLOVA, R. N., GORYACHENKOVA, E. V., WILLHARDT, I. H., AND BRAUNSTEIN, A. E. (1975) *Eur. J. Biochem.* **53,** 429–436.
26. BRAUNSTEIN, A. E. (1972) *in* Enzymes: Structure and Function, FEBS Symposium Vol. 29 (8th FEBS Meeting, Amsterdam), pp. 135–150, North-Holland, Amsterdam.
27. DAVIS, L., AND METZLER, D. E. (1972) *in* The Enzymes (Boyer, P. D., ed.), Ed. 3, Vol. 7, pp. 33–74, Academic Press, New York.
28. VEDERAS, J. C., SCHLEICHER, E., TSAI, M.-D., AND FLOSS, H. G. (1978) *J. Biol. Chem.* **253,** 5350–5354.