Catalytic Action of L-Methionine γ-Lyase on Selenomethionine and Selenols†

Nobuyoshi Esaki, Hidehiko Tanaka, Sakae Uemura, Tetsuya Suzuki, and Kenji Soda*

ABSTRACT: We examined the catalytic action of L-methionine γ-lyase (EC 4.4.1.11) on selenomethionine (2-amino-4-(methylseleno)butyric acid), methaneselenol, 1-hexaneselenol, and benzeneselenol. The enzyme catalyzes α,γ-elimination of selenomethionine to yield α-ketobutyrate, ammonia, and methaneselenol, and also its γ-replacement reaction with various thiols to produce S-substituted homocysteines. Selenomethionine is an even better substrate than methionine in α,γ-elimination but is less effective in γ-replacement. In addition, L-methionine γ-lyase catalyzes γ-replacement reaction of methionine and its derivatives with selenols to form the corresponding Se-substituted selenohomocysteines, although selenols are less efficient substituent donors than thiols. This is the first proven mechanism for the incorporation of selenium atom into amino acids.

Selenium is highly toxic but is also an essential trace element for animals and bacteria. Its biological role has received considerable attention as reviewed by Stadtman (1973) and Shrift (1973). Proteins and enzymes which contain selenium as an essential component were isolated from animals and microorganisms, though the form of selenium is not known except for the selenium moiety of protein A of a glycine reductase complex from *Clostridium sticklandii* and glutathione peroxidase of rat liver–selenocysteine (2-amino-3-hydroselenopropionic acid) (Cone et al., 1976; Forstrom et al., 1978).

Several enzymatic processes that cannot distinguish between selenium and sulfur have been reported (Stadtman, 1973; Shrift, 1973). The selenium analogues of sulfur-containing amino acids such as methionine, cystathionine, and *S*-methylcysteine occur in higher plants and microorganisms. It is suggested that some of the selenium compounds, e.g., selenomethionine (2-amino-4-(methylseleno)butyric acid) and selenocysteine, are produced through the biosynthetic pathways of the corresponding sulfur amino acids, although the mechanism has not been investigated in detail. ATP:L-methionine *S*-adenosyltransferase (Greene, 1969) and *S*-adenosylmethionine methyltransferase (Pegg, 1969) act on selenomethionine and *Se*-adenosylselenomethionine, respectively.

Recently, we have purified L-methionine γ-lyase (EC 4.4.1.11) to homogeneity from *Pseudomonas putida* (*Pseudomas ovalis*)[1] (IFO 3738) and showed that the enzyme catalyzes α,γ-elimination and γ-replacement reactions of L-methionine and its derivatives and also α,β-elimination and β-replacement reactions of S-substituted L-cysteines (Tanaka et al., 1977).

In this paper, we describe the α,γ-elimination and γ-replacement reactions of selenomethionine and also γ-replacement reaction of methionine and its derivatives with selenols by L-methionine γ-lyase, the first proven mechanism for the incorporation of selenium into amino acids.

Experimental Procedures

Materials. L-Methionine γ-lyase was purified to homogeneity from a cell-free extract of *Pseudomonas putida* (IFO 3738) as reported previously (Tanaka et al., 1976). DL-Selenomethionine, sodium α-ketobutyrate, and crystalline lactate dehydrogenase of bovine heart were purchased from Sigma, crystalline glutamate dehydrogenase of bovine liver from Boehringer Mannheim Gmbh, dimethyl diselenide from Alfa Division-Ventron, pyridine solution of *N*,*N*-dimethylformamide di-*n*-butyl acetal (Butyl 8) from Pierce Chemical, and D_2O (99.75%) from Merck. DL-Vinylglycine (2-amino-3-butenoate) was kindly provided by Dr. R. R. Rando (1974).

Benzeneselenol was synthesized by the borohydride reduction of benzeneselenocyanate which was prepared by the method of Uemura et al. (1975). Sodium borohydride (0.68 g, 18 mmol) in ethanol was added dropwise over 40 min to benzeneselenocyanate (2.73 g, 15 mmol) in ethanol (a final volume of 20 mL) in a stream of N_2. Concentrated HCl (5 mL) was added slowly to the mixture, followed by addition of 200 mL of water. After extraction with ether and drying over Na_2SO_4, the product was distilled under reduced pressure and N_2. The product was identified as benzeneselenol by ^1H NMR. Thus, we obtained 0.9 g of benzeneselenol, whose boiling point is 63 °C (20 mmHg). 1-Hexaneselenol was synthesized in the same way. Sodium borohydride (0.57 g, 15 mmol) and 1-hexaneselenocyanate (2.5 g, 13 mmol) were allowed to react in 20 mL of ethanol under N_2. The product was identified as 1-hexaneselenol by ^1H NMR. The yield was 0.5 g. Methaneselenol was prepared in a form of gas, since it is very volatile as reported by Baroni (1930) and Coates (1953). To 10 mL of dimethyl diselenide (3 g, 16 mmol) in ethanol, 10 mL of sodium borohydride (1.8 g, 48 mmol) in ethanol was added slowly at 30 °C. Evolved methaneselenol was introduced into the enzyme reaction mixture with N_2 as a carrier gas. The other chemicals were analytical grade reagents.

Enzyme Assay. The enzymatic α,γ-elimination of selenium or sulfur amino acids was routinely followed by determining α-ketobutyrate, a product, with 3-methyl-2-benzothiazolone hydrazone hydrochloride (MBTH) (Soda, 1968). The γ-replacement reaction was determined by measuring the amount of amino acids formed with ninhydrin after separation by paper chromatography (Soda et al., 1961). The standard assay systems and conditions were described in the previous paper (Tanaka et al., 1977; methods A and B). DL-Selenomethionine and selenols were substituted for L-methionine and thiols, respectively. Reactions with selenols were carried out in sealed tubes in which air was displaced with N_2. The pH dependence

† From the Laboratory of Microbial Biochemistry, Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611 (N.E. and K.S.), the Laboratory of Petroleum Chemistry, Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611 (S.U.), the Research Institute for Food Science, Kyoto University, Uji, Kyoto-Fu 611 (T.S.), and the Laboratory of Biochemistry, Kyoto College of Pharmacy, Yamashina, Kyoto-Fu 607, Japan (H.T.). *Received August 16, 1978.*

[1] *Pseudomonas ovalis* was recently reclassified as *Pseudomonas putida* (Buchanan & Gibbons, 1974).

0006-2960/79/0418-0407$01.00/0

of the α,γ-elimination and γ-replacement reactions was examined in the following buffers (a final concentration of 50 mM): sodium acetate, pH 5.0–6.0; potassium phosphate, pH 6.5–8.0; sodium pyrophosphate, pH 8.0–9.5; $NaHCO_3$–Na_2CO_3, pH 9.5–10.5; and K_2HPO_4–KOH, pH 11.0–12.0.

The incubation mixture for the stoichiometric studies consisted of 20 μmol of potassium phosphate buffer (pH 8.0), 0.02 μmol of pyridoxal-P,[2] 1.8 μmol of DL-selenomethionine, and enzyme in a final volume of 1.0 mL. Water was substituted for selenomethionine in a blank. After incubation at 37 °C for 0.5, 1, and 3 h, the residual DL-selenomethionine was determined by paper chromatography as described above. Ammonia was assayed spectrophotometrically by determining NADH at 25 °C. The reaction mixture contained 60 μmol of potassium phosphate buffer (pH 8.0), 25 μmol of sodium α-ketoglutarate, 0.3 μmol of NADH, a sample solution, and 30 units of glutamate dehydrogenase in a final volume of 1.0 mL. α-Ketobutyrate was determined spectrophotometrically with lactate dehydrogenase as well. The assay mixture in a final volume of 1.0 mL consisted of 40 μmol of potassium phosphate buffer (pH 8.0), 0.3 μmol of NADH, a sample solution, and 24 units of lactate dehydrogenase. The reaction was carried out at 25 °C for 30 min, and a change in A_{340} was read. Methaneselenol was determined with DTNB by use of a molar absorption coefficient of 13 600 M^{-1} cm^{-1} at 412 nm (Ellman, 1959; Nashef et al., 1977). The reaction was conducted at 30 °C for 3 h under anaerobic conditions in a Thunberg tube containing the reaction mixture in the main compartment and a mixed solution (2 mL) of 20 μmol of DTNB and 40 μmol of potassium phosphate buffer (pH 8.0) in the head compartment. After the DTNB solution was tipped into the enzyme reaction mixture, the absorbance at 412 nm was measured.

Selenols and thiols were determined with DTNB just before use, and the same amounts were added to the reaction mixture in the experiment where their γ-replacement reactions were compared. Concentrations of selenium amino acids other than selenomethionine were expressed in terms of selenomethionine concentration on the basis of its ninhydrin color yield. Specific activity of the enzyme was expressed as the amount of amino acid formed (μmol) per minute per milligram of enzyme.

Gas Chromatography–Mass Spectrometry. Gas chromatography–mass spectrometry was performed with a Shimadzu-LKB 9000 gas chromatograph–mass spectrometer. Ionization voltage, acceleration voltage, and trap current were 70 eV, 3.5 kV, and 60 μA, respectively. The ion source was kept at 290 °C. Gas chromatography was run on a coiled glass column (3 mm × 2 m) packed with 3% SE-52 on Chromosorb W (60–80 mesh; acid washed and silanized). Helium was used as a carrier gas at a flow rate of 30 mL/min. Injection port was kept at 280 °C. The column was programmed from 100 to 270 °C at a rate of 6 °C/min.

Other Instrumentation. Spectrophotometric measurements were made with a Carl-Zeiss PMQ II spectrophotometer or a Union SM 401 spectrophotometer with a 1.0-cm light path. ^1H NMR spectra were taken with a JEOL JNM-FX 100 spectrometer operated at 99.65 MHz in the Fourier transform mode. Sodium 4,4-dimethyl-4-silapentane-5-sulfonate was used as an internal standard, and chemical shifts are reported in δ values (ppm).

Results

α,γ-Elimination Reaction of Selenomethionine. When the enzyme reacted with DL-selenomethionine under the conditions described previously for L-methionine (Tanaka et al., 1977), we identified the products as α-ketobutyrate, ammonia, and methaneselenol as follows. Methaneselenol was identified by ^1H NMR after oxidation with DTNB. The reaction was carried out in a Thunberg tube as described under Experimental Procedures except that all compounds were dissolved in D_2O. The enzyme solution in D_2O was prepared by repeated concentrations and dilutions with 10 mM potassium phosphate buffer in D_2O (pD 7.6) with an Amicon 202 ultrafiltration unit. After the tube was evacuated and flushed with N_2 three times, the reaction was started by tipping DL-selenomethionine into the main compartment and performed at 37 °C for several hours. The DTNB solution in the head compartment was colored yellow (λ_{max} 412 nm), suggesting that volatile methaneselenol formed from selenomethionine reacted with DTNB to form 3-carboxylato-4-nitrothiophenolate anion (Ellman, 1959). The ^1H NMR spectrum of the yellow solution showed a new singlet peak at δ 2.50, which probably arises from methyl proton, in addition to signals of HDO (δ 4.75) and phenyl protons (δ 7.50–8.12). When DL-selenomethionine was replaced by L-methionine, a singlet signal at δ 2.45 also appeared. The product from L-methionine is methanethiol as reported previously (Tanaka et al., 1977), and methanethiol is certainly oxidized by DTNB to dimethyl disulfide or 3-carboxylato-4-nitrophenyl methyl disulfide under the conditions. Therefore, the singlet signal at δ 2.45 is assigned to the methyl proton of the oxidized product from methanethiol. The chemical shift of the methyl proton of dimethyl diselenide is δ 2.548 as reported by Lardon (1970). This value is very consistent with that observed for the oxidized product from selenomethionine. Thus, the direct product from selenomethionine is methaneselenol. The formation of methaneselenol was confirmed also by the experiments in which solutions of mercuric acetate, lead acetate, silver nitrate, and cupric sulfate were used instead of the DTNB solution. Yellow precipitates were formed with gaseous methaneselenol in the head compartment of the Thunberg tubes. The precipitate was not formed under aerobic conditions. Baroni (1930) observed that the salts of methaneselenol formed with metal solutions are yellow.

Table I: Stoichiometry of Reaction[a]

time (min)	seleno-methionine disappeared (μmol)	α-keto-butyrate formed[b] (μmol)	ammonia formed (μmol)	methane-selenol formed (μmol)
30	0.670	0.665	0.660	
60	0.755	0.750	0.756	
180	0.891	0.900	0.902	0.868

[a] The reactions and assays were carried out as described in the text. [b] α-Ketobutyrate was determined with MBTH and also with lactate dehydrogenase.

α-Ketobutyrate was identified paper chromatographically and enzymatically as described previously (Tanaka et al., 1977). The formation of ammonia was confirmed by the glutamate dehydrogenase method (Kun & Kearney, 1974). Balance studies show that 1 mol of selenomethionine is converted into equimolar amounts of α-ketobutyrate, ammonia, and methaneselenol (Table I). The maximum reactivity was found at pH 8.0 for the α,γ-elimination reaction of selenomethionine. This value is closely similar to that observed for the α,γ-elimination of methionine (Tanaka et al., 1976).

Kinetics. The kinetic parameters for DL-selenomethionine and L-methionine were determined under the standard assay conditions. K_m values were 0.51 mM for selenomethionine and

[2] Abbreviations used: pyridoxal-P, pyridoxal 5′-phosphate; DTNB, bis(5-carboxy-4-nitrophenyl) disulfide; NMR, nuclear magnetic resonance.

1.33 mM for methionine. We showed that D-methionine and D-cysteine are not substrates (Tanaka et al., 1977). Therefore, the value for selenomethionine was estimated on the assumption that only the L form is active. The V_{max} values for the α,γ-elimination reactions of selenomethionine and methionine were 2.27 and 1.88 (μmol/mg)/min, respectively.

γ-Replacement Reaction of Selenomethionine. We found that L-methionine γ-lyase catalyzes γ-replacement reaction between the selenomethyl group of selenomethionine and various thiols, e.g., ethanethiol, 1-propanethiol, and benzenethiol. The products were identified as the corresponding S-substituted homocysteines in the same manner as described previously (Tanaka et al., 1977). The enzyme showed an optimum reactivity at about pH 8.5 when examined in the DL-selenomethionine–1-propanethiol system. The rates of γ-replacement reactions of selenomethionine with ethanethiol, 1-propanethiol, and benzenethiol were approximately 24, 10, and 18%, respectively, lower than those of methionine.

Reactivity of Selenols as Substituent Donors. (1) Formation of Selenomethionine. Gaseous methaneselenol was introduced into the reaction mixture (2.2 mL) containing 100 μmol of DL-methionine sulfone, 200 μmol of potassium phosphate buffer (pH 8.0), 0.04 μmol of pyridoxal-P, and 1.2 mg of enzyme. The reaction mixture was incubated at 30 °C for 2 h and heated at about 100 °C for 5 min, followed by centrifugation. The supernatant solution was chromatographed at 25 °C on Toyo No. 51 filter paper with 1-butanol–acetic acid–water (12:3:5 v/v/v) as a solvent. The area corresponding to the product (R_f 0.52) 0.52) was cut off. The product was eluted with water and evaporated to dryness. To the residue was added 100 μL of a pyridine solution of *N,N*-dimethylformamide di-*n*-butyl acetal (2 mequiv/mL), which reacts with both carboxylic and amino groups of amino acids to form *N*-(*N,N*-dimethylaminomethylene) *n*-butyl ester (DMAM-BE) derivatives (Thenot & Horning, 1972). The reaction was carried out in a microvial with a Teflon cap liner at 60 °C for 20 min. The reaction mixture was subjected directly to gas chromatographic–mass spectrometric analysis. The derivative, whose retention time was 20 min under the conditions used, was identical by gas chromatography with the same derivative of authentic selenomethionine. The mass spectrum of DMAM-BE derivative of the product showed characteristic selenium isotope pattern at *m/e* 308 (M^+), 293 (M − 15), 264 (M − 44), and 251 (M − 57) (Agenäs, 1973). It coincided with that of authentic DL-selenomethionine. Thus, the product was identified as selenomethionine.

(2) Formation of Other Se-Substituted Selenohomocysteines. L-Methionine was incubated with the enzyme in the presence of benzeneselenol. The formation of a new amino acid, which reacts with a platinum reagent (Toennis & Kolb, 1951), was also observed by paper chromatography. To isolate and identify the amino acid, the reaction mixture on a four-times larger scale was incubated at 30 °C for 3 h under anaerobic conditions. After deproteinization the product was isolated and analyzed in the same manner as described for selenomethionine. The DMAM-BE derivative of the product gave a peak at 31.5 min by gas chromatography. The existence of Se atom in the molecule was suggested, on the basis of the relative abundance of each molecular ion peak and fragment, by mass spectrometry as described for selenomethionine. The spectrum was closely similar to that of the derivative of selenomethionine. The same fragment ion peaks were observed at *m/e* 44, 57, 84, 111, 112, 129, 170, 186, and 199 in both derivatives, and several other ion peaks of the derivative of product [e.g., molecular ion peak (*m/e* 370) and fragments at *m/e* 326 (M − 44) and *m/e* 269 (M − 101)] are higher than those of selenomethionine by 62 atomic mass units (amu). This difference is ascribed to the difference between the methyl group (15 amu) of selenomethionine and the phenyl group (77 amu) of the product. The results show that the product is *Se*-phenylselenohomocysteine.

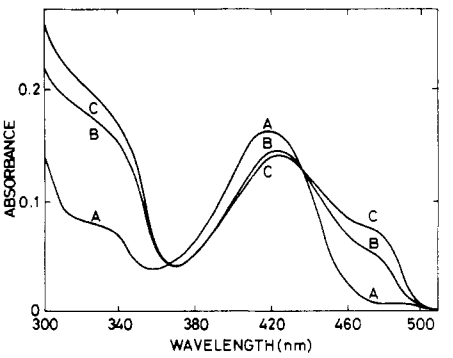

FIGURE 1: Spectral change of L-methionine γ-lyase with DL-selenomethionine and DL-vinylglycine. The reaction mixture contained, in a final volume of 0.7 mL, 1.06 mg of L-methionine γ-lyase and 140 μmol of potassium phosphate buffer (pH 8.0). The reaction was started by addition of 70 μmol of the substrate and performed at 25 °C. Curve A, control, water was substituted for the substrate; curve B, the absorption spectrum was scanned over a 10-s period immediately after addition of DL-selenomethionine; curve C, DL-vinylglycine was used instead of DL-selenomethionine as in curve B.

The formation of *Se*-*n*-hexylselenohomocysteine was shown in the same way with the reaction system in which benzeneselenol was replaced by 1-hexaneselenol.

When the derivatives of methionine (e.g., homocysteine) which are substrates for the α,γ-elimination reaction were incubated with benzeneselenol or 1-hexaneselenol, the corresponding Se-substituted selenohomocysteines were produced. DL-Vinylglycine also served as a substrate in γ-addition reaction with both selenols in addition to the thiols (Esaki et al., 1977), and the products were isolated and identified in the same manner.

(3) Other Properties. The reactivity of selenols was compared with that of thiols in a reaction system in which L-methionine was used as a substrate. Specific activities of the enzyme in γ-replacement reaction with benzeneselenol and 1-hexaneselenol were 0.094 and 0.167, respectively, whereas those with benzenethiol and 1-hexanethiol were 0.900 and 0.211, respectively. The pH optimum of the enzyme in γ-replacement reaction with selenols was between 8.5 and 9.0.

Absorption Spectral Change of the Enzyme with Selenomethionine. Absorption spectrum of the enzyme is characterized by a peak at 420 nm and a shoulder at 330 nm (Figure 1A). Immediately after the addition of DL-selenomethionine to the enzyme, a new absorption shoulder around 480 nm appeared, and the absorption at 420 nm was quenched slightly and shifted to 430 nm (Figure 1B) as observed for the reaction with DL-vinylglycine (Figure 1C). The spectrum gradually returned to its original form as the substrate concentration decreased. However, the absorption below 370 nm did not return, apparently because the product, α-ketobutyrate, absorbs greatly below 370 nm.

Discussion

Certain enzymes that participate in the metabolism of methionine are capable of acting on selenium analogues. Selenomethionine serves as a better substrate than methionine for ATP:L-methionine *S*-adenosyltransferase of *Saccharomyces cerevisiae*, *Escherichia coli*, rabbit liver, and rat liver (Mudd & Cantoni, 1957; Greene, 1969). *Se*-Adenosyl-

selenomethionine formed by the yeast enzyme also was shown to be an efficient methyl donor in various methylation systems (Coch & Greene, 1971). Hoffman et al. (1970) showed that methionyl-tRNA synthetase of *E. coli* works on both methionine and selenomethionine with almost the same affinity. The evidence presented here shows that selenomethionine is a substrate of L-methionine γ-lyase with higher affinity and reactivity than methionine in α,γ-elimination, although is a little less reactive in γ-replacement reaction.

Pyridoxal-P dependent enzymes which catalyze replacement reactions play an important role in biosynthesis of amino acids such as homocysteine, cysteine, and tryptophan. The formation of cysteine from *O*-acetylserine and H_2S by *O*-acetylserine sulfhydrylase has been demonstrated in microorganisms (Kredich & Thomkins, 1966; Wiebers & Garner, 1967) and higher plants (Giovanelli & Mudd, 1967). Chen et al. (1970) reported that serine is used as an intermediate in the biosynthesis of selenocysteine and *Se*-methylselenocysteine in *Astragalus bisulcatus* and suggested that *O*-acetylserine sulfhydrylase reacts with H_2Se to produce selenocysteine through β-replacement. No evidence, however, has been obtained for the incorporation of Se atom into the selenium amino acids. We showed here that L-methionine γ-lyase can replace the γ-substituent of methionine and its derivatives with selenoalkyl or selenoaryl groups to form the corresponding Se-substituted selenohomocysteines. This is the first evidence showing that selenols are incorporated into selenium-containing amino acids. This enzymatic replacement reaction facilitates the synthesis of various selenium amino acids labeled with ^{14}C, ^{75}Se, and ^{3}H.

Snell & Di Mari (1970) and Davis & Metzler (1972) proposed that β- and γ-replacement reactions by pyridoxal-P enzymes proceed through nucleophilic addition of a substituent donor to the intermediate derived from the substrate amino acid. It is most likely that selenols are more reactive as substituent donors than thiols in the replacement reactions, since selenols are more nucleophilic (Klayman, 1973). We, however, found that selenols are less efficient substituent donors than thiols in the γ-replacement reaction. L-Methionine γ-lyase was slowly inactivated and the reaction rate decreased to 70% of the initial one when incubated at 25 °C for 1 h in the reaction mixture containing benzeneselenol. The low reactivity of selenols is not attributable to this partial inactivation because the replacement reaction rate was determined as the initial velocity. Some other properties of selenols and thiols such as solubility and dissociation constant of ionization may affect their reactivity in the enzyme reaction, although further investigation is needed.

We suggested previously (Esaki et al., 1977) that α,γ-elimination and γ-replacement reactions by the enzyme proceed via a common key intermediate of a pyridoxal-P Schiff base of vinylglycine as first proposed for the cystathionine γ-synthase reaction (Guggenheim & Flavin, 1969). The addition of selenomethionine to the enzyme resulted in appearance of a new absorption shoulder around 480 nm as observed previously for the reaction with vinylglycine and sulfur amino acids which are substrates for α,γ-elimination reaction (Esaki et al., 1977). Thus, selenomethionine also probably undergoes α,γ-elimination through an intermediate derived from a pyridoxal-P Schiff base of vinylglycine. The γ-addition reaction of vinylglycine with selenols to produce Se-substituted selenohomocysteines supports the proposed reaction mechanism.

Acknowledgments

We thank Drs. T. Yamamoto and H. Imahara for their helpful advice. Thanks are also due to Dr. R. R. Rando for supplying DL-vinylglycine.

References

Agenäs, L. B. (1973) in *Organic Selenium Compounds: Their Chemistry and Biology* (Klayman, D. L., & Günther, W. H. H., Eds.) pp 963–988, Wiley, New York.
Baroni, A. (1930) *Atti Accad. Naz. Lincei, Mem. 12*, 234; (1931) *Chem. Abstr. 25*, 2687.
Buchanan, R. E., & Gibbons, N. E., Eds. (1974) in *Bergey's Manual of Determinative Bacteriology, 8th Ed.*, pp 222, Williams and Wilkins, Baltimore, MD.
Chen, D. M., Nigam, S. N., & MacConnell, W. B. (1970) *Can. J. Biochem. 48*, 1278.
Coates, G. E. (1953) *J. Chem. Soc.* 2839.
Coch, E. H., & Greene, R. C. (1971) *Biochim. Biophys. Acta 230*, 223.
Cone, J. E., Del Rio, R. M., Davis, J. N., & Stadtman, T. C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2659.
Davis, L., & Metzler, D. E. (1972) *Enzymes, 3rd Ed. 7*, 33.
Ellman, G. L. (1959) *Arch. Biochem. Biophys. 82*, 70.
Esaki, N., Suzuki, T., Tanaka, H., Soda, K., & Rando, R. R. (1977) *FEBS Lett. 84*, 309.
Forstrom, J. W., Zakowski, J. J., & Tappel, A. L. (1978) *Biochemistry 17*, 2639.
Giovanelli, J., & Mudd, S. H. (1967) *Biochem. Biophys. Res. Commun. 27*, 150.
Greene, R. C. (1969) *Biochemistry 8*, 2255.
Guggenheim, S., & Flavin, M. (1969) *J. Biol. Chem. 244*, 6217.
Hoffman, J. L., MacConnell, K. P., & Carpenter, D. R. (1970) *Biochim. Biophys. Acta 199*, 531.
Klayman, D. L. (1973) in *Organic Selenium Compounds: Their Chemistry and Biology* (Klayman, D. L., & Günther, W. H. H., Eds.) pp 67–171, Wiley, New York.
Kredich, N. M., & Thomkins, G. M. (1966) *J. Biol. Chem. 241*, 4955.
Kun, E., & Kearney, F. B. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., Ed.) 2nd ed., Vol. 4, pp 1802, Academic Press, New York.
Lardon, M. (1970) *J. Am. Chem. Soc. 92*, 5063.
Mudd, S. H., & Cantoni, G. L. (1957) *Nature (London) 180*, 1052.
Nashef, A. S., Osuga, D. T., & Feeney, R. E. (1977) *Anal. Biochem. 79*, 394.
Pegg, A. E. (1969) *Biochim. Biophys. Acta 177*, 361.
Rando, R. R. (1974) *Biochemistry 13*, 3859.
Shrift, A. (1973) in *Organic Selenium Compounds: Their Chemistry and Biology* (Klayman, D. L., & Günther, W. H. H., Eds.) pp 763–814, Wiley, New York.
Snell, E. E., & Di Mari, S. J. (1970) *Enzymes, 3rd Ed. 2*, 335.
Soda, K. (1968) *Anal. Biochem. 25*, 228.
Soda, K., Tochikura, T., & Katagiri, H. (1961) *Agric. Biol. Chem. 25*, 811.
Stadtman, T. C. (1973) *Science 183*, 915.
Tanaka, H., Esaki, N., Yamamoto, T., & Soda, K. (1976) *FEBS Lett. 66*, 307.
Tanaka, H., Esaki, N., & Soda, K. (1977) *Biochemistry 16*, 100.
Thenot, J. P., & Horning, E. C. (1972) *Anal. Lett. 5*, 519.
Toennis, G., & Kolb, J. J. (1951) *Anal. Chem. 23*, 823.
Uemura, S., Toshimitsu, A., Okano, M., & Ichikawa, K. (1975) *Bull. Chem. Soc. Jpn. 48*, 1925.
Wiebers, J. L., & Garner, H. R. (1967) *J. Biol. Chem. 242*, 5644.