and 2-hydroxy-4-pentenoic acid need to be determined. Furthermore, in the absence of a peptide with a sequence overlapping the β-alanine residue, inversion of the sequence during partial acid hydrolysis is a possibility, although an unlikely one. Therefore the sequence of this part of the molecule is not yet unequivocal. One approach to this problem would be to isolate the hydroxyl acyl peptide resulting from alkaline hydrolysis of roseotoxin B (Engstrom et al., 1975) and determine the C-terminal amino acid.

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Peroxide Oxidation Products of Homocystine and Lanthionine

Samuel H. Lipton

Isolation of DL-homocysteic acid, DL-homolanthionine sulfone, and DL-homolanthionine as crystalline products of peroxide oxidation of DL-homocystine in HCl solution confirmed structures deduced from amino acid analysis. DL-Homocysteic acid, produced in 4.8 N HCl, was easily isolated because it was not adsorbed by Dowex-50-H^+ resin. Similar oxidation in 0.8 N HCl produced DL-homolanthionine sulfoxide. This sulfoxide intermediate was heated in 6 N HCl to form DL-homolanthionine sulfone which crystallized from NH_4OH solution. Reduction of the sulfoxide by dimethyl sulfide in 6 N HCl formed DL-homolanthionine which crystallized from NH_4OH solution following ion-exchange chromatography. This is a simple new preparation of DL-homolanthionine from inexpensive DL-homocystine. *S*-Methyl-L-cysteine and *S*-ethyl-L-cysteine were oxidized by peroxide in 0.8 N HCl to sulfones. Thus these thioethers resembled methionine in yielding sulfones, unlike *meso*-lanthionine which mainly yielded sulfoxides in 0.8 N HCl. However, the main oxidation product of *meso*-lanthionine in 4.8 N HCl solution was the sulfone which crystallized from NH_4OH solution.

Peroxide oxidation of the sulfur amino acids cystine, lanthionine, and homocystine formed products that varied with reaction conditions, particularly hydrochloric acid concentration (Lipton et al., 1977). An especially interesting feature of the peroxide oxidation of the disulfide amino acids was the loss of 1 mol of sulfur. The formation of homolanthionine sulfoxide and sulfone by peroxide oxidation of homocystine was first reported by Clopath and McCully (1976). Homocystine oxidation was further studied and the analogous formation of lanthionine sulfoxide and sulfone from peroxide oxidation of cystine was reported (Lipton et al., 1977). These chemical losses of sulfur from disulfide amino acids are of interest because lanthionine is formed in heated proteins (Hurrell et al., 1976). In the present study, peroxide oxidations were on a larger scale so that products could be purified and the assigned structures (Lipton et al., 1977) could be confirmed. DL-Homocysteic acid, DL-homolanthionine sulfone,

Protein Nutrition Laboratory, Nutrition Institute, Federal Research, Science and Education Administration, Agricultural Research Center—East, U.S. Department of Agriculture, Beltsville, Maryland 20705.

and DL-homolanthionine were isolated from peroxide oxidation of DL-homocystine, and *meso*-lanthionine sulfone was isolated from peroxide oxidation of *meso*-lanthionine. Peroxide oxidation products of the thioether amino acids *S*-methyl-L-cysteine and *S*-ethyl-L-cysteine were studied on the amino acid analyzer.

EXPERIMENTAL SECTION

Materials. Amino acids and other chemicals were from the sources reported (Lipton et al., 1977) with two additions. *S*-Methyl-L-cysteine and *S*-ethyl-L-cysteine were from Sigma Chemical Co.

Amino Acid Analyses. A Phoenix Biolyzer, Model 3000, was used as described (Lipton et al., 1977). Amino acid analyses were used to select reaction conditions suitable for isolation of specific products, to evaluate fractionation procedures, and to assess the purity of the isolated products.

Preparation of DL-Homocysteic Acid by Oxidation of DL-Homocystine. DL-Homocystine (1.34 g, 5 mmol) was dissolved in 10 mL of 6 N HCl in a 40-mL conical centrifuge tube. When 2.5 mL of 30% H_2O_2 was added and the mixture was stirred with a glass rod, a vigorous reaction shortly ensued with production of heat and gas. For completion of the oxidation, the tube was then heated for 10 min in a waterbath at 100 °C. A sample analyzed on the 60-cm column of the amino acid analyzer indicated that the main product at about 16 min was homocysteic acid, with lesser amounts of homolanthionine sulfone and several other amino acid products. After transfer to a round-bottom flask, the reaction mixture was concentrated in a vacuum rotary evaporator to a thick syrup. This syrup was then further dried in a vacuum desiccator over containers of P_2O_5 and NaOH pellets. The almost glassy residue was dissolved in water, 1 N HCl was added to assure a strong reaction to Congo Red, and it was diluted to 40 mL with water. This solution was passed through a column (3.5 cm deep and 4.3 cm diameter) of Dowex-50-X8-H^+ form resin, 100–200 mesh, supported in a coarse sintered glass funnel (Pyrex No. 36060) at a flow rate of 3–4 mL/min. A 12-in. glass tube (4 mm diameter), attached to the funnel outlet, served as a "hydrostatic leg" and flow rate was controlled by adjustment of screw clamp on a short section of rubber tubing. Wash water was passed through the column at 3–4 mL/min. The combined effluent and wash, totalling 150 mL, was sampled for analysis on the amino acid analyzer. The only peak was due to homocysteic acid because the cation exchange resin absorbed all other amino acids. The effluent and wash fractions were dried as described above (weight of crude product was 1.59 g) and crystallized from about 4 mL of water. For analysis the product was recrystallized several times from water. Anal. Calcd for $C_4H_9NSO_5$: C, 26.22; H, 4.95; N, 7.75; S, 17.50. Found: C, 26.24; H, 5.20; N, 7.61; S, 17.26. (All microanalyses were run by Galbraith Laboratories, Knoxville, Tenn.)

Preparation of DL-Homolanthionine Sulfone by Oxidation of DL-Homocystine. DL-Homocystine (2.68 g, 10 mmol) was heated for 10 min at 100 °C in a mixture of 20 mL of 1 N HCl and 5.0 mL of 30% H_2O_2. The triple peaks from analysis on the amino acid analyzer indicated that homolanthionine sulfoxide isomers (see Lipton et al., 1977) were the main oxidation product. The corresponding sulfone and lesser amounts of other amino acid products were also present. After 25 mL of 12 N HCl was added, the reaction mixture was heated at 100 °C for 5 min to oxidize the sulfoxides to the sulfone. Amino acid analysis now confirmed that the main peak was due to homolanthionine sulfone. The reaction mixture was evaporated and dried as described above. After the dried residue was dissolved in dilute ammonium hydroxide, it was evaporated in a rotary evaporator to about 8 mL and crystallization was induced by scratching the walls of the container with a glass rod. The preparation was refrigerated overnight and then centrifuged, washed with cold ethanol, and dried in a vacuum desiccator. Only small amounts of amino acid impurities were present in the 1.6 g of dried crystals. After three recrystallizations from water with ammonium hydroxide to aid in solution, the product was chromatographically pure. Anal. Calcd for $C_8H_{16}N_2O_6S$: C, 35.82; H, 6.01; N, 10.44; S, 11.95. Found: C, 35.34; H, 5.96; N, 10.43; S, 12.02.

Preparation of DL-Homolanthionine from DL-Homocystine. DL-Homocystine (1.34 g, 5 mmol) was oxidized by heating for 10 min at 100 °C in a mixture of 10 mL of 1 N HCl and 2.5 mL of 30% H_2O_2. Amino acid analysis indicated that the main product was the mixture of isomers of homolanthionine sulfoxide. For reduction, this mixture was transferred to a 200-mL Wheaton pressure bottle (No. 1768-V10, Arthur H. Thomas, Philadelphia, Pa.) and cooled in an icebath; 12.5 mL of 12 N HCl and 10 mL of dimethyl sulfide were added, and the bottle was immediately sealed. The sealed bottle was stored at room temperature and occasionally shaken by hand. After about 18 h, amino acid analysis indicated that the main product was homolanthionine, which was eluted in the chromatogram at the norleucine position. Other amino acids included homocysteic acid and homolanthionine sulfone. The crude reaction mixture was evaporated to a syrup and further dried as described above. Efforts to crystallize the product as a hydrochloride failed. The amorphous product was dissolved in water, with acidification to Congo red by 1 N HCl, diluted to 40 mL, and passed through a column of Dowex-50-H^+ form resin by the procedure described above. After the homocysteic acid was washed through the column with water, elution was with aqueous HCl as follows: five fractions of 30 mL of 1 N HCl, then six fractions of 30 mL of 2 N HCl, and finally six fractions of 30 mL of 4 N HCl. Analysis of individual fractions on an amino acid analyzer showed that fractions 18–20 contained the homolanthionine. For isolation from these pooled fractions, the aqueous acid was evaporated and the residue was dried as described above. The dried residue was dissolved in ammonium hydroxide, the solution was evaporated to about 4 mL, and crystallization was induced. The weight of dried product was 0.50 g. For analyses, the sample was recrystallized three times from water, with ammonium hydroxide to aid in solution. Anal. Calcd for $C_8H_{16}N_2O_4S$: C, 40.66; H, 6.83; N, 11.86; S, 13.57. Found: C, 40.58; H, 6.61; N, 11.68; S, 13.41.

Peroxide Oxidation of *S*-Methyl-L-cysteine and *S*-Ethyl-L-cysteine. These amino acids, in 0.1 mmol amounts, were separately oxidized in 12-mL conical centrifuge tubes. They were heated for 10 min at 100 °C in mixtures of 0.2 mL of 1 N HCl and 0.05 mL of 30% H_2O_2, cooled in ice, and diluted to 1.0 mL with water; 10-μL samples were analyzed on the 60-cm column of the amino acid analyzer. Also analyzed were the unoxidized amino acids and samples of oxidized amino acids that were subsequently reduced with dimethyl sulfide for detection of sulfoxides. These samples were reduced with dimethyl sulfide by the published procedure (Lipton et al., 1977).

Preparation of *meso*-Lanthionine Sulfone by Peroxide Oxidation of *meso*-Lanthionine. *meso*-Lanthionine (0.52 g, 2.5 mmol) was dissolved in 5.0 mL of 6 N HCl in a 40-mL conical centrifuge tube and 1.25 mL of 30% H_2O_2 was added with stirring. The tube was then

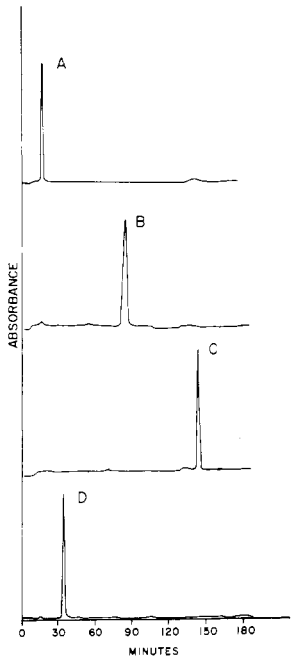


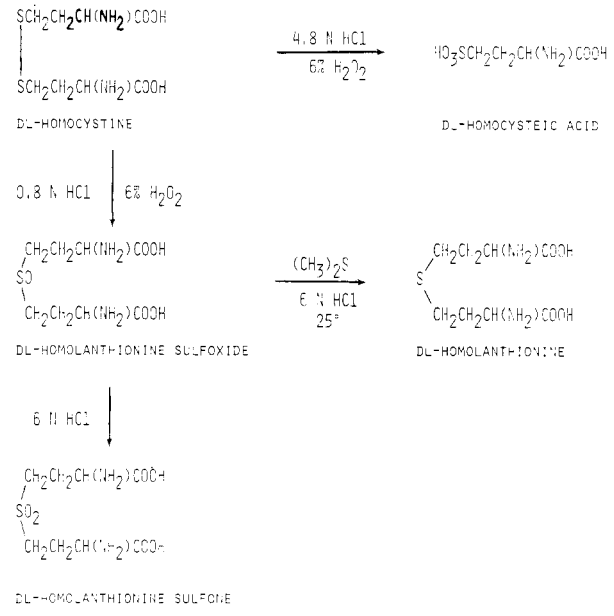
Figure 1. Amino acid analyzer chromatograms of acidic and neutral amino acids regions showing purified amino acids isolated from oxidation mixtures. Chromatogram identities are: (A) DL-homocysteic acid, (B) DL-homolanthionine sulfone, (C) DL-homolanthionine, (D) *meso*-lanthionine sulfone.

heated for 10 min at 100 °C; the reaction was vigorous and gas evolved. A sample analyzed on the amino acid analyzer had one main product at ca. 35 min. The product was isolated by evaporation in a rotary evaporator and the syrup was dried as described for the other preparations. The hydrochloride could not be crystallized, so the crude product was dissolved in aqueous ammonium hydroxide. This solution was evaporated to about 4 mL and crystallization of the free sulfone was induced by scratching with a glass rod. The weight of dried product was 0.42 g. For analysis, recrystallization was from aqueous ammonium hydroxide solution. Anal. Calcd for $C_6H_{12}N_2O_6S$: C, 29.99; H, 5.04; N, 11.66; S, 13.35. Found: C, 29.95; H, 5.17; N, 11.55; S, 13.24.

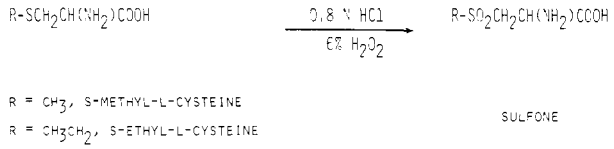
RESULTS AND DISCUSSION

The main purpose of this study was to confirm the structural assignments that had been based on chromatography (Lipton et al., 1977). By preparative scale peroxide oxidation of DL-homocystine, three pure products were isolated and microanalyses confirmed their structures. The ion-exchange chromatograms of the crystalline products indicated that they were chromatographically homogeneous, except for only traces of amino acid impurities (Figure 1). The main product was DL-homocysteic acid from oxidation in 4.8 N HCl (see Scheme I). This product was easily separated from the other amino acids because it alone was not absorbed by Dowex-50 cation-exchange resin. From oxidation in 0.8 N HCl, however, the main product was DL-homolanthionine sulfoxide. The sulfoxide was not purified but was further oxidized to the sulfone by increasing the HCl concentration to 6 N and heating at 100 °C. The DL-homolanthionine sulfone that was thus formed was isolated by direct crystallization from an ammonium hydroxide solution of the crude mixture. On the other hand, dimethyl sulfide in the presence of 6 N HCl at room temperature was used to reduce the sulfoxide of the crude mixture and thus the main product was DL-homolanthionine. In this case, the DL-homolanthionine was isolated by ion-exchange chromatography by elution

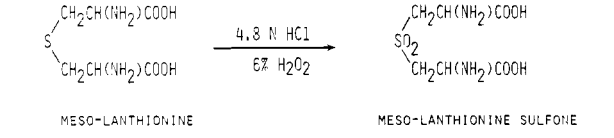
Scheme I



Scheme II



Scheme III



with increasing concentrations of aqueous HCl. Crystallization was from an ammonium hydroxide solution of the fraction eluted with 4 N HCl.

Stekol (1948) reported a synthesis of DL-homolanthionine but this newer route from the inexpensive commercially available DL-homocystine is simpler. It also would be suitable for preparation of L-homolanthionine from L-homocystine and would be simpler than the published method (Weiss and Stekol, 1951). The natural occurrence of homolanthionine was referenced (Lipton et al., 1977). It also accumulated in methionine-requiring mutants of *Aspergillus nidulans* (Paszewski and Grabski, 1975). Study of the metabolic relationship between homolanthionine and methionine should be stimulated by the increased accessibility of homolanthionine afforded by the present study.

The thioether amino acids, *S*-methyl-L-cysteine and *S*-ethyl-L-cysteine, were oxidized by peroxide to their respective sulfones, even at the low 0.8 N HCl concentration (see Scheme II). The products were not isolated, but were deduced to be sulfones, rather than sulfoxides, because their positions in the amino acid chromatograms did not shift after treatment with dimethyl sulfide in 6 N HCl in a pressure tube. The oxidation product of methionine in 0.8 N HCl was identified tentatively as the sulfoxide, but later as methionine sulfone because it was not reduced by dimethyl sulfide. Thus, lanthionine differed from the other thioethers and resembled instead the disulfides in that sulfoxides were the main product from oxidation in the presence of 0.8 N HCl. However, when the HCl was increased to 4.8 N, the main oxidation product

of *meso*-lanthionine was the sulfone (see Scheme III). Isolated *meso*-lanthionine sulfone was eluted in the amino acid chromatogram at about 35 min; thus it accounted for the peak that had been designated as unidentified (Lipton et al., 1977). This sulfone was previously reported by Zahn and Osterloh (1955).

Smith et al. (1974) identified *S*-methylcysteine sulfoxide as the toxic factor in kale and other brassica crops (Whittle et al., 1976) that causes hemolysis in ruminants. On the other hand, *S*-methylcysteine sulfoxide lowered the cholesterol in the plasma of rats (Itokawa et al., 1973). *S*-Methylcysteine sulfoxide was identified in cabbage (Synge and Wood, 1956). The high consumption of cruciferous crops in Japan (estimated human consumption of *S*-methylcysteine sulfoxide is 300 mg person^{-1} day^{-1}) has been suggested to protect against heart disease (see Whittle et al., 1976).

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Effects of Ionic Dextran Derivatives on Heat Precipitation of Protein

Kunihiko Gekko* and Hajime Noguchi

The effects of concentration, ionic or hydrophobic group density, and molecular weight of ionic polysaccharides on the heat precipitation of bovine plasma albumin (BPA) were investigated. Carboxymethyldextran (Cm-dextran), carboxymethylbenzyldextran (Cm-Bzl-dextran), dextran sulfate, and chondroitin sulfate were used as ionic polysaccharides. The heat denaturation of BPA was promoted by all ionic polysaccharides added, but there existed a critical point in the dependence of concentration, charge density, and molecular weight of ionic dextran derivatives on the heat precipitation of BPA. The kinetic studies showed that the heat precipitation of BPA in the presence of Cm-dextran and Cm-Bzl-dextran is accelerated by activation enthalpy change rather than activation entropy change, but in the presence of dextran sulfate its acceleration is mainly caused by the large increase of activation entropy. These results were discussed from the viewpoint of the different interaction behaviors of these dextran derivatives with BPA molecule.

The interaction between proteins and polysaccharides has recently been shown to be an essential element in the study of food texture or processing (Morris, 1973). Such investigations can present much valuable information for utilization of polysaccharides in precipitation systems of proteins (Hildago and Hansen, 1969; Hill and Zadow, 1974, 1975). For example, the interaction between milk proteins and carboxymethylcellulose (Cm-cellulose) at pH 3–4 has been used as a means of precipitating whey proteins (Hansen et al., 1971), while at pH 4–5 the interaction has been used to prevent the precipitation of proteins in fruit-flavored milk drinks (Asano, 1966). The effect of polysaccharides on protein solubility may be analyzed as a sum of three contributions: (1) the excluded volume effect by polysaccharide itself, (2) the salting in or out effect by an electrostatic field of polysaccharide, and (3) the intermolecular interaction between protein and polysaccharide through the electrostatic force, hydrophobic bond, and hydrogen bond. When the solubility of protein decreases on thermal denaturation, though such a system is common in food processing, the effect of protein–polysaccharide interaction on protein stability may be important to its solubility. However, only a small amount of fundamental research (Elbein and Mitchell, 1975; Imeson et al., 1977) has been carried out in this field because the system is very complicated to analyze. A possible approach to analyze such complicated systems may be to estimate systematically each contribution as mentioned above. From these points of view, in the present paper, the effect of dextran derivatives on thermal precipitation of bovine plasma albumin is systematically investigated as a model system because the thermal denaturation of bovine plasma albumin (Levy and Warner, 1954; Warner and Levy, 1958; Štokrová and Šponar, 1963; Petersen and Foster, 1965a), the properties of dextran derivatives (Noguchi et al., 1973; Gekko and Noguchi, 1974, 1975), and the interaction between them (Gorter and Nanninga, 1953; Noguchi, 1956, 1960; Bettelheim et al.,

Department of Food Science and Technology, Faculty of Agriculture, Nagoya University, Nagoya, Japan.

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