Lactoperoxidase-Catalyzed Incorporation of Thiocyanate Ion into a Protein Substrate†

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ABSTRACT: Lactoperoxidase catalyzed the peroxide-dependent incorporation of thiocyanate ion into a protein substrate. This activity was studied as a model for the peroxidase-dependent antithyroid and antimicrobial action of thiocyanate. Two types of incorporation were observed. When the amount of peroxide added did not exceed the amount of protein sulfhydryls, the sulfur and carbon portions of thiocyanate were incorporated in a one-to-one ratio. This type of incorporation was eliminated when protein sulfhydryls were blocked by reaction with *N*-ethylmaleimide. These results indicated incorporation of the intact thiocyanate moiety into a derivative of cysteine residues. Incubation with a sulfhydryl compound such as dithiothreitol resulted in release of the bound thiocyanate moiety. A second form of incorporation was observed when peroxide exceeded protein sulfhydryls. Incorporation of carbon exceeded that of sulfur. Blocking of protein sulfhydryls or addition of a sulfhydryl compound had no effect on this form of incorporation. Studies using amino acid and poly(amino acid) substrates indicated that modification of tyrosine, tryptophan, and histidine residues accounted for this incorporation. Similar modification of protein sulfhydryls and aromatic amino acids was obtained with the thiocyanate analogue of the halogens, thiocyanogen. These results suggest that lactoperoxidase-catalyzed oxidation of thiocyanate yields thiocyanogen, which modifies proteins in a manner similar to halogenation.

Lactoperoxidase and myeloperoxidase catalyze the oxidation of thiocyanate ion (SCN^-) by peroxide (H_2O_2) (Oram and Reiter, 1966a,b; Sorbo and Ljunggren, 1958). The combination of either of these peroxidases with H_2O_2 and SCN^- has been shown to have antimicrobial activity in vitro (Oram and Reiter, 1966b; Klebanoff et al., 1966). Lactoperoxidase, H_2O_2, and high concentrations of SCN^- are found in milk and saliva, and this system may contribute to the antimicrobial activity of these fluids (Zeldow, 1963; Morrison and Steele, 1968; Hamon and Klebanoff, 1973). In addition, SCN^- is an antithyroid substance and its oxidation is catalyzed by thyroid peroxidase (Barker, 1936; Wood and Williams, 1949; Coval and Taurog, 1967). The antimicrobial and antithyroid activities of SCN^- may have the same chemical basis.

Sulfate, carbon dioxide, and ammonia are the final products of SCN^- oxidation (Oram and Reiter, 1966b). None of these substances has antithyroid or antimicrobial activity. Therefore, these biological activities are probably due to intermediates in the oxidation of SCN^-. Cyanide may be an intermediate and may contribute to the toxic effects of SCN^- (Chung and Wood, 1970). The initial product of peroxidase-catalyzed oxidation of SCN^- has not been identified.

Wood and Williams (1949) detected incorporation of the sulfur portion of SCN^- into an acid-precipitable form in the thyroid. Also, bacterial enzymes are inhibited upon incubation of bacteria with lactoperoxidase, H_2O_2, and SCN^- (Oram and Reiter, 1966a; Mickelson, 1966). Therefore, products of SCN^- oxidation may react with protein. The aim of this study was to determine whether lactoperoxidase would catalyze the incorporation of SCN^- into derivatives of protein functional groups. Such incorporation could indicate which functional groups are modified and their relative reactivity. Because SCN^- has many of the properties of a halide ion (Hughes, 1975), and peroxidases can oxidize halides to halogens, the action of the peroxidase system was compared to that of the "pseudohalogen", thiocyanogen, $(SCN)_2$.

Materials and Methods

All materials were of reagent grade. Lactoperoxidase was purified from bovine milk (Morrison and Hultquist, 1963). Catalase, BSA,[1] DTT, and *N*-ethylmaleimide were obtained from Sigma, $K^{35}SCN$ (15.6 Ci/mol) from Amersham/Searle, and $NaS^{14}CN$ (10 Ci/mol) from ICN.

Synthesis of $(SCN)_2$ was achieved by reaction of lead thiocyanate with bromine (Wood, 1946). Lead thiocyanate was prepared by addition of 4.5 g of lead nitrate in 10 mL of water to 2.98 g of KSCN in 10 mL of water at 0 °C. The precipitate was collected by filtration, washed with cold water, and dried in vacuo over calcium chloride. Freshly prepared lead thiocyanate (0.17 mmol) was added to 10 mL of dry carbon tetrachloride (CCl_4) at 0 °C. Bromine (0.15 mmol) was added in small aliquots, allowing decolorization of bromine to occur before another aliquot was added. The clear, colorless solution of $(SCN)_2$ in CCl_4 was decanted from the remaining solids and used on the day of preparation. Radioactive $(SCN)_2$ was prepared on a smaller scale by adding bromine in CCl_4 to radioactive lead thiocyanate, which was obtained by adding lead nitrate to mixtures of KSCN and $NaS^{14}CN$ or $K^{35}SCN$ and then decanting the supernatant and lyophilizing the precipitate.

The concentration of $(SCN)_2$ was determined by measuring absorbance at 295 nm and assuming an extinction coefficient of 140 M^{-1} cm^{-1} (Bacon and Irwin, 1958). The concentration was also determined by iodometry (Wood, 1946). An aliquot of $(SCN)_2$ in CCl_4 was added to 10 mL of 0.1 M potassium iodide with vigorous shaking. Iodine was titrated with 10 mM sodium thiosulfate using starch as an indicator. These two methods gave identical values for freshly prepared $(SCN)_2$,

† From the Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38101. *Received April 19, 1977.* This work was supported by Research Grant No. DE 04235, Research Center Grant No. CA 08480, and Multidisciplinary Training Grant No. CA 05176 from the National Institutes of Health, and by American Cancer Society Grant No. IN-99.

[1] Abbreviations used are: BSA, bovine serum albumin; DTT, dithiothreitol; MalNEt, *N*-ethylmaleimide.

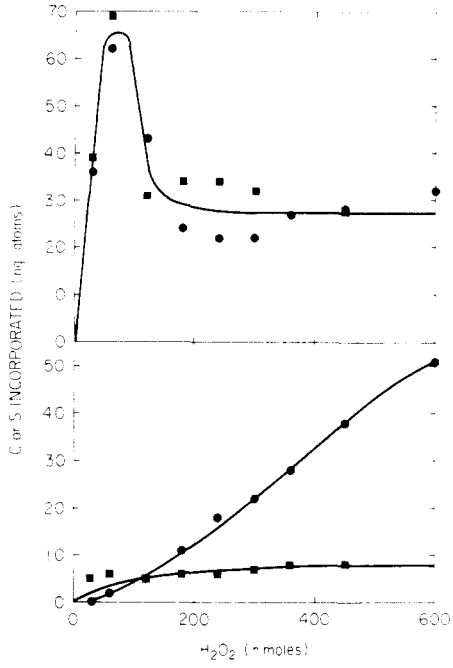

FIGURE 1: Lactoperoxidase-catalyzed incorporation of the carbon and sulfur portions of SCN^- into BSA (DTT-reversible and DTT-nonreversible forms). BSA (150 nmol; 90 nmol of sulfhydryl) was incubated with 0.1 nmol of lactoperoxidase, 1 μmol of $NaS^{14}CN$ (●) or $K^{35}SCN$ (■), and the indicated amounts of H_2O_2 in 1-mL total volume. The upper figure shows the difference between incorporation measured before and after addition of 10 μmol of DTT. The lower figure shows incorporation measured after adding DTT.

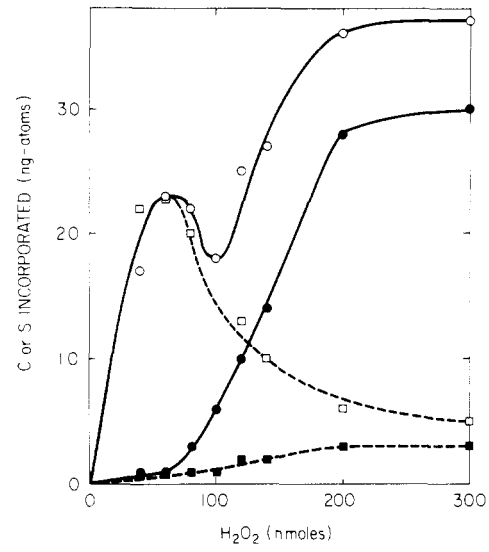

FIGURE 2: Lactoperoxidase-catalyzed incorporation of the carbon and sulfur portions of SCN^- into BSA (total incorporation and DTT-nonreversible forms). BSA (150 nmol; 80 nmol of sulfhydryl) was incubated with 0.1 nmol of lactoperoxidase, 50 nmol of $NaS^{14}CN$ (○, ●) or $K^{35}SCN$ (□, ■), and the indicated amounts of H_2O_2 in 1-mL total volume. Incorporation was measured before (○,□), or after (●,■) the addition of 10 μmol of DTT.

which was equal to that calculated from the amount of bromine added.

Incorporation of Radioactivity. Amino acids (1 mg/mL), poly(amino acids) (1 mg/mL), or BSA (10 mg/mL; 150 μM protein; 60 to 90 μM protein sulfhydryls) were incubated with $NaS^{14}CN$ or $K^{35}SCN$ and 0.1 μM lactoperoxidase in 10 mM potassium phosphate buffer (pH 6.6) with 5 mM potassium EDTA at 25 °C. Additions of H_2O_2 were made at 1-min intervals, with each addition sufficient to give a final concentration of 10 μM. After the final addition of H_2O_2, incubation was continued for 15 min. Alternatively, aliquots of $(S^{14}CN)_2$ or $(^{35}SCN)_2$ in CCl_4 were added directly to solutions of amino acids or BSA with rapid mixing.

Acid Precipitation. Poly(amino acids) and BSA were precipitated by adding 1 mL of cold 10% (w/v) trichloroacetic acid to 0.1 mL of reaction mixture, followed by filtration through nitrocellulose filters (Millipore Corp.) and washing with 5% trichloroacetic acid. Filters were dissolved in scintillation fluid prepared according to Bray (1960), and radioactivity was determined in a liquid scintillation spectrometer.

Thin-Layer Chromatography. Chromatography of amino acid derivatives was performed on cellulose sheets (Eastman Kodak Co.) in four solvent systems (Block and Bolling, 1951; Piez et al., 1956): solvent A, *tert*-amyl alcohol-2,6-lutidine-water, 19:19:12; solvent B, *tert*-butyl alcohol-formic acid-water, 14:3:3; solvent C, *sec*-butyl alcohol-*tert*-butyl alcohol-0.3% ammonium hydroxide, 4:1:3; and solvent D, ethanol-water-diethylamine, 77:23:2. Radioactive compounds were visualized by autoradiography and then removed from the cellulose sheets and radioactivity was determined as described above.

Results

Modification of BSA by Lactoperoxidase, H_2O_2, and SCN^-. Figure 1 shows lactoperoxidase-catalyzed incorporation of the carbon and sulfur portions of SCN^- into BSA with increasing amounts of H_2O_2. Incorporation was of two types. The first type (upper figure) consisted of incorporation of carbon and sulfur in a 1:1 ratio. This indicated incorporation of the SCN moiety as an intact unit. Incorporation of the SCN moiety reached a maximum when the amount of H_2O_2 was equal to the amount of protein sulfhydryls. This maximum amount of incorporation was about equal to the amount of sulfhydryls. Blocking of sulfhydryls by reaction with *N*-ethylmaleimide eliminated this type of incorporation. These results suggested that one SCN moiety per molecule of H_2O_2 was incorporated into one molecule of a derivative of protein sulfhydryls. When the sulfhydryl compound DTT was added, the bound SCN moiety was released.

A second type of incorporation (lower figure) increased sharply as H_2O_2 exceeded sulfhydryls. Incorporation of the carbon portion of SCN^- greatly exceeded that of sulfur, and the amount of incorporation was low relative to the amount of H_2O_2. This type of incorporation was not released upon addition of DTT. Total incorporation into BSA was the sum of that shown in the upper and lower portions of Figure 1.

Results shown in Figure 1 were obtained with SCN^- at a higher concentration than any of the other components in the system. Figure 2 shows incorporation obtained with an amount of SCN^- less than the largest amount of H_2O_2 added, and less than the amount of protein sulfhydryls. The pattern of incorporation was similar to that obtained with higher concentrations of SCN^-. Carbon and sulfur were incorporated together into a form released upon addition of DTT, and this form of incorporation reached a maximum when H_2O_2 was about equal to protein sulfhydryls. However, the amount of the bound SCN moiety was less than that obtained at high SCN^- concentrations, and less than the amount of sulfhydryls. The amount of bound SCN decreased as H_2O_2 exceeded sulfhydryls.

Carbon incorporation began to rise again as H_2O_2 exceeded sulfhydryls, and this incorporation was not released upon addition of DTT. With this lower SCN^- concentration, nonreversible incorporation reached a plateau level, rather than continuing to increase as more H_2O_2 was added. At the plateau

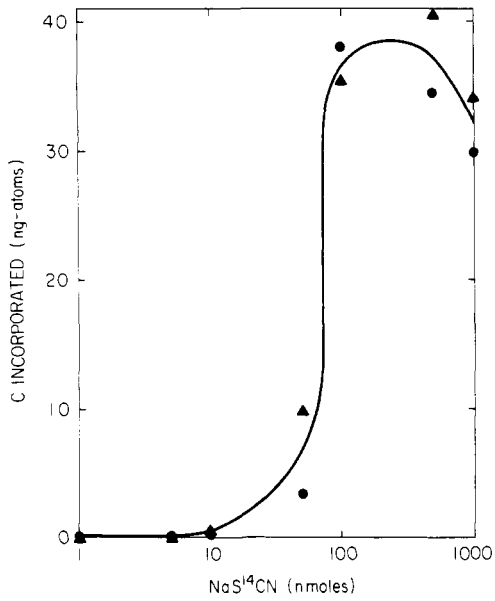

FIGURE 3: Effect of SCN^- concentration on lactoperoxidase-catalyzed incorporation of the carbon portion of SCN^- into BSA (DTT-nonreversible forms). BSA was incubated with a tenfold molar excess of *N*-ethylmaleimide at 37 °C for 1 h, then precipitated with acetone, and lyophilized to yield MalNEt-BSA. Lactoperoxidase (0.1 nmol), 200 nmol of H_2O_2, and the indicated amounts of $NaS^{14}CN$ were incubated with 150 nmol of BSA (70 nmol of sulfhydryl) (●) or MalNEt-BSA (5 nmol of sulfhydryl) (▲). Incorporation was measured after addition of 10 μmol of DTT.

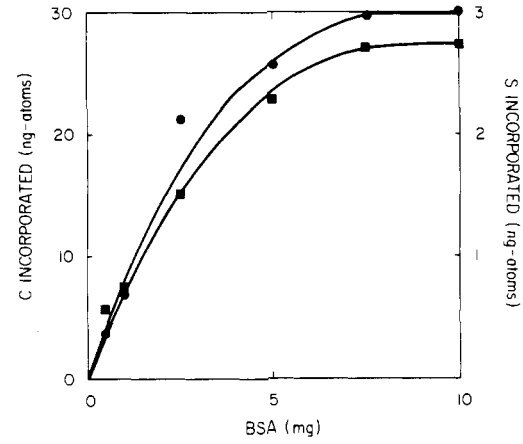

FIGURE 4: Effect of BSA concentration on lactoperoxidase-catalyzed incorporation of the carbon portion of SCN^- (DTT-nonreversible forms). The indicated amounts of BSA (7.5 to 150 nmol) were incubated with 0.1 nmol of lactoperoxidase, 50 nmol of $NaS^{14}CN$ (●) or $K^{35}SCN$ (■), and 200 nmol of H_2O_2 in 1-mL total volume. Incorporation was measured after addition of 10 μmol of DTT.

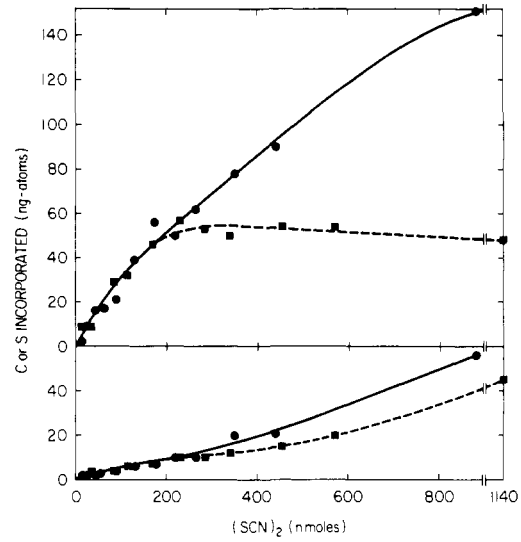

FIGURE 5: Incorporation of the carbon and sulfur of $(SCN)_2$ (DTT-reversible and -nonreversible forms). BSA (150 nmol; 80 nmol of sulfhydryl) was incubated with the indicated amounts of $(S^{14}CN)_2$ (—●) or $(^{35}SCN)_2$ (- - - ■) in 1-mL total volume. The upper figure shows the difference between incorporation measured before and after addition of 10 μmol of DTT. The lower figure shows incorporation measured after adding DTT.

level, 74% of the total carbon label was incorporated into protein. The amount of incorporation and the pattern of incorporation were independent of lactoperoxidase concentration over the range from 10 nM to 1 μM.

Figure 3 shows the nonreversible type of carbon incorporation as a function of SCN^- concentration. The amount of H_2O_2 was held constant at a level much higher than the concentration of protein sulfhydryls. Incorporation increased sharply at $Na^{14}SCN$ concentrations of about 50 μM. Also shown are results obtained with BSA that had been reacted with *N*-ethylmaleimide to block sulfhydryls (MalNEt-BSA). Nearly identical results were obtained with BSA and MalNEt-BSA, indicating that sulfhydryls were not required to obtain the nonreversible type of incorporation.

The amount of nonreversible incorporation depended on substrate protein concentration, as well as on H_2O_2 and SCN^- concentrations. Figure 4 shows that concentrations of BSA greater than 7 mg/mL were required for maximum incorporation. A high ratio of carbon to sulfur incorporation was observed at all concentrations of BSA. The amount of incorporation was less than one atom of carbon or sulfur per molecule of BSA.

Modification of BSA by $(SCN)_2$. Figure 5 shows incorporation of the carbon and sulfur portions of $(SCN)_2$ into BSA. Two types of incorporation were similar in most respects to those obtained with the peroxidase system: (1) a DTT-reversible type in which carbon equaled sulfur, and (2) a nonreversible type in which carbon exceeded sulfur and which increased after the DTT-reversible type reached a maximum. Results differed in that DTT-reversible incorporation continued to increase as $(SCN)_2$ exceeded the amount of protein sulfhydryls. Also, the ratio of carbon to sulfur in the DTT-nonreversible form was not as high as that obtained with the peroxidase system.

A third type of incorporation was obtained with $(SCN)_2$ that was not observed with the peroxidase system. This incorporation was DTT reversible, but contained only carbon. The addition of H_2O_2 almost completely eliminated this incorporation. Therefore, reaction with the H_2O_2 added in the peroxidase system could have eliminated this type of incorporation.

Modification of Amino Acid and Poly(amino acid) Substrates. Table I compares results obtained by incubating individual amino acids with the peroxidase system or with $(SCN)_2$. Derivatives of the amino acids containing radioactive carbon or sulfur, or both, were separated and quantitated by thin-layer chromatography. Identical derivatives were obtained with the peroxidase system and $(SCN)_2$. A higher yield of tyrosine derivatives was obtained with the peroxidase system. The yield of derivatives was not stoichiometric with either H_2O_2 or $(SCN)_2$.

Two radioactive derivatives of tyrosine, one of tryptophan, and two of histidine were obtained. One of the derivatives of histidine was the only major derivative that contained both the

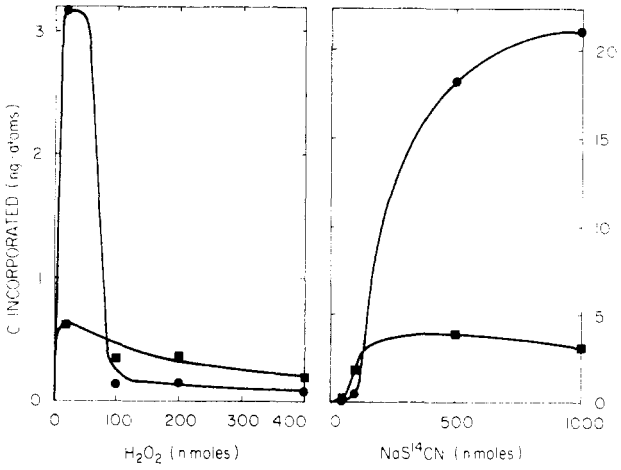

FIGURE 6: Effect of H_2O_2 or SCN^- concentration on lactoperoxidase-catalyzed incorporation of the carbon portion of SCN^- into L-histidine. L-Histidine (6.4 μmol) was incubated in 1-mL total volume with 0.1 nmol of lactoperoxidase and with either 50 nmol of $NaS^{14}CN$ and the indicated amounts of H_2O_2 (left figure), or with 400 nmol of H_2O_2 and the indicated amounts of $NaS^{14}CN$ (right figure). Thin-layer chromatography with solvent A was used to separate the radioactive derivatives (R_f 0.57 (●) and R_f 0.35 (■)).

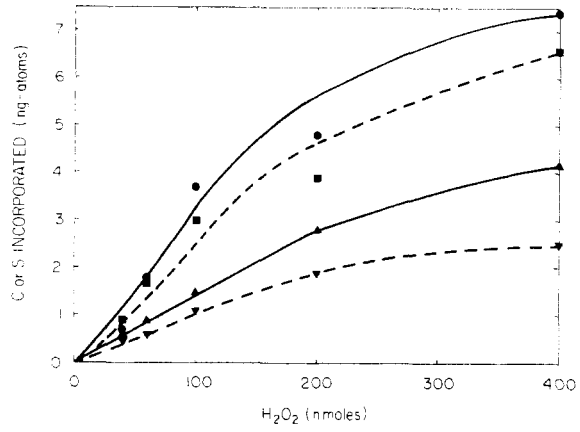

FIGURE 7: Lactoperoxidase-catalyzed incorporation of the carbon and sulfur portions of SCN^- into poly(L-histidine) and -(L-tyrosine). The poly(amino acids) (1 mg) were incubated in 1-mL total volume with 0.1 nmol of lactoperoxidase, 50 nmol of $NaS^{14}CN$ or $K^{35}SCN$, and the indicated amounts of H_2O_2 to obtain incorporation of radioactivity from $S^{14}CN^-$ into poly(L-histidine) (—●) or poly(L-tyrosine) (—▲), or from $^{35}SCN^-$ into poly(L-histidine) (- - - ■) or poly(L-tyrosine) (- - - ▼).

carbon and sulfur portions of SCN^- or $(SCN)_2$. Trace amounts of derivatives of tyrosine or tryptophan were observed that contained only sulfur, or both carbon and sulfur. No radioactive derivatives of other amino acids were observed. Cysteine was oxidized to cystine.

The components that were identified as amino acid derivatives were not due to contaminants or oxidation products of SCN^-. Sulfur- or carbon-labeled SCN^- gave only one radioactive spot in each of four chromatography systems. The major sulfur-containing component obtained from the peroxidase system or $(SCN)_2$ in the presence or absence of amino acids was $^{35}SO_4^{2-}$. There was loss of carbon label, possibly due to formation of $^{14}CO_2$. The amino acid derivatives were cleanly separated from SCN^-, SO_4^{2-} and the unmodified amino acids in all four chromatography systems. The R_f values of SCN^-, SO_4^{2-}, tyrosine, tryptophan, and histidine in solvent A were 0.86, 0.08, 0.35, 0.51, and 0.10, respectively.

Figure 6 shows the yields of the two derivatives of histidine as a function of H_2O_2 or SCN^- concentrations. The highest

TABLE I: Derivatives of Amino Acids.[a]

	Lactoperoxidase, H_2O_2, $S^{14}CN^-$		$(S^{14}CN)_2$	
	R_f	Yield (nmol)	R_f	Yield (nmol)
Tyrosine	0.59	0.4	0.58	1.6
	0.40	8.3	0.38	2.6
Tryptophan	0.61	3.7	0.67	3.6
Histidine	0.35	2.7	0.35	2.0
	0.57	17.3	0.55	19.0
	Lactoperoxidase, H_2O_2, $^{35}SCN^-$		$(^{35}SCN)_2$	
Histidine	0.58	19.8	0.55	17.6

[a] Amino acids (1 mg) were incubated with 0.1 nmol of lactoperoxidase, 1 μmol of radioactive SCN^-, and 400 nmol of H_2O_2 or with 400 nmol of radioactive $(SCN)_2$. Radioactive products were separated by thin-layer chromatography with solvent A.

yields of both derivatives were obtained when the ratio of SCN^- to H_2O_2 was about two. As H_2O_2 exceeded SCN^-, the yield of both derivatives decreased, indicating that attack by H_2O_2 or by oxidation products of SCN^- resulted in loss of sulfur and carbon from these derivatives. The major product at low ratios of SCN^- to H_2O_2 was the derivative containing carbon. A similar dependence on H_2O_2 and SCN^- concentrations was observed with derivatives of tyrosine. At low ratios of SCN^- to H_2O_2 only one derivative of tyrosine was observed (the low-yield derivative in Table I).

Figure 7 shows incorporation of carbon and sulfur into polyhistidine and polytyrosine upon incubation with the peroxidase system. The incorporated radioactivity was not released upon addition of DTT. Results obtained with the poly(amino acids) were similar in most respects to those obtained with the individual amino acids; incorporation of carbon exceeded that of sulfur, yield was low relative to the amount of H_2O_2 added, and incorporation into polyhistidine exceeded that into polytyrosine. Results differed in that incorporation continued to increase as H_2O_2 exceeded SCN^-, and the ratio of incorporation of carbon to sulfur was much higher with polytyrosine than with tyrosine.

No radioactive incorporation was observed with poly(L-alanine), -(L-lysine), -(L-arginine), -(L-asparagine), -(L-glutamic acid), -(L-serine), or -(L-*O*-acetyltyrosine). The low solubility of polytryptophan prevented study in this system. Dimethyl sulfoxide was added to assist solubilization of poly(amino acids). The presence of dimethyl sulfoxide had no effect on incorporation into BSA.

Discussion

Modification of BSA by products of lactoperoxidase-catalyzed oxidation of SCN^- was sequential. There was preferential incorporation of the intact SCN moiety into a derivative of cysteine residues. When this incorporation was complete, carbon and sulfur were incorporated into other derivatives, presumably of tyrosine, tryptophan, and histidine residues. Similar sequential modification of BSA and formation of identical amino acid derivatives were obtained with $(SCN)_2$. These results suggest that lactoperoxidase catalyzed the oxidation of SCN^- to $(SCN)_2$:

$$2SCN^- + H_2O_2 + 2H^+ \xrightarrow{\text{lactoperoxidase}} (SCN)_2 + 2H_2O$$

This reaction is analogous to the peroxidase-catalyzed oxidation of a halide ion to a halogen.

Consistent with the halogen analogy, sequential modification first of sulfhydryls and then of aromatic amino acid residues is observed during the reaction of iodine with proteins (Cohen, 1968). Iodine oxidizes protein sulfhydryls and then iodinates tyrosine and histidine residues. Oxidation of tryptophan by iodine has also been reported (Alexander, 1974).

Although $(SCN)_2$ has often been postulated as the product of nonenzymatic oxidation of SCN^- (Hughes, 1975), $(SCN)_2$ hydrolyzes rapidly and has not been demonstrated in water at neutral pH. Hydrolysis of $(SCN)_2$ yields SCN^- and hypothiocyanous acid, HOSCN (Hughes, 1975). Accumulation of HOSCN is observed during peroxidase-catalyzed oxidation of SCN^- (Aune and Thomas, submitted for publication). Whereas similar modification of aromatic amino acids was obtained with $(SCN)_2$ or the lactoperoxidase system, HOSCN does not react with tyrosine or tryptophan (Aune and Thomas, unpublished results). This observation suggests that $(SCN)_2$ rather than HOSCN is the first product of lactoperoxidase-catalyzed oxidation of SCN^-. An enzyme-bound oxidized form of SCN^- such as SCN^+ may be the precursor of $(SCN)_2$. However, the sulfhydryl of BSA would not be accessible to the active site of lactoperoxidase (Thomas and Aune, 1977), so that a diffusible product such as $(SCN)_2$ or HOSCN would be required for modification of this sulfhydryl.

Reaction of $(SCN)_2$ with sulfhydryls, phenols, and heterocyclic aromatic compounds has been described previously (Wood, 1946; Bacon, 1961). The SCN moiety of $(SCN)_2$ is introduced, to give the sulfenyl thiocyanate derivative of sulfhydryls and the aryl thiocyanate derivatives of aromatic compounds. The DTT-reversible incorporation of the SCN moiety into BSA is due to formation of the sulfenyl thiocyanate derivative of protein sulfhydryls (Aune and Thomas, 1976). Of the aromatic amino acids, only histidine reacted to give a major derivative containing both carbon and sulfur. The preferential incorporation of carbon into aromatic amino acid residues may result from reaction with breakdown products of $(SCN)_2$. For example, sulfur dicyanide (NC-SCN; cyanogen thiocyanate) may be formed in small amounts during the decomposition of $(SCN)_2$ (Hughes, 1975). Alternatively, loss of sulfur from aryl thiocyanates may result from oxidation by excess H_2O_2 or $(SCN)_2$. Further investigations will be required to clarify the mechanism of these reactions.

Several differences were observed between results obtained with the lactoperoxidase system and with $(SCN)_2$. First, incorporation of the SCN moiety continued to increase when $(SCN)_2$ exceeded the amount of protein sulfhydryls. The sulfenyl thiocyanate derivative of BSA is in equilibrium with sulfenic acid and free SCN^- (Aune and Thomas, 1976) and SCN^- is one of the products of $(SCN)_2$ hydrolysis. Therefore, the hydrolysis of excess $(SCN)_2$ would shift the equilibrium toward incorporation of the SCN moiety. Second, the yield of incorporation of carbon into tyrosine was higher with the lactoperoxidase system than with $(SCN)_2$. Tyrosine binds at the active site of lactoperoxidase (Morrison and Schonbaum, 1976), which may facilitate the reaction between tyrosine and products of SCN^- oxidation. Third, a DTT-reversible type of carbon incorporation and a greater incorporation of sulfur were obtained in the reaction of $(SCN)_2$ with BSA. In the lactoperoxidase system, these types of incorporation may have been eliminated by reaction with H_2O_2.

Results presented here suggest that the basis of the antimicrobial and antithyroid action of SCN^- could be the peroxidase-catalyzed incorporation of SCN^- into derivatives of biological components. In the thyroid, SCN^- may be incorporated into thyroglobulin or other proteins in place of iodide. A high ratio of incorporation of the carbon moiety of SCN^-, compared to the sulfur moiety, would be consistent with the reported accumulation of the sulfur moiety as sulfate (Wood and Williams, 1949). Modification of cysteine, tyrosine, tryptophan, and histidine residues of proteins could inactivate enzymes and disrupt cellular structure. Therefore, these modifications would be sufficient to account for the antimicrobial action of the peroxidase system.

Acknowledgments

We gratefully acknowledge helpful discussions with Dr. G. Schonbaum and the technical assistance of Ms. Kate Pera.

References

Alexander, N. M. (1974), *J. Biol. Chem. 249,* 1946.
Aune, T. M., and Thomas, E. L. (1976), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 35,* 1630.
Bacon, R. G. R. (1961), in Organic Sulfur Compounds, Vol. 1, Kharasch, N., Ed., London, Pergamon Press, p 306.
Bacon, R. G. R., and Irwin, R. S. (1958), *J. Chem. Soc.,* 778.
Barker, M. H. (1936), *Am. Med. Assoc. J. 106,* 762.
Block, R. J., and Bolling, D. (1951), in The Amino Acid Composition of Proteins and Foods, Springfield, Ill., Charles C. Thomas, p 400.
Bray, G. A. (1960), *Anal. Biochem. 1,* 279.
Chung, J., and Wood, J. L. (1970), *Arch. Biochem. Biophys. 141,* 73.
Cohen, L. A. (1968), *Annu. Rev. Biochem. 37,* 711.
Coval, M. L., and Taurog, A. (1967), *J. Biol. Chem. 242,* 5510.
Hamon, C. B., and Klebanoff, S. J. (1973), *J. Exp. Med. 137,* 438.
Hughes, M. N. (1975), in Chemistry and Biochemistry of Thiocyanic Acid and its Derivatives, Newman, A. A., Ed., London, Academic Press, p 1.
Klebanoff, S. J., Clem, W. H., and Luebke, R. G. (1966), *Biochim. Biophys. Acta 117,* 63.
Mickelson, M. N. (1966), *J. Gen. Microbiol. 43,* 31.
Morrison, M., and Hultquist, D. E. (1963), *J. Biol. Chem. 238,* 2847.
Morrison, M., and Schonbaum, G. (1976), *Annu. Rev. Biochem. 45,* 861.
Morrison, M., and Steele, W. F. (1968), in Biology of the Mouth, Person, P., Ed., Washington, D. C., AAAS, p 89.
Oram, J. D., and Reiter, B. (1966a), *Biochem. J. 100,* 373.
Oram, J. D., and Reiter, B. (1966b), *Biochem. J. 100,* 382.
Piez, K. A., Irreverre, F., and Wolff, H. H. (1956), *J. Biol. Chem. 223,* 687.
Sorbo, B., and Ljunggren, J. G. (1958), *Acta Chem. Scand. 12,* 470.
Thomas, E. L., and Aune, T. M. (1977), *Biochemistry 16,* 3581.
Wood, J. L. (1946), *Org. React. 3,* 240.
Wood, J. L., and Williams, E. F., Jr. (1949), *J. Biol. Chem. 177,* 59.
Zeldow, B. J. (1963), *J. Immunol. 90,* 12.