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Peroxidase-Catalyzed Oxidation of Protein Sulfhydryls Mediated by Iodine†

Edwin L. Thomas* and Thomas M. Aune

ABSTRACT: Lactoperoxidase, myeloperoxidase, or horseradish peroxidase catalyzed the peroxide-dependent oxidation of protein sulfhydryls in the presence of iodide. Sulfhydryl oxidation was mediated by the oxidation of iodide to iodine. About 1 mol of sulfhydryls of bovine serum albumin or β-lactoglobulin was oxidized per mol of peroxide or iodine. Sulfhydryls were oxidized to the sulfenyl iodide derivative as indicated by the loss of iodide from solution and formation of a derivative that reacted with the sulfenyl-specific reagent, 4,4′-bis(dimethylamino)thiobenzophenone (thio-Michler's ketone). Peroxidase-catalyzed oxidation of sulfhydryls was proportional to peroxide and independent of iodide concentration over a wide range. Therefore, each iodide ion could participate in the oxidation of a number of sulfhydryls. At low iodide concentrations, the yield of sulfenyl derivatives also exceeded the amount of iodide. These results indicated that sulfenyl iodide was in equilibrium with sulfenic acid and free iodide ion. At low iodide concentrations, release of iodide from sulfenyl iodide permitted reoxidation of iodide to iodine. When the initial sodium iodide concentration was less than 1 μM, no sulfhydryl oxidation was detected regardless of peroxide or peroxidase concentrations. Also, over a narrow range of iodide concentrations, sulfhydryl oxidation was not proportional to peroxide. Under these conditions, depletion of iodide and competition by electron donors other than iodide appeared to limit iodine formation. Although a lower limit for iodide turnover was observed, only small amounts of iodide were required for oxidation of large amounts of protein sulfhydryls. Therefore, in comparing the effects of iodine with those of the peroxidase system, the valid quantitative comparison can be between iodine and peroxide concentrations, rather than between iodine and iodide concentrations.

Peroxidase-catalyzed iodination has a role in resistance to viral and microbial infection (Klebanoff, 1975), and in thyroid hormone synthesis (Morrison, 1973). Also lactoperoxidase-catalyzed iodination has been used to introduce radioactive label into proteins (Morrison et al., 1971a) and as a vectorial probe of biological membrane structure (Phillips and Morrison, 1970, 1971; Morrison et al., 1974) and protein structure (Morrison and Schonbaum, 1976).

Peroxidases can oxidize iodide (I^-) to iodine (I_2), which in turn can iodinate biological components. However, lactoperoxidase catalyzes iodination of accessible tyrosine residues by a mechanism that does not involve a diffusible intermediate such as I_2 (Morrison and Bayse, 1970; Morrison and Schonbaum, 1976). It has been suggested that peroxidase-catalyzed antimicrobial and antiviral activity is not due to I_2 (Klebanoff, 1967, 1975), and that iodination of thyroglobulin may involve protein-bound iodinating agents (Maloof and Soodak, 1963; Jirousek and Cunningham, 1968).

Sulfhydryls of BSA[1] and β-lactoglobulin are located in clefts

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[1] Abbreviations used: BSA, bovine serum albumin; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); TNB, 5-thio-2-nitrobenzoic acid; TMK, 4,4′-bis(dimethylamino)thiobenzophenone (thio-Michler's ketone); $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid.

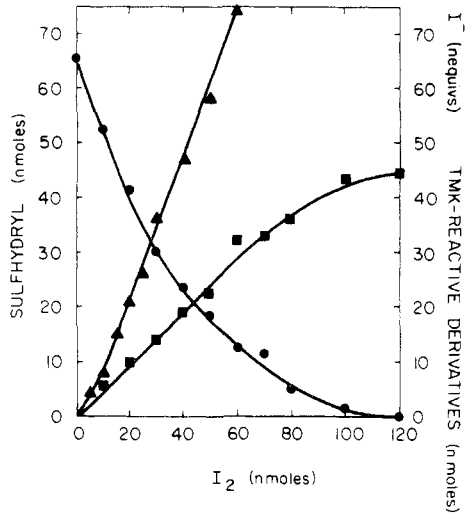


FIGURE 1: Oxidation of sulfhydryls by I_2. BSA was incubated with the indicated amounts of I_2. Concentrations of sulfhydryl (●), I^- (▲), and TMK-reactive derivatives (■) were measured as in Materials and Methods.

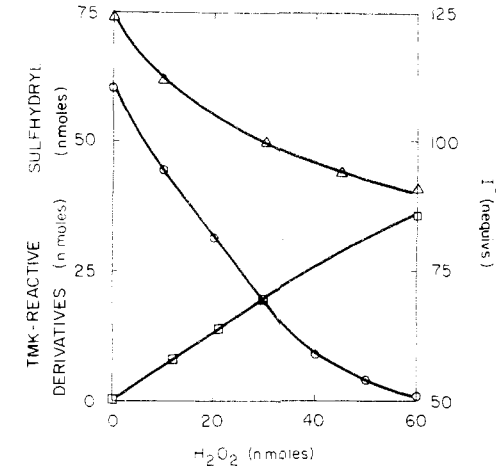


FIGURE 2: Lactoperoxidase-catalyzed oxidation of protein sulfhydryls with excess I^-. BSA was incubated with 0.01 nmol of lactoperoxidase, 0.12 μmol of NaI, and the indicated amounts of H_2O_2. Concentrations of sulfhydryl (○), I^- (△), and TMK reactive derivatives (□) were measured as in Materials and Methods.

within the proteins and are not accessible to bulky reagents (Griffith and McConnell, 1966; Noel and Hunter, 1972; Hull et al., 1975). Therefore, peroxidase-catalyzed oxidation of these sulfhydryls would appear to require a diffusible intermediate such as I_2. Protein sulfhydryls are oxidized by I_2 to yield sulfenyl iodide (Fraenkel-Conrat, 1955; Cunningham and Nuenke, 1959, 1960, 1961; Cunningham, 1964; Jirousek and Pritchard, 1971), sulfenic acid (Trundle and Cunningham, 1969; Parker and Allison, 1969), disulfide (Mora et al., 1969), or sulfonic acid (Hughes and Straessle, 1950) derivatives. Peroxidase-catalyzed oxidation of BSA and β-lactoglobulin sulfhydryls was studied and compared with oxidation by I_2.

Materials and Methods

Lactoperoxidase purified from bovine milk (Morrison and Hultquist, 1963) was provided by Dr. M. Morrison. Myeloperoxidase purified from human leukemic granulocytes was provided by Drs. M. Morrison and J. Naskalski. Horseradish peroxidase (type VI), BSA, β-lactoblogulin, and DTNB were obtained from Sigma, and TMK from Eastman Kodak. Horseradish peroxidase, BSA, and β-lactoglobulin were dialyzed against 10 mM potassium phosphate buffer (pH 6.6) containing 5 mM potassium EDTA (pH 7.0).

Saturated solutions of I_2 in water were prepared at 25 °C, and the concentration established as 1 mM by adding an aliquot to 0.1 M NaI, measuring absorbance at 350 nm, and assuming a molar extinction coefficient of 21 900 for I_3^- (Morrison et al., 1971b). Concentration of H_2O_2 was determined by measuring absorbance at 240 nm, and assuming a molar extinction coefficent of 43.6 (Beers and Sizer, 1952). Concentration of I^- was determined with an I^--selective electrode (Orion).

Modification by Peroxidase Systems and I_2. Reactions were carried out at 25 °C in 1 mL of total volume containing BSA (10 mg) or β-lactoglobulin (2.5 mg). Incubation was continued for 15 min after addition of H_2O_2 or I_2, and then 2.5 ng of catalase was added and incubation continued for 5 min.

Determination of Sulfenyl Derivatives (TMK-Reactive Derivatives). Protein sulfenyl derivatives were determined by reaction with TMK to yield the blue-green mixed disulfide derivative (Jirousek and Soodak, 1974). Reaction mixtures containing BSA were diluted with 0.1 mL of 1 M acetate buffer, pH 5.1, 0.01 mL of 10% (w/v) $NaDodSO_4$, and 0.2 mL of 2 mM TMK in dimethyl sulfoxide, and then incubated 15 min at 0 °C. Reaction mixtures containing β-lactoglobulin were treated similarly but received 0.05 mL of 10% $NaDodSO_4$ and were incubated at 37 °C. The mixtures were passed over Sephadex G-25 columns (1.5 × 40 cm) equilibrated and eluted with 0.1 M acetate buffer, pH 5.1, and the blue-green product was collected. After chromatography, 0.05 mL of 10% $NaDodSO_4$ was added to fractions containing β-lactoglobulin. Absorbance at 630 nm was measured and a molar extinction coefficient of 70 720 was assumed (Jirousek and Soodak, 1974). Absorbance at 530 nm was less than 10% of the absorbance at 630 nm, indicating that the oxidized form of TMK (Feigl's disulfide) did not make a significant contribution to absorbance at 630 nm.

Results

Equivalence of I_2 and H_2O_2. Figure 1 shows loss of BSA sulfhydryls upon incubation with I_2. Loss of sulfhydryls was nearly proportional to I_2. Slightly more than 1 mol of sulfhydryls was lost per mol of I_2 at high sulfhydryl concentrations, and less than 1 as sulfhydryl concentration approached zero. Figure 1 also shows that TMK-reactive derivatives were formed, indicating oxidation of sulfhydryls to a sulfenyl derivative. Oxidation of sulfhydryls resulted in reduction of I_2 to I^-. However, the amount of I^- formed was less than the number of iodine atoms added, indicating that iodine atoms were incorporated into BSA. The amount of incorporated iodine atoms was about equal to the yield of TMK-reactive derivatives. These results were consistent with formation of the sulfenyl iodide derivative of protein sulfhydryls.

Figure 2 shows that similar results were obtained with lactoperoxidase, H_2O_2 and I^-. In this experiment, the initial I^- concentration was at least twice that of H_2O_2 or sulfhydryl, so that the amount of I^- would not limit I_2 formation or sulfhydryl oxidation. Loss of sulfhydryls was nearly proportional to H_2O_2. The I^- concentration decreased and the change in concentration was about equal to the yield of TMK-reactive derivatives. These results were consistent with peroxidase-catalyzed oxidation of I^- to I_2, followed by reaction of I_2 with sulfhydryls to yield sulfenyl iodide.

Similar results were obtained with β-lactoglobulin as substrate. Identical loss of sulfhydryls and formation of TMK-reactive derivatives was obtained with I_2 and the peroxidase system. The β-lactoglobulin used in these studies had a sulf-

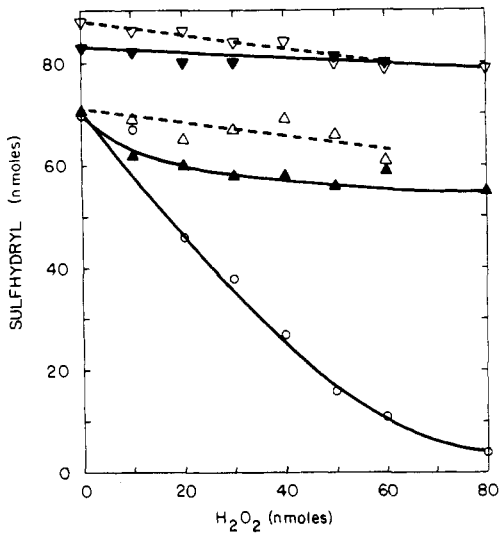


FIGURE 3: Lactoperoxidase-catalyzed oxidation of sulfhydryls with low I^- concentrations. BSA was incubated with the indicated amounts of H_2O_2 and no other additions (▽), with 120 nmol of NaI (▼), with 0.01 nmol of lactoperoxidase (△), with 1 nmol of NaI and 0.01 nmol of lactoperoxidase (▲), or with 10 nmol of NaI and 0.01 nmol of lactoperoxidase (○).

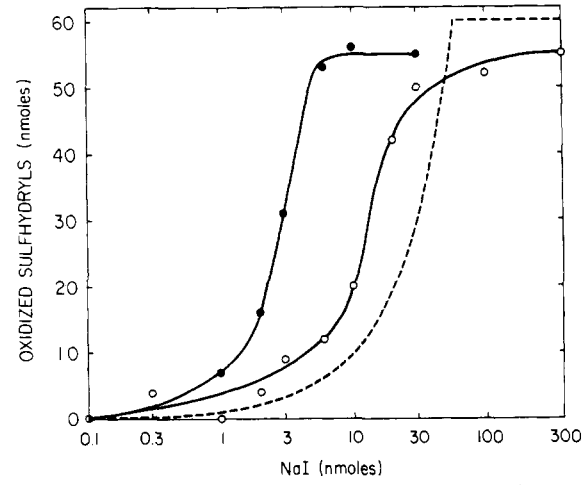


FIGURE 4: Lactoperoxidase-catalyzed oxidation of sulfhydryls as a function of I^- concentration. BSA (●) and β-lactoglobulin (○) were incubated with 0.01 nmol of lactoperoxidase, 60 nmol of H_2O_2, and the indicated concentrations of I^-. The dashed line was obtained by setting the amount of oxidized sulfhydryls equal to I^- but not exceeding H_2O_2.

hydryl content of 1.5 mol/mol of protein. Maximum yield of TMK-reactive derivatives was 0.9 mol/mol of protein.

Oxidation of 2 mol of sulfhydryl per mol of I_2 indicates disulfide formation. Therefore, oxidation of more than 1 mol of sulfhydryl per mol of H_2O_2 or I_2 suggested a limited amount of disulfide formation. Disulfide formation was also suggested by the difference between the amount of oxidized sulfhydryls and the yield of sulfenyl iodide. Increasing the sulfhydryl content of BSA by reduction of disulfides with dithiothreitol did not increase the yield of sulfenyl iodide when the reduced BSA was incubated with I_2. Instead, about 2 mol of the extra sulfhydryls were oxidized per mol of I_2.

The low ratio of sulfhydryls oxidized per mol of H_2O_2 or I_2 at low sulfhydryl concentrations appeared due to competing reactions that consume H_2O_2 or I_2. As sulfhydryl concentration approached zero, there was a sharp increase in incorporation of I^- into derivatives such as iodinated tyrosine residues. However, at the highest concentrations of H_2O_2 or I_2 used in the experiments reported in Figures 1 and 2, less than 3 nequiv of I^- was incorporated into forms that were not released upon incubation with TMK.

Turnover of I^-. The lower curve in Figure 3 shows lactoperoxidase-catalyzed oxidation of BSA sulfhydryls with 10 μM NaI and increasing amounts of H_2O_2. This concentration of I^- was almost an order of magnitude lower than sulfhydryl concentration. Therefore, if all the I^- were oxidized to I_2, the concentration of I_2 would be less than that of protein sulfhydryls. Nevertheless, results were similar to those obtained with excess I^-. About 1 mol of sulfhydryl was oxidized per mol of H_2O_2, and complete oxidation of sulfhydryls was obtained. The concentration of I^- decreased as increasing amounts of H_2O_2 were added. However, when the initial concentration of NaI was about 10 μM or greater, sulfhydryl oxidation was proportional to H_2O_2 and independent of I^- concentration.

Sulfhydryl oxidation was not independent of I^- concentration when the initial NaI concentration was less than about 10 μM. Figure 3 shows that little oxidation of sulfhydryls was obtained with 1 μM NaI. Under these conditions, I^- concentration decreased by more than 90% upon addition of 10 μM H_2O_2. About 30% of this decrease could be accounted for by incorporation of I^- into iodinated tyrosine residues. Therefore, depletion of I^- could in part account for inability of the peroxidase system to oxidize sulfhydryls. Figure 3 shows that little or no sulfhydryl oxidation was obtained with H_2O_2 alone, with H_2O_2 and I^-, or with lactoperoxidase and H_2O_2.

Figure 4 shows the effect of varying the initial NaI concentration on lactoperoxidase-catalyzed oxidation of BSA and β-lactoglobulin sulfhydryls. The amount of oxidized sulfhydryls was equal to H_2O_2 at I^- concentrations lower than the concentration of H_2O_2 or oxidized sulfhydryls. Therefore, each iodide ion could participate in oxidation of many sulfhydryls. Figure 4 also shows a theoretical curve based on the assumption that oxidation of 1 mol of sulfhydryls requires 1 mol each of H_2O_2 and NaI. Compared with this curve, the experimental curves were shifted to lower I^- concentrations by factors of about 10 and 2 for BSA and β-lactoglobulin, respectively. Therefore, under the conditions of these experiments, about 10 sulfhydryls of BSA and 2 of β-lactoglobulin could be oxidized per I^- ion.

Figure 5 shows lactoperoxidase-catalyzed oxidation of sulfhydryls to TMK-reactive derivatives under the same experimental conditions. At the lower I^- concentrations, the yield of TMK-reactive derivatives exceeded the amount of I^- by a factor of about 4 for BSA and 2 for β-lactoglobulin. Sulfenyl iodide alone could not account for the yield of TMK-reactive derivatives because the yield was greater than the amount of I^- present. These results indicated formation of a sulfenyl derivative that did not contain a bound iodine atom. This derivative was probably sulfenic acid.

Lower Limit of I^- Concentration for Sulfhydryl Oxidation. When the initial NaI concentration was less than 1 μM, no sulfhydryl oxidation was observed. Also, the amount of oxidized sulfhydryls was not equal to H_2O_2 over the range of initial NaI concentrations of about 1 μM to 10 μM. Increasing the lactoperoxidase concentration from 10 nM to 1 μM, or extending the incubation from 5 min to 4 h did not increase the amount of oxidized sulfhydryls. The dependence on I^- concentration was not altered by varying pH over the range of 6 to 8.

When either myeloperoxidase or horseradish peroxidase was substituted for lactoperoxidase, a similar dependence on I^- concentration was observed, and the amount of oxidized sulfhydryls could exceed the amount of I^-. However, longer times of incubation or higher concentrations of myeloperoxidase or horseradish peroxidase were required to obtain the same amount of oxidized sulfhydryls.

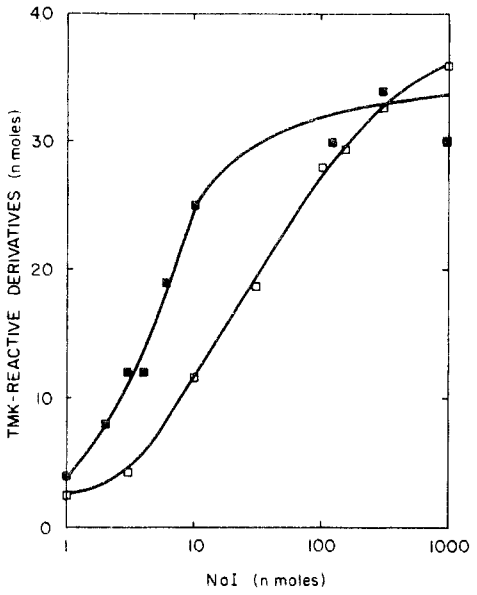


FIGURE 5: Lactoperoxidase-catalyzed formation of TMK-reactive derivatives. BSA (■) and β-lactoglobulin (□) were incubated with 0.01 nmol of lactoperoxidase, 60 nmol of H_2O_2, and the indicated concentrations of I^-.

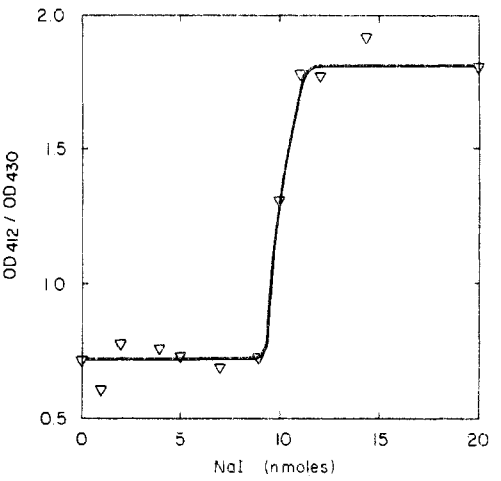


FIGURE 6: Effect of I^- concentration on appearance of lactoperoxidase compound II. BSA was mixed with 0.1 nmol of lactoperoxidase and the indicated concentration of NaI. Following addition of 40 nmol of H_2O_2, the absorption spectrum from 400 to 450 nm was recorded within 15 to 30 s.

When BSA was incubated with 10 nM lactoperoxidase, 40 μM H_2O_2, and 1 μM NaI for 1 h, only a small amount of sulfhydryls was oxidized. If 1 mM NaI was added at the end of this incubation, sulfhydryl oxidation resumed and the amount of oxidized sulfhydryls was about equal to the amount of H_2O_2 added initially. Therefore, inability to oxidize sulfhydryls at low I^- concentrations was not due to irreversible inactivation of lactoperoxidase, or to competing reactions that consume H_2O_2. However, consumption of H_2O_2 could have been a contributing factor at high peroxidase concentrations. About half the H_2O_2 was consumed during a 1-h incubation of 1 μM lactoperoxidase, myeloperoxidase, or horseradish peroxidase with BSA and 40 μM H_2O_2.

The inability of the peroxidases to catalyze oxidation of sulfhydryls at very low I^- concentrations appeared related to conversion of the peroxidases to the compound II form. Incubation of the peroxidases with H_2O_2 and BSA or β-lactoglobulin resulted in a diminution and shift in the Soret band from 412 nm to 430 nm for lactoperoxidase, 430 nm to 458 nm for myeloperoxidase, and 403 nm to 418 nm for horseradish peroxidase. The minor absorption maxima characteristic of the compound II form were observed at 538 nm and 570 nm for lactoperoxidase and 530 nm and 560 nm for horseradish peroxidase.

Figure 6 shows the amount of I^- required to prevent formation of the compound II form of lactoperoxidase in the presence of BSA. There was a sharp transition with increasing I^- concentration. The concentration of NaI at which compound II was not observed (10 μM) was equal to the concentration of NaI required to obtain sulfhydryl oxidation proportional to H_2O_2. At I^- concentrations below this transition concentration, compound II persisted for periods from 5 min at the higher I^- concentrations to more than 4 h at the lower I^- concentrations. At I^- concentrations above the transition concentration, compound II could not be observed within the shortest interval employed, which was 15 to 30 s. The transition concentration was independent of lactoperoxidase concentration over the range of 0.1 μM to 1 μM.

A similar curve was obtained in the presence of β-lactoglobulin. The transition concentration was shifted to a higher NaI concentration (35 μM), consistent with the higher I^- concentrations required for oxidation of sulfhydryls of β-lactoglobulin. Although the transition concentrations depended on the identity of the protein substrate, they were independent of substrate protein concentration over the range of 2.5 to 25 mg/mL.

In contrast to results obtained with lactoperoxidase, the compound II form of myeloperoxidase or horseradish peroxidase was observed at all NaI concentrations tested (up to 1 mM). The time required for these enzymes to return to the ground state decreased with increasing I^- concentrations and was the same as the time required to obtain sulfhydryl oxidation proportional to H_2O_2. The formation of compound II at high I^- concentrations and the slow return to the ground state was consistent with the slower oxidation of sulfhydryls catalyzed by these enzymes.

Discussion

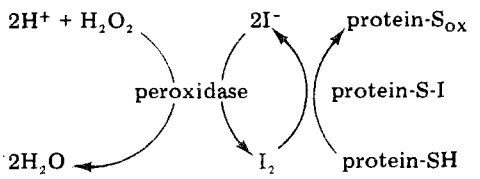
Oxidation of protein sulfhydryls by peroxidase, H_2O_2, and excess I^- appeared identical with oxidation by I_2, comparing oxidation per mol of H_2O_2 with that per mol of I_2. Oxidation of 1 mol of sulfhydryl per mol of H_2O_2 or I_2 to sulfenyl iodide is consistent with the equations:

$$2H^+ + H_2O_2 + 2I^- \xrightarrow{\text{peroxidase}} 2H_2O + I_2$$

$$\text{protein-SH} + I_2 \rightarrow \text{protein-S-I} + I^- + H^+$$

where protein-SH represents the sulfhydryl form of the protein substrate and protein-S-I represents the sulfenyl iodide derivative. Results presented here do not indicate that I_2 is the oxidizing species released from the peroxidase active site. The same stoichiometry would be obtained if sulfhydryls were oxidized by a species with the same number of oxidizing equivalents as I_2, or if I_2 were generated by reaction of such a species with I^-.

At low I^- concentrations, oxidation of sulfhydryls appeared consistent with the reaction sequence:



where protein-S_{ox} represents oxidized forms of protein sulfhydryls. In this sequence of reactions, oxidation of I^- yields I_2, which reacts with a sulfhydryl to yield I^- and an oxidized sulfhydryl. When I^- is released, it can be reoxidized and participate in oxidation of another sulfhydryl. In this way, I^- can function as a cofactor in peroxidase-catalyzed oxidation of sulfhydryls.

Peroxidase-catalyzed oxidation of I^- can result in iodination of biological components to yield either stable derivatives or unstable derivatives that decompose to yield I^- and the oxidized derivative. The concentration of I^- sets a limit to the yield of stable iodinated derivatives, whereas oxidation is not limited by I^- concentration provided that I^- is rapidly released from the unstable derivative. Therefore, in comparing the chemical or biological effects of I_2 with those of peroxidase, H_2O_2 and I^-, the valid quantitative comparison may be between I_2 and I^- concentrations, or between I_2 and Hinfn2O_2 concentrations.

Release of I^- from sulfenyl iodide may occur through hydrolysis to yield the sulfenic acid derivative (Allison, 1976):

$$\text{protein-S-I} + H_2O \rightleftarrows \text{protein-S-OH} + I^- + H^+$$

Sulfenic acid and sulfenyl iodide have the same oxidation state of sulfur (Kharasch, 1961), so that both would be expected to react with TMK. The reaction of sulfenyl halides and sulfenic acids with the thioketone compound, thiourea, has been used to quantitate these derivatives (Allison, 1976). At high I^- concentrations, the yield of TMK-reactive derivatives was consistent with formation of sulfenyl iodide, whereas at low I^- concentrations, the yield of TMK-reactive derivatives exceeded the amount of iodine atoms present. Therefore, hydrolysis appeared reversible such that release of I^- was favored at low I^- concentrations. Oxidation of sulfhydryls of creatine kinase to sulfenic acid by reaction with I_2 has been proposed (Trundle and Cunningham, 1969), based on oxidation of 1 mol of sulfhydryl per mol of I_2 and the observation that the modified protein contained no bound iodine atoms after free I^- was removed. Removal of I^- may favor hydrolysis of the sulfenyl iodide derivative of glyceraldehyde-3-phosphate dehydrogenase (Parker and Allison, 1969). Although hydrolysis is consistent with results presented here, the possibility has not been excluded that reactive groups adjacent to sulfenyl iodide may participate in displacement of the bound iodine atom.

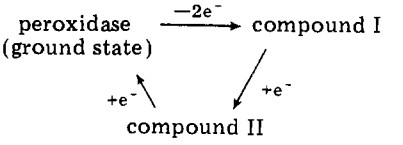
Release of I^- may also occur through disulfide formation:

$$\text{protein-S-I} + \text{protein-SH} \rightarrow \text{protein-S-S-protein} + I^- + H^+$$

Sulfhydryls of BSA and β-lactoglobulin are sterically restrained from forming intramolecular disulfides or disulfides involving two protein molecules (Griffith and McConnell, 1966; Noel and Hunter, 1972; Hull et al., 1975). However, sulfhydryls of the BSA and β-lactoglobulin used in these studies appeared heterogeneous. Iodination and oxidation of the proteins may have produced the apparent heterogeneity by promoting denaturation. The population of sulfhydryls capable of disulfide formation was sufficiently small that the stoichiometry of sulfhydryl oxidation approached 1 mol per mol of H_2O_2 or I_2. Reduction of a few of the many disulfides of BSA increased the population of sulfhydryls capable of disulfide formation with little effect on the population that yielded sulfenyl iodide.

Although results presented here provide evidence for turnover of I^-, the amount of sulfhydryls oxidized was not completely independent of I^- concentration. When the initial I^- concentration was low, the addition of H_2O_2 resulted in depletion of I^-. Incorporation sulfenyl iodide could deplete the system of I^- and limit I_2 formation.

Also, under conditions such that sulfhydryl oxidation depended on I^-, the peroxidases were observed to be in the compound II form. Oxidation of a number of substrates by peroxidases proceeds through two one-electron transfers with two oxidized forms of the peroxidase (compounds I and II) as intermediates in the catalytic cycle (Morrison and Schonbaum, 1976).



On the other hand, compound II oxidizes I^- at low rates compared with compound I, indicating that I^- oxidation proceeds by a mechanism that does not involve compound II (Yamazaki and Yokota, 1973). Conversion of the peroxidase to compound II indicates that reduction of compound II is rate limiting. Therefore, the substrate oxidized by compound I is not I^-. Competition by electron donors other than I^- would be favored as I^- is depleted. Competition by the alternative electron donors would lower the rate of oxidation of I^-, and would convert most of the enzyme to compound II, which would further lower the rate of I^- oxidation. Also, H_2O_2 would be consumed in reactions that do not oxidize sulfhydryls. The protein substrates may have provided the alternative electron donors. Peroxidases can oxidize certain amino acid residues if the residues are accessible to the peroxidase active site (Morrison and Schonbaum, 1976).

In addition to these considerations, different mechanisms of iodination may be favored at low I^- concentrations. Lactoperoxidase-catalyzed iodination of accessible tyrosine residues is thought to involve competition between I^- and tyrosine residues to be the acceptor of an enzyme-bound oxidized form of I^- (Morrison and Schonbaum, 1976). High I^- concentrations would favor I_2 formation, whereas competition by tyrosine residues would be favored at low I^- concentrations and high substrate protein concentrations (Morrison et al., 1974). Although no sulfhydryl oxidation was observed at NaI concentrations below 1 μM, lactoperoxidase-catalyzed iodination of tyrosine residues was readily observed. In fact, when the amount of H_2O_2 added was less than sulfhydryls, the amount of I^- incorporated into BSA was highest at 1 μM NaI, and less incorporation was obtained at higher concentrations (Thomas and Aune, unpublished results). These results suggest that tyrosine iodination was the preferred reaction at low I^- concentrations, and that sulfhydryl oxidation mediated by I_2 was the preferred reaction at high I^- concentrations.

Acknowledgments

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One- and Two-Electron Redox Chemistry of 1-Carba-1-deazariboflavin†

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ABSTRACT: 1-Carba-1-deazariboflavin is a purple, nonfluorescent analogue of riboflavin that should prove useful in flavin coenzyme chemical and enzymatic studies. The two-electron-reduced form dihydro-1-deazariboflavin is readily produced by reduction with dithionite, hydrogen over platinum, or light in the presence of (ethylenedinitrilo)tetraacetic acid, but not by sodium borohydride. The hydrogen at position 1 of dihydro-1-deazariboflavin exchanges slowly with solvent protons. Unlike the analogue dihydro-5-carba-5-deazariboflavin, dihydro-1-deazariboflavin is rapidly oxidized by molecular oxygen with appreciable production of superoxide anion. The redox potential of 1-deazariboflavin is −280 mV, some 70 mV more negative than riboflavin. 1-Deazariboflavin is bound to egg-white flavin-binding apoprotein with a dissociation constant of 1.6 nM. Titration of protein-bound dihydro-1-deazariboflavin with oxygenated buffer yields a long-wavelength-absorbing species, which spectral and electron paramagnetic resonance evidence suggests is the bound 1-deazariboflavin semiquinone. Similar semiquinone species are produced from 5-methyl-1-deazariboflavin and the 1-deazariboflavin cation. In contrast to 5-deazaflavins, 1-deazaflavins resemble the parent flavins in possession of readily accessible one- *and* two-electron redox chemistry. An accompanying manuscript describes the coenzymatic activity of 1-deazaflavins with several flavoenzymes.

The use of structurally modified substrates and structurally modified coenzyme analogues has been enlightening in the development of chemical insight of how electrons are transferred at flavoenzyme active sites (Bright and Porter, 1975; Bruice, 1976; Hemmerich, 1976; Walsh, 1977; Walsh et al., 1977a,b). The major barrier to broader application of mechanistically useful flavin analogues has been the lack of suitably modified flavin isosteres. The dearth has been remedied by syntheses of a variety of aza- and deazaflavin analogues by the synthetic chemical group at Merck (Ashton et al., 1977). We report here and in the following paper of this issue some of the chemical properties and enzymatic redox activities of one of these new analogues, 1-carba-1-deazariboflavin, and its coenzyme forms 1-deazaFMN[1] and 1-deazaFAD. A priori, this analogue is of special interest in that it is complementary

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