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Radioprotective Effect of Ergothioneine on γ-Irradiation of Metmyoglobin: Comparison with Cysteine on Sulfmyoglobin-Formation

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The radioprotective effect of ergothioneine, 2-mercaptoimidazole, and 2-mercapto-1-methylimidazole, containing mercaptoimidazole ring as thione form, has been compared with that of the aminothiols such as cysteine and cysteamine. Irradiation of the brown metmyoglobin only causes the formation of the bright red myoglobin which has ferryl structure. The conversion is inhibited by the addition of a 10- and 30-fold molar excess of ergothioneine and cysteine to metmyoglobin, respectively. The addition of cysteine exceeding the radioprotective concentration forms the green color sulfmyoglobin, which is an abnormal blood pigment. Ergothioneine allows the spectral change of metmyoglobin only by γ-irradiation in the addition of a 1000-fold molar excess, but no sulfmyoglobin forms. The spectrum in the presence of ergothioneine shows simply the formation of oxymyoglobin. The mechanism for the formation of ferrylmyoglobin, oxymyoglobin and sulfmyoglobin is also discussed.

Keywords——ergothioneine; cysteine; metmyoglobin; sulfmyoglobin; γ-irradiation; radioprotective effect

The study of γ-irradiation effect on the aqueous myoglobin solution have demonstrated to affect both the heme prothetic group and the protein portion of the macromolecule.[2–6] On the other hand, it has been known that cysteamine and its related aminothiols are radioprotective in various chemical and biological system.[7–12] However, these thiol compounds are cytotoxic.[13–15] We have previously observed that ergothioneine containing a mercaptoimidazole ring is also radioprotective on γ-irradiation of metmyoglobin.[16] Ergothioneine is present in most mammalian tissues including erythrocytes, liver, kidney and heart. Of particular interest is a role of the sulfur atom in the relationship between the biological and physicochemical properties of ergothioneine. Its mercaptoimidazole ring exists in thione form.[17] The present study on irradiated metmyoglobin in O_2 containing aqueous solution,

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2) E.S.G. Barron and P. Johnson, *Radia. Res.*, **5**, 290 (1956).
3) R. Clarke and J.F. Richards, *J. Agr. Food Chem.*, **19**, 170 (1971).
4) L.D. Satterlee, M.S. Wilhelm, and H.M. Barnhart, *J. Food Sci.*, **36**, 549 (1971).
5) G.G. Giddings and P. Markakis, *J. Food Sci.*, **37**, 361 (1972).
6) P. Paul and U.S. Kumta, *Radia. Res.*, **56**, 238 (1973).
7) H.J. Heitmann and H.C. Sturde, *Strahlen Therapy*, **139**, 91 (1970).
8) H. Loman, S. Voogd, and J. Block, *Radia. Res.*, **42**, 437 (1970).
9) M. Swartz, E.S. Copeland, and E.C. Richardoson, *Radia. Res.*, **45**, 542 (1971).
10) M.M. Grenan and E.S. Copeland, *Radia. Res.*, **47**, 387 (1971).
11) G. Nucifora, *Radiat. Res.*, **49**, 96 (1972).
12) M. Shinoda, S. Ohta, T. Hino, M. Chiba, and S. Akaboshi, *Yakugakuzasshi*, **93**, 25 (1973).
13) O. Vos, L. Burke, and A.J. Vergroesen, *Int. J. Radiat. Biol.*, **5**, 543 (1962).
14) S. Sawada and S. Okada, *Radia. Res.*, **44**, 116 (1970).
15) Y. Takagi, M. Shikita, T. Terashima, and S. Akaboshi, *Radia. Res.*, **60**, 292 (1974).
16) N. Motohashi, I. Mori, Y. Sugiura, and H. Tanaka, *Radioisotopes*, **22**, 451 (1973).
17) N. Motohashi, I. Mori, and Y. Sugiura, *Chem. Pharm. Bull.* (Tokyo), **24**, 1737 (1976).

was made to define the redox process of the iron and to compare the radioprotective and toxic effects of the aminothiols with those of ergothioneine. This approach enabled us to study the mechanism for the formation of sulfmyoglobin by the aminothiols.

Experimental

Materials

Metmyoglobin from equine skeletal muscle and catalase from beef liver were Sigma's crystalized materials. The purity of metmyoglobin was checked by the absorption spectrum. Ergothioneine was purchased from Sigma Chemical Company. 2-Mercaptoimidazole and 2-mercapto-1-methylimidazole were obtained from K & K Laboratories. L-Cysteine, cysteamine, DL-penicillamine, thiourea, and potassium ferricyanide were reagent grade materials. Carbon monoxide and oxygen were obtained from Takachifo Company. Silica glass distilled water was adjusted to about pH 7.0 with Tris buffer (1/10 M Tris-(hydroxy-methyl)-aminometane–1/10 M hydrochloride).

Methods

Gamma Irradiation——All γ-irradiation was carried out with 54000 rads in a 100Ci-^{137}Cs source at room temperature in an air atomosphere and the dark. The dose rate, 53 rads/min, was determined with a Fricke dosimeter. Metmyoglobin solutions were prepared to the concentration of 0.05 mM in 0.1 M Tris buffer (pH 7.0).

Spectrophotometry——The absorption spectra were measured in the range 350—750 nm with a Shimazu recording spectrophotometer, model Double UV-200. The visible spectra of the samples were immediately measured after irradiation. A few samples were treated with ferricyanide or saturated with CO just before the measurements of the spectra. Excess ferricyanide was eliminated through Sephadex G-25 column, 1×36 cm. For the measurement of the Soret band, the sample was diluted to one tenth concentration.

Judgement of Radioprotective Effect——When the spectrum of metmyoglobin irradiated in the presence of the mercaptocompound was consistent with that of the reference unirradiated, it was judged that the mercaptocompound has radioprotective effect on metmyoglobin.

Preparation of Sulfmyoglobin and Metsulfmyoglobin——These proteins were obtained by the methods of Berzofsky, *et al.*[18] To the metmyoglobin was added a 4-fold molar excess of H_2O_2, producing an immediate change of color from brown to bright red of ferrylmyoglobin. Catalase was added to destroy the unreacted H_2O_2. A 1.5-fold molar excess of $(NH_4)_2S$ was added, producing an immediate change to the deep bluish green color of ferrous sulfmyoglobin. Metsulfmyoglobin was prepared by passage of sulfmyoglobin through the column of Sephadex G-25, previously layered with ferricyanide. When the green sulfmyoglobin was treated with ferricyanide, it turned to the brown color of metsulfmyoglobin. Metsulfmyoglobin reached the bottom of the column, and it was separated from ferricyanide.

Results

Effect of γ-Irradiation on Metmyoglobin

When metmyoglobin (brown color) was irradiated with 54000 rads, the bright red color myoglobin appeared. The brown metmyoglobin is high spin ferric heme and its spectrum shows absorption maxima at 409, 503, and 630 nm. While the red myoglobin has the spectrum characterized by absorption maxima at 412, 543, and 580 nm (Fig. 1). The latter myoglobin is similar to oxymyoglobin, which is low spin ferrous heme and has the absorption peaks at 543 and 580 nm, but differs from oxymyoglobin in the Soret band at 412 nm. Oxymyoglobin gives the Soret band at 416 nm. The spectrum of the red myoglobin was reversed to that of original brown metmyoglobin to some extent with time. When metmyoglobin was irradiated in the presence of catalase, the spectrum was changed slightly from that of the original metmyoglobin (Fig. 1). This observation indicates that catalase, which decomposes hydrogen peroxide, decreases the effect of γ-irradiation on metmyoglobin. The spectrum of the bright red myoglobin was neither affected by bubbling CO gas, nor the addition of ferricyanide. The fact suggests that the bright red color compound differs from oxymyoglobin, which is altered to carboxymyoglobin by bubbling CO and is oxidized to metmyoglobin by the addition of ferricyanide. Giddings and Markakis[5] have described that the spectrum of O_2 containing beef myoglobin irradiated with 40000 rads is consistent with

18) J.A. Berzofsky, J. Peisach, and W.E. Blumberg, *J. Biol. Chem.*, **246**, 3367 (1971).

that of metmyoglobin treated with H_2O_2, and that the bright red color product of the reaction has a ferryl, (+4), oxidation state. In our study, the spectrum of the radiation-generated red myoglobin is also similar to that of metmyoglobin treated with H_2O_2. In addition, the decrease of the irradiation-generated red myoglobin in the presence of catalase suggests that the red myoglobin in identical with the product by the myoglobin-H_2O_2 reaction.

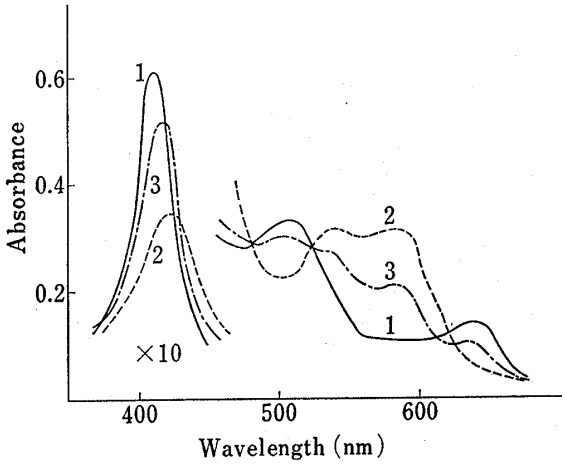

Fig. 1. Effect of γ-Irradiation on Metmyoglobin

The spectra were measured with 0.05 mM metmyoglobin at pH 7.0.
Curve 1, control (unirradiated); curve 2, immediately after irradiated with 54000 rads; curve 3, 54000 rads in the presence of catalase.

Table I. Radioprotective Concentration of Ergothioneine and Its Related Compounds on γ-Irradiation of Metmyoglobin

Compound	Concentration (mM)
a) Thiol Structure	
Cysteamine	1.5
Cysteine	1.5
b) Thione Structure	
Thiourea	3.5
Ergothioneine	0.5
2-Mercaptoimidazole	0.5
2-Mercapto-1-methylimidazole	3.5

All irradiation was carried out with 54000 rads. The concentration of metmyoglobin dissolved in 0.1M Tris-HCl buffer at pH 7.0 was 0.05mM.

Radioprotective Effect of Ergothioneine and Its Related Compounds

Table I shows radioprotective concentration of several mercaptocompounds on γ-irradiation of metmyoglobin. Ergothioneine and 2-mercaptoimidazole protected metmyoglobin from γ-irradiation in 0.5 mM in a molar ratio of 10:1 to metmyoglobin, while cysteine and cysteamine were radioprotective in 1.5 mM. In general, thiols such as cysteine and cysteamine,[7–12] and thione such as thiourea[19] reduce the radiation-induced damage. Ergothioneine is also present as thione form in an aqueous solution.[17] The activity of ergothioneine and 2-mercaptoimidazole was three times that of cysteine and cysteamine on the radiation-protection of metmyoglobin. 2-Mercapto-1-methylimidazole showed a lowering of activity with the substitution by methyl group at a nitrogen atom.

Table II. Spectral Data on γ-Irradiation of Metmyoglobin with Sulfur Compounds

Sulfur compound	Absorption maxima, nm (Extinction coefficients, $\times 10^3$)					
	Soret		Visible			
None (unirradiated)	409(120)	503(7.0)	—	—	—	630(2.9)
None	412 (64)	—	543(6.6)	580(6.6)	—	—
Ergothioneine	409(120)	503(7.0)	—	—	—	630(2.9)
2-Mercaptoimidazole	409(120)	503(7.0)	—	—	—	630(2.9)
Cysteamine	417 (63)	—	543(7.4)	581(6.9)	617(6.3)	—
Cysteine	417 (67)	—	543(7.0)	581(7.0)	617(7.4)	—
Penicillamine	410(108)	505(6.8)	543(7.4)	580(6.7)	617(6.9)	—

All irradiation was carried out with 54000 rads. The concentration of metmyoglobin and sulfur compounds dissolved in 0.1M Tris-HCl buffer at pH 7.0 were 0.05mM and 5.0mM respectively.

19) S.B. Reddy, *Canad. J. Genet. Cytol.*, **12**, 685 (1970); *idem*, *Strahlentherapie*, **149**, 194 (1975).

Effect of Sulfur Compounds on γ-Irradiation of Metmyoglobin

Table II summarizes spectral data of metmyoglobin irradiated in the presence of 5 mM sulfur compound in a molar ratio of 100: 1 metmyoglobin. When cysteine, cysteamine, and penicillamine were added to the metmyoglobin solution in the concentration of more than 5 mM, the green color slightly appeared and became very intense by γ-irradiation. The green myoglobin was similar to oxymyoglobin in its absorption at 543 and 581 nm, but different from that in the peaks at 617 nm and the Soret band. Whereas, the spectra of metmyoglobin irradiated in the presence of ergothioneine and 2-mercaptoimidazole, were not changed from that of the original metmyoglobin. When ergothioneine was added to the metmyoglobin solution in the concentration of as high as 50 mM, its spectrum was markedly changed after γ-irradiation (Fig. 2). The red color appeared and the spectrum consisted of the Soret

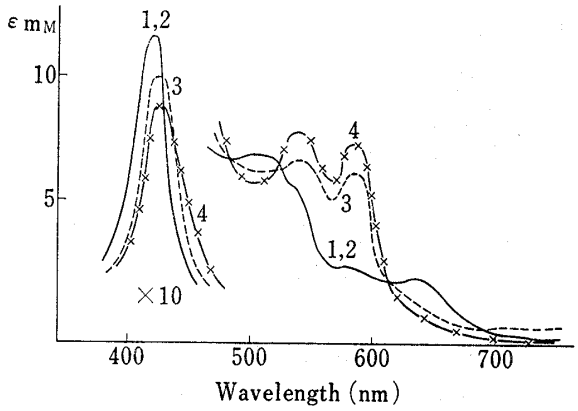

Fig. 2. Effect of Catalase on Absorption Spectra of Metmyoglobin Irradiated in the Presence of Ergothioneine

The spectra were measured with 0.05 mM metmyoglobin at pH 7.0.
Curve 1, unirradiated metmyoglobin in the presence of 50 mM ergothioneine; curve 2, "1" added catalase; curve 3, "1" irradiated with 54000 rads; curve 4, "2" irradiated with 54000 rads.

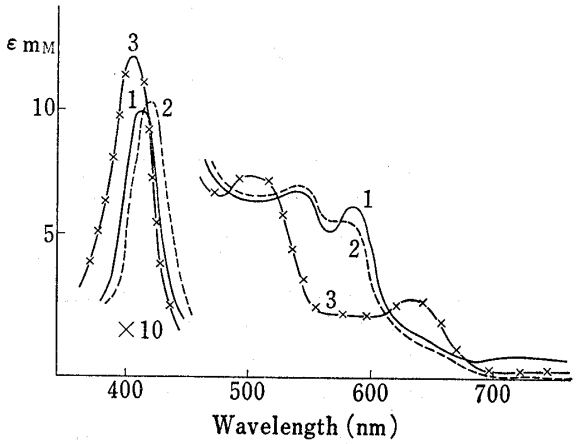

Fig. 3. Effects of Carbon Monoxide and Ferricyanide on Absorption Spectra of Metmyoglobin irradiated in the Presence of Ergothioneine

The spectra were measured with 0.05 mM metmyoglobin at pH 7.0.
Curve 1, irradiated with 54000 rads in the presence of 50 mM ergothioneine; curve 2, "1" treated with CO; Curve 3, "1" treated with ferricyanide.

absorption at 410 nm and prominent two peaks at 542 and 581 nm. When catalase was added to the sample solution prior to γ-irradiation, the prominent two peaks furthremore increased, and then red color became more intense (Fig. 2). On the other hand, the spectrum of the red myoglobin solution was changed by bubbling CO as shown in Fig. 3. The visible absorptions at 542 and 581 nm were shifted to 541 and 578 nm, respectively. The spectrum is similar to that of carboxymyoglobin which has the visible absorptions at 540 and 578 nm and is a low spin ferrous heme. When potassium ferricyanide was added, the red color was immediately lost and turned to the brown color. The spectrum shows absorption maxima at 503 and 630 nm, and is characteristic of metmyoglobin (Fig. 3). These facts indicate that the red myoglobin formed by γ-irradiation of metmyoglobin in the presence of 50 mM ergothioneine behaves identically to oxymyoglobin and differs from the bright red color compound formed by γ-irradiation of metmyoglobin only.

Fig. 4 shows the spectra of metmyoglobin γ-irradiated in the presence of 5 mM cysteine. The unirradiated spectrum has the weak peak at 617 nm and prominent two peaks at 543 and 581 nm, resembling to that of oxymyoglobin. When the same sample solution was irradiated, the green color became very intense and the peak at 617 nm remarkably increased. If the solution was irradiated in the presence of catalase, however, the characteristic peak at 617 nm decreased markedly, contrary to an increase of the absorption at 543 and 581 nm. The addition of catalase to the unirradiated metmyoglobin-cysteine solution also caused an

increased in the absorptions at 543 and 581 nm, compared with that of the unirradiated sample solution without catalase. These results suggest that hydrogen peroxide generates in the metmyoglobin solution in the presence of cysteine.

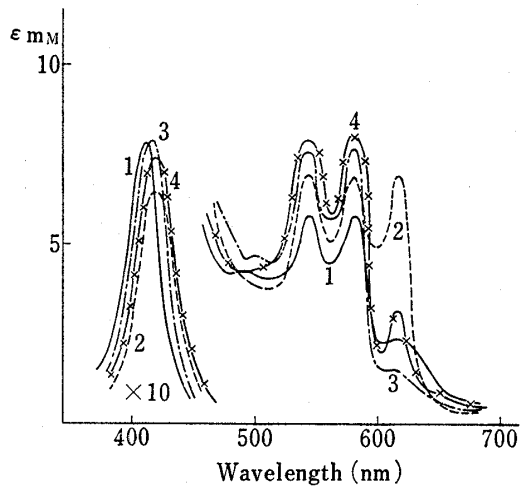

Fig. 4. Effect of Catalase on Absorption Spectra of Metmyoglobin irradiated in the Presence of Cysteine

The spectra were measured with 0.05 mM myoglobin at pH 7.0.
Curve 1, unirradiated metmyoglobin in the presence of 5 mM cysteine; curve 2, "1" irradiated with 54000 rads; curve 3, unirradiated metmyoglobin in the presence of 5 mM cysteine and catalase; curve 4, "3" irradiated with 54000 rads.

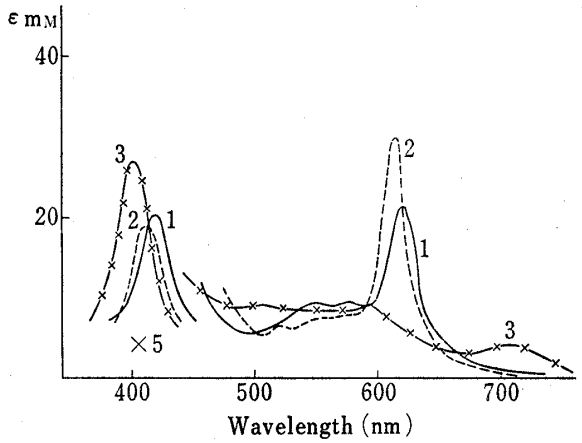

Fig. 5. Absorption Spectra of Sulfmyoglobin, Carboxysulfmyoglobin and Metsulfmyoglobin

Curve 1, sulfmyoglobin; curve 2, carboxysulfmyoglobin; curve 3, metsulfmyoglobin.

Fox,[20] *et al.* have assigned the characteristic absorption (616 nm) of the irradiated metmyoglobin containing cysteine to the formation of sulfmyoglobin. A deep green colored sulfmyoglobin has been prepared by the reaction of metmyoglobin peroxide, prepared from metmyoglobin and H_2O_2, with inorganic sulfide.[18,21,22] In the preparation of sulfmyoglobin oxymyoglobin has always appeared as the major contaminant.[18] Sulfmyoglobin which is a ferrous heme, binds CO at the sixth coordination position of heme to be converted into carboxysulfmyoglobin,[23] and turns to the brown metsulfmyoglobin by the ferricyanide oxidation.[18] The spectrum of sulfmyoglobin is shown in Fig. 5, together with those of carboxy- and met-sulfmyoglobin. Sulfmyoglobin is characterized optically by a strong band at 617 nm and the Soret band at 420 nm. Carboxysulfmyoglobin obtained by saturating the sulfmyoglobin solution with CO gas, shows the shorter wave shifts to 613 and 413 nm. While, the spectrum of metsulfmyoglobin consists of the peaks at 717 and 595 nm, and the Soret band at 405 nm. When the irradiation-generated green myoglobin in the presence of a 100-fold molar excess of cysteine was treated with CO and ferricyanide, the spectral changes as shown in Fig. 6 were observed. By bubbling CO, the characteristic peak at 617 nm was shifted to 613 nm, and the visible absorptions at 543 and 581 nm which is identical with that of oxymyoglobin, was slightly shifted to 542 and 578 nm, respectively. The result is indicative of

20) J.B. Fox Jr., T. Strehler, C. Berzofsky, and B.S. Schweigert, *J. Agr. Food Chem.*, **6**, 692 (1958).
21) B.P. Nicholls, *Biochem. J.*, **81**, 374 (1961).
22) D.B. Morell and Y. Chang, *Biochim. Biophys. Acta*, **136**, 121 (1967).
23) J.A. Berzofsky, J. Peisach, and J.O. Alben, *J. Biol. Chem.*, **247**, 3774 (1972).

the formation of carboxymyoglobin. By the ferricyanide oxidation, in addition, the green color was immediately lost and a brown color appeared. The new peak was observed at 717 nm and the absorption at 543 and 581 nm were no longer present. These observations in the irradiation-generated green myoglobin are consistent with the optical properties of sulfmyoglobin and oxymyoglobin. Based on these evidences, the red myoglobin formed by the γ-irradiation of metmyoglobin with a 1000-fold molar excess of ergothioneine is assigned to oxymyoglobin. It is considered that the green myoglobin solution formed by γ-irradiation with a 100-fold molar excess of cysteine exists in two species of sulfmyoglobin and oxymyoglobin.

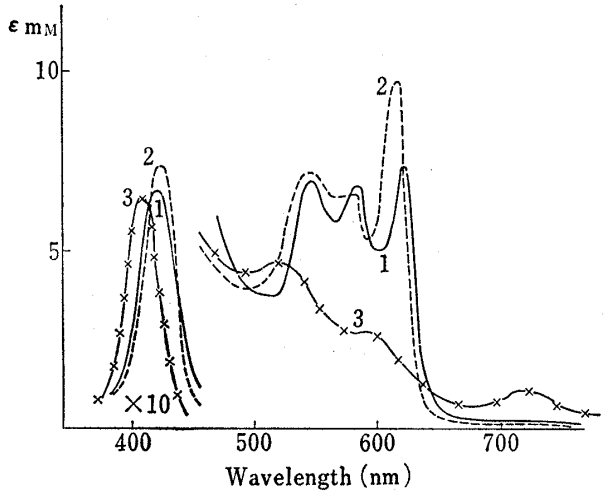

Fig. 6. Effects of Carbon Monoxide and Ferricyanide on Absorption Spectra of Metmyoglobin irradiated in the Presence of Cysteine

The spectra were measured with 0.05 mM metmyoglobin at pH 7.0.
Curve 1, irradiated with 54000 rads in the presence of 5 mM cysteine; curve 2, "1" treated with CO; curve 3, "1" treated with ferricyanide.

Discussion

In the γ-irradiated aqueous solution, the radical and molecular products of water decomposition are shown in the following reactions.

$$H_2O \text{ -}\!\!\text{W}\!\!\rightarrow e^-_{aq}, H, H_2, H_3O^+, \cdot OH, H_2O_2 \quad (1)$$

In the radiolysis of oxygen-containing neutral water, superoxide anion (O_2^-) is formed predominantly from the reaction of hydrated electron with molecular oxygen, and the bimolecule of O_2^- produces hydrogen peroxide[24] (reactions 2 and 3).

$$e^-_{aq} + O_2 \longrightarrow O_2^- \quad (2)$$

$$2O_2^- + 2H_2O \longrightarrow H_2O_2 + O_2 + 2OH^- \quad (3)$$

It has been also reported that the products in the interaction of ferri hemoprotein with H_2O_2 have a ferryl, (+4), oxidation state.[25-29] Peisach, *et al.*[30] had presented that the ferrylmyoglobin iron has four 3*d* valence electrons and an effective spin of 1, complexed to an oxygen atom. Therefore, the formation of ferrylmyoglobin (bright red color) in the irradiated metmyoglobin solution is attributed to the reaction of metmyoglobin with hydrogen peroxide formed in the water radiolysis as shown in reaction 4. The radiation-protection of cysteamine and ergothioneine analogues for metmyoglobin is achieved by decomposing the produced hydrogen peroxide according to reaction 5. As shown in Table I, the fact that ergothioneine and

$$\underset{\text{(metmyoglobin)}}{MbFe^{III}\cdot H_2O} + H_2O_2 \longrightarrow \underset{\text{(ferrylmyoglobin)}}{MbFe^{IV}\cdot O} + 2H_2O \quad (4)$$

$$2RSH + H_2O_2 \longrightarrow RSSR + 2H_2O \quad (5)$$

2-mercaptoimidazole are more effective than cysteamine and cysteine in the radiation-protection, suggests to be dependent on the unstability of mercaptoimidazole disulfide in neutral

24) G. Czapski and L.M. Dorfman, *J. Phys. Chem.*, **68**, 1169 (1964).
25) P. George and D.H. Irvine, *Biochem. J.*, **60**, 596 (1955); *idem*, *J. Phys. Chem.*, **63**, 415 (1959).
26) D. Dolphin, A. Forman, D.C. Borg, J. Fajer, and R.H. Felton, *Proc. Nat. Acad. Sci.*, **68**, 614 (1971).
27) N.K. King and M.E. Winfield, *J. Biol. Chem.*, **238**, 1520 (1963).
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29) E. Mochan and P. Nicholls, *Biochem. J.*, **121**, 69 (1971).
30) J. Peisach, W.E. Blumberg, B.A. Wittenberg, and J.B. Wittenberg, *J. Biol. Chem.*, **243**, 1871 (1968).

solution. Ergothioneine which is not a typical thiol, is more resistant against oxidation. Although ergothioneine oxidized to the disulfide form by hydrogen peroxide in strong acid solution, the disulfide is unstable in water or alkaline solution.[31] Therefore, ergothioneine disulfide formed by irradiation-generated hydrogen peroxide is reduced immediately to ergothioneine in neutral solution, and then the regenerated ergothioneine reacts with hydrogen peroxide again.

As shown in Table II, the thione compounds such as ergothioneine and 2-mercaptoimidazole, are distinct from the thiol compounds. When the thiol compounds are added beyond their radioprotective concentration, the characteristic absorption at 617 nm assigned to sulfmyoglobin is always generated. A significant spectral change of metmyoglobin with ergothioneine is observed only by γ-irradiation in a 1000-fold molar excess of ergothioneine, and its spectrum shows no absorption maximum at 617 nm (Fig. 2). On the other hand, the spectral features of metmyoglobin with 5 mM cysteine shown in Fig. 4 and 5 are characteristic of the mixture of oxymyoglobin and sulfmyoglobin. All cases of the formation of sulfheme protein *in vivo*[32] and *in vitro*,[33,34] involve the intermediate higher oxidation state iron (+4) species, which reacts with HS^-. In addition, it has been known that superoxide anions generate during the metal-catalyzed oxidation of thiols.[35,36] Accordingly, the following reactions may be provided to interpretate the data described here.

$$RS^- + MbFe^{III}\cdot H_2O \longrightarrow \underset{\text{(deoxymyoglobin)}}{MbFe^{II}} + RS\cdot + H_2O \quad (6)$$

$$MbFe^{II} + O_2 \longrightarrow MbFe^{II}\cdot O_2 \quad (7)$$

$$\Updownarrow$$

$$MbFe^{III}\cdot O_2^- \rightleftharpoons MbFe^{III}\cdot H_2O + O_2^- \quad (8)$$

$$RS^- + RS\cdot + O_2 \longrightarrow RSSR + O_2^- \quad (9)$$

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2 \quad (3)$$

$$RS^- + RS\cdot + H^+ \longrightarrow RSSR + H \quad (10)$$

$$H + RSH \longrightarrow H_2 + 0.5RSSR \quad (11)$$

$$\searrow H_2S + R\cdot \quad (12)$$

$$MbFe^{IV}\cdot O + HS^- \longrightarrow \underset{\text{(sulfmyoglobin)}}{SMbFe^{II}} + OH^- \quad (13)$$

At room temperature, thiols reduce metmyoglobin to deoxymyoglobin, and deoxymyoglobin produces oxymyoglobin by the binding of molecular oxygen (reactions 6 and 7). The electronic charge distribution on the iron in oxymyoglobin is substantially the same as that in low spin ferric hemoproteins,[37–39] and the autoxidation of oxymyoglobin to metmyoglobin generates O_2^-[40] (reaction 8). Superoxide anion is also generated by the reaction of O_2 with a thiyl radical (reaction 9), and hydrogen peroxide is produced according to reaction 3. Hydrogen atoms formed in reaction 10 react with thiols in two mechanisms (reaction 11 and 12). The latter reaction generates hydrogen sulfide. The generation of H_2O_2 and H_2S from a series of reactions in the unirradiated metmyoglobin solution with thiol leads to the formation of sulfmyoglobin. In the irradiated metmyoglobin solution with thiol, the formation of sulf-

31) H. Heath and G. Toenies, *Biochem. J.*, **68**, 204 (1958).
32) A.W. Nichol, I. Hendry, D.B. Morell, and P.S. Clezy, *Biochem. Biophys. Acta.*, **156**, 97 (1968).
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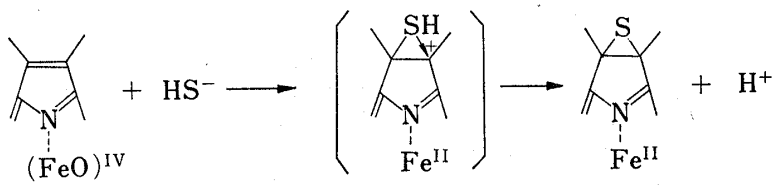
35) P.C. Jocelyn, "Biochemistry of SH Group," Academic Press, New York, 1972, p. 99.
36) H.P. Misra, *J. Biol. Chem.*, **249**, 2151 (1974).
37) T. Yamamoto, G. Palmer, D. Gill, I.T. Salmeen, and L. Rimai, *J. Biol. Chem.*, **248**, 5211 (1973).
38) T.G. Spiro and T.C. Strekas, *J. Am. Chem. Soc.*, **96**, 338 (1974).
39) L. Rimai, I. Salman, and D.H. Petering, *Biochemistry*, **14**, 378 (1975).
40) H.P. Misra and I. Fridovich, *J. Biol. Chem.*, **247**, 6960 (1972).

myoglobin is further promoted by the reaction products of the water radiolysis (e^-_{aq}, H and H_2O_2), because hydrogen atoms and hydrated electrons further produce HS^- according to reaction 12 and 14,[41] respectively.

$$e^-_{aq} + RSH \longrightarrow HS^- + R \quad (14)$$

$$\searrow \text{ other products} \quad (15)$$

As shown in reaction 13, sulfmyoglobin is isolated as the product of the reaction of one mol of inorganic sulfide with 1 mol of the higher oxidation state derivative of myoglobin, ferrylmyoglobin.[42] This reaction is viewed as the nucleophilic attack of HS^- on a β carbon atom of a pyrrole as shown in reaction 16. Here, a charge on the β carbon of the pyrrole is stabilized by the allylic position.[42]



(16)

The lowering of electrondensity at the iron of the prothetic group of sulfmyoglobin causes the decrease in O_2 and CO affinity, as compared to deoxymyoglobin. Oxygen[43] and carbon monoxide[23] affinities of sulfmyoglobin are 2500- and 1500-fold lower than those of deoxymyoglobin under the same conditions, respectively.

Thus, the presence of excess thiol more than the radioprotective concentration in γ-irradiation of the metmyoglobin solution caused the formation of sulfmyoglobin which is one of the nonfunctional abnormal pigment. While, the interaction of metmyoglobin with ergothioneine was scarcely observed, and ergothioneine was more effective than cysteine and cysteamine on the radiation-protection of metmyoglobin. These facts indicate strongly that ergothioneine, containing mercaptoimidazole ring, may be available in the radiation-protection, and that the aminothiols such as cysteine and cysteamine, should be further investigated on the paradoxical dose-dependence of the radioprotective and toxic effects.

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