Bull Yamaguchi Med Sch 40(1-2) : 11-20, 1993

Radiolysis of Mouse Collagen Type I *in vitro*

Kazuyu Ebe, Kazuyuki Nakamura**, Kazusuke Takeo**, Taishi Nakada* and Takashi Nakanishi**

*Department of Radiology and **Department of Biochemistry, Yamaguchi University School of Medicine, Ube 755, Japan.
(Recieved February 8, revised May 25, 1993)

ABSTRACT The molecules of mouse collagen type I were degraded by X-ray irradiation in a dose dependent manner under aerobic condition *in vitro*. Dose-response curves were depicted with a scanning densitometer on SDS-polyacryamide gels. An oxygen enhancement ratio of 7.2 was obtained. The radioprotector WR-1065 · 2HCI(5mM) showed the dose modifying factor(DMF) of 5.7 under aerobic conditions. The hypoxic cell radiosensitizer misonidazole(5mM) showed no sensitization under anaerobic condition, but radioprotection under aerobic condition with a DMF of 11.5. Amino acid analysis showed the numbers of His, Arg, Lys, and Pro residues were decreased and that of OH-Pro residues was increased. The degradation may be caused by cleavage of the collagen chain at the site of OH-prolyl residues generated by the irreversible oxidation of prolyl residues by active oxygen species. The significance of oxidation of histidyl residue and arginyl residue is not clarified yet. Furthermore, irradiated collagen enhanced the reactivity with 2, 4-dinitrophenyl hydrazine, which suggested the generation of reactive carbonyl group on the collagen molecule. These findings may provide pathophysiological significance of active oxygen species in radiation-induced tissue damages *in vivo*.

Key Words : Radiolysis, Mouse collagen type I, WR-1065, Misonidazole, Hydroxyproline

INTRODUCTION

Many preliminary reports concerning a modification of protein structure and function caused by irradiation have been reported since the 1950's. A mechamism of protein degradation by active oxygen species generated in oxygenated aqueous solution by irradiation has been recently postulated from the observations of the change of molecular weight of the proteins[1)2)] and alterations in their conformation to modulate their biological activity [3)4)5)6)]. The susceptibility of collagen to degradation by active oxygen species generated by mixed function oxidase system[7)8)] and gamma-irradiation[9)10)11)12)] was also examined. Pathophysiological significance of free radicals in the cause of radiation-induced tissue damage was discussed[13)]. On the other hand, it has been reported that free radicals were involved in the hydroxylation of prolyl and lysyl residue by prolyl and lysyl hydroxylases[14)15)].

We report here the degradation of collagen molecules by X-ray irradiation under aerobic and anaerobic conditions. Hypoxic cell radiosensitizer misonidazole and radioprotector WR-1065 · 2HCI were also investigated for a modification of the radiosensitivity of collagen molecules.

MATERIALS AND METHODS

Materials

Purified mouse collagen type I (Cellmatrix I-A, 3mg/ml, pH3.0) was purchased from Nitta gelatin Co. Ltd. Biochemical Institute (Osaka, Japan). Misonidazole (MISO)* or WR-1065 · 2HCl* (WR), which is the SH form of WR-2721, was dissolved in phosphate-buffered saline (PBS). Sodium dodecyl Sulfate (SDS), 2-mercaptoethanol, Tris-(hydroxymethyl) aminomethane, 2,4-dinitrophenyl hydrazine, guanidine chloride, Coomassie blue R-250, hydroxylproline, acrylamide, and bisacrylamide were of the highest grade commercially available.

Methods

Irradiations

Mouse collagen type I (3mg/ml, pH3.0) was exposed to 300 Gy of X-rays at 200kVp, 20mA, 0.5mm Cu + 1.0mm Al added filtration. This produced a beam with a half-value layer of 1.5mm Cu. The dose rate was 0.91 Gy/min at a source-to-specimen distance of 50cm. The Fricke chemical dosimeter was used to measure the dosage. The procedures of irradiation were carried out at room temperature (22℃) as described in our previous paper[16]. Hypoxia was induced by a stream of 1.0 L/min N_2 blown across the surface of the collagen solution in a volume of 0.5ml for one hour. The sample tubes were sealed during the irradiation. MISO or WR in PBS was added before irradiation at a final concentration of 5mM.

Polyacrylamide gel electrophoresis in SDS (SDS-PAGE)

Samples of collagen for electrophoresis were prepared at a protein concentration of 1.5mg/ml in 67mM Tris-hydrochloric acid buffer (pH6.7) containing 2% SDS, 10% glycerol, and 5% 2-mercaptoethanol. The samples were heated at 100℃ for 5 min prior to electrophoresis. Electrophoresis was carried out at room temperature by Laemmli's method[17] using 7.5% polyacrylamide slab type gel (6×8.5×0.1cm) until a dye marker (bromophenol blue) had reached the bottom of the gel. After electrophoresis, the gel was stained with 0.25% Coomassie blueR-250 in 10% acetic acid containing 25% methanol overnight. The gel was then destained in 7.5% acetic acid containing 5% methanol until the background became colorless. The concentration of collagen of the sample solutions was estimated from the content of hydroxyproline which was determined from the absorbance at 440 nm by amino acid analysis of acid hydrolysate of the sample solutions using standard solutions of 4-hydroxyproline[18]. The amount of collagen in the collagen bands (gamma-, beta-, alpha-, and fragmented-collagen chain) was estimated from their optical densities, which were determined by a densitometer (Densitoron PAN-802, Joko Co. Ltd., Tokyo, Japan) using a wave length of 610 nm[19]

Amino acid analysis

Amino acid analyses of collagen were carried out by an automated high performance amino acid analyser (Model 835, Hitachi Ltd., Tokyo, Japan) to estimate the content of each amino acid from the absorbance at 570 nm except proline and hydroxyproline from the absorbance at 440 nm using a standard mixture of amino acids and a standard solution of hydroxyproline in 0.02 N hydrochloric acid. A half ml of collagen (3mg/ml) samples were mixed with an equal volume of concentrated hydrochloric acid in bench top screw capped glass vials (3.5 ml). Oxygen was removed from the mixture by supplying nitrogen gas in the vials sealed with silicone on the bench top prior to heating the mixture at 110℃ for 24h in heating module (Reacti Thermo Heating Module, Pierce, Rockford, IL). The hydrolysates were completely dried by evaporation under reduced pressure to be neutralized with 0.5 ml of 0.01 N NaOH and followed by the addition of 1.5 ml of 0.02 N hydrochloric acid to apply 50 μl of the hydrolysate to the amino acid analyser.

The concentrations of individual amino

*WR-1065 · 2HCl was supplied by the Yamanouchi Pharmaceutical Co., Ltd., Japan.

*Misonidazole was supplied by Roche Products Japan.

acids in a protein hydrolysate will not be equal, as they are in the calibrating standard. Depending on the method of integration, this could introduce small errors in the analysis of the protein hydrolysate. One shoud be able to avoid this potential problem in comparing the amino acid composition of the ative collagen with the irradiated collagen. An effective remedy is to use the hydrolysate of the native collagen as the calibrating standard for the integrator, setting the concentration of each amino acid to 100%. Differences in the volume of sample applied to the analyzer would change the value of 100%, but it will be constant for all amino acids. In this study, the concentration of valine residue was set to 100% because of no difference between native collagen and irradiated collagen.

Derivatives with 2,4-dinitrophenyl hydrazine

Collagen samples were mixed with 5 volumes of 2,4-dinitrophenyl hydrazine (DNPH) reagent which was prepared with a final concentration of 0.425 mM DNPH in 1 N hydrochloric acid containing 6M guanidine[20)21)22)]. The mixtures were allowed to stand for 30 min at room temperature with gentle shaking, and the absorbance at 387 nm was determined by a spectrophotometer (Model 200-20, Hitachi Ltd, Tokyo, Japan).

RESULTS

Degradation of mouse collagen type I

Fig.1 shows the electrophoretogram of X-ray irradiated collagen type I under aerobic condition on SDS-PAGE. Trimer(γ band), dimer(β band), and monomer(α_1 and α_2) of the collagen chain exhibited a decrease in density of their stained bands with increasing radiation dose, in contrast to the increase of the density of anodal bands with molecular weight less than 10 kdalton(e.g., fragmented bands). Furthermore, patterns of these bands became blurry, and the density of the background between the identified collagen bands became stronger(lane 5 and lane 6). Dose-response curves are depicted in figure. The optical density of the γ band was decreased to 70% at 25 Gy, 55% at 50 Gy, 37% at 100 Gy, 15% at 200 Gy, and 3.5% at 300 Gy.

This decrease of optical density was larger than that of the β band and α bands. In

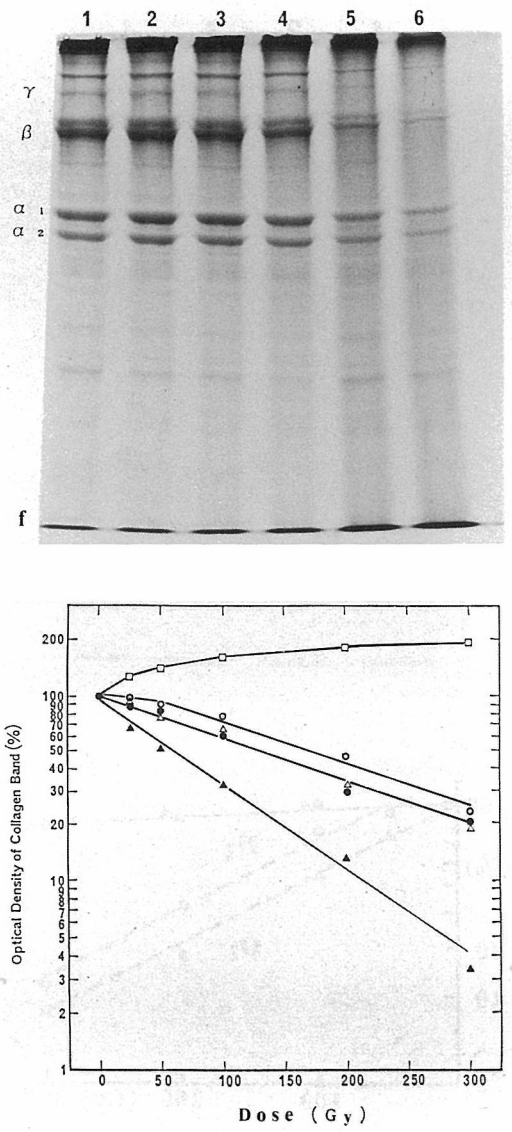


Fig.1. Upper panel shows electrophoretograms of collagen chains under aerobic conditions. Each lane shows at a dosage of 0 Gy(lane 1), 25 Gy (lane 2), 50 Gy(lane 3), 100 Gy(lane 4), 200 Gy(lane 5), and 300 Gy(lane 6) respectively. The collagen chains are trimer(γ), dimer(β), monomers (α_1, α_2), and fragmented(f). Lower panel shows dose-response curves derived from optical densities of collagen bands Which are γ (▲), β(△), α_1 (○), α_2 (●), and f(□).

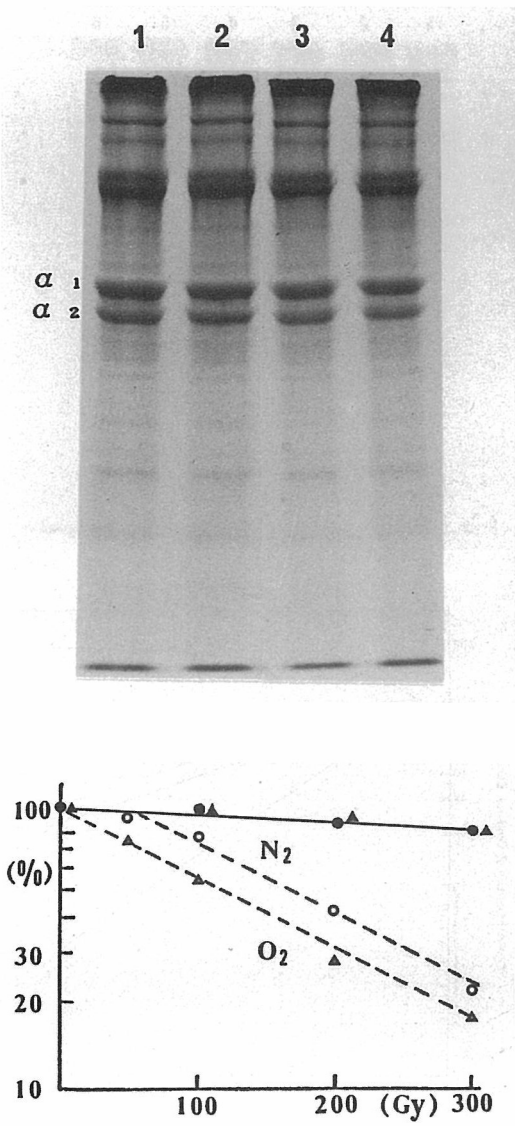


Fig.2. Upper panel shows electrophoretograms of collagen chains under anaerobic condition. Each lane shows at a dosage of 0 Gy(lane 1), 100 Gy(lane 2), 200 Gy(lane 3), and 300 Gy(lane 4) respectively. Lower panel shows dose-response curves derived from optical densities of collagen bands of α_1 (○, ●) and α_2 (△, ▲). Open symbols show under aerobic condition and closed symbols to anaerobic condition. The oxygen enhancement ration was calculated to be 7.2 for $\alpha_1$band.

contrast, the optical density of the the fragmented band was increased to 200% by irradiation of 300 Gy. Fig.2 shows the electrophoretogram and dose-response curve under anaerobic condition. Collagen molecules were less radio-sensitive in the absence of oxygen. An oxygen enhancement ratio(OER) of 7.2 was estimated for the α_1 band.

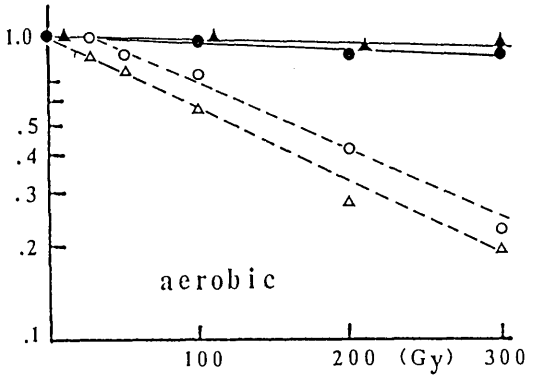
Modification by WR or MISO

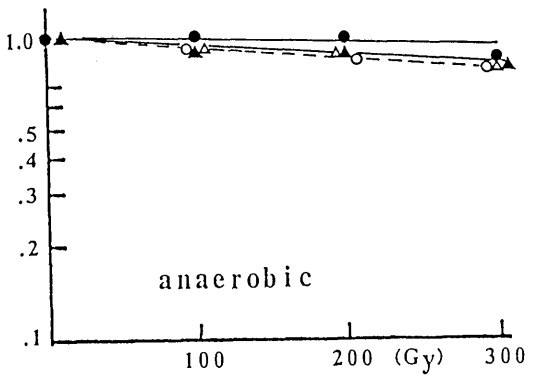
The degradation of collagen was strongly inhibited by adding a radioprotector WR under aerobic condition in a dose dependent manner(Fig.3). The dose modifying factor of 5.7 was obtained with 5 mM of WR on α_1 band. The intensity of fragmented collagen band decreased. The dose-response curve with 5mM of WR under anaerobic condition was almost similar to the control. WR protected the collagen markedly only under aerobic condition.

The radiosensitizing effect of MISO was examined under aerobic and anaerobic conditions(Fig.4). Unexpectedly, radioprotection by MISO(5 mM) was found with the DMF of 11.5 on α_1 band under aerobic condition in a dose dependent manner. MISO protected collagen molecules from X-ray irradiation. On the other hand, radiosensitization was not observed under hypoxic condition in this study.

Amino acid analysis

Amino acid analyses of hydrolysate of collagen, which was irradiated with a series of radiation doses under aerobic condition, showed a loss of amino acids, proline, lysine, histidine, and arginine, in contrast with the gain of hydroxyproline as summarized in Table I. Irradiation at a dosage less than 50 Gy increased the number of hydroxyproline and decreased the number of proline(Fig.5). The 25-Gy irradiated collagen gained 10 residues of hydroxyproline and lost 12 residues of proline from a total of 1,000 amino acid residues consisting in the collagen monomer chain. The number of hydroxyproline was then gradually decreased at doses above 50 Gy, which caused severe degradation. The collagen also lost one residue of lysine, one





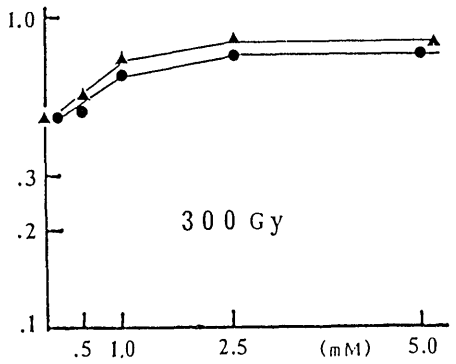
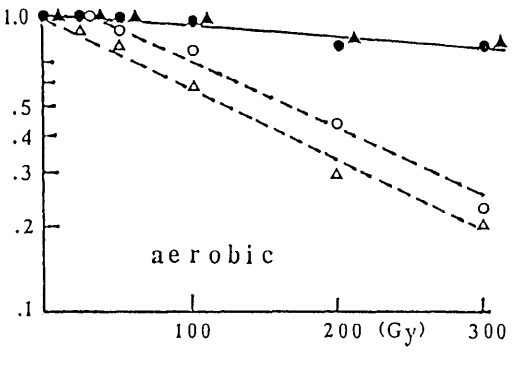
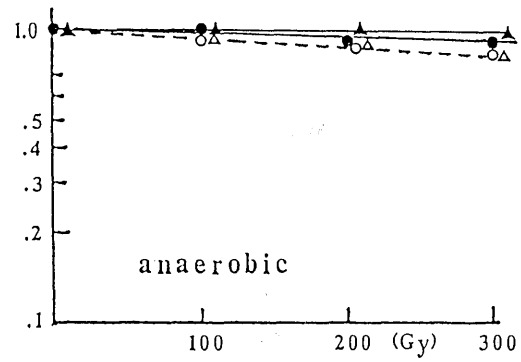


Fig.3. Modification by WR-1065. 2HCl under aerobic condition(upper panel), under anaerobic condition(middle panel), and its dose dependency under aerobic condition(lower panel). Dose-response curves derived from optical densities of collagen bands of α_1 (○, ●) and α_2 (△, ▲). Open symbols show without WR and closed symbols show with WR. The dose modifying factor of 5.7 was obtained with 5 mM of WR under aerobic condition for α_1 band.





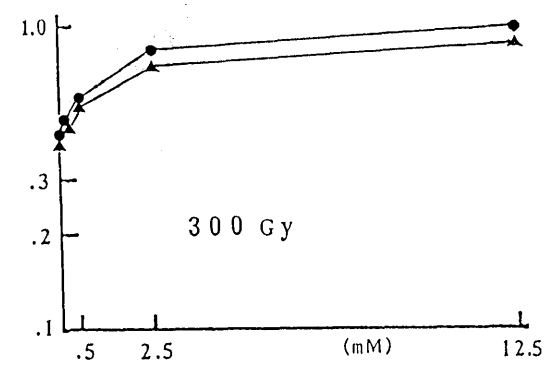


Fig.4. Modification by misonidazole under aerobic condition(upper panel), under anaerobic condition(middle panel), and its dose dependency under aerobic condition(lower panel). Dose-response curves derived from optical densities of collagen bands of α_1 (○, ●) and α_2 (△, ▲). Open symbols show without MISO and closed symbols show with MISO. Radioprotection of MISO(5 mM) was found with the dose modifying factor of 11.5 under aerobic condition for α_1 band. Radiosensitization was not observed under hypoxic condition.

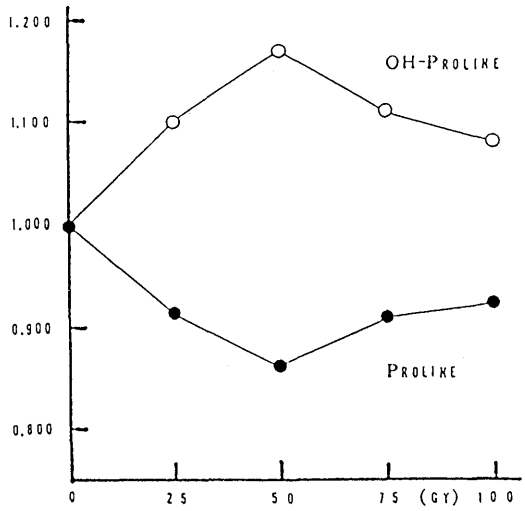


Fig.5. Hydroxylation of prolyl residues in collagen chains by irradiation. The ratio of the numbers of hydroxyprolyl residues(○) and prolyl residues(●) in irradiated mouse collagen type I to those of untreated collagen is plotted versus radiation dose.

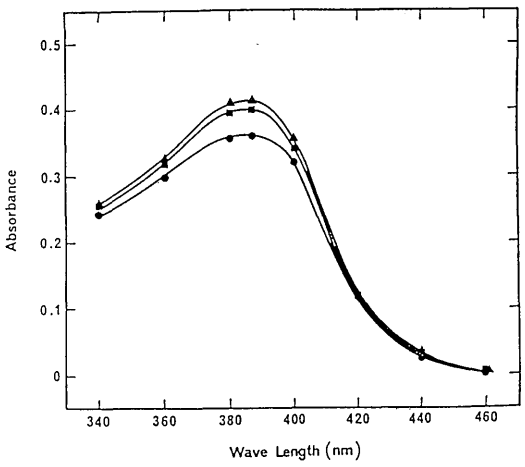


Fig.6. Reactivity of irradiated collagen with 2, 4-dinitrophenyl hydrazine. The collagen samples were reacted with 2,4-dinitrophenyl hydrazine as described in Materials and Methods. The absorbance of the reaction mixture is plotted versus wave length. The samples were irradiated at a dosage of 0 Gy (●), 50 Gy(▲), and 75 Gy(■) under aerobic condition.

residue of histidine, and three residues of arginine. But the number of hydroxylysine was not significantly increased. Other amino acid residues showed no significant change. Proline was hydroxylated by X-ray irradiation.

Fig.6 shows spectra of the irradiated collagens derivatized with DNPH. Absorbances were increased at 387 nm compared with derivatized intact collagen. However, the absorbance did not correlate to the radiation dosage.

DISCUSSION

X-ray irradiation of mouse collagen type I in aqueous solution under aerobic condition caused degradation of the collagen in a dose dependent manner(Fig.1). The kinetics of the degradation showed that the trimeric form of collagen(γ band) was more susceptible to the degradation than a monomer. This may be explained as follows. The cleavage of the collagen chain loosend its triple helix to dissociate into dimer and monomer. Furthermore, X-rays broke not only a strand but cross-linkings between each strand since amino acid analysis showed a loss of lysine and histidine which participate in intermolecular cross-linkings of collagen[23]. This degradation essentially required molecular oxygen with an OER of 7.2. It was strongly inhibited by a free radical scavenging thiol compound WR-1065 · 2HCl, which is the SH form of WR-2721[24]. These results indicate that active oxygen species such as superoxide anion and hydroxyl radical may be involved in causing the cleavage of the collagen chain, since such a degradation of collagen has been reported in mixed function oxidation systems with ozone and Fenton's reagent[10] and xanthine oxidase system[11]. In addition, such active oxygen species may also be involved in hydroxylation of prolyl residues in the collagen molecule[14)15]. Those active oxygen species specifically oxidized a histidyl residue at the active site of several enzymes, causing irreversible inactivation of the enzymes[21)22)25)26)27)28].

Careful analyses of amino acid composition of irradiated collagen, as summarrized in Table I, showed that irradiation with less

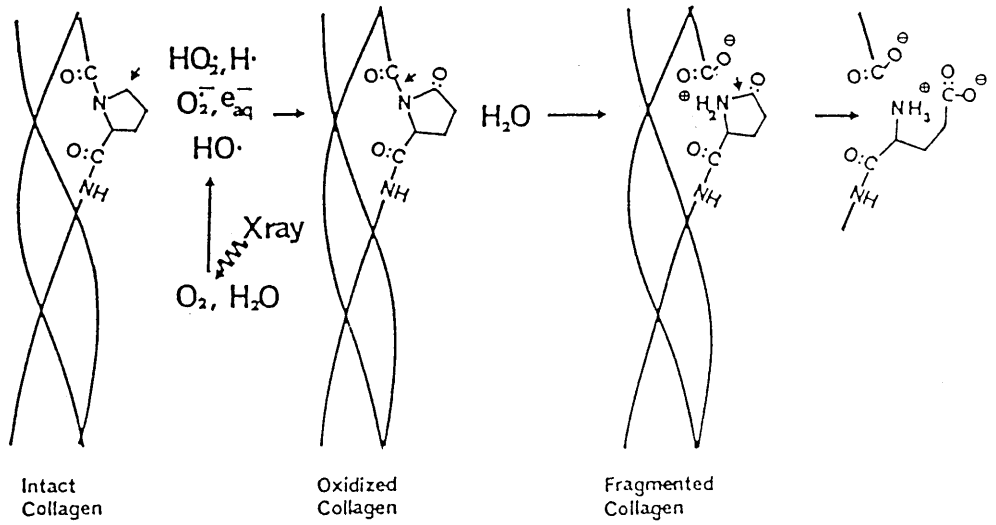


Fig.7. Possible mechanism involved in degradation of collagen chains by irradiation under aerobic condition.

Table 1 Ratio of the number of amino acids in acid hydrolysate of collagen chains after irradiation to before irradiation

Amino acids		Ratio of the number of amino acids (after irradiation/before irradiation)			
		Radiation dose (Gy)			
		25	50	75	100
Asp	(47)	1.021	1.010	1.021	0.987
Thr	(17)	1.023	0.988	1.023	1.011
Ser	(33)	1.003	0.998	1.003	0.931
Glu	(79)	1.005	0.991	1.000	0.975
Gly	(304)	1.008	0.993	0.993	1.003
Ala	(97)	1.023	1.005	1.003	1.017
Val	(23)	1.000	1.000	1.000	1.000
Met	(6)	0.935	1.064	1.048	1.064
Ile	(11)	1.009	0.981	1.009	1.028
Leu	(30)	1.003	0.980	1.003	0.980
Tyr	(6)	0.984	0.952	0.935	0.968
Phe	(13)	0.993	1.000	1.018	0.993
OH-Lys	(8)	1.033	1.012	1.026	1.033
Lys	(28)	0.957	0.932	0.943	0.939
His	(6)	0.757	0.788	0.818	0.727
Arg	(56)	0.959	0.897	0.954	0.946
OH-Pro	(103)	1.100	1.170	1.110	1.080
Pro	(131)	0.910	0.865	0.910	0.925

Numbers in parenthesis indicate the number of residues in a collagen chain consisted of 1,000 amino acid residuses.

than 50 Gy caused hydroxylation of prolyl residues and further irradiation caused the loss of hydroxyprolyl residues with severe degradation of the collagen chain. Furthermore, the irradiation enhanced the reactivity of the collagen with DNPH to form hydrazone derivatives(Fig.6), which suggested generation of reactive carbonyl groups on the collagen molecules[21)22)28)29)]. Our results suggest that one of cleavage sites of collagen may be hydroxyprolyl residues which were generated by active oxygen mediated hydroxylation of prolyl residues. Such a mechanism of active oxygen mediated damage of the chain was also postulated in the case of radiation-induced fragmentation of proteins[1)2)5)13)] including bovine serum albumin and some enzymes. However, which amino acid residues were responsible for the modification that caused the fragmentation of collagen have not been elucidated yet.

In addition, it has not been suggested that the oxidation of histidyl residue by irradiation is essential for the cleavage of the collagen molecule since no significant fragmentation of proteins was observed by oxidative modification of histidyl residues of the proteins by mixed function oxidase systems[21)22)25)26)27)28)], though the loss of histidyl residues was found in our study. By the way, dose response curves for α_1 band and $\alpha_2$band show a difference in the susceptibility to the degradation(Fig.1). This may be due to the difference in their content of prolyl residues[30)] which are oxidized by active oxygen species generated by irradiation. Our results so far strongly suggest that the hydroxylation of prolyl residues by active oxygen species was responsible for the fragmentation of collagen by X-ray irradiation. It has been reported also that the hydroxylation of ribose moiety in DNA by active oxygen species may cause the fragmentation of the DNA strand[31)]. Radioprotective efficacy of WR-1065, which is the SH form of WR-2721, has been reported on cultured cells in vitro[24)32)]. The critical role of oxygen concentration was described for the radio-protection[33)]. Our results show radioprotection by WR-1065 · 2HCI with a DMF of 5.7 under aerobic conditioning of collagen molecules(Fig.2). This indicates that oxygen tension is very important for the mechanism of radioprotection by WR. It seems likely that WR acted not only as a radical scavenger of $OH^{\cdot}$, e_{aq}, and O_2^- but also as a hydrogen donor which competed with oxygen for target radicals on the collagen molecules induced by irradiation[34)]. Misonidazole, which is one of hypoxic cell radiosensitizers, showed no sensitization under anaerobic condition but radioprotection under aerobic condition in a dose dependent manner(Fig.4) as well as WR. These results suggest that MISO has a double role: a sensitizing one and a protective one. A radioprotective efficiency of oxygen was shown in a study on radiation-induced inactivation of penicillinase[35)]. It seems to be fascinating to investigate the mechanism of a double role of MISO and other 2-nitroimidazole derivatives. It is conclued that: 1)collagen is degraded by X-ray irradiation in the presence of molecular oxygen with an OER of 7.2. This degradation may be mediated by the specific cleavage at the site of hydroxyprolyl residues in the collagen chain which are generated by irradiation., 2) WR-1065. 2HCI(5mM) showed the DMF of 5.7 under aerobic condition., 3) Misonidazole(5mM) showed no sensitization under anaerobic condition but radioprotection with the DMF of 11.5 under aerobic condition.

REFERENCES

1) Wolff, S.P. and Dean, R.T.: Fragmentation of proteins by free radicals and its effect on their susceptibility to enzymic hydrolysis. *Biochem.J.*, **234** : 399-403, 1986.
2) Davies, K.J.A.: Protein damage and degradation by oxygen radicals. I. General aspects. *J. Biol. Chem.*, **262** : 9895-9901, 1987.
3) Saito, M.: Depression of substrate inhibition in gamma irradiated lactic dehydrogenase. *Radiat. Res.*, **56** : 334-344, 1973.
4) Rokusika, S.: Effects of gamma irradiation on the function and comformation of ribonuclease A in dilute solution. *Radiat. Res.*, **57** : 349-357, 1974.
5) Davies, K.J.A.: Intracellular proteolytic systems may function as secondary antioxidant defenses:An hypothesis. *J. Free Radical Biol. Med.*, **2** : 155-173, 1986.
6) Dean, R.T.,Thomas, S.M. and Garner, A.:

Free-radical-mediated fragmentation of monoamine oxidase in the mitochondrial membrane. *Biochem. J.*, **240** : 489-494, 1986.
7) Monboisse, J.C.,Braquet, P.,Randoux, A. and Borel, J.P.: Non-enzymatic degradation of acid-soluble calf skin collagen by superoxide ion: protective effect of flavonoids. *Biochem. Pharmacol.*, **32** : 53-58, 1983.
8) Curran, S.F.,Amoruso, M.A., Goldstein, B.D. and Berg, R.A.: Degradation of soluble collagen by ozone or hydroxyl radicals. *FEBS Letters,* **176** : 155-160, 1984.
9) Wellings, L. and Bakerman, S.: Effects of low-dosage gamma-radiation on soluble collagen. *Nature.*, **210** : 495-496, 1964.
10) Labout, J.J.M.: Gamma-radiation in collagen solutions influence of solutes on the gelation dose. *Int. J. Radiat. Biol.*, **21** : 483-492, 1972.
11) Friedberg, F., Kominami, S., Nandedkar,A. K.N. and Riesz, P.: Effects of metal ions on the formation of chain breaks and radicals in γ-irradiated collagen and polyadenylic acid. *Radiat. Res.*, **61** : 55-62, 1975.
12) Bougta, G. and Dancewicz, A.M.: Radiolytic and enzymatic dimerization of tyrosyl residues in insulin, ribonuclease, papain and collagen. *Int.J.Radiat. Biol.*, **43** : 249-265, 1983.
13) Dean, R.T.: A mechanism for accelerated degradation of intracellular proteins after limited damage by free radicals. *FEBS Letters,* **220** : 278-282, 1987.
14) Bhatnagar, R.S. and Liu,T.Z.: Evidence for free radical involvement in the hydroxylation of proline: Inhibition by nitro blue tetrazolium. *FEBS Letters,* **26** : 32-34, 1972.
15) Myllyla, R., Schubotz, L.M., Weser, U. and Kivirikko, K.I.: Involvement of superoxide in the prolyl and lysyl hydroxylase reactions. *Biochem. Biophys. Res. Comm.*, **89** : 98-102, 1979.
16) Ebe, K.: Responses to radiation of Ehrlich ascites tumor cells in the presence of YM-08310(=WR-2721) and/or Ro-07-0582(In vivo, In vitro assay). *Nippon Acta Radiol.*, **47** : 307-317, 1987.
17) Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* **227** : 680-685, 1970.
18) Nagai, y. and Fujimoto, D.: *Collagen Jikkenhou (in Japanese).* Koudansha Scientific, Tokyo, 1988.
19) Nakamura, K., Inoue, S., Abiko, S., Aoki, H. and Takeo, K.: Improved separation of alpha chains of collagen type I, type III, and type V by non-interrupted electrophoresis using thioglycolic acid as a negatively charged reducer. *Electrophoresis,* **10** : 29-33, 1989.
20) Fields, R. and Dixon, H.B.F.: Micro method for determination of reactive carbonyl groups in proteins and peptides, using 2,4-dinitrophenyl hydrazine. *Biochem. J.*, **121** : 587-589, 1971.
21) Levine, R.L.: Oxidative modification of glutamine synthetase. I. Inactivation is due to loss of one histidine residue. *J.Biol. Chem.*, **258** : 11823-11827, 1983.
22) Levine, R.L.: Oxidative modification of glutamine synthetase. II. Characterization of the ascorbate model system. *J. Biol. Chem.*, **258** : 11828-11833, 1983.
23) Tanzer, M.L.: Cross-linking of collagen. *Science,* **180** : 561-566, 1973.
24) Purdie, J.W. and Inhaber, E.R.: Interaction of cultured mammaliam cells with WR-2721 and its thiol, WR-1065: implications for mechanisms of radioprotection. *Int. J. Radiat. Biol.*, **43** : 517-527, 1983.
25) Levine, R.L., Oliver, C.N., Fulks, R.M. and Stadtman, E.R.: Turnover of bacterial glutamine synthetase: Oxidative inactivation precedes proteolysis. *Proc. Natl. Acad. Sci. USA.*, **78** : 2120-2124, 1981.
26) Fucci, L., Oliver, C.N., Coon, M.J. and Stadtman, E.R.: Inactivation of key enzymes by mixed-function oxidation reactions: Possible implication in protein turnover and ageing. *Proc. Natl. Acad. Sci. USA.*, **80** : 1521-1525, 1983.
27) Nakamura, K., Oliver, C.N. and Stadtman, E. R.: Inactivation of glutamine synthetase by a purified rabbit liver microsomal cytochrome P-450 system. *Arch. Biochem. Biophys.*, **240** : 319-329, 1985.
28) Farber, J.M. and Levine, R.L.: Sequence of a peptide susceptible to mixed -function oxidation. Probable cation binding site in glutamine synthetase. *J. Biol. Chem.*, **261** : 4574-4578, 1986.
29) Jelenska, M.M. and Dancewicz, A.M.: Thermal aggregation of tropocollagen solutions irradiated with low doses of ionizing radiation. *Int. J. Radiat. Biol.*, **16** : 193-196, 1969.
30) Yamakawa, T.: *Seikagaku Databook (in Japanese),* Tokyo Kagaku Dojin, Tokyo, 1979.
31) Teoule, R. Radiation-induced DNA damage and its repair. *Int. J. Radiat. Biol.*, **51** : 573-589, 1987.
32) Antoku, S.: Protection of cultured mam-

malian cells by S-2-(3-amino- propylamino) ethylphosphothioic acid (WR-2721). *Int. J. Radiat. Oncol. Biol. Phys.*, **9** : 1481-1485, 1983.
33) *Denekamp, J., Michael, B.D., Rojas, A. and Stewart, F.A.: Thiol radioprotection in vivo: the critical role of tissue oxygen concentration. Brit. J. Radiol.*, **54** : 1112-1113, 1981.
34) Quintiliani, M.: The oxygen effect in radiation inactivation of DNA and enzymes. *Int. J. Radiat. Biol.*, **50** : 573-594, 1986.
35) Samuni, A., Kalkstein, A. and Czapski, G.: Does oxygen enhance the radiation-induced inactivation of penicillinase?. *Radiat. Res.*, **82** : 65-73, 1980.