— **Review** —

Strategy in a Living Body to Protect against Oxidative Stress-Induced Damage

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Oxidative stress derived from reactive oxygen species and/or heavy metal ions is believed to cause random damage to components in a living body, as well as aging and/or various disorders. A variety of systems that protect the living body from oxidative stress-induced damage are known; the present author elucidated three new defense systems against oxidative stress. Firstly, although it has been thought that lipid peroxidation in the living body promotes oxidative stress and increases the degree of oxidative damage, it was found that lipid peroxidation attenuated the DNA damage induced by such stress. This was determined by careful estimation of in situ lipid peroxidation. Secondly, red blood cells suffered from oxidative stress during their circulation, and membrane band 3 became aggregated and clustered so that anti-band 3 IgG and macrophages attached to it through sialylated poly-*N*-acctyllactosaminyl (PL) sugar chains. The PL sugar chain attachment to macrophages was stimulated by oxidative stress in the macrophages. Thirdly, the presence of oxidized protein hydrolase (OPH) that preferentially hydrolyzed proteins damaged by oxidative stress was found, and this enzyme may constitute a secondary defense system of the cells against such stress.

Key words —— oxidative stress, lipid peroxidation, thiobarbituric acid-reactive substance, DNA damage, red blood cell aging, oxidized protein hydrolase

INTRODUCTION

Oxidative stress derived from reactive oxygen species and/or heavy metal ions is believed to cause random damage to components in a living body, and also to cause aging and/or various disorders. Systems that protect the living body from oxidative stress-induced damage are known: enzymes that remove reactive oxygen species, superoxide dismutase, catalase and glutathione peroxidase; proteins that mask the heavy metal ions, ferritin and transferrin; antioxidant systems including vitamin E and vitamin C; repair enzyme systems for damaged DNA; and enzyme systems that remove damaged proteins, ubiquitin-proteasome.

The present author elucidated three new defense systems against oxidative stress, which are described in this review. Firstly, lipid peroxidation in the living body has been thought to promote oxidative stress which increases the degree of oxidative damage, it was found that lipid peroxidation attenuated the DNA damage induced by this stress. This result was obtained by careful estimation of in situ lipid peroxidation. Secondly, red blood cells sustained oxidative stress during their circulation, and membrane band 3 became aggregated: anti-band 3 IgG and macrophages then attached to it through sialylated poly-*N*-acetyllactosaminyl (PL) sugar chains. The PL sugar chain attachment to macrophages was stimulated by oxidative stress in the macrophages, and also increased reactive oxygen species and activated nuclear factor kappa B (NF-κB) in the cells to increase cytokine and chemokines. Thirdly, the presence of oxidized protein hydrolase (OPH) that preferentially hydrolyzed proteins damaged by oxidative stress was found, and this enzyme may constitute a secondary defense system of the cells against this stress.

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1. Lipid Peroxidation Induced by Oxidative Stress in a Living Body

Lipid peroxidation is initiated by heat, light and radical initiators under atmospheric oxygen (Fig. 1). In the initial stage, hydrogen atom is eliminated from the active methylene group of polyunsaturated fatty acid (PUFA) residue by heat, light and radical initiators to create the carbon radical (R•). Molecular oxygen ($^3O_2$) adds to R• to generate the reactive peroxyl radical (ROO•), which in turn eliminates hydrogen atom from PUFA residue to form the hydroperoxide (ROOH) and initiate the radical chain reaction. Under the atmospheric oxygen the radical chain reaction progresses. The hydroperoxide is then decomposed in the presence of a trace amount of a heavy metal ion to afford the secondary peroxidation products, aldehydes, hydrocarbons, fatty acids and so on. Proteins or DNA would be modified by the reactive aldehydes formed.

In a living body, lipid peroxidation has so far been thought to be similarly initiated by oxidative stress including reactive oxygen species, and to proceed through the radical chain reaction. However, because oxygen tension in a living body is much lower than that in the atmosphere, lipid peroxidation due to the radical chain reaction may be more restricted in a living body. We thus investigated the consequence of lipid peroxidation in a living body.

1.1. Damage to Proteins Through Lipid Peroxidation

In vitro studies have demonstrated that peroxidized lipids prepared under atmospheric conditions react with proteins to form blue fluorescence and cross-links. During 1970–1990 much attention was paid to bifunctional malonaldehyde (MA) as a candidate to create fluorescence and cross-links in proteins. MA chemistry studies we performed elucidated that free MA reacted with amines under physiological conditions to give fluorescence at 460 nm due to 1,4-dihydropyridine-3,5-dicarbaldehyde derivatives and cross-links resulting from a non-fluorescent conjugated Schiff base (Fig. 2).[1–15] However, characteristics of the fluorescence due to MA were not identical with those due to peroxidized lipids.[16–19] In contrast, monofunctional aldehydes produced fluorescence at 430–440 nm, whose characteristics were close to those of peroxidized lipids.[18,20–24] Moreover, it was found that monofunctional aldehydes formed cross-links in proteins.[19] Analysis of the reaction products between 1-butanal and benzylamine gave the bifunctional tetrameric aldehyde shown in Fig. 3.[15,25] Blue fluorescent substances extracted from rat tissues by sodium dodecylsulfate (SDS) and purified by gel filtration showed characteristics similar to those of the fluorescence from the reaction between amines with peroxidized lipids and monofunctional aldehydes.[18,26,27] Hence, it was concluded that protein

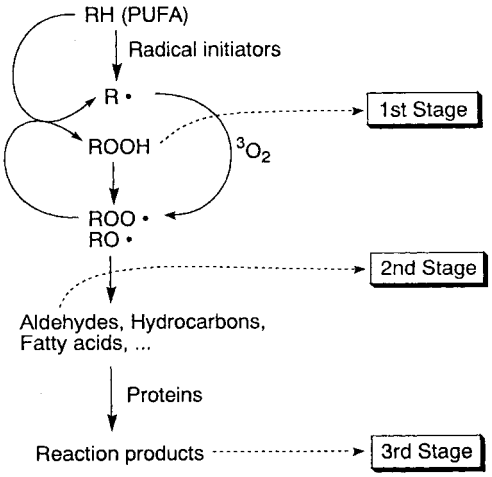


Fig. 1. Scheme of Lipid Peroxidation under Atmospheric Conditions

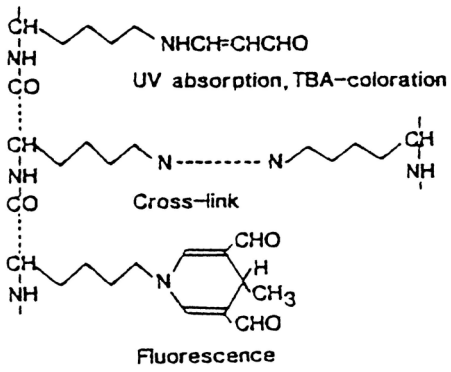


Fig. 2. Fluorescence and Cross-Links Formed in the Reaction of Proteins with MA under Physiological Conditions[15]

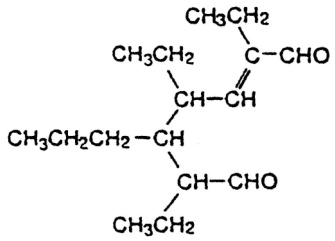


Fig. 3. Proposed Structure of Bifunctional Aldehyde Formed by Reaction of 1-Butanal with Benzylamine[15]

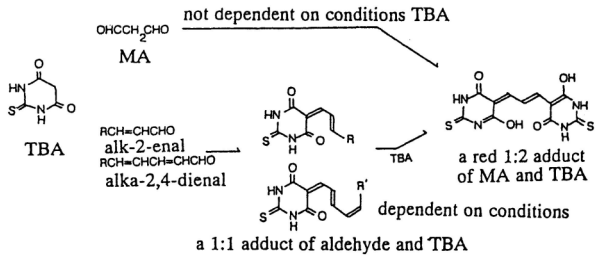
damage due to lipid peroxidation was caused mainly by monofunctional aldehydes and little by free MA.

It has been shown that yellow fluorescent lipofuscin is detected in microscopic observation of aged tissues, and is believed to be derived from lipid peroxidation. Our studies, however, showed that yellow fluorescence at 620 nm isolated from aged rat kidney was not derived from lipid peroxidation.[28–33]

1.2. Establishment of Thiobarbituric Acid (TBA) Assay as a Method to Measure Lipid Peroxidation

The thiobarbituric acid (TBA) assay was developed in 1948 as a method for use in lipid peroxidation studies, and since then it has been widely recognized as a simple and sensitive technique. The red color at 532 nm developed by heating the mixture of peroxidized lipid sample and TBA in an acidic medium is measured as the degree of lipid peroxidation. Because the spectrum of the red color was the same as that of 1 : 2 adduct of MA and TBA, TBA-reactive substances (TBARS) are believed to be free MA and the MA derivatives that release free MA on acid treatment. A wide variety of variations of the assay developed during 1960–1980. However, the assay fell out of favor owing to a lack of specificity to MA. Nevertheless, many researchers used it, because each of the other assay methods had both a benefit and a drawback.[34,35]

Our studies in TBA chemistry established the TBA assay procedure for lipid peroxidation studies. We found that alkenals/alkadienals (and their derivatives) produced the red 1 : 2 adduct of MA and TBA, and the pigment formation is highly dependent on the reaction conditions in a yield of 30% at the highest, whereas the pigment formation from MA (and its derivatives) was quantitative and not dependent on the assay conditions (Fig. 4).[35–42] For the assay of samples isolated from a living body, it was requisite to add antioxidant butylated hydroxytoluene (BHT) to the assay mixture in order to prevent lipid peroxidation during heating. Red color development from alkenals/alkadienals (and their derivatives) was optimal at pH 3.5, stimulated in the presence of Fe ion and completely inhibited in the presence of EDTA. In the assay at pH 3.5 with EDTA, MA (and its derivatives) were exclusively determined as TBARS, and in the assay at pH 3.5 without EDTA both MA (and its derivatives) and akenals/alkadienals (and their derivatives) were determined as TBARS.[43–55] Using the assays with and without



TBARS	Reaction condition						Yield of a red pigment (%)
	Water	Dissolved oxygen	Optimal pH	t-BuOOH	Fe(III) ion	EDTA	
MA	–	–	< 5	–	–	–	100
Alk-2-enal	↑	↑	> 5	↑	↑	↓	< 5
Alka-2,4-dienal	↑	↑	3-4	↑	↑	↓	< 30

Fig. 4. Formation of a Red 1 : 2 Adduct of MA and TBA from MA, Alkenal/Alkadienal Derivatives in the TBA Reaction[37]

EDTA, TBARS of vegetable oils were found to be alkenals/alkadienals (and their derivatives),[51] those of rat tissues were to be both MA (and its derivatives) and alkenals/alkadienals (and their derivatives),[53] and those of human urine were to be MA (and its derivatives).[54,56,57] TBA assay of plasma or serum did not reflect either of these aldehyde groups, and thus did not reflect lipid peroxidation.[50]

1.3. Oxidative Stress-Induced Damage of DNA is Attenuated by Lipid Peroxidation

Many earlier studies have demonstrated that supplementation of oils composed of PUFA residues with higher unsaturation to experimental animals causes a higher degree of lipid peroxidation in the body. The methods for measurement of lipid peroxidation employed in most cases, however, seemed inadequate, because TBARS in tissues have been estimated without addition of an antioxidant in the assay mixtures. We carefully re-examined the effect of supplementing oil containing PUFA residues with higher unsaturation on lipid peroxidation in a living body.

Rats were supplemented with diets containing 5% safflower oil or fish oil for 6 weeks. Safflower oil is rich in n-6 PUFA residue and fish oil is rich in n-3 PUFA residue, and the vitamin E content is the same and sufficient. Fish oil was much more readily oxidized than safflower oil by a lipid soluble radical initiator under atmospheric conditions. Red blood

cells, brain, heart, lung and liver were isolated from the rats. Fatty acid composition of these tissues showed that n-6 PUFA was rich in the safflower oil diet group and n-3 PUFA was rich in the fish oil diet group. Vitamin E levels of the tissues of the fish oil group were only slightly lower than those of the safflower oil group. TBARS of the tissues was estimated by the TBA assay with and without EDTA in the presence of BHT, and phospholipid hydroperoxides were estimated by the HPLC-chemiluminescence method developed by Miyazawa *et al*.[58] It was found that TBARS levels and phospholipid hydroperoxide levels of each tissue of the two groups was the same.[59,60] When red blood cells of both groups were oxidized with Fe(III) ion under atmospheric conditions, TBARS levels and phospholipid hydroperoxide levels of the fish oil group increased to a greater extent. The results indicated that supplementation of PUFA with higher unsaturation did not cause a higher degree of lipid peroxidation, the results being different from those of earlier studies. Differences between the earlier and our observations may be due to the difference of the assay methods employed.

Oxidative stress was induced in rats by intraperitoneal injection of ferric nitrilotriacetate (Fe-NTA). The TBARS levels of liver of the fish oil group increased to a greater extent than those of the safflower oil group, whereas the levels of hydroperoxides of liver of the two groups increased to a similar extent. Liver vitamin E level of the fish oil group was remarkably decreased (Fig. 5), indicating that the oxidative stress caused higher lipid peroxidation in this organ containing PUFA with higher unsaturation. The degree of oxidative DNA damage of liver cells was assessed by comet type characterization in alkaline single cell gel electrophoresis and 8-hydroxy-2′-deoxyguanosine levels. The degree of DNA damage of both groups was increased by the oxidative stress, but the increased level of the fish oil group was remarkably lower than that of the safflower oil group (Fig. 6).[61] When rat liver cells isolated from liver of both groups were exposed to oxidative stress with hydrogen peroxide *in vitro*, the degree of lipid peroxidation of the fish oil group was higher and the degree of DNA damage was lower than those of the safflower oil group.[62] Thus, fish oil supplementation did not enhance but suppressed oxidative stress-induced DNA damage, and lipid peroxidation did not enhance but lowered this damage.

It was found that unoxidized linoleic acid and

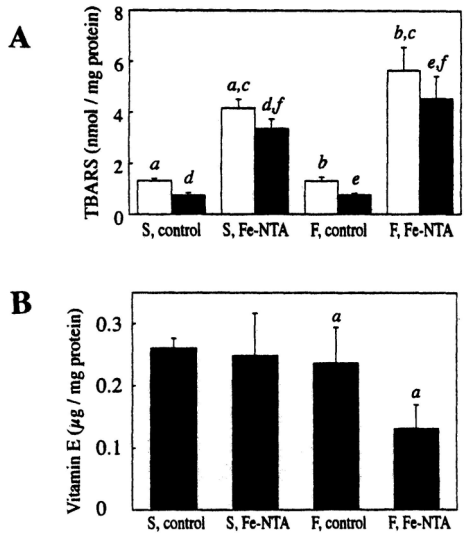


Fig. 5. TBARS (A) and Vitamin E (B) Levels of Liver Homogenate of Rats Treated with Intraperitoneal Iinjection of Fe-NTA[61]

S and F indicate liver of safflower oil and fish oil diet group, respectively. A: TBA assay was performed in the presence of BHT, with (■) and without EDTA (□). *a*,*b*,*d*,*e*) $p < 0.001$, *c*) $p < 0.03$, and *f*) $p < 0.05$. B: *a*) $p < 0.03$.

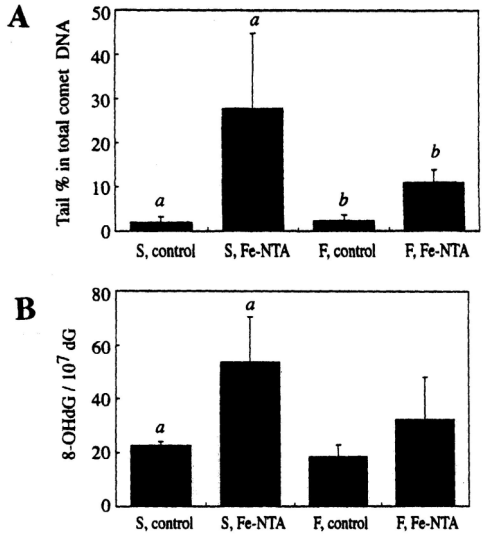


Fig. 6. Tail DNA% in Total DNA in Comet Assay of Liver Cells (A) and 8-OHdG Levels of Liver Homogenate (B) of Rats Treated with Intraperitoneal Injection of Fe-NTA[61]

S and F indicate liver of safflower oil and fish oil diet group, respectively. A: *a*) $p < 0.03$ and *b*) $p < 0.002$. B: *a*) $p < 0.02$.

low density lipoprotein (LDL) suppressed free radical-induced supercoiled plasmid DNA single strand breaks.[63] Unoxidized linoleic acid suppressed DNA strand breaks induced by the free radicals generated from hydrogen peroxide/Fe(II) ion, 2′-azobis(2-amidinopropane)hydrochloride (AAPH), and 4-

(hydroxymethyl)benzene diazonium salt. Although linoleic acid hydroperoxide caused DNA strand breaks as has already been shown, its strand breaking activity was observed only at higher concentrations. Unoxidized LDL inhibited ascorbic acid/Cu(II) ion-, ascorbic acid/Fe(II) ion-, peroxynitrite- and AAPH-induced DNA strand breaks. The TBARS of LDL were increased by treatment with the agents. LDL oxidized with Cu(II) ion did not cause DNA strand breaks. The results indicated that the potency of the free radicals to cause DNA strand breaks was attenuated by the fatty acid and the lipoprotein through lipid peroxidation, and the observations supported the concept that lipid peroxidation attenuated oxidative stress-induced DNA damage.

2. Oxidative Stress-Induced Human Red Blood Cell Aging and Macrophage Recognition of the Cells

Human red blood cells (RBC) become senescent and are removed by macrophages 120 days after the circulation. In earlier studies, the hypothesis that macrophages directly recognize asialylated sugar chains on the senescent cell prevailed. However, since it was found that senescent RBC are phagocytosed by macrophages depending on the cell surface bound IgG antibodies, specific antibodies including anti-band 3 IgG (for fragmented band 3), anti-α-galactosyl IgG and anti-asialoglycophorin IgG were shown to be possible candidates. We demonstrated the dynamic senescence process of RBC involving oxidative stress.

2.1. Senescent or Oxidized RBC Bind to Ant-Band 3 IgG through Sialylated PL Sugar Chains

Whole RBC collected from several donors were separated into young and old RBC by Percoll density gradient centrifugation. Free iron ion contents of old RBC from 5 donors were much higher than those of young RBC.[64] Levels of phosphatidyl ethanolamine hydroperoxides and TBARS of the membrane lipid fraction of old RBC were higher than those of young RBC,[65] indicating that RBC suffered from oxidative stress during aging in the circulation.

Whether oxidative modification of RBC caused generation of the binding sites for autologous IgG on the cell surface was examined in *in vitro* studies.[66,67] Unseparated whole RBC were briefly oxidized by incubation with an iron catalyst, ADP/Fe(III) chelate, or a thiol specific oxidizing agent, diamide, at 37°C for 3 hr. Autologous IgG bound better to the oxidized RBC. There were optimum Fe(III) ion and diamide concentrations for effective binding. When the oxidized RBC were treated with dithiothreitol, autologous IgG did not bind to the cells, suggesting that disulfide formation was responsible for generation of the binding sites.

Anti-band 3 IgG obtained by fractionation of whole IgG efficiently bound to the Fe(III) ion-oxidized RBC, whereas anti-band 3 depleted IgG did not at all, indicating that band 3 was an antigen.[66] Among glycoproteins, band 3 and lactoferrin effectively inhibited the binding of whole IgG to the oxidized RBC.[68] While the inhibitory effects of band 3 and lactoferrin were unchanged when treated with trypsin, chymotrypsin or pronase, their inhibitory effects diminished on treatment with endo-β-galactosidase or neuraminidase. Whole oligosaccharides isolated from band 3 were also inhibitory against the binding of whole IgG to the oxidized RBC, which diminished on treatment with endo-β-galactosidase or neuraminidase. Sialylated PL sugar chains commonly present in band 3 and lactoferrin that are cleaved by endo-β-galactosidase and neuraminidase, were determined to be the antigenic sites (epitope) of band 3 on the surface of the oxidized RBC. The cell-free binding studies between anti-band 3 IgG and band 3 or lactoferrin digested with *N*-glycosidase F, endo-β-galactosidase and neuraminidase showed that nearly 70% of total anti-band 3 IgG antibodies bound to the sialylated PL sugar chains of band 3 and lactoferrin, but the remaining 30% may have been directed to the peptide region of the glycoproteins.[69]

When RBC were oxidized with Fe(III) ion, the amount of non ionic detergent $C_{12}E_8$-insoluble aggregates of the membrane proteins was increased, and subsequent SDS-polyacrylamide gel electrophoresis (PAGE) and Western blotting analysis using rabbit anti-band 3 IgG showed that the amount of band 3 aggregates was increased by the oxidation.[68–70] Treatment of RBC with acridine orange[70] and sesamol,[71] nonoxidizing membrane protein aggregating agents, resulted in the increase of these aggregates and also the increased binding of anti-band 3 IgG. These results indicate that, upon oxidation, RBC membrane band 3 became aggregated or clustered so as to bind to anti-band 3 IgG more readily (Fig. 7).

We found that the amount of autologous IgG antibodies bound to old RBC was greater than that bound to young RBC by using ^{125}I-labeled anti-human IgG as a secondary antibody.[72] The radioactivity on old RBC was released when the cells were

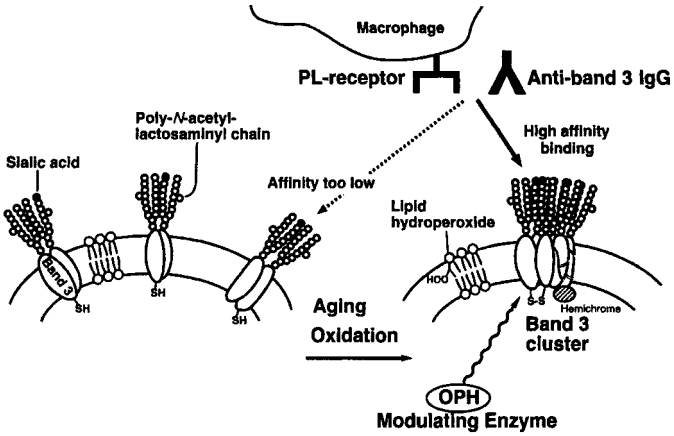


Fig. 7. A Proposed Mechanism of Binding of Anti-Band 3 IgG and Macrophages to Senescent or Oxidized RBC through PL Sugar Chains and its Modulation by OPH

incubated with band 3 oligosaccharides but not in incubation with those pretreated with endo-β-galactosidase or neuraminidase. The amount of aggregates of band 3 on old RBC membranes was greater than that in young RBC membranes.[70] The results indicated that senescence of RBC in the circulation proceeded through oxidative stress induced by released iron ion, and that the sialylated PL sugar chain epitope of band 3 became clustered so as to bind to anti-band 3 IgG.[73–79]

2.2. Oxidized RBC Bind to Macrophages through PL Sugar Chains

Mouse macrophages bound better to oxidized RBC in the absence of serum.[80–84] Human monocytic leukemia cell line THP-1 differentiated into macrophages by phorbol myristate acetate bound better to the Fe(III) ion-oxidized RBC, and the binding was inhibited by band 3 and its oligosaccharides, and by lactoferrin and its oligosaccharides.[85–87] Removal of the non-reducing terminal region of the sugar chains of band 3 on RBC surface by treatment of the cells with endo-β-galactosidase showed a preventive effect against subsequent oxidation and macrophage adhesion, whereas removal of sialyl residues from the sugar chains by neuraminidase showed no effect. Hence, human macrophages directly bound to PL sugar chains of band 3 on oxidized RBC, indicating that band 3 aggregation induced by oxidative stress may lead to the removal of old RBC from the circulation by direct binding to macrophages (Fig. 7). Fe ion-oxidation of polymorphonuclear leucocytes[88] and lymphocytes[89] resulted in an increase in PL aggregates due to CD43, to which anti band 3 IgG and macrophages bound better. Aggregates of PL sugar chains were created in apoptotic lymphocytes to which anti band-3 IgG bound better.[90]

2.3. Macrophage Recognition of Oxidized RBC is Augmented by Oxidative Stress in Macrophage Cells

Scavenger receptor activity of mouse macrophages to bind oxidized LDL was found to be inhibited by a wide variety of antioxidants.[91–96] Various antioxidants inhibited mouse macrophage protein phosphorylation,[95] and specific inhibitors of protein kinase C, protein tyrosine kinase and protein serine/threonine kinase inhibited while specific inhibitors of tyrosine phosphatase and serine/threonine phosphatase augmented the macrophage binding,[94] suggesting that oxidative stress was involved in the macrophage binding of the oxidized lipoprotein. It was also shown this binding was dependent on oxygen tension (unpublished results). When oxygen tension was reduced from normoxia (20%) to hypoxia (5%), the macrophage binding was suppressed, and then was recovered by normoxia, supporting that oxidative stress plays an important role in macrophage binding of the oxidized lipoprotein.

It was found that the antioxidants suppressed mouse macrophage binding of oxidized RBC,[97] indicating that oxidative stress in macrophages is involved in the macrophage binding of oxidized RBC through PL sugar chains. The presence of lectin-like proteins (PL receptor) on the surface of THP-1 macrophages that recognize PL sugar chains on the oxidized cell surface was shown.[98] Moreover, it was

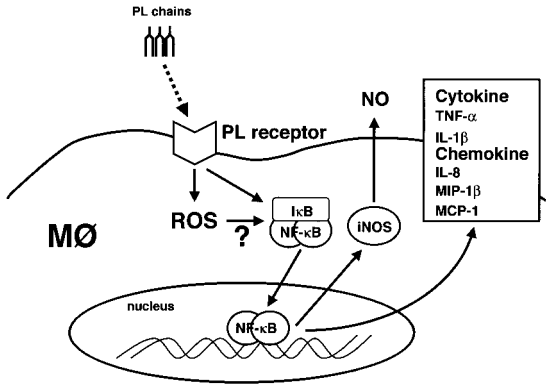


Fig. 8. Generation of Reactive Oxygen Species (ROS) and NF-κB Activation in Macrophages (MØ) by PL Sugar Chains

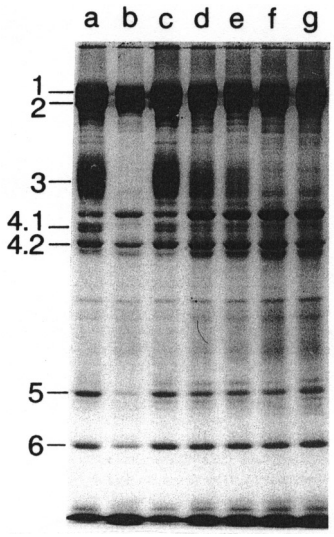


Fig. 9. SDS-PAGE of Membranes Isolated from Xanthine/Xanthine Oxidase/Fe(III)-Oxidized RBC after the 2nd Incubation in the Presence of Protease Inhibitors[99]

Membranes isolated from RBC oxidized at 37°C for 3 hr (lane a) were incubated at 37°C for 6 hr in the presence of α-tocopherol with no protease inhibitor (lane b), 1 mM DFP (lane c), 0.2 mM PMSF (lane d), 1 mM EDTA (lane e), 10 μg.ml leupeptin (lane f) and 50 μM pepstatinA (lane g). Bands were stained with coomassie brilliant blue.

found that treatment of THP-1 cells and mouse macrophages with PL sugar chains induced reactive oxygen species and activated NF-κB in the cells (unpublished results). Inducible nitric oxide synthase (iNOS) was induced and generated NO, and cytokines and chemokines were also induced in the cells (Fig. 8). The results indicated that oxidative stress was induced in macrophages by binding of PL sugar chains, suggesting that such binding induced oxidative stress in the cells, which may stimulate PL sugar chain binding.

3. Presence of a New Enzyme, OPH, in Cells

It has been shown that proteins modified by oxidative stress in the cells are removed by ubiquitin-proteasome systems. We found a new enzyme. OPH, that preferentially degraded oxidized proteins in a wide variety of cells, and rescued the cells from oxidative stress.

3.1. Discovery of OPH in RBC

RBC were oxidized mildly with xanthine/xanthine oxidase/Fe(III) ion at 37°C for 3 hr, and the membranes were incubated at 37°C for 6 hr in the presence of α-tocopherol with and without protease inhibitors. SDS-PAGE of the oxidized membranes revealed that the membrane proteins including band 3 disappeared in the absence of protease inhibitors (lane b) whereas the disappearance was inhibited in the presence of serine protease inhibitors, diisopropyl fluorophosphate (DFP) (lane c) and phenyl methylsulfonyl fluoride (PMSF) (lane d) (Fig. 9).[99] Similar incubation of unoxidized RBC membranes did not cause membrane protein degradation, suggesting that oxidized membrane proteins became susceptible by a certain serine protease present in the membranes.

The presence of 80 kDa serine protease in RBC membranes and cytosol that could be labeled by [^{3}H]DFP was shown[100] This enzyme was termed OPH. The enzyme present in RBC cytosol was found to move to the membranes when the cells were oxidized to remove oxidized membrane proteins.[101] RBC membrane proteins became aggregated to bind to anti-band 3 IgG and macrophages during aging in the circulation by oxidative stress as shown above (Fig. 7), and OPH may modulate the bindings by removing the aggregated membrane protein aggregates.[102]

3.2. Isolation and Identification of OPH

[^{3}H]DFP-labeled OPH was isolated and purified from RBC cytosol.[101] Homology search of the amino acid sequences of the OPH peptide fragments prepared by lysylpeptidase digestion showed that OPH highly coincided with human liver acylpeptide hydrolase (ACPH)[103] whose biological activity has been hitherto unknown.

Recombinant ACPH (rACPH) was prepared from cDNA of the human erythroleukemic cell line K-562. rACPH showed both ACPH activity and OPH activity, and OPH showed both ACPH activity and OPH activity[103] Analysis of the OPH digest of

bovine serum albumin oxidized with hydrogen peroxide/peroxidase on SDS-PAGE revealed that several peptide fragments appeared, whereas unoxidized bovine serum albumin was not digested by OPH.[104] OPH acted toward the oxidized protein as an endopeptidase and the cleaving site was found to be Leu218-219Ser, Tyr410-411Thr and Phe596-597Thr, which may be buried in the unoxidized protein and may be exposed by oxidation. OPH showed chymotrysin-type peptidase activity.

Using anti-OPH IgG it was found that OPH was present not only in RBC but in a wide variety of cells and tissues.[105] It was ubiquitous and may act to remove oxidized proteins thus rescuing cells from oxidized stress.

3.3. Role of OPH in Cells Exposed to Oxidative Stress

The role of OPH in intact cells exposed to oxidative stress was examined using COS-7, a cell line derived from African green monkey kidney. COS-7-OPH cells that stably overexpressed OPH were established. When these cells were exposed to oxidative stress induced by hydrogen peroxide (Fig. 10) and paraquat, the accumulation of protein carbonyls in the cells was apparently lower than that of parental COS-7 cells, and COS-7-OPH cells were significantly resistant in oxidative stress compared with parental COS-7 cells.[106] The results indicated that OPH acted to prevent accumulation of oxidized proteins and to rescue the cells from oxidative stress. It is interesting to note that the majority of overexpressed OPH in the cells was found to be located uniformly in cytosol, and its location was not altered by hydrogen peroxide-induced oxidative stress.

Roles of OPH and proteasome in the COS-7-OPH cells in which oxidative stress had been induced with paraquat were compared using a specific inhibitor of OPH, acetylleucine chloromethyl ketone (ALCK), a specific inhibitor of proteasome, epoxomicine (epox), and the inhibitor of both the enzymes, lactacystine (LC) (unpublished results). In ALCK-treated COS-7-OPJ cells, accumulation of protein carbonyls increased depending on the concentration of paraquat, and the cell resistance to the stress decreased. In contrast, in epox-treated COS-7-OPH cells, protein carbonyls did not accumulate with the paraqauat treatment. In ALCK- and epox-treated COS-7-OPH cells, protein carbonyls accumulated depending on the paraquat concentration; this was also true in LC-treated COS-7-OPH cells.

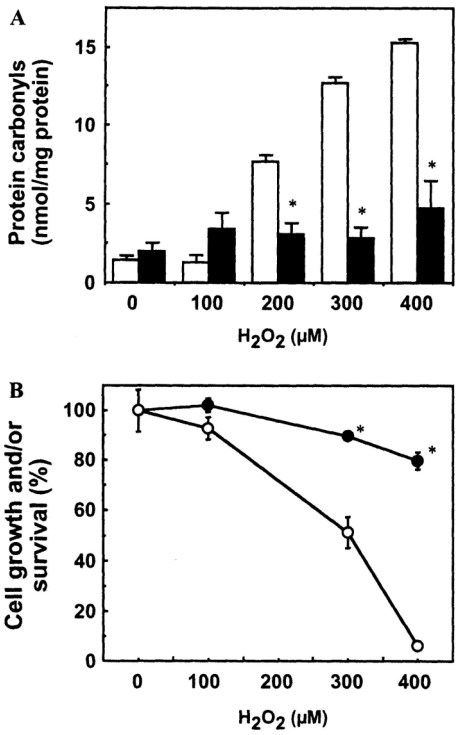


Fig. 10. The Effect of Hydrogen Peroxide Damage on COS-7 and COS-7-OPH Cells[106]

A: Accumulation of protein carbonyls in trichloroacetic acid-precipitable proteins in COS-7 (□) and COS-7-OPH (■) cells treated with hydrogen peroxide at the indicated concentrations at 37°C for 24 hr. B: Cell growth and/or survival of COS-7 (○) and COS-7-OPH (●) cells treated with hydrogen peroxide at the indicated concentrations at 37°C for 24 hr. *Significantly different from parental COS-7 cells at $p < 0.01$.

The results indicated that overexpressed OPH in COS-7-OPH cells more effectively removed oxidized proteins in the cells as compared with proteasome. OPH may constitute a secondary defense system of cells against oxidative stress.

CONCLUDING REMARKS

The present author elucidated three new defense systems against oxidative stress. Lipid peroxidation attenuated the DNA damage induced by oxidative stress. Red blood cells became senescent by suffering from oxidative stress during the circulation, and aged or oxidized cells were recognized by macrophages in the intracellular oxidative stress. The presence of OPH that preferentially hydrolyzed proteins damaged by oxidative stress was found, and may constitute a secondary defense system of the cells against this stress.

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