Effect of Ferritin on λ DNA Strand Breaks in the Reaction System of Alloxan Plus NADPH-Cytochrome P450 Reductase: Ferritin's Role in Diabetogenic Action of Alloxan

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The incubation of λ DNA in the reaction system of alloxan plus NADPH-cytochrome P450 reductase (fp2) in the presence of ferritin caused strand breaks after a lag time of about 5 min. Addition of ferritin to the reaction system at concentrations below 50 μ g/ml caused the strand breaks of DNA in a concentration-dependent fashion. Catalase, scavengers of hydroxyl radicals ($\text{HO}\cdot$) and iron-chelators almost completely inhibited the DNA strand breaks, but superoxide dismutase (SOD) did not, suggesting that the strand breaks are induced by the generation of $\text{HO}\cdot$ via the reaction of H_2O_2 and Fe(II), namely, the Fenton reaction. When the ferritin was incubated in the reaction system of alloxan plus fp2, the iron release from ferritin increased with incubation time depending on the amount of fp2. The addition of increasing concentrations of ferritin to the reaction system resulted in progressive increase in the iron release and a decrease in the electron spin resonance signal intensity of alloxan radical ($\text{HA}\cdot$), the one electron reduced form of alloxan, suggesting that $\text{HA}\cdot$ generated in the reaction system is capable of releasing iron from ferritin. These results support the possibility that the iron released from ferritin may be involved in the diabetogenic action of alloxan.

Key words ferritin; alloxan; DNA strand break; NADPH-cytochrome P450 reductase; hydroxyl radical; alloxan radical

Alloxan is widely used in studies of experimental diabetes because this agent destroys pancreatic β -cells with a specific selectivity.^{1,2)} Since this diabetogenic action is inhibited by superoxide dismutase (SOD), catalase or hydroxyl radical ($\text{HO}\cdot$) scavengers such as thiourea, dimethylsulfoxide (DMSO) and *N,N*-dimethyl urea,^{3–5)} the action of alloxan is believed to involve oxygen radicals. In the reaction system of alloxan with reduced glutathione (GSH) in the presence of Fe(III)-ethylenediaminetetraacetic acid (EDTA), we have proposed⁶⁾ that alloxan causes the rise of superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) during the redox cycling between alloxan and alloxan radical ($\text{HA}\cdot$) (reaction 1–3) and that $\text{HO}\cdot$ is generated by $\text{HA}\cdot$ through a Fenton reaction (reaction 4 and 5).

Several researchers have shown,^{7,8)} with experimental systems using isolated pancreatic islets of rats, that the primary step of alloxan toxicity is the generation of $\text{HO}\cdot$ by which the DNA of pancreatic islets is attacked to produce strand breaks. These findings suggest that $\text{HO}\cdot$ which was generated by $\text{HA}\cdot$ through a Fenton-type reaction initiates the DNA strand breaks in pancreatic islet cells.

The involvement of iron in the diabetogenic action of alloxan has also been indicated by several lines of studies, *in vivo*^{9,10)} and *in vitro*.¹¹⁾ In a biological system, the production of $\text{HO}\cdot$ was catalyzed by transition metals such as iron.¹²⁾ However, there was no direct evidence for the existence of free iron capable of inducing this toxicity under normal physiological conditions, because the metabo-

lism of iron is tightly controlled and regulated by the iron-binding proteins, transferrin and ferritin.¹³⁾ Most intercellular iron except for heme iron is stored within ferritin from which various reducing agents such as O_2^- , ascorbate and GSH can liberate it.^{14–16)} The iron released from ferritin can produce $\text{HO}\cdot$ from H_2O_2 through the Fenton reaction (reaction 5).^{17,18)}

We established¹⁹⁾ that the generation of $\text{HA}\cdot$ was catalyzed by NADPH-cytochrome P450 reductase [E.C.1.6.2.4] (fp2) purified from rat liver microsomes. To clarify the mechanism of DNA strand breaks by alloxan in islet cells, we examined the effect of ferritin on λ DNA strand breaks in the $\text{HA}\cdot$ -generating system of alloxan plus fp2.

MATERIALS AND METHODS

Materials Alloxan, FeSO_4 (Fe(II)), DMSO and ferritin (from horse spleen) were purchased from Wako Pure Chemical Industries, Ltd., Japan. A solution of Fe(II) was made up freshly just before use in dilute HCl purged with N_2 gas for 5 min. SOD (from bovine erythrocytes), catalase (from bovine liver, thymol-free) and apotransferrin (from human) were obtained from Sigma Co., U.S.A. The apotransferrin was dissolved in 10 mM sodium phosphate buffer, pH 7.4, containing 5 mM NaHCO_3 . λ DNA (M.W. 31.5×10^6 daltons, 48502 base pairs) was from Nippon Gene, Ltd., Japan; phenobarbital (PB) from Sankyo Ltd., Japan, bathophenanthroline disulfonate from Dojindo Laboratories Co. Ltd., Japan; dimethylthiourea (DMTU) from Aldrich Chemical Co., Inc., U.S.A. and desferrioxamine from CIBA Geigy, Switzerland. Other reagents used in the experiments were of the highest grade.

Detection of DNA Strand Breaks To remove the Tris-HCl buffer and EDTA, the commercial λ DNA solution was dialyzed against sterilized 10 mM sodium

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phosphate buffer, pH 7.4, containing 0.15 M NaCl at 4 °C for 2 d with three changes of the same buffer. After the incubation of DNA (20 μ g/ml) in 10 mM sodium phosphate buffer, pH 7.4, containing 1 mM alloxan, 0.5 mM NADPH, 50 μ g/ml ferritin, 0.15 unit/ml fp2 and 0.15 M NaCl at 37 °C for various periods of time, the DNA strand breaks were observed as described previously by using the electrophoresis method.²⁰⁾ and were photographed in ultraviolet light.

Purification of fp2 Male Wistar strain rats weighing about 250 g (7 to 8 weeks old) were used in this experiment. PB was injected intraperitoneally to rats at the dose of 80 mg/kg body weight daily for 4 d. Liver microsomes were prepared by ultracentrifugation as described by Ernster *et al.*²¹⁾ and washed once with 0.15 M KCl. The fp2 was prepared from this microsomal preparation according to the method of Yasukochi and Masters,²²⁾ with a minor modification. Emulgen 911 (Kao Tokyo, Japan) at 30% concentration was used as a substitute for Renex 690. The fp2 solution obtained here contained 25% glycerol which has been proposed to react with HO $\cdot$. To remove the glycerol, the fp2 solution was diluted with cold sterilized 10 mM sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl, and then concentrated with ultrafiltration (an Amicon Centricon-30). This process was repeated three times. The concentration of glycerol in the fp2 solution obtained here was not more than 3 μ M. The homogeneity of fp2 obtained was established by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The activity of fp2 was measured at 25 °C by monitoring the rate of reduction of cytochrome c at 550 nm (using an extinction coefficient of 21.1 $\text{mm}^{-1}\cdot\text{cm}^{-1}$). One unit of activity was defined as 1 μ mol of cytochrome c reduced per min/ml. The activity of fp2 used in this experiment was 67 unit/mg of protein.

Preparation of Ferritin and Iron Release Commercial ferritin was incubated in 10 mM sodium phosphate buffer, pH 7.4, containing 10 mM EDTA at 4 °C and the solution was applied to a Sepharose 6B (Pharmacia Fine Chemicals, Sweden) column (2.6 $\times$ 80 cm) previously equilibrated with 10 mM sodium phosphate buffer, pH 7.4. Ferritin fractions were collected and concentrated by ultrafiltration. The iron content of ferritin was determined to be 0.22 mg Fe(III)/mg of ferritin with a polarized Zeeman atomic absorption spectrophotometer (Hitachi 180-80). The iron release from ferritin was determined in the reaction system of alloxan plus fp2 at 37 °C spectrophotometrically using the ferrous iron chelator bathophenanthroline sulfonate, the increase in absorbance at 530 nm ($\epsilon = 22.14 \text{ mm}^{-1}$) was monitored with a Hitachi model U-2000 spectrophotometer.¹⁵⁾

Detection of Alloxan Radical The generation of HA $\cdot$ in the reaction system of alloxan plus fp2 was detected as described previously by using an ESR (JEOL model JES-RE1X) at room temperature.⁶⁾ The relative intensity of HA $\cdot$ was determined by measuring the peak height at the center hyperfine line of the ESR spectrum.

RESULTS

Effect of Ferritin on DNA Strand Breaks in the Reaction System of Alloxan Plus fp2 As shown in Fig. 1, when the DNA was incubated in the reaction system of alloxan plus fp2 in the absence of ferritin, the strand breaks were not

Fig. 1. Effect of Ferritin-Concentrations on DNA Strand Breaks in the Reaction System of Alloxan Plus fp2

The reaction mixture consisted of 1 mM alloxan, 0.5 mM NADPH, 0.15 unit/ml fp2, 0.15 M NaCl, 20 μ g/ml λ DNA and various concentrations of ferritin in 10 mM sodium phosphate buffer, pH 7.4. After the incubation for 20 min at 37 °C, the DNA strand breaks were detected by the method described in Materials and Methods. The concentrations of ferritin were: track 1, 0 μ g/ml; 2, 10 μ g/ml; 3, 20 μ g/ml; 4, 30 μ g/ml; 5, 50 μ g/ml; 6, 70 μ g/ml and 7, 100 μ g/ml.

observed (track 1). The addition of ferritin to the reaction system at concentrations below 50 μ g/ml (as Fe(III) 0.19 mM) caused DNA strand breaks in a concentration-dependent fashion (tracks 2—5). The addition of ferritin to the reaction system above 70 μ g/ml resulted in a decrease in the extent of the DNA strand breaks (tracks 6 and 7). Although data are not shown, the strand breaks were not observed without either alloxan, NADPH or fp2 in the reaction system in the presence of ferritin. These results indicate that ferritin is required for the DNA strand breaks in the reaction system of alloxan plus fp2.

As shown in Fig. 2, the incubation of DNA in the reaction system in the presence of 50 μ g/ml ferritin caused DNA strand breaks after a lag time of about 5 min (A). When the DNA was incubated in the reaction system in the presence of 10 μ M Fe(II), the strand breaks of DNA increased with incubation time (B). These results indicate the requirement for longer incubation time in the presence of ferritin than Fe(II) to induce the strand breaks, and suggest that the iron released from ferritin catalyzes these strand breaks.

Release of Iron from Ferritin As shown in Fig. 3, when ferritin was incubated in the reaction system of alloxan plus fp2, the amount of released iron from ferritin increased with incubation time in the range from 0.03 to 0.15 unit/ml depending on fp2 amount. When the fp2 was omitted from the reaction system, little release of iron was observed.

As shown in Table I, the rate of iron release from ferritin was 8.8 μ M/20 min under the experimental conditions used here. When alloxan or NADPH was omitted from the reaction system, very little iron release was seen. The addition of SOD to the reaction system had no significant effect on the rate of the iron release while catalase brought about a slight increase. These results indicate that O $_2^-$ is not directly involved in the release in the reaction system of alloxan plus fp2, and suggest that H $_2$ O $_2$ generated in the reaction system reoxidizes Fe(II) to Fe(III).

Fig. 2. Time Course of DNA Strand Breaks in the Reaction System of Alloxan Plus fp2 in the Presence of Ferritin or Fe(II)

The reaction mixture consisted of 1 mM alloxan, 0.5 mM NADPH, 0.15 unit/ml fp2 and 0.15 M NaCl in 10 mM sodium phosphate buffer, pH 7.4. λ DNA (20 μ g/ml) was incubated at 37 °C in the reaction mixture in the presence of 50 μ g/ml ferritin (A) or 10 μ M Fe(II) (B). The incubation times were: track 1, 0 min; 2, 1 min; 3, 3 min; 4, 5 min; 5, 10 min; 6, 20 min and 7, 30 min.

Fig. 3. Time Course of Release of Iron from Ferritin in the Reaction System of Alloxan Plus fp2

The reaction mixtures consisted of 1 mM alloxan, 0.5 mM NADPH, 0.5 mg/ml ferritin, 1 mM bathophenanthroline sulfonate and various concentrations of fp2 in 10 mM sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl. The iron release was determined as described in Materials and Methods. The amounts of fp2 are indicated on the graph.

TABLE I. Iron Release from Ferritin in the Reaction System of Alloxan Plus fp2

Condition	Iron release (μ M/20 min)	Percent of control (%)
Reaction system	8.8 ± 0.4	100
– Alloxan	1.5 ± 0.1	17
– NADPH	0.1 ± 0.0	1
+ 100 U/ml SOD	7.7 ± 0.4	88
+ 140 U/ml catalase	10.1 ± 0.3	116

The reaction system consisted of 1 mM alloxan, 0.5 mM NADPH, 0.15 unit/ml fp2, 50 μ g/ml ferritin, 1 mM bathophenanthroline sulfonate and 0.15 M NaCl in 10 mM sodium phosphate buffer, pH 7.4. The iron release was determined as described in the Materials and Methods. Each value represents the mean $\pm$ S.E. of triplicate experiments.

As previously reported,^{23,24)} in the reaction system of alloxan with GSH, the iron release from ferritin occurred through a direct reduction of ferritin iron by HA $\cdot$, the one electron reduced form of alloxan. Therefore, we

Fig. 4. Effect of Ferritin on Iron Release and Generation of HA $\cdot$

The release of iron from ferritin was carried out in the reaction mixtures consisting of 1 mM alloxan, 0.5 mM NADPH, 0.15 unit/ml fp2, 1 mM bathophenanthroline sulfonate and various concentrations of ferritin in 10 mM sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl. The determination of signal intensity of HA $\cdot$ was performed in the reaction mixtures without bathophenanthroline sulfonate. Other conditions were as described in Materials and Methods. Each point represents the mean $\pm$ S.E. of triplicate experiments.

investigated the possible participation of HA $\cdot$ in the iron release from ferritin in the reaction system of alloxan plus fp2. As shown in Fig. 4, the addition of increasing concentrations of ferritin to the reaction system resulted in progressive increase in the iron release with decreasing of the ESR signal intensity. The ESR spectrum obtained here agreed with that of the HA $\cdot$ reported in the previous paper ($a^N = a^H = 0.45$ G, $g = 2.005$).⁶⁾ These results suggest that HA $\cdot$ generated in the reaction system of alloxan plus fp2 is capable of releasing iron from ferritin.

Effect of Oxygen Radical Scavengers and Iron-Chelators on DNA Strand Breaks We next investigated the effect of SOD, catalase, HO $\cdot$ scavengers and iron-chelators on the DNA strand breaks in the reaction system of alloxan plus fp2 in the presence of ferritin. As shown in Fig. 5, catalase (track 3) and HO $\cdot$ scavengers (tracks 4 and 5) almost completely inhibited the DNA strand breaks, but SOD had little effect (track 2), indicating the possible participation of H₂O₂ and HO $\cdot$ in these breaks.

Fig. 5. Effect of SOD, Catalase, HO $\cdot$ Scavengers and Iron-Chelators on DNA Strand Breaks

The experimental conditions were the same as given in Fig. 2, except that the incubation time was 20 min. Track 1, control; 2, +110 U/ml SOD; 3, +140 U/ml catalase; 4, +10 mM DMTU; 5, +10 mM DMSO; 6, +4 mg/ml apotransferrin; 7, +0.2 mM desferrioxamine and 8, +0.2 mM bathophenanthroline sulfonate.

An addition of apotransferrin (4 mg/ml), a Fe(III)-binding protein which has 100 μ M Fe(III)-binding capacity, to the reaction system did not inhibit the DNA strand breaks (track 6). However, desferrioxamine, a Fe(III)-chelator, and bathophenanthroline, a Fe(II)-chelator, strikingly inhibited then (tracks 7 and 8). Although the data are not shown, when ferritin was replaced by Fe(II) or Fe(III) in the reaction system, almost the same results were obtained. These findings suggest that both Fe(II) and Fe(III) are involved in DNA strand breaks under the experimental conditions presented here.

DISCUSSION

Alloxan induces DNA strand breaks in rat pancreatic islet cells *in vivo* and *in vitro*.^{7,25)} The strand breaks seem to be important in the development of diabetes mellitus. Previous studies in this laboratory have demonstrated that λ DNA strand breaks result in the HA $\cdot$ -generating system of alloxan with GSH in the presence of Fe(III),²⁶⁾ and that the generation of HA $\cdot$ is catalyzed by fp2 in the islet cell.¹⁹⁾ In the present study, we showed that the strand breaks of λ DNA were caused by the reaction system of alloxan plus fp2 in the presence of ferritin (Fig. 1) and were strongly inhibited by catalase, HO $\cdot$ scavengers and iron-chelators, but not effectively by SOD (Fig. 5). These results indicate that H₂O₂ and HO $\cdot$ together with Fe are involved in the strand breaks. Although the participation of ferritin itself in the reaction of HO $\cdot$ -generating is unclear,^{17,27)} several studies have confirmed that the iron released from ferritin can catalyze the generation of HO $\cdot$ in the reaction system of xanthine with xanthine oxidase.^{17,18)} The present study showed that the release of iron from ferritin was caused by the HA $\cdot$ generated in the reaction system of alloxan plus fp2 (Fig. 4). From these results, we concluded that the strand breaks of DNA in the presence of ferritin were directly induced by the generation of HO $\cdot$ via the HA $\cdot$ -dependent Fenton reac-

tion.

Incubation of DNA in the reaction system in the presence of ferritin resulted in the strand breaks after a lag time of about 5 min (Fig. 2). Under the same experimental conditions, with 5 min incubation, the amount of released iron was about 3 μ M (Fig. 3). The strand breaks were also observed by the addition more than 3 μ M of Fe(II) to the reaction system (data not shown). These results suggest that the lag time can be attributed to the length of time required to release the iron from ferritin and that the release of iron is a rate-limiting step in the strand breaks of DNA in the reaction system.

Since the DNA strand breaks were inhibited by either Fe(II)- or Fe(III)-chelators in the reaction system of alloxan plus fp2 in the presence of ferritin (Fig. 5), the redox-cycling of released iron appears to occur during the reaction of strand breaks. Although apotransferrin has been reported to inhibit the reaction of iron-dependent HO $\cdot$ generation in the reaction system of xanthine with xanthine oxidase,²⁸⁾ it does not inhibit the DNA strand breaks in the reaction system of alloxan plus fp2 in the presence of ferritin (Fig. 5). In our previous studies,²⁶⁾ we showed that the apotransferrin did not remove iron from the DNA-Fe(III) complex which was reduced by HA $\cdot$ to DNA-Fe(II) complex, suggesting that the Fe bound to DNA catalyzes the site-specific generation of HO $\cdot$ via the Fenton-type reaction (reaction 6).

These findings indicate that the iron (Fe(II) or Fe(III)) bound to DNA involved in the DNA strand breaks which are caused by the site-specific generation of HO $\cdot$ through the Fenton reaction in the reaction system of alloxan plus fp2 in the presence of ferritin.

One possible reason why the adding of an excess (more than 70 μ g/ml) of ferritin inhibited the strand breaks of DNA in the reaction system (Fig. 1) is as follows: the HA $\cdot$ which has been reported to reduce O₂ by one electron to O₂⁻.^{6,29)} may be predominantly consumed by the releasing reaction of iron by an interaction with excess ferritin. In fact, the ESR signal of HA $\cdot$ could not be detected in the presence of 100 μ g/ml ferritin (data not shown).

The results of the present study provide evidence that the HA $\cdot$ generated by fp2 induces the strand breaks of DNA. Several lines of evidence³⁰⁻³²⁾ have indicated that the various quinone antibiotics such as adriamycin and bleomycin which are structurally similar to alloxan, are reduced to the semiquinone radicals by fp2, *in vivo* and *in vitro*. The semiquinone radicals react rapidly with oxygen to produce O₂⁻, H₂O₂ and HO $\cdot$.^{32,33)} In biological system, HO $\cdot$ reacts with cellular DNA, thereby the strand breaks of DNA are induced.^{32,34)} These events of quinone antibiotics may ultimately cause the tumor cell death. We have demonstrated that the generation of HA $\cdot$ in the islet cell is catalyzed by the fp2.¹⁹⁾ In addition, alloxan accumulates rapidly and preferentially in islet.³⁵⁾ Therefore, we inferred that the HA $\cdot$ generated by fp2 was involved in the process of the pancreatic β -cell death and the diabetes mellitus.

MacDonald *et al.*³⁶⁾ have shown that β -cell of rat pancreatic islet contains a high amount of ferritin. Other

workers³⁷⁻³⁹ demonstrated that ferritin and fp2 were found in nuclear of the rat liver cell. These findings suggest a possibility that ferritin and fp2 play an important role in damaging DNA by alloxan in pancreatic islet cells. To explain the exact mechanism of the diabetogenic action of alloxan, more studies are required to clarify the existence of fp2 in pancreatic cell, especially in β -cell.

REFERENCES

- 1) I. Lundquist, C. Rerup, *Eur. J. Pharmacol.*, **2**, 35 (1967).
- 2) R. E. Heikkilä, B. Winstor, G. Cohen, H. Barden, *Biochem. Pharmacol.*, **25**, 1085 (1976).
- 3) K. Grankvist, S. Marklund, I. B. Täljedal, *Nature* (London), **294**, 158 (1981).
- 4) K. Grankvist, S. Marklund, J. Sehlin, I. B. Täljedal, *Biochem. J.*, **182**, 17 (1979).
- 5) L. J. Fischer, S. A. Hamburger, *Diabetes*, **29**, 213 (1980).
- 6) K. Sakurai, T. Miura, T. Ogiso, *Chem. Pharm. Bull.*, **38**, 993 (1990).
- 7) Y. Uchigata, H. Yamamoto, A. Kawamura, H. Okamoto, *J. Biol. Chem.*, **257**, 6084 (1982).
- 8) N. Takasu, I. Komiya, Y. Nagasawa, T. Yamada, *J. Biol. Chem.*, **266**, 2112 (1991).
- 9) K. Grankvist, S. Marklund, *Life Sci.*, **33**, 2535 (1983).
- 10) A. El-Hage, E. H. Herman, G. C. Yang, *Res. Commun. Chem. Pathol. Pharmacol.*, **52**, 341 (1986).
- 11) L. A. Idahl, A. Lernmark, J. Sehlin, I. B. Täljedal, *Biochem. J.*, **162**, 9 (1977).
- 12) S. D. Aust, L. A. Morehouse, C. E. Thomas, *J. Free Radic. Biol. Med.*, **1**, 3 (1985).
- 13) P. Aisen, I. Listowsky, *Annu. Rev. Biochem.*, **49**, 357 (1980).
- 14) D. L. Jacobs, G. D. Watt, R. B. Frankel, G. C. Papaefthymiou, *Biochemistry*, **28**, 1650 (1989).
- 15) C. E. Thomas, S. D. Aust, *J. Biol. Chem.*, **261**, 13064 (1986).
- 16) H. P. Monteiro, C. C. Winterbourn, *Arch. Biochem. Biophys.*, **271**, 536 (1989).
- 17) G. Carlin, R. Djursater, *FEBS Lett.*, **177**, 27 (1984).
- 18) P. Biemond, A. J. G. Swaak, C. M. Beindorff, J. F. Koster, *Biochem. J.*, **239**, 169 (1986).
- 19) K. Sakurai, T. Ogiso, *Biol. Pharm. Bull.*, **17**, 1451 (1994).
- 20) K. Sakurai, K. Haga, T. Ogiso, *Chem. Pharm. Bull.*, **40**, 2147 (1992).
- 21) L. Ernster, P. Siekevitz, G. E. Palade, *J. Cell Biol.*, **15**, 541 (1977).
- 22) Y. Yasukochi, B. S. S. Masters, *J. Biol. Chem.*, **251**, 5337 (1976).
- 23) D. W. Reif, V. M. Samokyszyn, D. M. Miller, S. D. Aust, *Arch. Biochem. Biophys.*, **269**, 407 (1989).
- 24) T. Miura, K. Sakurai, *Life Sci.*, **43**, 2145 (1988).
- 25) H. Okamoto, *Biomed. Biochim. Acta*, **44**, 15 (1985).
- 26) K. Sakurai, K. Haga, T. Ogiso, *Biol. Pharm. Bull.*, **17**, 227 (1994).
- 27) B. H. Halliwell, *FEBS Lett.*, **92**, 321 (1978).
- 28) B. Halliwell, J. M. C. Gutteridge (eds.), "Free Radicals in Biology and Medicine," Clarendon Press, Oxford, 1985.
- 29) C. C. Winterbourn, *Biochem. J.*, **207**, 609 (1982).
- 30) V. Berlin, W. A. Haseltine, *J. Biol. Chem.*, **256**, 4747 (1981).
- 31) L. Giloni, M. Takeshita, F. Johnson, C. Iden, A. P. Grollman, *J. Biol. Chem.*, **256**, 8608 (1981).
- 32) B. K. Sinha, *Chem.-Biol. Interact.*, **69**, 293 (1989).
- 33) C. A. Pritsos, A. C. Sartorelli, *Cancer Res.*, **46**, 3528 (1986).
- 34) J. H. Doroshov, *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 4514 (1986).
- 35) W. J. Malaisse, F. Malaisse-Lagae, A. Sener, D. G. Pipeleers, *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 927 (1982).
- 36) M. J. MacDonald, J. D. Cook, M. L. Epstein, C. H. Flowers, *FASEB J.*, **8**, 777 (1994).
- 37) W. W. Franke, B. Deumling, B. Ermen, E.-D. Jarash, H. Kleiing, *J. Cell Biol.*, **46**, 379 (1970).
- 38) A. G. Smith, P. Carthew, J. E. Francis, R. E. Edwards, D. Dinsdale, *Hepatology*, **12**, 1399 (1990).
- 39) L. M. Nutter, E. O. Ngo, G. R. Fisher, P. L. Gutierrez, *J. Biol. Chem.*, **267**, 2474 (1992).