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Ascorbate-mediated iron release from ferritin in the presence of alloxan

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Abstract

Release of iron from ferritin requires reduction of ferric to ferrous iron. The iron can participate in the diabetogenic action of alloxan. We investigated the ability of ascorbate to catalyze the release of iron from ferritin in the presence of alloxan. Incubation of ferritin with ascorbate alone elicited iron release (33 nmol/10 min) and the generation of ascorbate free radical, suggesting a direct role for ascorbate in iron reduction. Iron release by ascorbate significantly increased in the presence of alloxan, but alloxan alone was unable to release measurable amounts of iron from ferritin. Superoxide dismutase significantly inhibited ascorbate-mediated iron release in the presence of alloxan, whereas catalase did not. The amount of alloxan radical ($A^{\cdot -}$) generated in reaction systems containing both ascorbate and alloxan decreased significantly upon addition of ferritin, suggesting that $A^{\cdot -}$ is directly involved in iron reduction. Although release of iron from ferritin and generation of $A^{\cdot -}$ were also observed in reactions containing GSH and alloxan, the amount of iron released in these reactions was not totally dependent on the amount of $A^{\cdot -}$ present, suggesting that other reductants in addition to $A^{\cdot -}$ (such as dialuric acid) may be involved in iron release mediated by GSH and alloxan. These results suggest that $A^{\cdot -}$ is the main reductant involved in ascorbate-mediated iron release from ferritin in the presence of alloxan and that both dialuric acid and $A^{\cdot -}$ contribute to GSH/alloxan-mediated iron release.

Abbreviations: $A^{\cdot -}$ – Alloxan radical; AFR – Ascorbate free radical; BPS – bathophenanthrolinedisulfonic acid disodium salt; EDTA – ethylenediaminetetraacetic acid; ESR – electron spin resonance; GSH – glutathione reduced form; $O^{\cdot}{}_2^{-}$ – superoxide anion radical; PBS – phosphate buffered saline; ROS – reactive oxygen species; SOD – superoxide dismutase

Introduction

Iron-containing proteins are central to biological reactions such as respiration, transport of molecular oxygen to tissues and detoxification of peroxides. If it is not properly sequestered, however, free iron in biological systems can result in the generation of powerful oxidants that cause lipid peroxidation, protein oxidation, and DNA fragmentation (Halliwell & Gutteridge 1984; Sakurai *et al.* 1994; Egana *et al.* 2003). Indeed, several *in vivo* and *in vitro* studies have shown that free iron is involved in the onset of various diseases and cytotoxicity (Halliwell & Gutteridge 1984; Cederbaum 1992; Sakurai & Cederbaum 1998). There is no direct evidence, however, for the existence of free iron capable of inducing this toxicity under normal physiological conditions, because *in vivo* iron metabolism is tightly regulated by the iron-binding proteins, transferrin and ferritin.

Ferritin is a multimeric protein composed of 24 polypeptide subunits of both H and L types that is

capable of binding up to 4500 atoms of ferric iron per molecule. Release of iron from ferritin requires reduction of ferric to ferrous iron in the presence of a suitable iron chelator (Jones *et al.* 1978; Bienfait & van den Briel 1980). Physiological reducing agents such as ascorbate and sulfide can reductively release ferritin iron only at high concentration and at pH 5 or below (Harrison *et al.* 1974; Cassanelli & Moulis 2001). Superoxide anion radical ($O_2^{\cdot -}$), derived from stimulated polymorphonuclear leukocytes, the xanthine-xanthine oxidase system and the quinone redox system can also act as reductants (Biemond *et al.* 1984; Miura & Sakurai 1988; Reif 1992).

The diabetogenic agent, alloxan (2,4,5,6(1*H*, 3*H*)-pyrimidinetetrone), induces damage to the insulin-producing cells of pancreatic islets. Alloxan, a quinoidal compound, is a mild oxidant and is easily reduced by GSH or ascorbate (Reif et al. 1989; Sakurai *et al.* 1990; Katoh *et al.* 2002). Its reduced forms, alloxan radical ($A^{\cdot -}$) and dialuric acid, autoxidize to alloxan under aerobic conditions, generating $O_2^{\cdot -}$ and H_2O_2 (Sakurai *et al.* 1990; Bromme *et al.* 2001). Although the mechanism of alloxan cytotoxicity is not yet clearly understood, several studies have shown that its diabetogenic action is initiated by the generation of reactive oxygen species (ROS) (Fischer & Hamburger 1980; Sakurai *et al.* 2001). In preliminary studies, we demonstrated that islet cells isolated from mice were protected from ROS-induced cytotoxicity by the iron-chelator, dietylenetriaminepentaacetic acid (DETAPAC), and the iron-binding protein, apotransferrin (Sakurai *et al.* 1988). Previous studies have also demonstrated that some iron chelators are able to protect mice and islet cells from alloxan-mediated diabetogenesis (Fischer & Hamburger 1980; El-Hage *et al.* 1986). These results suggest that intracellular iron may be involved in the onset of alloxan-induced diabetes.

Several reductants are present in plasma and in interstitial and intracellular fluids (Prior & Cao 1999). One of these reductants, ascorbate, reduces alloxan to $A^{\cdot -}$ and ferric to ferrous iron (Reif *et al.* 1989). However, it is not clear whether ascorbate can enhance the production of powerful oxidants capable of initiating and propagating the cytotoxicity of alloxan. The goals of this study were to investigate the effect of ascorbate on iron release from ferritin in the presence of alloxan and to determine whether the mechanism of iron release by ascorbate and alloxan differs from that by GSH and alloxan. The results presented here show that ascorbate-mediated iron release from ferritin is significantly enhanced in the presence of alloxan. In reactions containing ascorbate and alloxan, $A^{\cdot -}$ and ascorbate caused iron release from ferritin. In reactions containing GSH and alloxan, $A^{\cdot -}$ and dialuric acid were implicated as potential causes of iron release.

Materials and methods

Materials

Alloxan, GSH, cysteine and sodium ascorbate were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Ferritin (from horse spleen), superoxide dismutase (SOD) (from bovine erythrocytes) and thymol-free catalase (from bovine liver) were obtained from Sigma Co., St. Louis, MO. Bathophenanthrolinedisulfonic acid disodium salt (BPS) was from Tokyo Kasei Industries, Ltd., Tokyo, Japan. All other chemicals were of analytical grade and were obtained from commercial suppliers.

Detection of iron release from ferritin

To remove traces of adventitious iron, commercial ferritin was incubated in phosphate-buffered saline (PBS), pH 7.4, containing 10 mM disodium EDTA for 1 h at 4 °C. After incubation in PBS without disodium EDTA for a further 3 h, the solution was applied to a 1.0×20 cm Sephadex G-25 column (Pharmacia Fine Chemicals, Sweden) previously equilibrated with 10 mM PBS, pH 7.4. Ferritin fractions were collected and concentrated by ultrafiltration using a centrifugation-based method ($3000 \times g$, 4 °C for 85 min). The total iron content of the purified ferritin was measured using a polarized Zeeman atomic absorption spectrometer (Hitachi 180-80, Tokyo, Japan) and was found to be 5.65–6.22 μmol Fe (III)/mg protein of ferritin. Total ferritin iron content was also determined according to the method of Bonomi & Pagani (1986) using sodium hydrosulfite and BPS. Results obtained using this method correlated well with those obtained by atomic absorption spectrometry.

Ferritin was incubated in PBS (pH 7.4) containing 1.0 mM alloxan and 1.0 mM BPS and either 1.0 mM ascorbate or 1.0 mM GSH. Iron release was initiated by alloxan addition at 37 °C and was continuously monitored at 534 nm. The amount of iron released was calculated from the increase in absorption, based on a coefficient of 22140 M^{-1} cm^{-1}. To decrease the concentration of O_2 in the reaction, the solution was purged with argon gas for 5 or 10 min prior to alloxan addition. The O_2 concentration was measured polarographically at 37 °C using a Clark-type oxygen electrode (YSI model 5300, Yellow Springs Instrument Co., Inc., Yellow Springs, OH).

Free radical measurements

Electron spin resonance (ESR) spectra were performed using a JEOL model JES RE1X spectrometer (JEOL, Tokyo, Japan). Experimental conditions were as described above for the studies of iron release from ferritin. Spectrometer settings for $A^{\cdot -}$ were as follows: magnetic field, 336.5 mT; microwave power, 5.0 mW; modulation frequency, 9.450 GHz; field modulation width, 0.020 mT; time constant, 0.3 s; and scan speed, 0.25 mT/min. After the addition of reductants, the samples were rapidly aspirated into the aqueous flat cell and the spectra recorded. Settings for ascorbate free radical (AFR) were as follows: magnetic field, 336.5 mT; microwave power, 5.0 mW; modulation frequency, 9.450 GHz; field modulation width, 0.020 mT; time constant, 0.3 s; and scan speed, 0.25 mT/min, as previously described (Sakurai *et al*. 1990; Katoh *et al*. 2002).

Detection of dialuric acid

Dialuric acid production was initiated by the addition of GSH to reaction solution containing alloxan at 37 °C. Absorbance at 273 nm was recorded at 2 min intervals for 10 min. The amount of dialuric acid produced was calculated based on the increase in absorption, using a coefficient of 16000 M^{-1} cm^{-1}.

Statistical analysis

Data are expressed as the mean ± S.D and were analyzed using the Student's *t*-test for paired data. $P < 0.05$ was considered statistically significant.

Results

Ascorbate-mediated iron release from ferritin with alloxan

Iron was released from ferritin in the presence of alloxan and ascorbate (Figure 1). After 10 min at 37 °C, 33 nmol/ml of iron was released from ferritin in the reaction containing only ascorbate. Addition of alloxan to the reaction increased the rate of iron release to 68 nmol/ml in 10 min. However, little iron was released in the presence of alloxan alone. It was observed that addition of increasing concentrations of ascorbate and alloxan progressively increased iron release, that the rates of iron release by ascorbate and alloxan, or by ascorbate alone, increased with increasing ferritin concentration, and that the rate of iron release was independent of the concentration of the iron chelator, BPS (data not shown). These results indicate that significantly more iron is released from ferritin in reactions containing both ascorbate and alloxan, compared with reactions containing ascorbate alone.

To determine the primary reductants in the reaction system containing ascorbate and alloxan, we investigated the effect of SOD and catalase on iron release. SOD had no effect on ascorbate-mediated iron release, and catalase alone and SOD/catalase increased the rate only slightly (Figure 2). In contrast, SOD alone and SOD/catalase significantly inhibited iron release by ascorbate with alloxan, and catalase had no effect. These results suggest that $O_2^{\cdot -}$ can act as a reductant of ferritin iron in reactions containing ascorbate and alloxan. Figure 3 shows the effect of O_2 concentration on iron release. When the reaction solution was purged with argon gas for 5 or 10 min, the concentration of O_2 decreased by about 30% (inset). The rate of ascorbate-mediated iron release with alloxan increased at a low concentration of O_2, but the release rate did not increase in the presence of ascorbate alone. These results suggest that products other than $O_2^{\cdot -}$ in reactions containing ascorbate and alloxan may act as the primary reductants of iron under aerobic or low O_2 conditions.

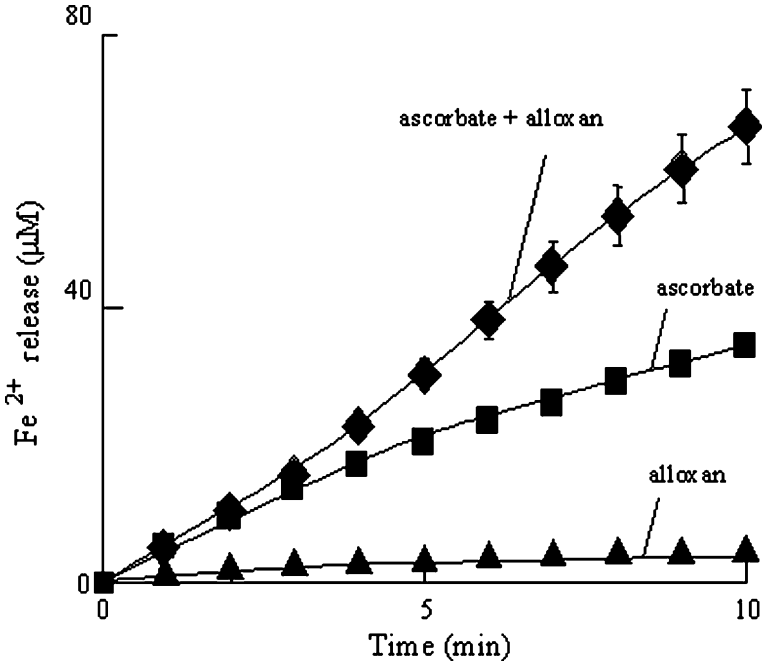

Figure 1. Ascorbate-induced iron release from ferritin in the presence of alloxan. Reactions contained ferritin (10μmol as Fe), BPS (1 mM), ascorbate (1 mM), and alloxan (1 mM) in PBS in a final volume of 2 ml. Reactions were initiated by the addition of ascorbate at 37 °C. The amount of iron released was determined as described in Materials and methods. Each point represents the mean ± S.D. of three experiments.

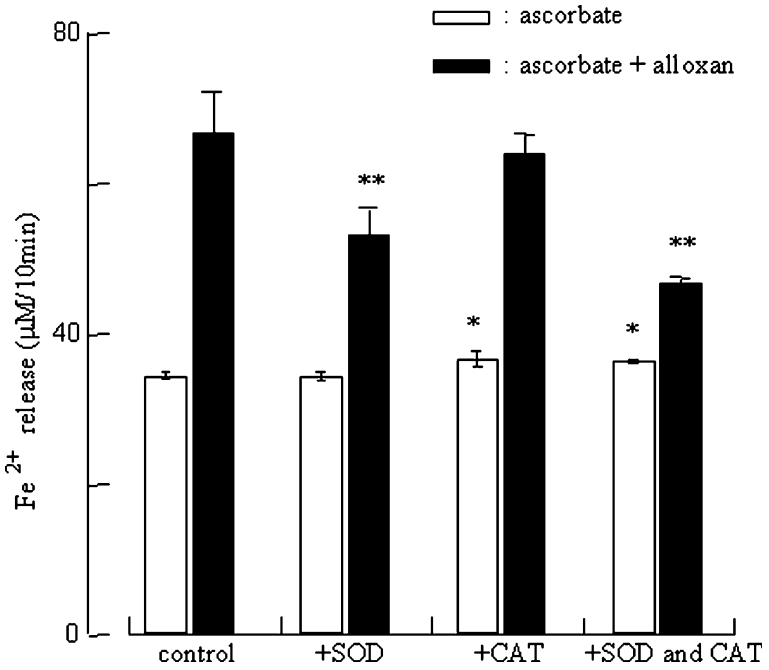

Figure 2. Effect of SOD and catalase on iron release from ferritin by ascorbate with or without alloxan. After addition of SOD (0.1 mg protein/ml) and/or catalase (0.01 mg protein/ml), ferritin was incubated in complete reactions as described in the legend to Figure 1. Each value represents the mean ± S.D. of three experiments. *$P < 0.05$ compared with control in the absence of alloxan. **$P < 0.05$ compared with control in the presence of alloxan.

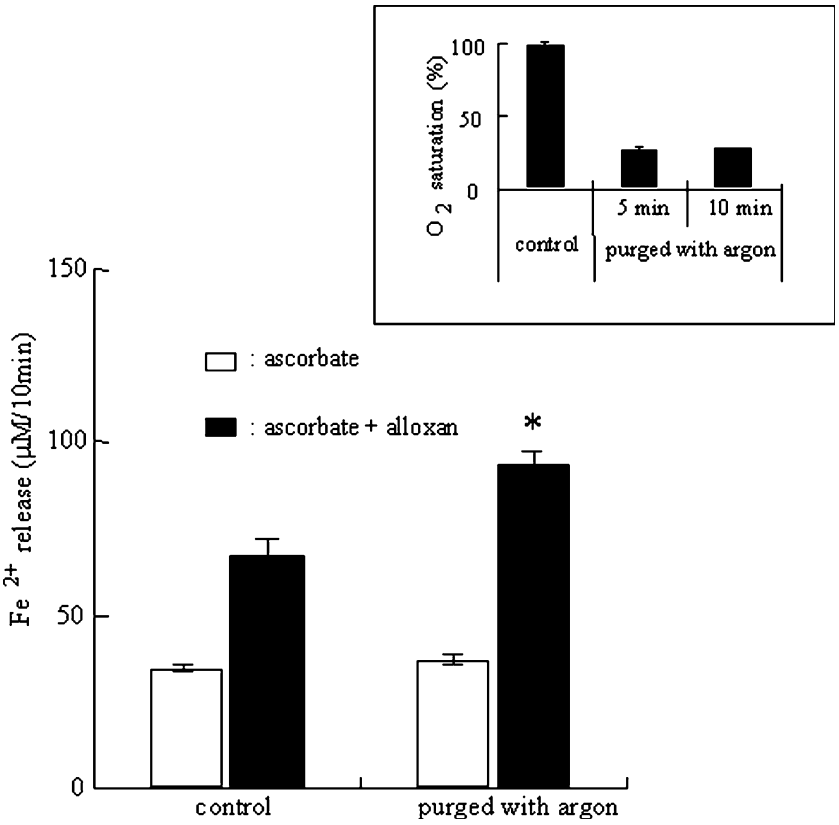

Figure 3. Effect of oxygen concentration on ascorbate-induced iron release from ferritin with or without alloxan. Experimental conditions were as described in the legend to Figure 1, except for O_2 concentration. Inset: The reaction solution was purged with argon gas for 5 or 10 min, and O_2 concentration was measured polarographically at 37 °C with a Clark-type oxygen electrode. Each value represents the mean ± S.D. of three experiments. $^*P < 0.05$ compared with reactions containing alloxan performed under aerobic conditions.

Effect of AFR and alloxan radicals on iron release from ferritin

Next, we investigated the roles of AFR and $A^{\cdot -}$ in the release of iron from ferritin using ESR spectrometry (Figure 4). An ESR spectrum indicative of AFR was not clearly detected in the solution containing ascorbate alone (spectrum 1). A clear signal for AFR was detected in the incubation of ascorbate with ferritin (spectrum 2), in agreement with a previous report (May *et al*. 1998), suggesting that ascorbate directly reduces ferritin iron. In accordance with our previous observations (Katoh *et al*. 2002), both $A^{\cdot -}$ and AFR were generated in the reaction containing both ascorbate and alloxan (spectrum 3). When ferritin was added to the system, the signal intensity of $A^{\cdot -}$ decreased markedly, but the AFR signal was unchanged (spectrum 4). These results suggest that $A^{\cdot -}$ is the main reductant of ferritin iron in reactions containing both ascorbate and alloxan. When SOD (spectrum 5) or both SOD and catalase (spectrum 7) were added, the $A^{\cdot -}$ signal was not detected clearly but that of AFR increased. Catalase did not have a clear influence on the intensity of the AFR and $A^{\cdot -}$ signals. These results suggest that ascorbate and/or $A^{\cdot -}$ participate in the release of iron from ferritin.

The amount of iron released from ferritin upon exposure to ascorbate and alloxan was then compared to that released in reactions containing GSH and alloxan. As shown in Figure 5, the rate of iron release in the system containing GSH and alloxan was about 6 nmol/min, or three times greater than the rate of release in reactions containing ascorbate and alloxan. However, as noted previously (Miura & Sakurai 1988; Reif *et al*. 1989), GSH alone was unable to release measurable amounts

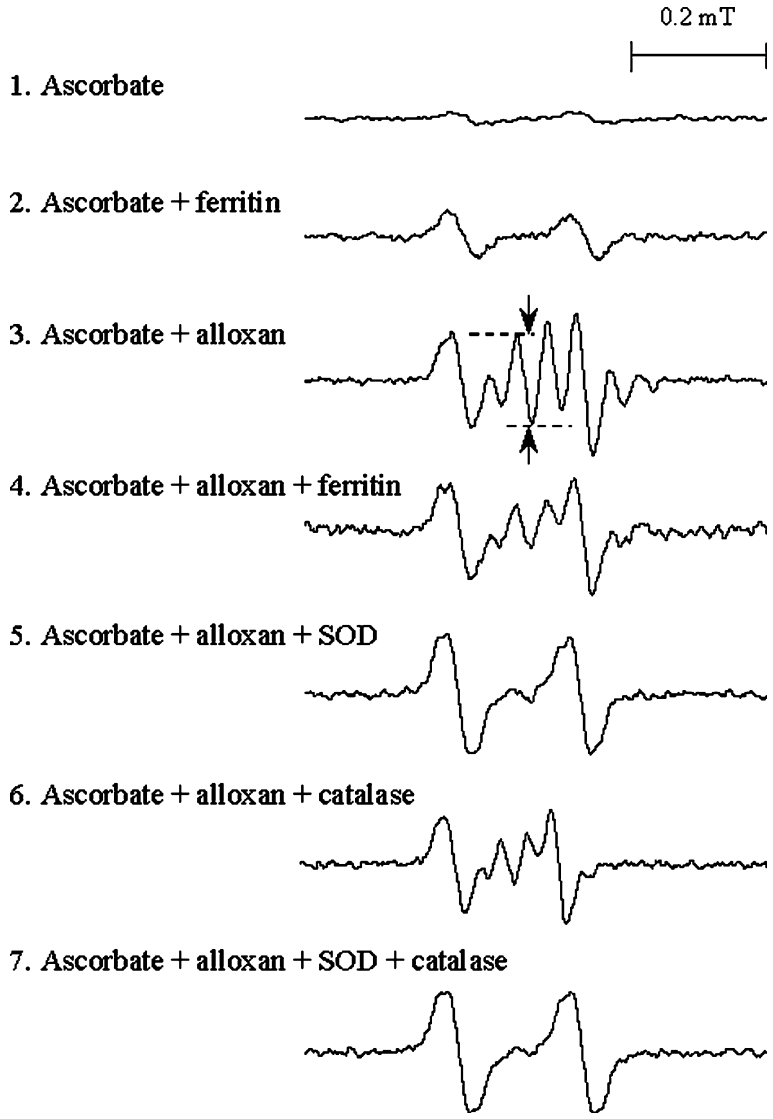

Figure 4. Generation of ascorbate free radical and alloxan radical in reactions containing ascorbate, alloxan and ferritin. Reactions contained 1 mM ascorbate, 1 mM alloxan and ferritin (10 μmol as Fe) in the presence or absence of SOD (0.1 mg protein/ml) and/or catalase (0.01 mg protein/ml). Other conditions were as described in the legend to Figure 1. AFR and alloxan radicals were detected as described in Materials and methods. Arrows indicate the site at which the peak intensity of the alloxan radical was determined. The experimental results matched the ESR spectrum simulated with the following parameters: hyperfine coupling constant $a^H = 0.186$ mT and g value = 2.0054 for AFR; hyperfine coupling constant $a^H = a^N = 0.045$ mT and g value = 2.0052 for the alloxan radical. Typical results from one experiment are shown. Similar results were obtained in triplicate experiments.

of iron from ferritin (Figure 5b). In the present study, we sought to perform a quantitative comparison of the amount of $A^{\cdot -}$ generated in reaction systems containing either ascorbate or GSH with alloxan, because the $A^{\cdot -}$ generated in reactions containing GSH and alloxan could cause the release of iron from ferritin. Figure 6 shows the relationship between iron release and $A^{\cdot -}$ signal intensity. In both systems, the rate of iron release increased in proportion to the intensity of the $A^{\cdot -}$ signal, suggesting that $A^{\cdot -}$ may directly interact with the iron in ferritin. However, the comparison between alloxan/GSH and alloxan/ascorbate at the same intensity of $A^{\cdot -}$ signal revealed that the rate of iron release in the GSH/alloxan reaction was greater than that in the ascorbate/alloxan

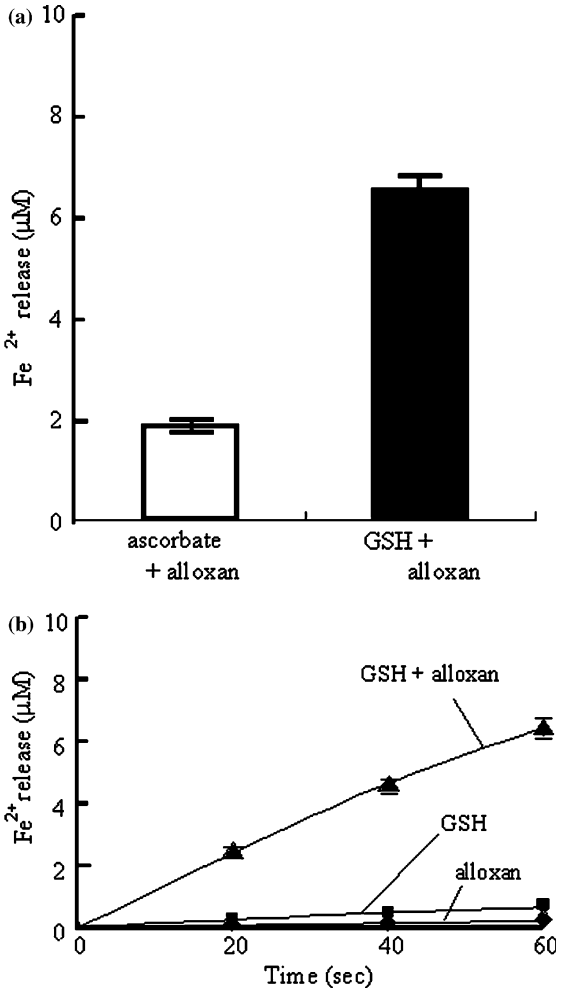

Figure 5. Iron release by ascorbate or GSH in the presence of alloxan. (a) Ferritin (10 μmol as Fe) was incubated with ascorbate (1 mM) or GSH (1 mM) in the presence of alloxan (1 mM). (b) Reactions consisted of 1 mM GSH, 1 mM alloxan, 1 mM BPT in PBS, pH 7.4, at 37 °C. The reaction was initiated by the addition of GSH. Other reaction conditions were as described in the legend to Figure 1. Each value represents the mean ± S.D. of three experiments.

reaction. These results suggest that the mechanism of iron release by alloxan with ascorbate is not the same as that by alloxan with GSH.

Generation of dialuric acid

Dialuric acid is a two-electron reduction product of alloxan. To investigate the involvement of dialuric acid in the release of iron, we used Fe^{3+}-EDTA rather than ferritin because ferritin absorbs strongly at 273 nm, the wavelength of maximal absorption of dialuric acid (Sakurai & Ogiso 1991). As shown in Figure 7, an increase in the production of dialuric acid was observed in reactions containing alloxan and GSH. This increase was clearly inhibited when Fe^{3+}-EDTA was added to the system. However, no dialuric acid was detected in reactions containing ascorbate and alloxan, even under conditions of excess ascorbate (data not shown). These results suggest that GSH reduces alloxan to $A\cdot^-$ and dialuric acid, which then induce the reduction of Fe^{3+}-EDTA.

Discussion

Under physiological conditions, labile iron can potentiate the toxicity induced by ROS. Most intracellular iron, except for heme iron, is stored in ferritin. The release of iron from ferritin requires reduction of the ferric iron. It has been suggested that iron is released from ferritin by reductants under pathological conditions (Halliwell & Gutteridge 1984; Cederbaum 1992). It has also been shown that $O_2\cdot^-$ produced by stimulated polymorphonuclear leukocytes can cause release of iron from ferritin (Biemond *et al.* 1984). Several *in vitro* and *in vivo* studies have also suggested a role for iron in diabetogenesis (Fischer & Hamburger 1980; Oberley 1988). We and other researchers demonstrated that the diabetogenic agent, alloxan, induces the release of iron from ferritin in the presence of GSH, causing lipid peroxidation in liposomes (Miura & Sakurai 1988; Reif *et al.* 1989). The biological reductant, ascorbate, also has the ability to release iron from ferritin *in vivo* (Harrison *et al.* 1974). The present study was performed to evaluate whether alloxan acts as a potentiator of ascorbate-induced iron release from ferritin. Our results show that alloxan markedly enhances the release of iron by ascorbate.

We showed that incubation of ascorbate with ferritin generated AFR and caused a low rate of iron release (Figure 4). Boyer & McCleary (1987), using a different chelator, ferrozine, as well as different incubation temperature/times than in our study, proposed that $O_2\cdot^-$ generated during the iron-promoted oxidation of ascorbate acts as a reductant of ferritin iron. This process seems unlikely to account for our results. The reactivity of iron to O_2 and H_2O_2 changes remarkably with the

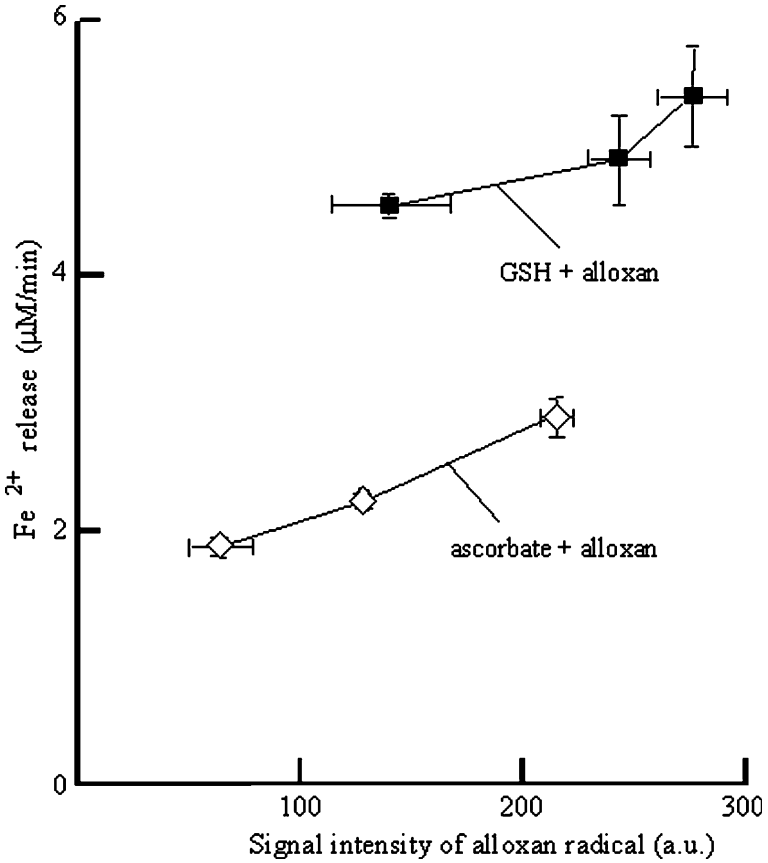

Figure 6. Iron release from ferritin as a function of $A^{\cdot -}$signal intensity. Iron release was measured under the conditions described in Figures 1 and 5b except that the concentration of alloxan varied from 1 to 3 mM. The generation of the alloxan radical was determined by ESR spectrometry. Each value represents the mean ± S.D. of three experiments.

local environment e.g. pH and O_2 level (Reif 1992; Lovstad 2003). The present study yielded the following results: (1) The rate and extent of iron release from ferritin in the presence of ascorbate scarcely decreases even under low O_2 concentrations (Figure 3); (2) SOD does not affect iron release under aerobic conditions (Figure 2); and (3) AFR is generated when ascorbate is incubated with ferritin (Figure 4). These results suggest that ascorbate may induce iron release through direct interaction with the iron in ferritin. Under aerobic conditions, addition of catalase caused a slight increase in iron release (Figure 2). Thus, we hypothesize that catalase inhibits the oxidation of Fe^{2+} by H_2O_2 in the Fenton reaction (reaction 1), thereby increasing the amount of the Fe^{2+}-BPT complex.

$$H_2O_2 + Fe^{2+} \rightarrow HO\cdot + HO^- + Fe^{3+} \qquad \text{(reaction1)}$$

The rate of ascorbate-induced iron release increased markedly in the presence of alloxan (Figure 1). In reactions containing ascorbate and alloxan, $A^{\cdot -}$, $O_2^{\cdot -}$ and AFR were generated as follows:

$$\text{ascorbate} + \text{alloxan} \rightarrow \text{AFR} + A\cdot^- \qquad \text{(reaction2)}$$

$$A\cdot^- + O_2 \rightleftharpoons \text{Alloxan} + O_2\cdot^- \qquad \text{(equilibrium1)}$$

The $A^{\cdot -}$ signal intensity was reduced by the addition of ferritin to reactions containing ascorbate and alloxan (Figure 4). In addition, we observed that the rate of iron release from ferritin was higher under low O_2 concentrations and that the $A^{\cdot -}$ signal intensity was stronger under anaerobic conditions, suggesting that $A^{\cdot -}$ directly causes the reduction of iron in ferritin. However, the

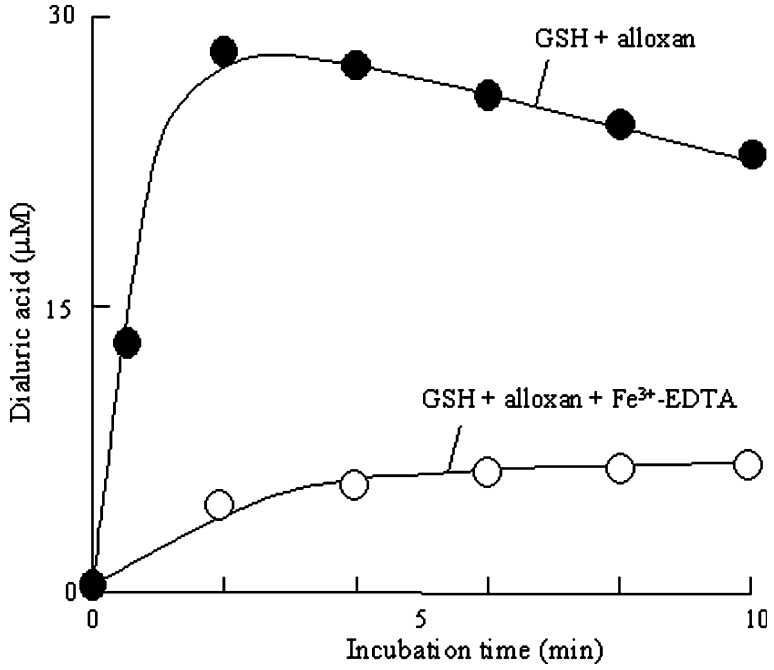

Figure 7. Effect of ferric iron on dialuric acid generation in reactions containing GSH and alloxan. Reactions contained 50 μM alloxan and 1 mM GSH in the presence or absence of 50 μM Fe^{3+}-EDTA. Other conditions were as described in the legend to Figure 1. Reactions were initiated by the addition of GSH. Each point represents the mean ± S.D. of three or four experiments.

addition of SOD to the system (with or without catalase) reduced both the rate of iron release (Figure 2) and the signal intensity of $A\cdot^-$ (Figure 4). SOD reduces the amount of $A\cdot^-$ by dismutating $O_2\cdot^-$ and moving equilibrium 1 to the right, which may result in a reduced level of iron release by $A\cdot^-$, as proposed by Winterbourn (1982). These results suggest that the $A\cdot^-$ generated in this reaction system is the main cause of the reduction of ferritin iron and that alloxan and $O_2\cdot^-$ do not directly participate in this process under aerobic conditions.

Iron release from ferritin was observed in reactions containing GSH and alloxan; however, GSH alone does not cause significant iron release (Miura & Sakurai 1988; Reif *et al.* 1989). $A\cdot^-$ could also directly reduce ferritin iron in the reaction system involving GSH and alloxan. If $A\cdot^-$ were solely responsible for this effect, then the rate of iron reduction would be dependent on the amount of $A\cdot^-$ generated. The iron release from ferritin observed in the GSH and alloxan reaction system was greater than that observed in reactions containing ascorbate and alloxan, even although the same amount of $A\cdot^-$ was generated in both systems (Figure 6). Dialuric acid can also be considered as a candidate for reducing iron in the GSH and alloxan reaction system (Sakurai & Ogiso 1991). Indeed, dialuric acid was generated in this system and was consumed by the addition of Fe^{3+} (Figure 7). In the presence of alloxan, an excess of ascorbate did not accelerate iron release and did not generate dialuric acid (data not shown). These results suggest that $A\cdot^-$ and dialuric acid are responsible for the reduction of ferritin iron in the GSH and alloxan reaction system.

The redox potential of ferritin is −190 mV. Thus, iron release requires compounds having a lower reducing potential. Ascorbate and GSH are mild reducing agents that are involved in various redox reactions under physiological conditions. The redox potential of GSH is lower than that of ascorbate (Harrison *et al.* 1974). At millimolar concentrations, both ascorbate and GSH can cause the reduction of alloxan. In the reaction containing ascorbate and alloxan, AFR and $A\cdot^-$ coexist comparatively stably in the absence of ferritin, suggesting that ascorbate is able to reduce the alloxan to $A\cdot^-$ but is not able to reduce $A\cdot^-$ to dialuric acid. In contrast, GSH reduces alloxan to $A\cdot^-$and dialuric acid (Figure 7). Dialuric acid, when produced, reduces the iron in

ferritin (Sakurai & Ogiso 1991). Therefore, we conclude that the amount of iron released by ascorbate and alloxan was not equal to that released by GSH and alloxan, even although the amount of $A^{\cdot -}$ generated was the same due to the presence of dialuric acid in the GSH/alloxan system.

$A^{\cdot -}$ can accumulate in plasma, where there is a high concentration of ascorbate. There is ferritin in plasma, and highly reactive ROS may be generated since $A^{\cdot -}$ reduces molecular oxygen to $O_2^{\cdot -}$, and Fe^{3+} to Fe^{2+}. This can initiate the Fenton reaction, generating hydroxyl radicals (reaction 1). Quinone derivatives such as emodine, dimethoxy-1,4-naphthoquinone as well as alloxan are also considered ROS generators because the properties of quinones and derived semiquinones allow the transfer of an electron to O_2 (Johnson Inbaraj *et al*. 1999; Yi *et al*. 2004). The present study demonstrates that ascorbate can reduce a quinone compound to a semiquinone radical, but not to a hydroquinone, and that GSH can induce the generation of both a semiquinone and a hydroquinone. These findings suggest the possibility that ascorbic acid, but not GSH, generates more ROS through the redox cycle of quinones under certain conditions. Indeed, when incubated with alloxan, ascorbic acid enhances the decrease in viability of the rat pancreatic β-cell line, INS-1 (unpublished data). Many studies have demonstrated that ascorbic acid has pro-oxidant effects on lipid peroxidation and cell damage in the presence of iron. The results reported here further clarify the mechanism whereby ascorbic acid functions as a pro-oxidant in the presence of iron and quinones.

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