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Ferritin-dependent inactivation of microsomal glucose-6-phosphatase

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Abstract

Glucose-6-phosphatase (G6Pase) is a microsomal enzyme which is very sensitive to inactivation by lipid peroxidation. Experiments were carried out to evaluate whether ferritin, which is the major storage form of iron within cells, could catalyze inactivation of G6Pase and to determine the mechanism responsible for this effect of ferritin. Incubation of microsomes with NADPH in the absence of ferritin led to decreased activity of G6Pase. Ferritin stimulated this inactivation of G6Pase in a time- and concentration-dependent manner. Ferritin did not stimulate G6Pase inactivation when NADH replaced NADPH as the microsomal reductant. Superoxide dismutase but not catalase or DMSO prevented the ferritin-stimulated inactivation of G6Pase suggesting a role for superoxide, but not H_2O_2 or hydroxyl radical, in the overall mechanism. Trolox, at concentrations which prevent lipid peroxidation, also prevented the ferritin-catalyzed inactivation of G6Pase. Inhibition of G6Pase by ferritin was further enhanced in the presence of ATP but was inhibited in the presence of EDTA or desferrioxamine; ferric-ATP stimulates, whereas ferric-EDTA inhibits microsomal lipid peroxidation. The redox cycling agent paraquat increased the ability of ferritin to inactivate G6Pase by a reaction prevented by superoxide dismutase, trolox, EDTA, and desferrioxamine, but not by catalase or DMSO. Ferritin stimulated microsomal light emission, a reaction reflecting lipid peroxidation, with time and concentration dependence, and sensitivity to scavengers (trolox, superoxide dismutase), iron chelators and paraquat, identical to the inactivation of G6Pase. These results indicate that one possible toxicological consequence of ferritin-catalyzed lipid peroxidation is inhibition of microsomal enzymes such as G6Pase.

Key words: Ferritin; Glucose-6-phosphatase; Lipid peroxidation; Paraquat; Chemiluminescence

1. Introduction

Ferritin is the major storage form of iron within cells [1,2]. Numerous reducing agents can liberate iron from ferritin, especially if a chelating agent is present to complex the released iron [3–10]. Superoxide anion radical, generated by a variety of systems such as the xanthine oxidase reaction, NADPH-cytochrome *P*-450 reductase, microsomes, radiation-induced radiolysis, and autooxidation of redox cycling agents, has been shown to mobilize iron from ferritin [11,12]. The iron released from ferritin was capable of catalyzing lipid peroxidation of isolated microsomes [13–16]; when xenobiotics such as paraquat were used to mobilize the iron from ferritin and promote lipid peroxidation, both of these reactions were sensitive to superoxide dismutase indicating the important role of superoxide in releasing the iron.

Release of iron from ferritin and the subsequent catalysis of lipid peroxidation is assumed to be of toxicological significance. Several enzymes present in the endoplasmic reticulum are sensitive to inhibition by lipid peroxidation processes [17–24]. Inhibition of enzyme activity is attributed to altered membrane structure or to effects of reactive products, e.g., malondialdehyde, 4-hydroxy-nonenal, produced during lipid peroxidation [25–27]. Microsomal G6Pase has been shown to be particularly sensitive to loss of activity when microsomes undergo either iron-dependent or haloalkane-catalyzed lipid peroxidation [28,29]. Glende et al. [23] showed that G6Pase activity was decreased during aerobic, but not anaerobic metabolism of CCl_4, suggesting inactivation by lipid peroxidation rather than

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by trichloromethyl radical or covalent binding of CCl_4 carbon adducts. Lipids extracted from peroxidizing, but not from non-peroxidizing microsomes inhibited G6Pase activity of control microsomes [22–26]; direct inhibition of enzyme activity by malondialdehyde and 4-hydroxynonenal has also been demonstrated [27,29].

The goal of the current study was to determine the effect of ferritin, on G6Pase activity of rat liver microsomes. The overall reaction scheme which was evaluated was that $O_2^{\cdot-}$, produced by microsomal electron transport, liberates iron from ferritin, the iron redox cycles and produces an initiator of lipid peroxidation, and products produced by the lipid peroxidation process inactivate G6Pase. The effect of radical scavengers and iron chelators on the ferritin-catalyzed inactivation was determined. Experiments were carried out in the absence or presence of paraquat, and the effects of ferritin on G6Pase activity were compared to its effects on microsomal lipid peroxidation in order to validate that the two reactions correlate with each other.

2. Materials and methods

Liver microsomes were isolated from male, Sprague-Dawley rats weighing 200–220 g. The liver was homogenized (1:4 dilution) in 0.25 M sucrose, 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 25 mM KCl, and 8 mM $CaCl_2$. The homogenate was then filtered and centrifuged at $9000 \times g$ for 15 min in a Sorvall RC5 centrifuge. The pellet was discarded, and the supernatant was diluted 1:6 with a solution containing 12.5 mM sucrose, 5 mM $MgCl_2$ and 8 mM $CaCl_2$. The supernatant was centrifuged at $1500 \times g$ for 10 min in the Sorvall centrifuge. This low speed centrifugation has been shown to be effective in separating ferritin from the microsomes [30]. The resultant supernatant was discarded and the pellet was resuspended in 20 mM Tris-HCl/150 mM KCl buffer (pH 7.5), and resedimented at $165\,000 \times g$ for 40 min in a Beckman ultracentrifuge. This washed pellet was then dispersed in the above Tris-KCl buffer and stored at −70°C usually at a protein concentration of about 15 mg per ml. Since microsomes isolated by differential centrifugation contain ferritin [31], even after sucrose gradient centrifugation [32], the calcium aggregation method to prepare microsomes was used to remove ferritin from microsomes [30].

Rat liver ferritin was prepared as previously described [33]. Ferritin was incubated on ice in 10 mM EDTA for 1 h and passed over a Sephadex G-25 column equilibrated with 0.3 M NaCl (pH 7.0) to remove loosely associated iron [14]. The iron content of the ferritin was usually about 1300 atoms per ferritin shell equivalent to 3.3 μmol nonheme iron per mg protein.

Isolated microsomes were incubated in 10 mM Tris-HCl (pH 7.4) in the absence or presence of ferritin and 1 mM NADPH over a 10–90-min time period either at 0°C (ice controls) or at 37°C. The reaction was stopped by rapid centrifugation of the mixture and the microsomal pellet was resuspended in 10 mM Tris-HCl (pH 7.4). The activity of glucose-6-phosphatase was determined by a second incubation of the microsomes with 11 mM glucose 6-phosphate for 20 min at 37°C. Enzyme activity was only determined at this 20-min time point which was within the linear time period, after an initial 2–3-min latency period. The effects of ferritin on latency of G6Pase was not determined in this experimental design. The amount of phosphate formed in the assay was determined essentially as described by Fiske and SubbaRow [34].

As an index of lipid peroxidation, the resuspended microsomes were assayed for chemiluminescence [35–37], in 100 mM Tris-HCl (pH 7.4) in the presence of 0.5 mM NADPH as cofactor. Reactions were carried out in 13×40 mm tubes placed inside glass scintillation vials, previously dark-adapted. The reactions were initiated by the addition of approximately 0.5 mg of microsomal protein and light emission was recorded for 20 min. Chemiluminescence was measured at room temperature in a LKB 1230 liquid scintillation counter in the out-of-coincidence mode with the discriminator adjusted for low level emission. Results are expressed as arbitrary units, obtained by quantification of areas under the curves of emission as a function of time.

All reagents were of the highest grade available. The buffers and the water used to prepare all solutions were passed through columns containing Chelex-100 to remove metal contaminatants. All results are from experiments carried out in duplicate and replicated with at least two or three different microsomal preparations. Where indicated, values refer to mean ± S.E. Statistical analysis was carried out using the statview SE + program for unpaired data (Abacus Concepts, Berkeley, CA).

3. Results

The addition of NADPH to microsomes caused a time-dependent decrease in G6Pase activity, compared to samples kept on ice during the first incubation with NADPH. G6Pase activity decreased about 25% after 10–30 min incubation, and about 50–60% after 45–90 min incubation (Fig. 1). Ferritin, at a concentration of 10 μg per mg microsomal protein, had little effect on inactivation of G6Pase activity over the NADPH inactivation after incubation times of 10 or 30 min. However, ferritin produced further inactivation at more prolonged incubation periods over the decreases produced by NADPH itself (Fig. 1). The inset of Fig. 1 shows

that after 90 min incubation of microsomes with NADPH, G6Pase activity could be further lowered by the presence of 5–15 μg ferritin per mg microsomal protein in the incubation mixture.

In general, NADPH is superior to NADH in promoting the generation of reactive oxygen species by microsomes [38–40], most likely reflecting the effectiveness of NADPH as a cofactor for the mixed function oxidase system. In the presence of NADH, microsomal G6Pase activity decreased 23% and 41% after 60 and 90 min incubation at 37°C, respectively, compared to decreases of 55% and 60% in the presence of NADPH. Ferritin, which further decreased G6Pase activity in the presence of NADPH, e.g., 70 and 81% loss of activity at 60 and 90 min incubation, respectively, did not cause a further loss of activity with NADH as the microsomal reductant (22% and 45% loss in G6Pase activity after 60 or 90 min incubation in the presence of NADH plus 10 μg ferritin per mg microsomal protein).

Effect of scavengers and chelating agents

The inactivation of G6Pase produced by NADPH in the absence of added ferritin was not prevented by catalase, SOD, or DMSO suggesting no major role for H_2O_2, O_2^-, or $^{\cdot}OH$ in the inactivation (Table 1). However, the vitamin E analogue, trolox, partially prevented the inactivation. The further loss of G6Pase activity caused by ferritin was also not prevented by catalase or DMSO but was partially prevented by trolox. In contrast to the inactivation found in the absence of ferritin, SOD, protected against the ferritin-dependent inactivation; rates of G6Pase activity in the presence of SOD were the same for samples incubated in the presence of NADPH alone or NADPH plus ferritin suggesting that SOD completely prevented the increased inactivation produced by ferritin (Table 1). Boiled SOD did not protect against the ferritin-stimulated inactivation (data not shown).

Table 1
Effect of scavengers and chelators on the inactivation of glucose-6-phosphatase

Addition	Activity of G6Pase (nmol/min per mg protein)	
	NADPH	NADPH + ferritin
A. None	90 ± 5 (47)	66 ± 1 (61) ‡
220 units catalase	80 ± 1 (53)	63 ± 2 (63) ‡
30 mM DMSO	90 ± 8 (47)	66 ± 4 (61) ‡
0.1 mM trolox	128 ± 9 (24) *	110 ± 9 (35) *
50 units SOD	93 ± 3 (45)	90 ± 6 (47) *
B. None	82 ± 10 (40)	59 ± 5 (57) ‡
0.5 mM ATP	48 ± 3 (65) *	26 ± 6 (81) *,‡
0.5 mM EDTA	109 ± 9 (20) *	112 ± 13 (18) *
0.5 mM desferrioxamine	98 ± 1 (28)	113 ± 7 (18) *

* $P < 0.05$ compared to the no addition control. ‡ $P < 0.05$ ferritin plus NADPH versus NADPH. Microsomes were incubated for 90 min at 37°C with either 1 mM NADPH or 1 mM NADPH plus 10 μg ferritin per mg microsomal protein in the absence or presence of the indicated additions. The microsomes were recovered after centrifugation and incubated with glucose-6-phosphate for 20 min and G6Pase activity determined by measuring the released P_i. Activity of G6Pase for microsomes kept on ice for the 90-min incubation period was 169 ± 23 and 137 ± 12 nmol/min per mg protein for experiments A and B, respectively. Numbers in parentheses refer to the percent inactivation of G6Pase and were calculated from the following: activity of G6Pase for microsomes kept on ice minus activity for microsomes kept at 37°C for 90 min, divided by the activity for the ice controls.

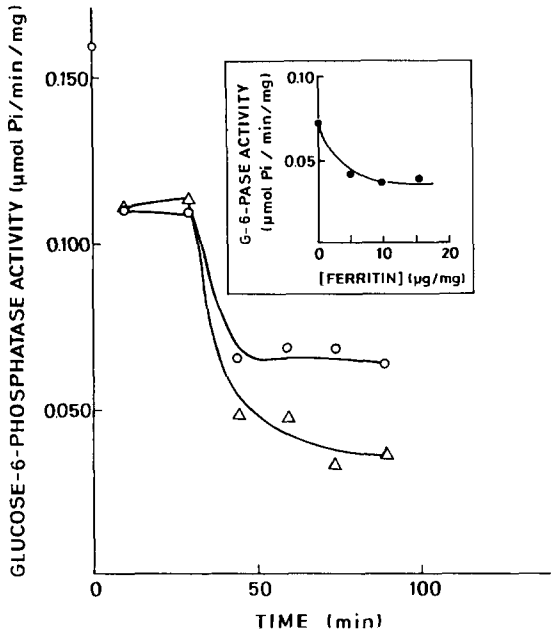

Fig. 1. Effect of ferritin on glucose-6-phosphatase activity. Microsomal glucose-6-phosphatase activity was measured after a 10–90-min incubation period at 37°C in the presence of 1 mM NADPH (○) or 1 mM NADPH plus 10 μg ferritin/mg protein (△). Inset: Dependence of glucose-6-phosphatase inactivation upon ferritin concentration. Activity for samples kept on ice during the 90-min incubation period was 160 ± 33 nmol/min per mg microsomal protein (value for 0 time incubated).

Numerous studies have shown that G6Pase activity is sensitive to products produced during microsomal lipid peroxidation [21–29]. Microsomal lipid peroxidation can be catalyzed by transition metals such as iron, and certain iron chelates, e.g., iron-ATP complexes are very reactive in promoting lipid peroxidation whereas other iron chelates, e.g., iron-EDTA are inhibitory [37,41]. To evaluate a role for iron in the inactivation of G6Pase by NADPH, or to demonstrate that iron released from ferritin played a role in the further inactivation produced by ferritin, the effects of ATP, EDTA, and desferrioxamine were determined. ATP caused a further decrease in G6Pase activity for both the NADPH and the NADPH plus ferritin incubation systems over values found in the absence of ATP, whereas EDTA and desferrioxamine were partially

protective in both incubation systems (Table 1). Similar to the effects observed with SOD, EDTA and desferrioxamine completely prevented the increased inactivation produced by ferritin.

Effect of paraquat

Redox cycling agents such as paraquat increase the production of reactive oxygen species by microsomes in the presence of NADPH, and increase the release of iron from ferritin [11,12]. It was therefore considered that paraquat would increase the ferritin-dependent inactivation of G6Pase. In the absence of ferritin, paraquat produced a small increase in the NADPH catalyzed inactivation of G6Pase (Fig. 2). In the presence of ferritin, paraquat increased the inactivation of G6Pase in a concentration-dependent manner (Fig. 2). A time course for the effects of paraquat is shown in the inset to Fig. 2; up to 20 min incubation, paraquat plus NADPH produced only a small inactivation of G6Pase in the absence of ferritin, however, significant loss of enzyme activity occurred in the presence of ferritin. An incubation period of 20 min and a paraquat concentration of 2 mM was chosen for additional experiments since a clear stimulation by ferritin of G6Pase

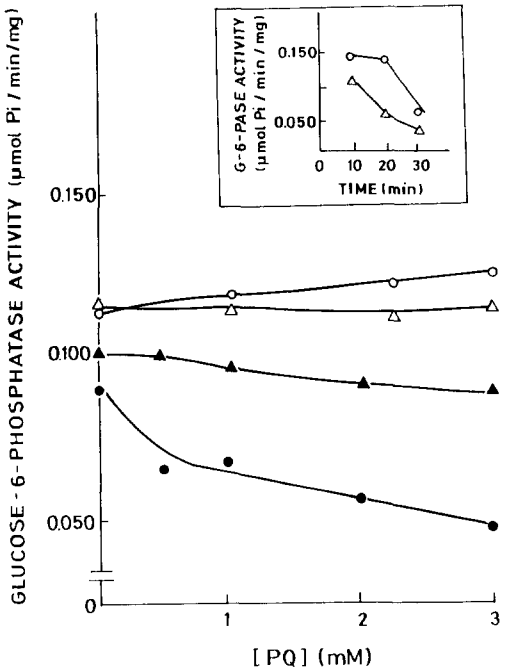

Fig. 2. Effect of the addition of paraquat and ferritin on glucose-6-phosphatase activity. Microsomal glucose-6-phosphatase activity was measured after a 20 min incubation under the following conditions: the absence of NADPH (△); absence of NADPH but presence of 10 μg ferritin/mg protein (○); 1 mM NADPH (▲) and 1 mM NADPH plus 10 μg ferritin/mg protein (●). Experiments were carried out in the presence of the indicated concentrations of paraquat. Inset: Dependence of glucose-6-phosphatase activity with incubation time, in the presence of either 1 mM NADPH plus 2 mM paraquat (○) or 1 mM NADPH plus 10 μg ferritin/mg protein plus 2 mM paraquat (△).

Table 2
Effect of scavengers and chelators on the inactivation of glucose-6-phosphatase in the presence of paraquat

Addition	Activity of G6Pase (nmol/min per mg protein)	
	NADPH + paraquat	NADPH + paraquat + ferritin
None	93 ± 3 (47)	42 ± 9 (76) ‡
220 units catalase	83 ± 3 (53)	47 ± 9 (73) ‡
30 mM DMSO	94 ± 1 (47)	53 ± 14 (70) ‡
0.1 mM trolox	134 ± 10 (24) *	154 ± 12 (13) *
50 units SOD	107 ± 14 (39)	131 ± 17 (26) *
0.5 mM ATP	81 ± 8 (54)	28 ± 6 (84) *,‡
0.5 mM EDTA	176 ± 9 (1) *	155 ± 13 (12) *
0.5 mM desferrioxamine	131 ± 7 (26) *	158 ± 9 (10) *

* $P < 0.05$ compared to the no addition control. ‡ $P < 0.05$ ferritin plus NADPH versus NADPH. Microsomes were incubated for 20 min at 37°C in the presence of 1 mM NADPH plus 2 mM paraquat or 1 mM NADPH plus 2 mM paraquat plus 10 μg ferritin per mg protein, in the absence or presence of the indicated additions. The ice control G6Pase activity was 176 nmol/min per mg microsomal protein. Numbers in parentheses refer to the percent inactivation of G6Pase.

inactivation could be found under these conditions. In the absence of NADPH, neither paraquat nor ferritin plus paraquat caused loss of G6Pase activity.

The effect of scavengers and chelators on the inactivation of G6Pase produced by NADPH, in the absence or presence of ferritin, in the paraquat incubation system was essentially the same as reported for the control incubation system (Table 1). Thus, catalase and DMSO did not prevent the NADPH plus paraquat nor the ferritin-stimulated NADPH plus paraquat inactivation of G6Pase, whereas trolox was protective (Table 2). ATP caused a further lowering of enzyme activity, whereas EDTA and desferrioxamine were protective with both incubation systems (Table 2). SOD, which was only slightly protective against the NADPH plus paraquat catalyzed inactivation, was strongly protective against the ferritin-catalyzed inhibition of G6Pase activity (Table 2). A titration curve for the protection by SOD is shown in Table 3; 4 units per ml of SOD showed some protection against G6Pase inactivation by a system containing NADPH, paraquat, and ferritin, whereas 50 units of SOD did not significantly protect against the inactivation produced by NADPH plus paraquat in the absence of ferritin. Concentrations of 50 or 100 units of SOD per ml completely prevented the increased inactivation of G6Pase caused by addition of ferritin (restoring rates to those found in the absence of ferritin); boiled SOD had no protective effect (Table 3).

Microsomal chemiluminescence

The inhibition of the ferritin-catalyzed inactivation of G6Pase by trolox or EDTA or desferrioxamine, but

Table 3
Effect of SOD on the inactivation of G6Pase

Reaction condition	Concentration of SOD (units per ml)	G6Pase activity (nmol/min per mg protein)
A. Minus ferritin	0	93 ± 3 (62)
	50	107 ± 9 (56)
B. Plus ferritin	0	21 ± 4 (91)
	4	66 ± 7 (73) *
	10	78 ± 1 (68) *
	50	90 ± 3 (63) *
	200	98 ± 16 (60) *
	50 (boiled)	23 ± 12 (91)

* $P < 0.05$ compared to no SOD control. Microsomes were incubated for 20 min at 37°C in the presence of NADPH plus 2 mM paraquat in the absence (A) or presence (B) of 10 μg ferritin per mg protein and the indicated concentrations of SOD. The ice control G6Pase activity was 245 ± 34 nmol/min per mg protein. Numbers in parentheses refer to the percent activation of G6Pase.

not by catalase or DMSO, suggested an important role for microsomal lipid peroxidation in the mechanism of the ferritin stimulation. Microsomal chemiluminescence was measured as an index of lipid peroxidation; we have previously shown that this reaction appears to be more sensitive than the production of thiobarbituric acid-reactive components in detecting stimulation of lipid peroxidation by ferritin [42]. In the presence of paraquat or ferritin but the absence of NADPH, microsomal light emission was low (Table 4). The addition of NADPH increased chemiluminescence and this light emission was further enhanced in the presence of either paraquat or ferritin; highest rates of microsomal chemiluminescence were produced in the presence of NADPH plus ferritin plus paraquat (Table 4), analogous to the extensive inactivation of G6Pase which occurs under these conditions (Table 2). A ferritin concentration curve for stimulating microsomal light emission in the presence of paraquat was compared to

Table 4
Effect of ferritin on microsomal chemiluminescence

Reaction condition	Chemiluminescence (arbitrary units/mg protein)		Effect of ferritin (%)
	control	plus ferritin	
Paraquat, minus NADPH	60 ± 20	80 ± 20	+33
NADPH	160 ± 20 *	340 ± 40 *	+113 †
NADPH plus paraquat	440 ± 20 *,‡	880 ± 40 *,‡	+100 †

* $P < 0.05$ compared to minus NADPH. ‡ $P < 0.05$ paraquat plus NADPH compared to NADPH. † $P < 0.05$ ferritin compared to minus ferritin. Microsomal light emission was determined over a 20-min reaction period as described in Materials and methods. Final concentrations of paraquat and ferritin were 2 mM and 10 μg per mg microsomal protein, respectively. Chemiluminescence was recorded as CPM every minute and the area from the resulting curve was weighed and the data expressed as arbitrary units.

Table 5
Effect of scavengers and chelators on microsomal chemiluminescence

Addition	Chemiluminescence (arbitrary units/mg protein)	
	NADPH + ferritin	NADPH + ferritin + paraquat
None	320 ± 20	780 ± 40 ‡
220 units catalase	300 ± 40 (−6)	740 ± 80 (−5) ‡
30 mM DMSO	280 ± 20 (−13)	700 ± 60 (−10) ‡
0.1 mM trolox	80 ± 20 (−75) *	200 ± 20 (−74) *,‡
50 units SOD	160 ± 20 (−50) *	400 ± 80 (−49) *,‡
0.5 mM ATP	1320 ± 160 (+312) *	1560 ± 120 (+100) *
0.5 mM EDTA	80 ± 20 (−75) *	200 ± 40 (−74) *,‡

* $P < 0.05$ compared to the no addition control. ‡ $P < 0.05$ ferritin plus paraquat compared to ferritin. Microsomal light emission was recorded over a 20-min reaction period in a mixture containing 0.5 mM NADPH plus 10 μg ferritin per mg microsomal protein in the absence or presence of 2 mM paraquat and the indicated additions. Numbers in parentheses refer to the effect of the addition.

that for inactivating G6Pase in the presence of paraquat. A close association between these two reactions as a function of ferritin concentration was observed (data not shown) as the concentration of ferritin which increased light emission two-fold (about 10 μg per mg microsomal protein) was very similar to the concentration which doubled the inactivation of G6Pase (about 12 μg per mg microsomal protein).

Results in Table 5 show that the effect of scavengers and chelators on the ferritin-catalyzed microsomal light emission were very similar to the effects of these agents on the ferritin-stimulated inactivation of G6Pase (Tables 1 and 2, ferritin columns). Catalase and DMSO did not inhibit the light emission or the enzyme inactivation, trolox, SOD, and EDTA were strongly protective in both reactions, and ATP increased the actions of ferritin in both reactions. Similar responses to the scavengers and chelators were noted in the absence or presence of paraquat (Table 5).

4. Discussion

Peroxidative damage of membrane enzymes, such as glucose-6-phosphatase, has been shown to be at least partially due to the formation of reactive aldehydic products [21–29]. The production of potent oxidizing species which initiate lipid peroxidation and other toxic reactions requires catalysis by metals such as iron and copper. In the mammalian cell, the potential toxicity of non-heme iron is reduced by storing the metal in proteins such as ferritin. Nevertheless, recent studies have shown that iron stored in ferritin is not necessarily an inert form of iron as the iron mobilized from ferritin is reactive in initiating lipid peroxidation [11,12]. Experiments were carried out to evaluate whether fer-

ritin could catalyze inactivation of G6Pase, and whether such inactivation correlated with ferritin-stimulated lipid peroxidation.

G6Pase inactivation occurred when microsomes were incubated with NADPH in the absence of ferritin. In view of the prevention of this inactivation by EDTA or desferrioxamine, it would appear that the loss of G6Pase activity was due to iron present in the reaction system. The lack of prevention by SOD (in contrast to the prevention by SOD of the increased inactivation produced by added ferritin) suggests that this source of iron does not appear to be residual ferritin associated with the microsomal preparation. Isolated microsomes contain small amounts of tightly bound non-heme iron, which can participate in catalyzing production of potent reactive oxygen species [43,44]. Inhibition of this ferritin-independent G6Pase inactivation by trolox suggests a role for lipid peroxidation in the NADPH-catalyzed reaction. The increased effectiveness of NADPH compared to NADH in promoting inactivation of G6Pase may reflect the higher rates of lipid peroxidation which occur with NADPH as the microsomal reductant [35–37,40]. For example, rates of NADH-dependent microsomal light emission were about 20% the rates with NADPH [42]. The ability of paraquat to increase the ferritin-independent NADPH-dependent inactivation of G6Pase is likely due to increased oxygen radical production in the presence of this redox cycling agent. Although the effects of paraquat on microsomal lipid peroxidation are complex, the clear stimulation of light emission by paraquat, and prevention of the increased inactivation of G6Pase and chemiluminescence by trolox, EDTA, and desferrioxamine, suggest that elevated peroxidation in the presence of NADPH plus paraquat is responsible for the ability of this redox cyling agent to increase inactivation of G6Pase.

Ferritin increased the NADPH-dependent inactivation of G6Pase and microsomal light emission in a time-dependent and concentration-dependent manner. The increases produced by ferritin, in contrast to the effects produced in the absence of ferritin, were sensitive to SOD. This suggests an important role for superoxide radical in mobilizing the iron from ferritin. Increased inactivation of G6Pase by ferritin in the presence of paraquat is due to increased production of superoxide since enhanced inactivation is completely sensitive to SOD. Although paraquat radical itself can mobilize iron from ferritin [14], superoxide appears to be the primary reductant responsible for releasing iron from ferritin under the aerobic conditions of these experiments. The sensitivity of the paraquat elevation of G6Pase inactivation to SOD, trolox, EDTA, and desferrioxamine indicates that paraquat radical itself is not directly responsible for the increased inactivation. The poor ability of NADH to promote the ferritin-dependent inactivation of G6Pase is likely due to the much lower rates of superoxide production by microsomes with NADH as reductant, as compared to NADPH. Under conditions in which ferritin stimulated NADPH-dependent production of thiobarbituric acid reactive components or microsomal chemiluminescence, there was no effect by ferritin on the NADH-dependent reactions [42]. NADH is also not effective as a reductant for the interaction of paraquat with microsomes [45,46].

In the current study, ferritin was found to stimulate the inactivation of G6Pase by a reaction which appeared to reflect ferritin-stimulated lipid peroxidation since a) the concentration of ferritin which stimulated light emission two-fold was similar to the concentration which doubled the rate of G6Pase inactivation; b) both ferritin-stimulated reactions showed the same response to scavengers, e.g., inhibition by SOD and trolox, lack of effect by catalase, and DMSO; c) both reactions also showed a similar response to chelating agents, e.g., stimulation of G6Pase inactivation or light emission by ATP, inhibition by EDTA or desferrioxamine; and d) the extent of inactivation of G6Pase and microsomal chemiluminescence were similarly enhanced by the addition of the redox cycling agent paraquat. These results indicate that one toxicological result of ferritin-catalyzed lipid peroxidation is inhibition of microsomal enzymes such as G6Pase, and support the concept that iron mobilized from ferritin via O_2^- may, under certain circumstances, catalyze the production of reactive oxygen species which result in cellular damage.

Acknowledgements

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