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RESTORATION OF RED CELL CATALASE ACTIVITY BY GLUCOSE METABOLISM AFTER EXPOSURE TO A VITAMIN K ANALOG

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Abstract—The relationship between catalase and reducing equivalents in the red cell was studied by first exposing cells to an oxidative insult and then adding glucose as an energy source to facilitate recovery from the oxidative insult. Oxidative damage was produced by incubation of red cells with either 2×10^{-6} M or 4×10^{-5} M 1,4-naphthoquinone-2-sulfonic acid, a vitamin K analog which generates superoxide in the presence of oxyhemoglobin. After a 90 min incubation, cellular catalatic activity decreased to about 30 per cent of the original activity, NADH content was decreased to about 40 per cent, and NADPH content decreased to 10 per cent. At 90 min, D-glucose was added to a concentration of 5 mM. After a further 90 min incubation, red cells originally treated with the low concentration of quinone recovered catalatic activity to 70 per cent of the original activity, NADH to 60 per cent of the starting content, and NADPH content rose above 100 per cent. Red cells incubated with the higher quinone concentration did not recover catalatic activity or reduced nucleotide content. The data imply that catalase accumulates as the peroxidatic intermediate, Compound II, in the absence of a sufficient concentration of reducing equivalents. If oxidative damage is mild enough so that the glycolytic pathway and hexose monophosphate shunt can still restore an adequate level of reducing equivalents after the addition of glucose, then catalatic activity will be restored as the concentrations of ferricatalase and Compound I increase. The results indicate that catalase may function both peroxidatically and catalatically in the red cell.

Previous studies [1,2] have shown that a decrease of cellular catalatic activity occurs when red cells are incubated with 1,4-naphthoquinone-2-sulfonic acid (NQ), a vitamin K analog which generates superoxide in the presence of oxyhemoglobin [3,4]. The presence of glucose in the incubation medium prevents the loss of catalatic activity. Experiments with intact red cells or with purified catalase all suggested that NQ might be causing the accumulation of catalase Compound II in the absence of an adequate concentration of reducing equivalents [1]. In this condition, the cellular catalase could not function to reduce H_2O_2 concentration. If cellular metabolic pathways remained intact, NADH and NADPH might be regenerated by addition of glucose, followed by conversion of Compound II to ferricatalase. To test this hypothesis, red cells were first exposed to NQ, causing catalatic activity and NADH and NADPH content to decrease. Later, glucose was added to the incubation medium and recoveries of catalatic activity, NADH and NADPH were measured. Methemoglobin content was also measured and compared with catalatic activity and reduced pyridine nucleotide content.

MATERIALS AND METHODS

Adult human blood was drawn daily into 3.8% sodium citrate solution. After centrifugation, plasma and white cells were removed, and red cells were washed three times with 0.9% NaCl and suspended in 144 mM NaCl, 0.1 mM EDTA, and 10 mM NaH_2PO_4 (pH 7.4), to a concentration of 0.7%. Red cell suspensions were incubated for 180 min at 37° and samples were taken for assay at 0, 90, 120 and 180 min. In some experiments, D-glucose was added to red cell suspensions at either 0 or 90 min to give a concentration of 5 mM. In some experiments, NQ was added at 0 min to give a final concentration of either 2×10^{-6} M or 4×10^{-5} M.

Catalatic activity was measured in red cell lysates by observing the decrease in absorbance at 240 nm of a hydrogen peroxide solution, as described by Beers and Sizer [5]. Since this assay measures only ferricatalase and Compound I, any accumulation of Compound II or other forms [6] will appear as a loss of catalatic activity.

For determination of NADH and NADPH content, samples were prepared for extraction by collecting cells from 100 ml of 0.7% suspensions by centrifugation, and resuspending them in 2 ml of 0.9% NaCl. Ethanolic extraction and fluorometric measurement of NADH and NADPH were carried out using the method of Sander *et al.* [7]. The cellular content of oxidized nucleotides, NAD^+ and $NADP^+$, was also measured. Increases or decreases in the content of reduced nucleotides were reflected by opposite changes in the content of oxidized nucleotides.

Oxyhemoglobin (HbO_2), methemoglobin (Hb^{+3}) and intact Hb (intact hemoglobin, defined as the sum, $HbO_2 + Hb^{+3}$) were measured as described by Harley and Mauer [8]. HbO_2 was measured by observing the increase in absorbance at 620 nm of red cell lysates after the addition of $Fe(CN)_6^{-3}$. Hb^{+3} was measured by observing the decrease in absorbance at 620 nm after addition of CN^-. Intact Hb was measured directly by observing the decrease in absorbance at 620 nm after addition of CN^- to lysates treated previously with $Fe(CN)_6^{-3}$. The results of direct measurement of intact Hb were checked with the calculated sum, $HbO_2 + Hb^{+3}$. A decrease of intact Hb during incuba-

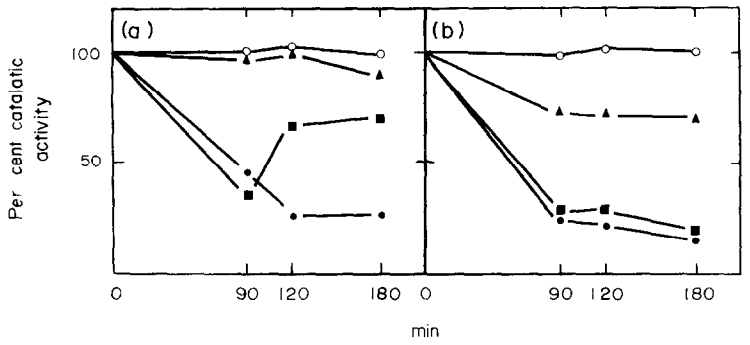

Fig. 1. Catalatic activity of red cells incubated with 2×10^{-6} M NQ (A) or 4×10^{-5} M NQ (B). Other additions: none (●); 5 mM D-glucose at 0 min (▲); and 5 mM D-glucose at 90 min (■). Control samples were incubated without NQ or glucose (○). Other samples incubated without NQ but with glucose added at 0 or 90 min showed no loss of catalatic activity.

tion of red cells was taken as indirect evidence for an accumulation of hemoglobin metabolites.

RESULTS

Figure 1 shows catalatic activity during various schedules of incubation with NQ and glucose. NQ caused a greater than 70 per cent loss of catalatic activity by 180 min with both doses of NQ. Glucose added at 0 min protected catalatic activity either completely or partially, depending on the NQ dose. Glucose added 90 min after incubation with NQ led to recovery of catalatic activity with the low NQ dose (2×10^{-6} M), but not with the high dose (4×10^{-5} M).

Red cell NADH was also measured during incubation with NQ and glucose (Fig. 2). In control incubations without NQ or glucose, NADH decreased to 40 per cent by 180 min (Fig. 2, panel A). Incubation with NQ caused no significant change in the final NADH content at 180 min (Fig. 2, panels B and C). When glucose was added to red cell suspensions, NADH was increased to 130 per cent in the absence of NQ, and maintained starting levels in the presence of the low NQ dose. In the presence of the high NQ dose, glucose had no effect on the NADH content. When glucose was added after 90 min of incubation, partial recovery of NADH content was observed with NQ-free suspensions and those incubated with the low NQ dose. Adding glucose at 90 min had no effect on NADH in cells treated with the high NQ dose.

NQ and glucose affected NADPH content differently than NADH (Fig. 3). As observed with NADH in red cells incubated in the absence of additions, NADPH decreased to 40 per cent by 180 min. On addition of NQ, however, NADPH decreased nearly to zero. When glucose was added at 0 min, NADPH levels increased with increasing NQ concentrations. In experiments where glucose was added at 90 min, NADPH content was completely reconstituted in the presence of the low NQ dose but not at all with the high NQ dose. Table 1 summarizes the relationships between glucose protection or glucose stimulated recovery of catalatic activity and NADH and NADPH content. It can be seen that increased protection or recovery of catalatic activity is proportional to the content of NADH and NADPH.

Figure 4 shows the per cent of Hb^{+3} formed during incubation with NQ and glucose. In the absence of glucose the formation of Hb^{+3} is proportional to the NQ concentration. Glucose protected against the formation of Hb^{+3} with the low NQ dose but not with the high dose. Addition of glucose at 90 min did not result in recovery of HbO_2.

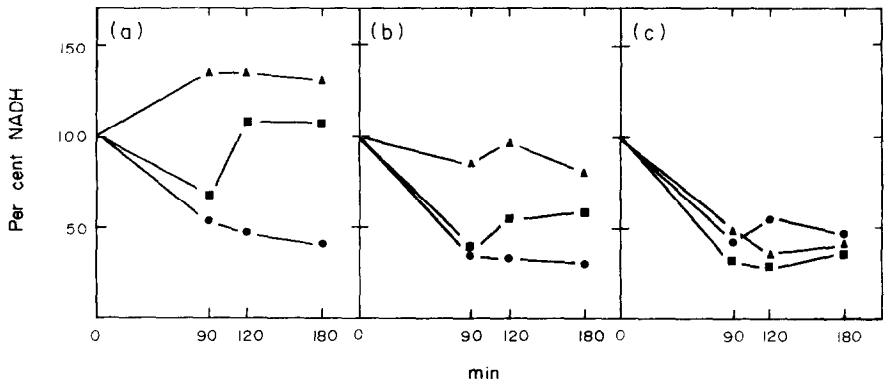

Fig. 2. NADH content of red cells incubated with 0 M NQ (A), 2×10^{-6} M NQ (B), or 4×10^{-5} M NQ (C). Other additions: none (●); 5 mM D-glucose at 0 min (▲); and 5 mM D-glucose at 90 min (■). The starting NADH concentration (100 %) was 8.4 nmoles/g Hb.

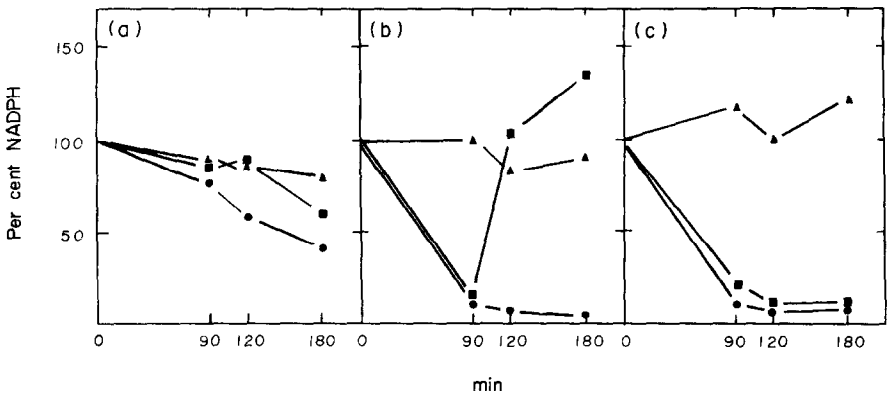

Fig. 3. NADPH content of red cells incubated with 0 M NQ (A), 2×10^{-6} M NQ (B), or 4×10^{-5} M NQ (C). Other additions: none (●); 5 mM D-glucose at 0 min (▲); and 5 mM D-glucose at 90 min (■). The starting NADPH concentration (100%) was 9.5 nmoles/g Hb.

DISCUSSION

The results show that a decrease in red cell catalatic activity caused by NQ can be partly restored by the addition of glucose to the medium. The recovery of catalatic activity was paralleled by the recovery of NADH and NADPH content. These results are consistent with the hypothesis that catalase accumulates as the peroxidatic intermediate, Compound II, in the absence of a sufficient concentration of reducing equivalents. When red cells were incubated with 2×10^{-6} M NQ, the oxidative damage was mild enough so that the glycolytic pathway and the hexose monophosphate shunt could restore the level of reducing equivalents when glucose became available. Under these conditions, Compound II could be reduced to ferricatalase, restoring catalatic activity. Experiments with purified catalase are consistent with this interpretation. It was observed that *in vitro* inhibition of catalatic activity by NQ could be prevented or reversed by NADH or NADPH [1].

Catalase is known to function either catalatically or peroxidatically *in vitro* [6]. Ferricatalase reacts with H_2O_2 to form the two-electron oxidation product, Compound I. Compound I may undergo either a concerted two-electron reduction by H_2O_2 with the formation of ferricatalase (catalatic activity) or two successive one-electron reductions by appropriate electron donors with the sequential formation of Compound II and ferricatalase (peroxidatic activity). Generally, catalatic activity predominates with high H_2O_2 concentrations, and peroxidatic activity predominates with low H_2O_2 concentrations and in the presence of a suitable electron donor. Evidence for or against a catalatic or peroxidatic function for catalase in a variety of tissues has been reviewed [9–12]. It has been suggested that the concentration of electron donors in the red cell is too low for catalase to function peroxidatically [13]. Indirect evidence that catalase may function peroxidatically in red cells is the observation that primaquine caused a rapid reduction of catalase activity in the red cells of glucose-6-phosphate dehydrogenase deficient volunteers, followed by recovery of activity in a few days [14]. This implies that in the glucose-6-phosphate dehydrogenase-deficient red cells there were enough internal electron donors to cause Compound II formation, but inadequate metabolic capacity for immediate further reduction to ferricatalase. Eaton *et al.* [15] hypothesized that NADPH, and possibly NADH and reduced glutathione, are internal electron donors which keep Compound II from accumulating in red cells. They showed that NADPH could restore catalatic activity as efficiently as ethanol in lysates of ascorbate-treated cells. In our experiments, red cells were exposed to NQ, a drug which generates superoxide, which in turn dismutates to H_2O_2. Catalase reacts with this H_2O_2 to form intracellular Compound I

Table 1. Relationship of protection or recovery of catalatic activity to NADH and NADPH content*

Schedule of additions		Level of protection or recovery†		
NQ dose	Glucose added at	Catalatic activity	NADH	NADPH
4×10^{-5} M	None	−	−	−
4×10^{-5} M	90 min	−	−	−
2×10^{-6} M	None	−	−	−
2×10^{-6} M	90 min	+	+	++
4×10^{-5} M	0 min	+	−	++
2×10^{-6} M	0 min	++	++	++

* Data are summarized from Figs. 1 to 3.

† No protection or recovery (−); partial protection or recovery (+); and nearly complete protection or recovery (++).

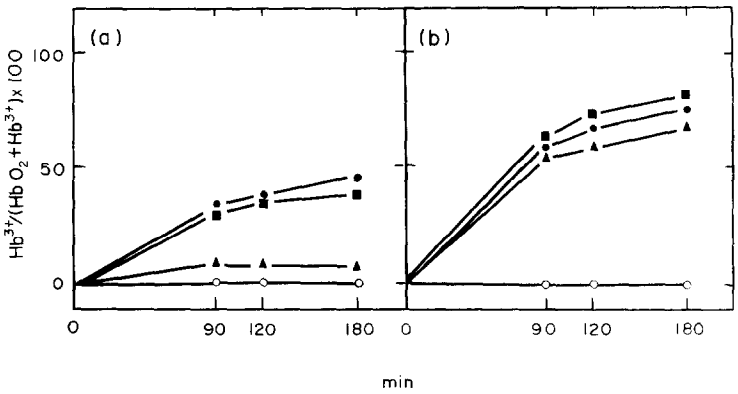

Fig. 4. Percent Hb^{+3} content of red cells incubated with 2×10^{-6} M NQ (A) or 4×10^{-5} M NQ (B). Other additions: none (●); 5 mM D-glucose at 0 min (▲); and 5 mM D glucose at 90 min (■). Control samples were incubated without NQ or glucose (○). Other samples incubated without NQ but with glucose added at 0 or 90 min had less than 1% Hb^{+3}. The highest decrease of intact Hb (HbO_2 + Hb^{+3}) in any experiment was 8 per cent.

as shown by the formation of an inactive complex between Compound I and 3-amino-1,2,4-triazole [2]. Compound I may react with intracellular electron donors to form Compound II and then ferricatalase. Therefore, during peroxidatic degradation of H_2O_2, the red cell will contain ferricatalase, Compound I, and Compound II. In the absence of glucose, the concentration of the electron donors, NADH and NADPH, will decrease and some catalase will be trapped as Compound II. We have observed this by measuring the simultaneous loss of catalase activity and NADH and NADPH content. When glucose was added to red cells, NADH and NADPH content was restored, allowing the reduction of Compound II and the recovery of catalatic activity. If the oxidative damage to red cells was too great, as was the case with 4×10^{-5} M NQ, then NADH and NADPH content was not restored and catalatic activity did not recover. The results indicate that catalase may remove H_2O_2 peroxidatically using electrons from NADH generated by glycolysis and NADPH generated by the hexose monophosphate shunt.

The Hb^{+3} reductase system does restore HbO_2 after the addition of glucose (data not shown) but this process requires between 24 and 48 hr for completion [16] and is not significant in the 90-min recovery period of this experiment. It was observed, however, that glucose added at zero time prevented Hb^{+3} formation in cells incubated with 2×10^{-6} M NQ. Hb^{+3} formation was directly proportional to loss of catalatic activity, as reported previously by Paniker and Iyer [17].

Of interest is the differential response of the red cell in maintaining NADH and NADPH content. The presence or absence of NQ had little effect on the final NADH content at 180 min in the absence of glucose, whereas the NADPH content fell to zero in cells incubated with either 2×10^{-6} M or 4×10^{-5} M NQ. When glucose was available to red cells from zero time, NADH content decreased with increasing NQ doses, whereas NADPH content increased with increasing NQ doses. These differences imply different responses to oxidative stress by the glycolytic pathway and the hexoxe monophosphate shunt. Generally, the hexose monophosphate shunt is capable of a much greater response than glycolysis, to oxidative stress [18,19].

The red cell has several mechanisms for protection of hemoglobin and membrane function. Among these are the methemoglobin reductase system, glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase. Glucose metabolism provides cofactors for the functioning of the methemoglobin reductase system, glutathione reductase, and glutathione peroxidase. Our results show that glucose metabolism also provides electrons for maintenance of catalatic activity and possibly for normal peroxidatic functioning of catalase.

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