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EFFECT OF MET- AND SULFHEMOGLOBIN ON GLYCOLYTIC ACTIVITY IN RED CELLS TREATED WITH NITRITE IN VITRO

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Abstract

The effect of nitrite-induced met-/sulfhemoglobin on glycolytic activity was examined in normal red cells under metabolic stress with sodium ascorbate. In the absence of ascorbate, the marked accumulation of methemoglobin was observed in red cells treated with nitrite, concomitant with increased concentration of pyruvate. In contrast, in the presence of ascorbate, the marked accumulation of sulfhemoglobin was seen with the normal level of methemoglobin, even after treatment with nitrite. Glycolytic intermediates, namely, FDP, DHAP, G-3-P and 2,3-DPG, were significantly increased in the nitrite pre-treated red cells in the presence of ascorbate. Thus, the higher concentration of methemoglobin and of sulfhemoglobin may affect glycolytic activity significantly in red cells, if stressed metabolically such as by ascorbate.

INTRODUCTION

Hemoglobin, energy metabolism including pentose phosphate pathway, and membrane functions have been known as the most important in red cell metabolism concerning its hemolysis. These three have been investigated extensively at their own fields for many years, but the exact mechanism of the interaction among hemoglobin, membrane and glycolysis in red cells have been a little elucidated even at the present time.

Concerning the interaction between hemoglobin and membrane, the mechanism of Heinz body formation in red cells of the patients with abnormal hemoglobins[1], and the close relationship between hemoglobin SS and the decreased membrane deformability in the patients with sickle cell anemia[2][3] have been described. In addition, increased hemolysis, that is, increased susceptibility of red cell membrane to its rupture has been reported in the patients with enzymopathies[4][5][6] such as glucose-6-phosphate dehydrogenase deficiency and pyruvate kinase deficiency, and it suggests that the enzyme-membrane interaction might be the most important factor on the hemolysis of these red cells.

In contrast, a close relationship between hemoglobin and glycolytic activity has not been studied extensively. In the patients with congenital methemoglobinemia[7] due to DPNH-dependent diaphorase deficiency, neither hemolysis nor critical abnormality in red cell metabolism has been known under the not-stressed condition.

On the other hand, the marked abnormality to the various extent has been reported in red cells of some patients with severe chronic uremia[8]. Although no abnormality has been detected at the resting state, the increased glycolytic activity[9] has been observed in vitro in these red cells incubated with sodium ascorbate concomitant with the increased formation of sulfhemoglobin and to the decreased activity of pentose phosphate pathway. Furthermore, the glycolytic intermediates, especially FDP to 3-PGA along the glycolytic pathway, have been found to be accumulated in association with the increased sulfhemoglobin formation.

Thus, in the present communication, the relationship between increased met- or sulfhemoglobin formation and glycolytic activity is examined in the nitrite-treated normal adult red cells incubated with or without sodium ascorbate.

MATERIALS AND METHODS

Blood specimens from normal individuals were centrifuged, and the buffy coats and plasma were removed, as described previously[10]. Red cell suspensions were prepared by washing with 0.153 M Na/K phosphate-buffered saline containing 14 mM glucose, and a hematocrit was adjusted to 30%. Red cells containing methemoglobin were prepared by incubating with various concentrations of sodium nitrite solution at 25°C for 10 minutes, and then washed five times with the buffer to remove the excessive nitrite. In other experiments, red cells were also incubated with 1% monochloramine solution at 25°C for 10 minutes to obtain the higher concentration of methemoglobin in the red cells. Nitrite- or monochloramine-treated red cells were further incubated with or without 12 mM sodium ascorbate. The concentrations of methemoglobin and sulfhemoglobin were determined spectrophotometrically by the method of Everyn and Malloy[11], and expressed as per cent of total hemoglobin concentration in specimens.

The ascorbate screening tests were performed to detect the abnormality of red cell metabolism by the modification[8] of the method of Jacob and Jandl[12]. The extent of the abnormality is expressed as the ratio of O.D. at 620 nm/O.D. at 540 nm. as described previously[9].

The concentrations of pyruvate andl actate[13], the glycolytic intermediates[14], and 2,3-DPG[15] were determined at zero time and after 3 hours of incubation at 37°C with or without sodium ascorbate.

Substrates and glycolytic enzymes were purchased from Sigma Chemicals (St. Louis, Mo., U.S.A.) and others were reagent-grade.

All experiments were performed in duplicate, and the mean values of three experiments are shown.

Abbreviations used in text are;

G-6-P; glucose-6-phosphate, F-6-P; Fructose-6-phosphate, FDP; fructose diphosphate, DHAP; dihydroacetone phosphate, G-3-P; glyceraldehyde-3-phosphate, 2,3-DPG; 2,3-diphosphoglyceric acid, 3-PGA; 3-phosphoglyceric acid, 2-PGA; 2-phosphoglyceric acid, PEP; phosphoenol pyruvate, Pyr; pyruvate, and DPNH; reduced nicotinamide adenine dinucleotide.

RESULTS

1. Effect of various concentrations of nitrite on the screens, and the methemoglobin and sulfhemoglobin content in red cells.

The ratio of the optical density at 620 nm to that at 540 nm increases according to the concentrations of nitrite used up to 0.5%, as shown in the upper part of Fig. 1. The ratio is markedly elevated in the presence of sodium ascorbate compared to that in the absence of ascorbate. In the absence of ascorbate, most of hemoglobin was converted to methemoglobin (the middle panel of Fig. 1). In contrast, the presence of sodium ascorbate did not produce methemoglobin even with nitrite up to 0.5%. Instead, the marked accumulation of sulfhemoglobin was observed in the presence of sodium ascorbate, as denoted in the lower panel of Fig. 1.

2. Effect of nitrite on the lactate and pyruvate formation.

As shown in Fig. 2, sodium nitrite in the presence of ascorbate increased the formation of lactate and also of pyruvate, and, thereby, the ratio of the concentration of lactate to that of pyruvate decreased significantly. Instead of that, nitrite alone did not change the extent of the lactate formation, but rather enhanced the pyruvate formation with the concomitant decrease of the ratio, lactate/pyruvate.

3. Effect of sodium ascorbate alone on the pyruvate and lactate formation.

The steady increase of lactate and pyruvate contents in red cells was observed during the in vitro incubation up to 3 hours with sodium ascorbate alone. In the absence of ascorbate, pyruvate was not accumu-

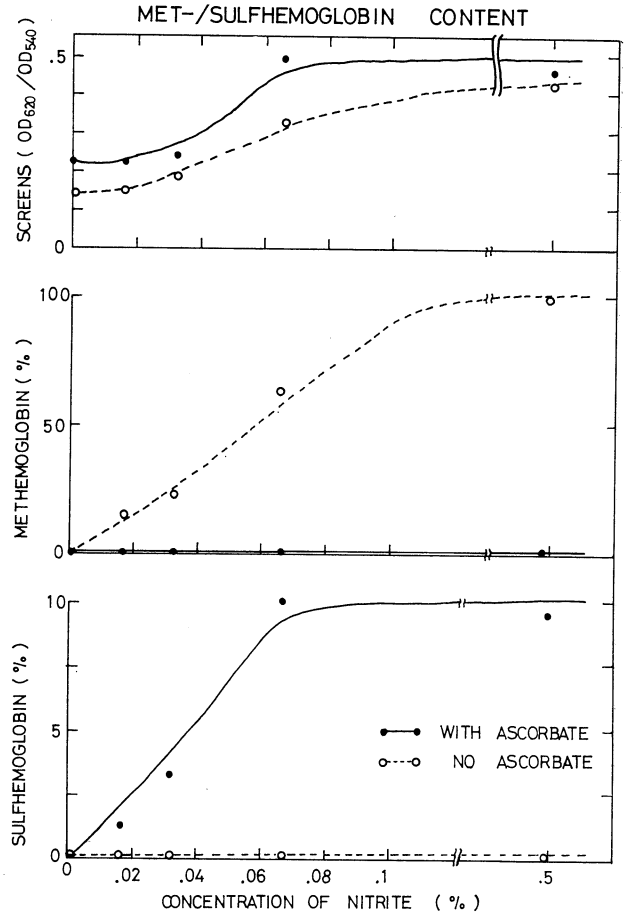

Fig. 1. Effect of nitrite on the screens and the concentrations of met-and sulfhemoglobin.

Red cells pretreated with various concentrations of nitrite were further incubated at 37°C for 3 hours in the absence (open circles) or the presence (closed circles) of 12 mM sodium ascorbate. Details of the experiments are in text.

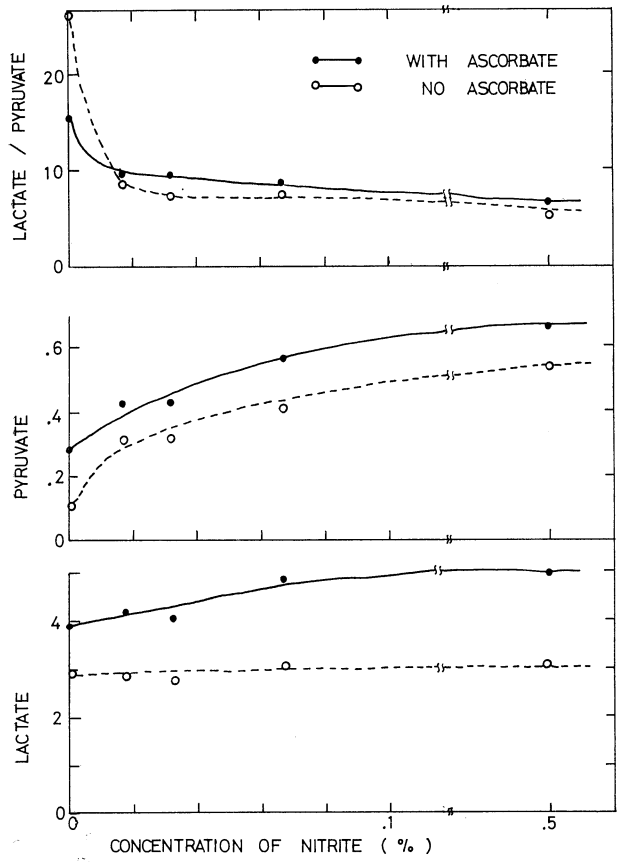

Fig. 2. Effect of nitrite on the pyruvate and lactate formation.

The nitrite-treated red cells were incubated with (closed circles) or without (open circles) 12 mM sodium ascorbate. The extents of the formation of lactate (the lower panel) and of pyruvate (the middle panel) are shown as μmoles/hour/ml red cells. At the top panel, the ratio lactate/pyruvate is shown.

lated significiantly, contrary to the steady increase of the lactate concentration during the timed incubation as shown in Fig. 3.

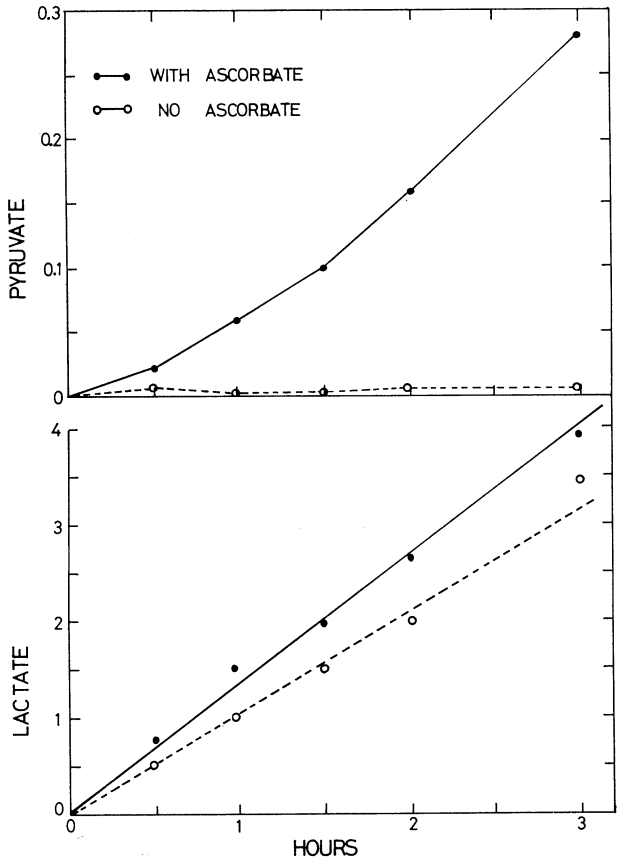

Fig. 3. Effect of ascorbate on the lactate and pyruvate formation.

Normal red cells were incubated with (closed circles) or without (open circles) 12 mM sodium ascorbate at 37°C for 3 hours. The concentration of lactate or pyruvate is expressed as μmoles/hour/ml red cells.

4. Effect of nitrite on the pattern of glycolytic intermediates in red cells incubated with or without sodium ascorbate.

After treatment with ascorbate alone, no significant increase of the concentrations of the glycolytic intermediates was observed except for 2,3-DPG, and pyruvate (the lower portion of Fig. 4). When the red cells

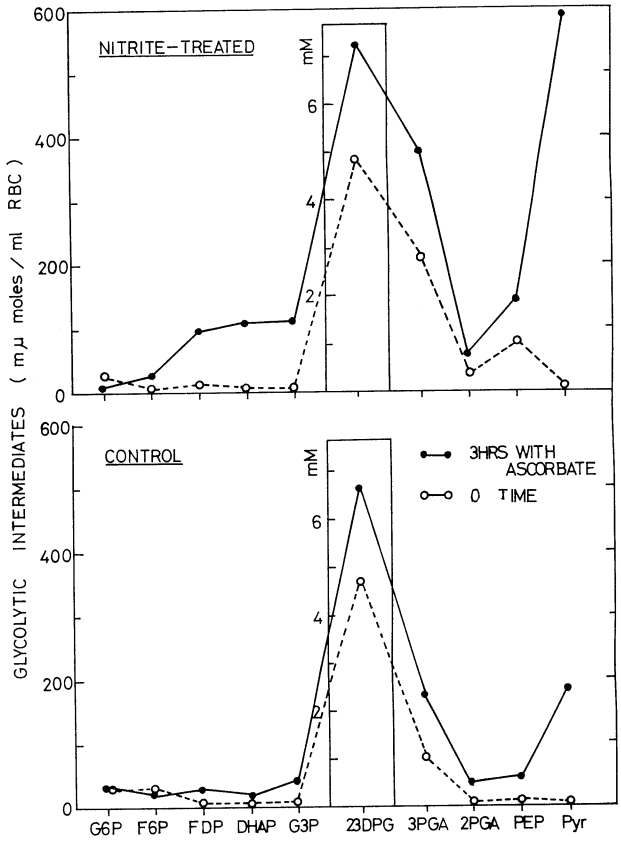

Fig. 4. Pattern of glycolytic intermediates in red cells pretreated with nitrite.

The concentrations of the glycolytic intermediates were determined in red cells pretreated with (the upper panel) or without nitrite (the lower panel) before (open circles) and after (closed circles) the incubation with 12 mM sodium ascorbate. Abbreviations for the glycolytic intermediates are described in text.

pretreated with nitrite were further incubated with ascorbate, the marked accumulationof intermediates, especially, FDP, DHAP, G-3-P, 2,3-DPG, 3-PGA and pyruvate, was observed, as depicted at the upper portion of Fig. 4. The change of the concentration of glycolytic intermediates is shown in Fig. 5. In the presence of ascorbate, it is clearly shown that nitrite increases the concentrations of FDP, DHAP, G-3-P, 2,3-DPG and pyruvate compared to those in the control without nitrite.

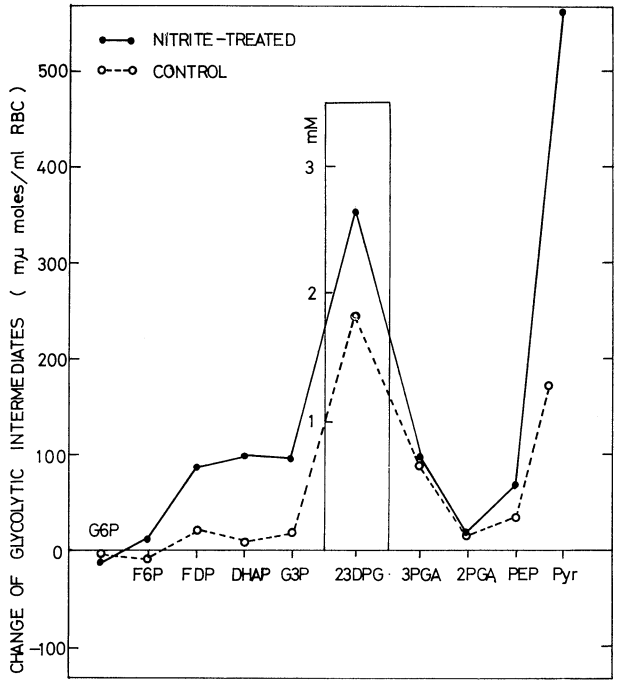

Fig. 5. The change of the glycolytic intermediates in red cells pretreated with nitrite in the presence of ascorbate.
The change of the concentrations of the glycolytic intermediates in red cells pretreated with nitrite (closed circles) in the presence of ascorbate is shown compared to those in control (open circles).

5. Effect of monochloramine on the pattern of the glycolytic intermediates in red cells incubated with or without sodium ascorbate.

The untreated red cells were incubated with ascorbate for 3 hours, and the concentrations of the glycolytic intermediates were determined at zero time and after 3 hours of incubation.

After incubation for 3 hours, ascorbate increased the concentrations of 2,3-DPG, 3-PGA, and pyruvate, compared to that at zero time before incubation (the lower panel of Fig. 6). Contrary to these results obtained at the untreated red cells, the treatment of monochloramine enhanced slightly the concentration of 3-PGA, 2-PGA and PEP even at zero time before the further incubation with ascorbate. In addition to those changes, the presence of ascorbate increased the concentrations of the glycolytic intermediates, such as FDP, DHAP, G-3-P, 2,3-DPG, 3-PGA, PEP and pyruvate, except for G-6-P and F-6-P (the upper panel of Fig. 6).

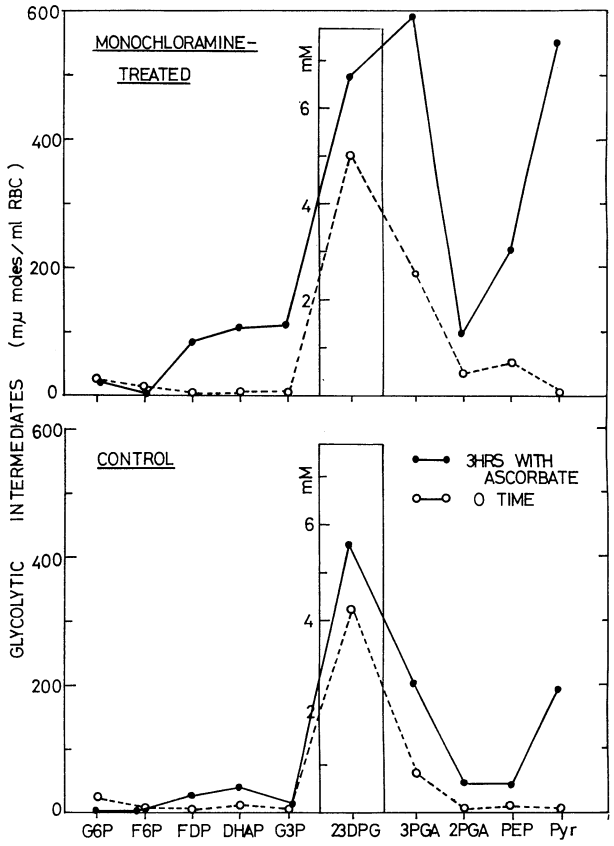

Fig. 6. Effect of monochloramine on the pattern of the glycolytic intermediates in red cells.

The concentrations of the glycolytic intermediates were determined in red cells pretreated with monochloramine (the upper panel) and in control (the lower panel). Details are shown in Fig. 4.

The change of the concentrations of these intermediates in red cells pretreated with monochloramine is summarized and depicted in Fig. 7. It is clearly shown that the glycolytic intermediates, namely FDP to 3-PGA and also PEP and pyruvate, were accumulated markedly under these conditions.

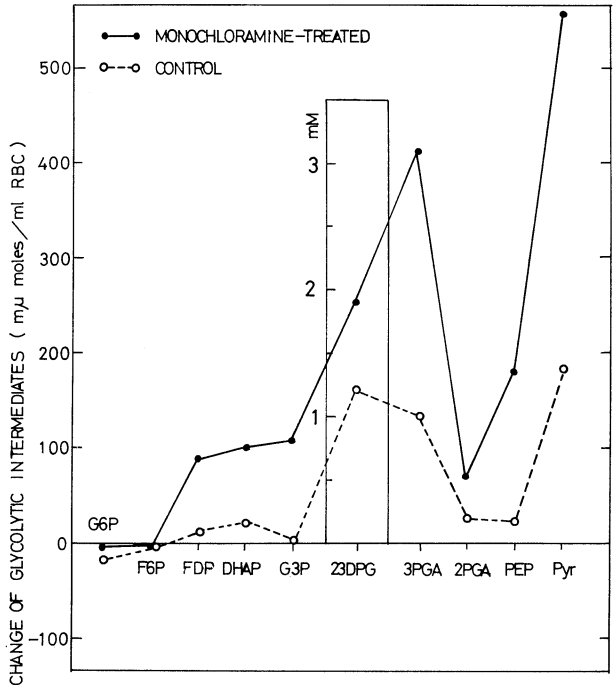

Fig. 7. The change of the glycolytic intermediates in red cells pretreated with monochloramine with sodium ascorbate. Details are shown in Fig. 5.

DISCUSSION

The relationship between hemoglobin and energy metabolism has been of increasing interest as well as that between glycolytic activity and membrane metabolism, or between hemoglobin and membrane functions. The interaction is particularly interesting in red cells of the patients with metabolic abnormalities concerning the exact machanism of hemolysis in these patients. These red cells are strikingly susceptible to the oxidant stress to get hemolyzed. The oxidants, such as sodium ascorbate, can produce the oxydative stress metabolically in these red cells, and even in normal red cells.

The concentration of methemoglobin or sulfhemoglobin increases drastically in red cells in the presence of nitrite. The abnormality in red cell energy metabolism can be detected by the ascorbate screening test[8)11)]. In the nitrite-treated red cells, the abnormality detected by the screening test is even more marked than that in normalred cells. Although ascorbate itself incre ases the formation of pyruvate in vitro, nitrite enhances further the extents of pyruvate and lactate formation.

Associated with the increased met-/sulfhemoglobin formation, the pattern of the glycolytic intermediates was changed markedly in red cells treated with nitrite, especially, in the presence of ascorbate. Fructose-diphosphate, DHAP, G-3-P and 2,3-DPG contents increased significantly. Thus, it might reflect the increased flow of glycolysis due to the marked activation of pentose phosphate pathway as shown previously[8], or due to the increased reduction of the abnormally accumulated met-/sulfhemoglobin by DPNH-dependent diaphorase.

The effect of nitrite itself on the glycolytic activity may not be specific. Monochloramine, another one of the most powerful volatile oxidant which can produce the higher concentration of methemoglobin, also shows the same pattern of glycolytic intermediates as nitrite does.

In conclusion, the increased met-/sulfhemoglobin formation does affect the activity of glycolysis, especially, in red cells stressed with ascorbate in vitro, although some possibilities that nitrite, monochlormine or sodium ascorbate might affect directly the glycolytic activity independent of the altered hemoglobin metabolism might exist.

ACKNOWLEDGMENTS

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