ELSEVIER

Biochimica et Biophysica Acta 1195 (1994) 205–210

BBA Biochimica et Biophysica Acta

Effects of chemical modification of membrane thiol groups on hemolysis of human erythrocytes under hydrostatic pressure

Takeo Yamaguchi *, Yoshitaka Murata, Junichi Kobayashi, Eiji Kimoto

Department of Chemistry, Faculty of Science, Fukuoka University, Jonan-ku, Fukuoka 814-80, Japan

Received 11 May 1994

Abstract

Membrane stability of the human erythrocyte under high pressure was examined by modifying membrane SH-groups with NEM or diamide. Hemolysis at 200 MPa of chemically modified erythrocytes was significantly suppressed by the prolonged incubation of them in a reagent-free medium above 30°C prior to the application of high pressure. However, there was no detectable change regarding membrane phospholipid distribution, CD spectra and SDS-PAGE of membrane proteins, and intracellular K^+ concentration during the incubation. On the other hand, the data of protein-spin labeling and SH-group content showed that the SH-groups buried in membrane proteins appeared on their surface by conformational changes of membrane proteins induced during the incubation. The extraction of peripheral proteins from NEM-treated membranes in 0.1 N NaOH was considerably suppressed by the incubation. These results suggest that, upon chemical modification of membrane SH-groups, protein–protein interactions are modulated during prolonged incubation above 30°C so that high pressure-induced hemolysis is suppressed.

Keywords: Erythrocyte; High pressure behaviour; Hemolysis; Thiol group; Spin labeling; Protein–protein interaction

1. Introduction

We have been studying how the membrane structure of human erythrocytes responses to high pressure. Upon exposure to high pressure, hemolysis [1] and vesiculation [2,3] take place. Such a hemolysis is largely suppressed by specific intermolecular cross-linking of membrane proteins [4] but enhanced by enzymatic digestion of glycopeptides or sialic acids from membrane surface [5]. Dynamics of membrane holes produced by high pressure are significantly different from those of the holes by a hypotonic medium [6]. The different behavior of hole dynamics in both membranes is ascribed to the difference of cytoskeletal structure, i.e., cytoskeletal network is partially destroyed in high pressure-treated erythrocytes [1]. On the other hand, high pressure-induced vesicles are also different from membrane vesicles produced by other methods regarding membrane protein composition [2,3]. Thus, the application of high pressure to red cells has provided unique information about the interactions among membrane components.

The red cell is exposed to higher oxygen tensions than other cells and is more susceptible to oxidative stress. Therefore, erythrocytes possess various means of preventing oxidative damage. Active oxygens such as superoxide radical and hydrogen peroxide are decomposed by intracellular enzymes such as superoxide dismutase, catalase, and glutathione peroxidase [7]. In addition, red cells contain high levels of reduced glutathione to prevent oxidation of the SH-groups of hemoglobin, and of the intramembranous and cytoskeletal proteins. Thus, membrane SH-groups are expected to play an important role in the maintenance of membrane structure and function of red cells. In order to understand the role of these SH-groups in more detail, it is of interest to examine how the membrane structure of red cells is perturbed by chemical modification of membrane SH-groups.

Abbreviations: DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonate; diamide, bis(*N*,*N*′-dimethylamide)diazinedicarboxylic acid; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); NEM, *N*-ethylmaleimide; PBS, phosphate-buffered saline (10 mM sodium phosphate, 150 mM NaCl, pH 7.4); PE, phosphatidylethanolamine; TNBS, 2,4,6-trinitrobenzenesulfonate.

* Corresponding author. Fax: +81 92 8656030.

SSDI 0005-2736(94)00187-1

In the present work we show that, from hemolytic properties under pressure, membrane protein–protein interactions in NEM- or diamide-treated erythrocytes are tightened by changes in the tertiary structure of SH-modified proteins induced during prolonged incubation above 30°C.

2. Materials and methods

2.1. Materials

Compounds were obtained from the following sources: bis(*N*,*N*′-dimethylamide)diazinedicarboxylic acid (diamide), Sigma; 4,4′-diisothiocyanatostilbene-2,2′-disulfonate (DIDS), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and *N*-ethylmaleimide (NEM), Wako; bovine serum albumin and 2,4,6-trinitrobenzenesulfonate (TNBS), Nacalai Tesque; 4-maleimide-2,2,6,6-tetramethylpiperidinooxyl, Syva. All other chemicals were of reagent grade.

2.2. Treatment of erythrocytes with diamide or NEM

Human blood which was drawn in citrate/phosphate/dextrose solution or in mannitol/adenine/phosphate solution was obtained from the Fukuoka Red Cross Blood Center. The blood was centrifuged at $750 \times g$ for 10 min at 4°C. The plasma and buffy coat were removed carefully. The erythrocytes were washed three times with phosphate-buffered saline (PBS; 10 mM sodium phosphate, 150 mM NaCl, pH 7.4). For treatment of erythrocytes with diamide (0.5–2 mM) or NEM (0.5–2 mM), red cells were suspended at 10% hematocrit in PBS containing SH-reactive agents and incubated for 30 min at 37°C and atmospheric pressure, unless stated otherwise. After incubation, the erythrocytes were washed twice with chilled PBS. Thus prepared erythrocytes were suspended at 0.3% hematocrit in PBS and incubated as a function of time or temperature (Fig. 2) prior to the application of 200 MPa.

2.3. Hemolysis

For high pressure-induced hemolysis, intact or chemically modified erythrocytes were suspended at 0.3% hematocrit in PBS and subjected to a pressure of 200 MPa for 30 min at 37°C as previously described [1,4]. After decompression, the erythrocyte suspension was centrifuged at $750 \times g$ for 10 min at 35°C. The concentration of hemoglobin in the supernatant was estimated from the absorbance at 542 nm. One hundred percent hemolysis was carried out by adding 10 μl of Triton X-100 (10%, v/v) to 3 ml of the erythrocyte suspension. For hypotonic hemolysis, red cells (6 μl) were added to 2 ml of a hypotonic medium (10 mM sodium phosphate, 46.9 mM NaCl, pH 7.4), incubated for 10 min at 37°C, and then centrifuged for 10 min at $750 \times g$. The degree of hemolysis was similarly determined. In order to prepare red ghosts, intact erythrocytes (0.5 ml) were hemolyzed in 0.5 ml of 5 mM sodium phosphate (pH 8.0) in the presence or absence of 1.5 mM $MgSO_4$ and then incubated for 15 min at 0°C [8,9]. To reseal the membrane, the hemolysates were made isotonic using NaCl and incubated for 45 min at 37°C. The red ghosts were washed three times in PBS and then subjected to a high pressure of 200 MPa.

2.4. Phospholipid distribution

To examine the distribution of phospholipids in the lipid bilayer, intact erythrocytes, NEM (2 mM)-treated ones, or resealed ghosts which were prepared in the absence of Mg^{2+} [8,9] were suspended at 10% hematocrit in PBS, incubated with 0.1 mM DIDS for 5 min at 20°C, and then with 10 mM TNBS for 30 min at 0°C. After incubation, the cells were washed once with 1% bovine serum albumin and three times with chilled Tris buffer (10 mM Tris, 150 mM NaCl, pH 7.4). Erythrocyte phospholipids were extracted using chloroform/isopropanol (7:11, v/v) from the cells thus prepared [10]. The extracted phospholipids were analyzed by two-dimensional thin-layer chromatography, as previously described [3,4].

2.5. ^{39}K-NMR measurement

The NEM (2 mM)-treated erythrocytes were suspended at 10% hematocrit in PBS, incubated for 60 min at 37°C, and then centrifuged at $750 \times g$ for 10 min. The pellets were mixed with equal volume of PBS. The ^{39}K-NMR spectra were recorded at 25°C with no spinning of a 10 mm round bottom NMR tube containing both the erythrocyte suspension (about 3 ml) and a small glass capillary filled with saturated KCl (reference signal). The ^{39}K-NMR spectra were run at 18.50 MHz on a JEOL GSX-400 spectrometer with the following instrument settings: 8192 data points, a 2-kHz spectral width, a 2.0-s pulse repetition, 200 scans and a 90° flip angle.

2.6. SDS-PAGE and CD spectra of chemically modified membrane proteins

Ghost membranes were prepared from diamide (2 mM)- or NEM (2 mM)-treated erythrocytes using 5 mM sodium phosphate (pH 8.0). SDS-PAGE of membrane proteins was performed by the method of Laemmli [11] using 2% acrylamide in the stacking gel and 5–10% acrylamide in the separation gel. The CD spectra of ghost membranes were recorded in the wavelength range 197–280 nm using a JASCO J-600 spectropolarimeter. A cylindrical quartz cell (0.1 cm path length) containing the samples was kept at 25°C. The concentration of membrane proteins in PBS was 50 μg/ml. Protein concentrations were determined by the method of Lowry et al. [12] with bovine serum albumin as a standard.

2.7. Spin labeling

Resealed ghosts (20% hematocrit in PBS) prepared from intact erythrocytes were treated with 0.5 mM NEM for 30 min at 37°C and washed three times in chilled PBS. The NEM-treated ghosts in PBS were incubated for 1 h at various temperatures (0–40°C) and then with maleimide spin-label for 2 h at 0°C [13]. Spin-labeled ghosts were washed three times in PBS and used for the EPR measurement. The EPR spectra were recorded at room temperature (20°C) on a JEOL JES-RE-1X spectrometer.

2.8. Membrane SH-groups

The erythrocytes pretreated with 2 mM NEM for 10 min at 37°C were incubated in PBS for 1 h at 0 or 37°C. After incubation, red cells (10% hematocrit) in PBS were treated with 0.1 mM NEM for 30 min at 0°C. Then, ghost membranes were prepared from these red cells by hypotonic hemolysis [8]. Membrane SH-groups in these ghosts were determined by using the reaction of a thiol with DTNB, as described previously [4].

2.9. Extraction of membrane proteins

As shown in spin labeling, resealed ghosts were treated with 0.5 mM NEM for 30 min at 37°C and then incubated for 1 h at 0 or 37°C in PBS. To extract cytoskeletal proteins, the ghosts (0.1 ml pellet) were incubated in 2 ml of 0.1 N NaOH for 30 min at 0°C. After incubation, the ghost suspension was centrifuged at 81 000 × g for 30 min at 4°C. The protein concentration in the supernatant was determined [12].

3. Results

3.1. Hemolytic properties of diamide- or NEM-treated erythrocytes by high pressure or hypotonic buffer

Effects of diamide and NEM on high pressure-induced hemolysis are shown in Fig. 1A. Upon subjection to a pressure of 200 MPa of diamide- or NEM-treated erythrocytes, the values of hemolysis were slightly increased for diamide but decreased for NEM, at higher reagent concentrations. On the other hand, when the erythrocytes were treated with diamide or NEM and then incubated for 1 h at 37°C in a reagent-free medium, the values of hemolysis at 200 MPa were decreased gradually with increasing reagent concentrations. These results indicate that the membrane structure of the erythrocytes in which membrane SH-groups were chemically modified becomes stable to high pressure upon prolonged incubation at 37°C.

Fig. 1B shows the effects of diamide and NEM on hypotonic hemolysis. In erythrocytes treated with diamide or NEM at any concentration, the values of hypotonic hemolysis were significantly unaffected upon prolonged incubation (1 h) at 37°C.

3.2. Effects of preincubation time and preincubation temperature on hemolysis at 200 MPa of diamide- or NEM-treated erythrocytes

Red cells treated with 2 mM diamide or 2 mM NEM were incubated as a function of times (0–2 h) in PBS prior to the application of high pressure. The values of hemolysis at 200 MPa of the erythrocytes thus obtained were decreased gradually with preincubation time, i.e., upon 2 h incubation, the values of hemolysis were decreased by

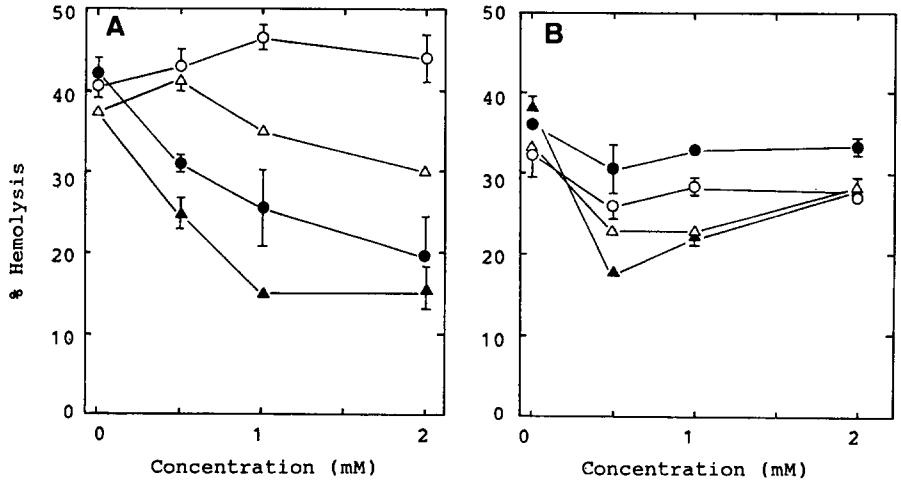

Fig. 1. Effects of NEM and diamide on hemolysis at 200 MPa (A) or in a hypotonic medium (B). Intact erythrocytes were treated for 30 min at 37°C with diamide (circle) or NEM (triangle). Then these erythrocytes were used for hemolytic experiments without prolonged incubation (open symbols) or after incubation in PBS for 1 h at 37°C (closed symbols). For high pressure hemolysis, red cells were subjected to a pressure of 200 MPa for 30 min at 37°C, whereas for hypotonic hemolysis red cells were incubated for 10 min at 37°C in a hypotonic medium. Values are the mean ± S.D. for two experiments.

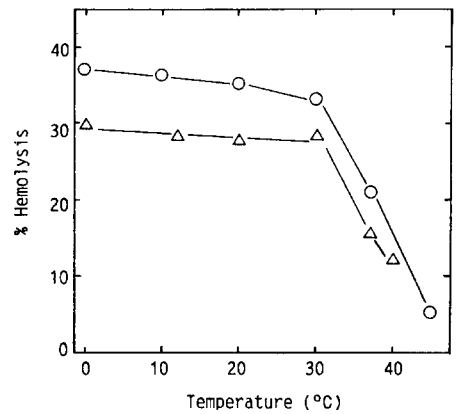

Fig. 2. Effects of preincubation temperature on hemolysis at 200 MPa of 2 mM NEM- or 2 mM diamide-treated erythrocytes. NEM(△)- or diamide (○)-treated erythrocytes were incubated for 1 h at various temperatures (0–45°C) prior to application of 200 MPa.

about 20%. Similarly, we examined the effect of preincubation temperature (0–45°C) on hemolysis at 200 MPa of 2 mM diamide- or 2 mM NEM-treated erythrocytes. The values of hemolysis were almost unaffected by preincubation temperature up to 30°C but were steeply decreased at higher temperatures (Fig. 2).

3.3. Phospholipid distribution in NEM-treated erythrocytes

We used TNBS as a reagent to detect the aminophospholipids in the outer leaflet of erythrocyte membranes. To prevent membrane permeation of TNBS via band 3, erythrocyte membranes were treated with an anion transport inhibitor, DIDS. Then DIDS-treated erythrocyte membranes were incubated with TNBS. The analysis of phospholipids in erythrocyte membranes thus obtained showed that about 18 or 54% of total phosphatidylethanolamine was located in the outer leaflet of the intact erythrocyte membrane or the resealed ghost membrane, respectively. Similar results were obtained concerning phosphatidylserine (data not shown). These results indicate that TNBS can detect the aminophospholipids in the outer leaflet under our conditions. Further, it is reported that there exist membrane holes in diamide-treated erythrocytes but no hole in NEM-treated ones [14]. So, we applied this method to NEM-treated erythrocytes. Under such conditions as high pressure-induced hemolysis is suppressed, the phospholipid distribution in NEM-treated erythrocytes was almost the same as that in intact erythrocytes (Table 1). Additionally, to confirm the effect of the phospholipid distribution on high pressure-induced hemolysis, red ghosts were prepared in the presence or absence of Mg^{2+}. Red ghosts obtained in the presence of Mg^{2+} have phospholipid asymmetry, compared to those prepared in the absence of Mg^{2+}. However, the value ($56.9 \pm 3.4\%$, $n = 5$) of hemolysis at 200 MPa of red ghosts obtained in the presence of Mg^{2+} was similar to that ($52.1 \pm 3.7\%$, $n = 5$) of red ghosts prepared in the absence of Mg^{2+}.

3.4. Conformational changes of membrane proteins in NEM-treated ghosts

To explain the hemolytic properties of diamide- or NEM-treated erythrocytes at 200 MPa, the conformational changes of membrane proteins were examined by the CD measurement. However, the CD spectra of NEM-treated ghosts did not change before and after the incubation (1 h) at 37°C (data not shown). Similar results were obtained in SDS-PAGE of NEM-treated ghosts (data not shown).

The spin-label method can sensitively detect the conformational changes in membrane proteins [1,13]. So, we attempted to examine the structural changes in NEM-treated ghosts by this method. The NEM (0.5 mM)-treated ghosts were incubated for 1 h at 0 or 40°C, and then labeled with a SH-reactive maleimide spin label. The EPR signal amplitude of ghosts incubated at 40°C was larger than that obtained at 0°C (Fig. 3A). Therefore, similar experiments were performed at various temperatures. In the temperature range of 0–30°C the EPR signal amplitude was weak, but at higher temperatures the signal amplitude was enhanced (Fig. 3B). This indicates that SH-groups which are buried in proteins appear on their surface upon incubation at higher temperatures (above 30°C). To confirm this hypothesis, 2 mM NEM-pretreated erythrocytes were incubated for 1 h at 0 or 37°C in a reagent-free medium and then treated with 0.1 mM NEM at 0°C. The concentration of membrane SH-groups in ghosts prepared from these erythrocytes was decreased by the incubation at 37°C than 0°C (Table 2).

3.5. Extraction of membrane proteins

NEM (0.5 mM)-treated ghosts were incubated for 1 h at 0 or 37°C in a reagent free medium and then cytoskeletal

Table 1
High pressure-induced hemolysis and phospholipid distribution in NEM-treated erythrocytes

Treatment		% Hemolysis	% PE in the outer leaflet [a]
1st (reagent)	2nd (incubation in buffer)		
(a) Intact erythrocyte			
None	37°C	36.5 ± 2.7 ($n = 3$)	18.3 ± 6.0 ($n = 5$)
2 mM NEM	0°C	29.8 ± 0.4 ($n = 3$)	15.4 ± 6.0 ($n = 2$)
2 mM NEM	37°C	15.9 ± 2.5 ($n = 3$)	20.0 ± 1.0 ($n = 2$)
(b) Resealed ghost			
None	37°C		53.8 ($n = 1$)

Intact erythrocytes were treated with 2 mM NEM for 30 min at 37°C and then incubated in PBS for 1 h at 0 or 37°C. For phospholipid distribution, intact erythrocytes, NEM-treated ones, or resealed ghosts were labeled with 0.1 mM DIDS and then with 10 mM TNBS. Values are expressed as means ± S.D.

[a] Results are given as a percentage of total phosphatidylethanolamine (PE).

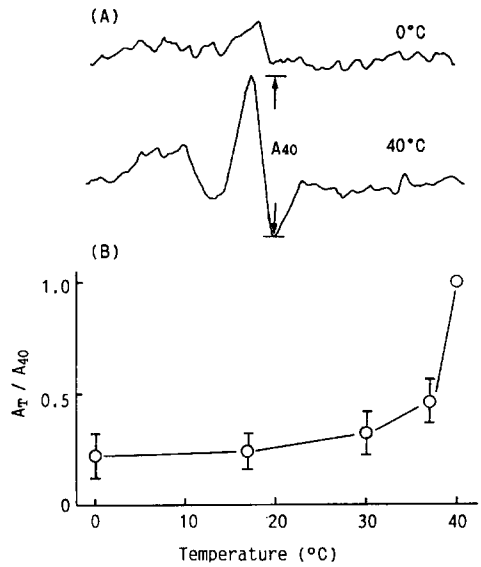

Fig. 3. EPR spectra of ghost membranes treated with NEM and then maleimide spin label (A), and the effect of incubation temperature on EPR signal amplitude (B). Resealed ghosts were treated with 0.5 mM NEM for 30 min at 37°C and then incubated for 1 h at various temperatures (0–40°C) in PBS. These ghosts were spin-labeled. A_T is the EPR signal amplitude of NEM-treated ghosts which were incubated at any temperature and then spin-labeled.

proteins were extracted from the ghost membrane by using 0.1 N NaOH. The amounts of extracted proteins from the ghosts incubated at 0 or 37°C were 39.2 ± 0.6% ($n = 2$) or 31.5 ± 2.4% ($n = 2$), respectively.

3.6. Intracellular K^+ concentration

The intracellular K^+ concentration of NEM (2 mM)-treated erythrocytes was examined by using ^{39}K-NMR. The NEM-treated erythrocytes were incubated for 1 h at 37°C in PBS. However, the intracellular K^+ concentration remained constant irrespective of the prolonged incubation (data not shown).

Table 2
Determination of membrane SH-groups as a function of temperature in NEM-treated ghosts

Treatment			SH- groups remaining (nmol/mg protein)
1st (reagent)	2nd (incubation in buffer)	3rd (reagent)	
None	0°C	None	79.7 ± 4.1($n = 3$)
2 mM NEM	0°C	None	51.7 ± 2.3($n = 3$)
2 mM NEM	37°C	None	51.7 ± 0.7($n = 3$)
2 mM NEM	0°C	0.1 mM NEM	53.0 ± 1.3($n = 2$)
2 mM NEM	37°C	0.1 mM NEM	44.8 ± 1.6($n = 3$)

Intact erythrocytes (10% hematocrit) were treated with 2 mM NEM for 10 min at 37°C, incubated in PBS for 1 h at 0 or 37°C, and finally treated with 0.1 mM NEM for 30 min at 0°C. Ghost membranes were prepared from these erythrocytes. Values are expressed as means ± S.D.

4. Discussion

In the present work we have demonstrated that the hemolysis at 200 MPa of NEM- or diamide-treated erythrocytes is significantly suppressed when these red cells are additionally incubated above 30°C in a reagent-free medium prior to the application of high pressure. To clarify the cause of such hemolytic properties, membrane phospholipid distribution, conformational changes of membrane proteins, extraction of cytoskeletal proteins from the membrane and K^+ efflux in chemically modified erythrocytes were examined.

Membrane phospholipids in intact erythrocytes are asymmetrically distributed, i.e., choline phospholipids such as phosphatidylcholine and sphingomyelin are mainly located in the outer leaflet, whereas aminophospholipids such as phosphatidylserine and phosphatidylethanolamine are predominantly distributed in the inner leaflet [15–17]. Such an asymmetry of phospholipids is maintained by the aminophospholipid translocase [18] and cytoskeletal proteins [19,20]. The activity of aminophospholipid translocase is inhibited by SH-reactive agents such as NEM and diamide [21]. Haest et al. reported that the aminophospholipids in diamide-treated erythrocytes are readily translocated from the inner leaflet to the outer leaflet [22]. On the other hand, Middelkoop et al. [23] showed that the translocation of aminophospholipids in diamide-treated erythrocytes occurs when the intracellular ATP content is decreased below 10% of its original value. In our case, when diamide (2 mM)-treated erythrocytes were incubated in PBS for 1 h at 37°C, the intracellular ATP content remained about 60% of its original level (Yamaguchi et al., unpublished data). This suggests that no translocation of aminophospholipids takes place during prolonged incubation of diamide-treated erythrocytes. In NEM-treated erythrocytes, similar results may be expected. Indeed, the phospholipid distribution in NEM-treated erythrocytes did not change upon prolonged incubation. These results suggest that there is no contribution of phospholipids to hemolytic properties described here.

Recently, we have found that high pressure-induced hemolysis is also affected by intracellular viscosity, or cell volume (Yamaguchi et al., unpublished data). For instance, upon increment of intracellular Ca^{2+} concentration, K^+ efflux occurs and intracellular viscosity increases [24,25]. In such erythrocytes, their membranes become more stable to high pressure. So, we have examined by using ^{39}K-NMR whether the K^+ efflux occurs during prolonged incubation of NEM-treated red cells. Intracellular K^+ concentration in NEM-treated erythrocytes remained constant irrespective of the incubation.

Previously, we showed that high pressure-induced hemolysis is greatly suppressed by cross-linking of transmembrane proteins with the cytoskeleton, but not by cross-linking of spectrin only [4]. When red cells are treated with low concentration (0.1–0.5 mM) of diamide at

atmospheric pressure, spectrin is mainly cross-linked. On the other hand, large-molecular-weight aggregates composed of transmembrane proteins and cytoskeleton are formed by treatment of red cells with diamide under pressure [4]. In the present work, high concentration of diamide (2 mM) was used at atmospheric pressure. When red cells were compressed without prolonged incubation after diamide treatment, the values of hemolysis at 200 MPa were increased, compared to that of intact erythrocytes. However, upon prolonged incubation, the hemolysis at 200 MPa was greatly suppressed. Similar results were obtained from NEM-treated erythrocytes. Therefore, these results suggest that cross-linking of spectrin is not directly associated with the decrease of hemolysis observed here. Thus, to analyze such hemolytic properties, NEM-treated erythrocytes are more appropriate than diamide-treated ones in that the effect of cross-linking of membrane proteins on hemolytic properties is excluded.

The CD spectra and SDS-PAGE of membrane proteins in NEM-treated ghosts did not change upon prolonged incubation. The EPR spectrum of SH-reactive maleimide spin label reflects sensitively conformational changes of proteins [13]. NEM-treated ghosts were incubated as a function of temperature and then spin-labeled. The EPR signal amplitude increased upon incubation above 30°C, as seen in temperature dependence of hemolysis (Fig. 2). One possible explanation for the EPR spectral changes is as follows. When NEM-treated ghosts are incubated at higher temperatures (above 30°C), conformational changes of membrane proteins occur so that the SH-groups which are buried in proteins are appeared on the surface. Thus, exposed SH-groups react readily with a maleimide spin label. This hypothesis was supported by determining the content of membrane SH-groups. These results suggest that the reorganization of membrane proteins in NEM-treated ghosts or erythrocytes occurs during the incubation above 30°C. Taking into account that membrane stability to high pressure is dependent on the interaction among membrane proteins, hemolytic properties seen in NEM- or diamide-treated erythrocytes may be explicable in terms of protein–protein interactions modulated by conformational changes of membrane proteins. Provided that there are the tight interactions among membrane proteins in such erythrocytes, it should be difficult to extract cytoskeletal proteins from the membrane by using an alkaline medium. Indeed, upon incubation at 37°C, the extraction of cytoskeleton from NEM-treated membranes was suppressed.

Properties of high pressure-induced hemolysis have been compared with those of osmotic hemolysis. The former is affected significantly by chemical and enzymatic modifications of membrane proteins but the latter is not so [4,5]. Thus, compared to osmotic hemolysis, high pressure-induced hemolysis is more sensitive to membrane protein–protein interactions. The difference of hemolytic properties seen upon prolonged incubation of NEM- or diamide-treated erythrocytes also suggests that the mechanism of hemolysis by both methods is quite different.

In summary, we have demonstrated that when the erythrocytes in which membrane SH-groups are modified with NEM or diamide are incubated above 30°C, the conformational changes of membrane proteins occur so that the interactions among membrane proteins are modulated (perhaps, tightened). Thus, the present data suggest what happens in the membrane structure if membrane SH-groups are oxidized, i.e., by reduction of intracellular glutathione level.

Acknowledgements

This work was partly supported by a grant from the Central Research Institute of Fukuoka University.

References

[1] Yamaguchi, T., Kawamura, H., Kimoto, E. and Tanaka, M. (1989) J. Biochem. 106, 1080–1085.
[2] Yamaguchi, T., Kajikawa, T. and Kimoto, E. (1991) J. Biochem. 110, 355–359.
[3] Yamaguchi, T., Saeki, T. and Kimoto, E. (1993) Biochim. Biophys. Acta 1147, 1–5.
[4] Kitajima, H., Yamaguchi, T. and Kimoto, E. (1990) J. Biochem. 108, 1057–1062.
[5] Yamaguchi, T., Matsumoto, M. and Kimoto, E. (1993) J. Biochem. 114, 576–581.
[6] Lieber, M.R. and Steck, T.L. (1982) J. Biol. Chem. 257, 11660–11666.
[7] Rice-Evans, C. (1990) in Blood Cell Biochemistry: Erythroid Cells (Harris, J.R., ed) Vol. 1, pp. 429–453, Plenum Press, New York.
[8] Dodge, J.T., Mitchell, C. and Hanahan, D.J. (1963) Arch. Biochem. Biophys. 100, 119–130.
[9] Steck, T.L. and Kant, J.A. (1974) Methods Enzymol. 31, 172–180.
[10] Rose, H.G. and Oklander, M. (1965) J. Lipid Res. 6, 428–431.
[11] Laemmli, U.K. (1970) Nature 227, 680–685.
[12] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[13] Yamaguchi, T., Koga, M., Takehara, H. and Kimoto, E. (1982) FEBS Lett. 141, 53–55.
[14] Deuticke, B., Poser, B., Lütkemeier, P. and Haest, C.W.M. (1983) Biochim. Biophys. Acta 731, 196–210.
[15] Verkleij, A.J., Zwaal, R.F.A., Roelofsen, B., Comfurius, P, Kastelijn, D. and Van Deenen, L.L.M. (1973) Biochim. Biophys. Acta 323, 178–193.
[16] Devaux, P.F. (1991) Biochemistry 30, 1163–1173.
[17] Schroit, A.J. and Zwaal, R.F.A. (1991) Biochim. Biophys. Acta 1071, 313–329.
[18] Seigneuret, M. and Devaux, P.F. (1984) Proc. Natl. Acad. Sci. USA 81, 3751–3755.
[19] Sato, S.B. and Ohnishi, S. (1983) Eur. J. Biochem. 130, 19–25.
[20] Rybicki, A.C., Heath, R., Lubin, B. and Schwartz, R.S. (1988) J. Clin. Invest. 81, 255–260.
[21] Connor, J. and Schroit, A.J. (1990) Biochemistry 29, 37–43.
[22] Haest, C.W.M., Plasa, G., Kamp, D. and Deuticke, B. (1978) Biochim. Biophys. Acta 509, 21–32.
[23] Middelkoop, E., Van der Hoek, E.E., Bevers, E.M., Comfurius, P., Slotboom, A.J., Op den Kamp, J.A.F., Lubin, B.H., Zwaal, R.F.A. and Roelofsen, B. (1989) Biochim. Biophys. Acta 981, 151–160.
[24] Eriksson, L.E.G. and Beving, H. (1993) Arch. Biochem. Biophys. 303, 296–301.
[25] Johnson, R.M. and Tang, K. (1992) Biochim. Biophys. Acta 1107, 314–318.