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Influence of 2,3-diphosphoglycerate on the deformability of human erythrocytes

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Effect of 2,3-diphosphoglycerate (2,3-DPG) on the deformability of human erythrocytes was examined with a rheoscope under shear stress of 8–82 dyn/cm^2. With increasing 2,3-DPG in erythrocytes (from 5 to 15 mM/l cells) by incubating with inosine and pyruvate in isotonic 50 mM phosphate-buffered saline, erythrocyte deformability under uniform shear stress was remarkably impaired. But reduction of 2,3-DPG (from 5 to 2.2 mM/l cells) did not affect the deformability. In 2,3-DPG-enriched erythrocytes, increased intracellular hemoglobin concentration (MCHC), decreased intracellular pH, and increased contents of ATP and IMP (and ITP) were observed. (1) When the MCHC (i.e., the internal viscosity) was normalized by suspending in hypotonic medium, the deformability of 2,3-DPG-enriched erythrocytes was greatly improved, but still decreased. (2) The change of intracellular pH between 6.5 and 7.5 (as compared adjusting to same MCHC) did not alter the deformability. (3) The changes of purine nucleotides, ATP (0.6–2.1 mM/l cells), IMP (0–0.9 mM/l cells) and ITP (0–0.5 mM/l cells) did not alter the erythrocyte deformability. In conclusion, decreased deformability of erythrocytes induced by augmentation of 2,3-DPG is due mainly to the increased internal viscosity and due partly to the increased membrane viscoelasticity.

Introduction

The physiological importance of 2,3-diphosphoglycerate (2,3-DPG) in human erythrocytes lies in the increase in oxygen delivery to tissues by decreasing the interaction of oxygen and hemoglobin. The rheologically important factor for oxygen delivery to tissues is blood flow. Decrease of erythrocyte deformability not only reduces the blood flow due to increasing the blood viscosity, but also impairs the capillary passage of erythrocytes [1]. The decreased deformability also makes diffusion barrier of oxygen around erythrocytes [2]. It is generally recognized that the increase of 2,3-DPG in erythrocytes increases oxygen delivery to tissues, but the rheological behavior of such erythrocytes is not well understood. Some rheological studies using erythrocyte ghosts (and/or intact erythrocytes) are still controversial in the effect of 2,3-DPG on the erythrocyte deformability [3,4].

In this paper, the decreased deformability of 2,3-DPG-enriched erythrocytes is observed with a rheoscope. The cause of the impaired deformability is examined for the following characteristics of 2,3-DPG-enriched erythrocytes prepared by incubating with inosine and pyruvate in isotonic 50 mM phosphate-buffered saline: (1) increased intracellular hemoglobin concentration, (2) increases of some purine nucleotides (ATP, IMP, ITP), and (3) decreased intracellular pH. The changes of internal viscosity and membrane viscoelasticity in 2,3-DPG-enriched erythrocytes are discussed with respect to the high shear deformability.

Materials and Methods

Preparation of 2,3-DPG-varied erythrocytes

Fresh human blood was collected from cubital vein of healthy adult donors and was heparinized. Plasma and buffy coat were discarded after centrifugation at $1000 \times g$ for 10 min at 4°C. The erythrocytes were washed four times with isotonic 50 mM phosphate-buffered saline (50 mM sodium phosphate, 90 mM NaCl, 5 mM KCl (pH 7.4); 285 mosM, as determined with Halbmikroosmometer (Knauer, type M, F.R.G.) by a freezing point depression method).

Abbreviations: DPG, diphosphoglycerate; MCV, mean corpuscular volume; MCH(C), mean corpuscular hemoglobin (concentration).

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To increase 2,3-DPG content in erythrocytes, washed erythrocytes (the hematocrit, 20%) were incubated with 1–20 mM inosine, 10 mM pyruvate and 5.5 mM D-glucose in 50 mM phosphate-buffered saline (pH 7.4; the osmolality was adjusted by decreasing the concentration of NaCl) for 90 min at 37°C with a modified method of Deuticke et al [5]. On the other hand, 2,3-DPG in erythrocytes was decreased by incubating washed erythrocytes (the hematocrit, 20%) in isotonic phosphate-buffered saline of pH 6.5 for 90 min. The control erythrocytes were obtained by incubating without inosine and pyruvate.

Determination of organic phosphates in erythrocytes

2,3-DPG. An enzymatic method of Maeda et al. [6] was adopted for the determination of 2,3-DPG.

Purine nucleotides. Purine nucleotides were extracted from a known amount of erythrocytes (0.2 ml packed cells) by treating with cold 10% trichloroacetic acid, according to the method of Bartlett [7]. Quantitative measurement of nucleotides was carried out with a liquid chromatograph (Shimadzu, Model LC-3A or LC-6A, Kyoto, Japan) essentially by a method of Maeda et al. [8], but a DEAE-2SW column (an anion-exchanger column, from Toso, Tokyo, Japan; column size, 4.6 mm × 25 cm) was used for the separation of nucleotides at 40°C. After applying a known amount of extract (40 μl) to the column equilibrated with 1 vol of acetonitrile + 9 vol. of 140 mM KH_2PO_4 (adjusted to pH 3.0 with phosphoric acid), nucleotides were eluted at a flow rate of 0.9 ml/min by a linear gradient of KH_2PO_4 (pH 3) at the increasing rate of 8 mM/min in the presence of 10% acetonitrile. The eluent was monitored at 254 nm. The peak area on the chromatogram was simultaneously calculated with a computer (Shimadzu, Chromatopack C-E1B, Kyoto, Japan). The content of purine nucleotides in the extract was calculated from the peak area by comparing with those of standard nucleotides.

Measurement of erythrocyte deformability

Erythrocyte deformability was measured at 24°C with a high-shear 'rheoscope' [9] consisting of an inverted microscope (Olympus Optics Co., Model IMT, Tokyo, Japan), a transparent 0.8° cone-plate viscometer (Tokyo Keiki, Model B, Tokyo, Japan), a flash light (Sugawara, Model PS-240, Tokyo) and a camera [10,11].

Erythrocytes were suspended in isotonic phosphate-buffered saline containing 16.5 g/dl Dextran T-40 (Pharmacia, Uppsala, Sweden). The final hematocrit was adjusted to 0.4% to observe cellular deformation. The shear stress was changed stepwise, ranging from 8 to 82 dyn/cm^2.

Lengths of the long axis (L) and the short axis (S) of more than 50 ellipsoidally deformed cells were measured on flash photographs by using a digitizer (Graphtec Lab., Mitablet-II, Tokyo, Japan). The degree of deformation of erythrocytes ('deformability') was expressed by the ratio, S/L.

Hematological examinations

Hematocrit was determined with a microhematocrit centrifuge (Kubota, Model KH-120MII, Tokyo, Japan), hemoglobin concentration by the CN-methemoglobin method [12], and number of erythrocytes with an automatic counter (Toa, Model CC-110, Kobe, Japan). On the basis of these values, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated. The volume of packed saline in the measurement of hematocrit was not corrected, since the value was less than 2%.

Measurement of intracellular pH

The pH in erythrocytes was determined with a microelectrode (Chemical Manufacturing Co., Model SE-1700 GC, Tokyo, Japan) by a freezing-thawing method of Enoki et al. [13].

Statistical analysis

Data are presented as mean ± S.D., and the statistical significance was evaluated by Student's t-test.

Results

Hematological and biochemical properties of 2,3-DPG-altered erythrocytes

2,3-DPG in erythrocytes was increased by incubating with various concentrations of inosine (0–20 mM) and 10 mM pyruvate in isotonic 50 mM phosphate-buffered saline (pH 7.4) for 90 min. The hematological and the biochemical alterations are summarized in Fig. 1.

2,3-DPG. With increasing the concentration of inosine in the incubation medium, 2,3-DPG in erythrocytes increased, but the value reached plateau at 5 mM inosine in this experimental condition. The prolonged incubation further increased the 2,3-DPG content, but the content did not increase more than 120% of the

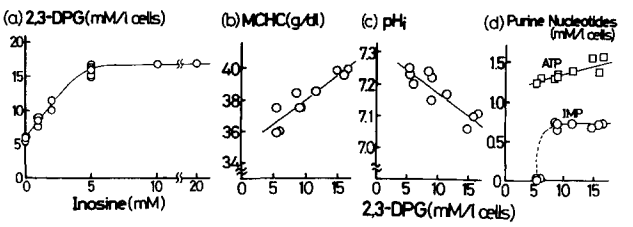

Fig. 1. Changes of 2,3-DPG in erythrocytes and the hematological and biochemical alterations of erythrocytes. (a) Change of 2,3-DPG in erythrocytes. Human erythrocytes (hematocrit, 20%) were incubated with 0–20 mM inosine and 10 mM pyruvate in isotonic phosphate-buffered saline (pH 7.4) at 37°C for 90 min. (b) Change of MCHC. (c) Change of intracellular pH (pH_i). (d) Changes of purine nucleotides: ATP (□), IMP (○).

value at 90 min incubation. 2,3-DPG content in control erythrocytes was not altered during incubation of 90 min.

Mean corpuscular hemoglobin concentration (MCHC). With increasing 2,3-DPG in erythrocytes, MCHC increased and MCV inversely decreased. A linear relation between MCHC (g/dl) and 2,3-DPG (mM/l cells), MCHC = 0.31 [2,3-DPG] + 34.9 ($r = 0.915$), was obtained. The MCHC of control cells in the present medium slightly increased in comparison with that of fresh erythrocytes (probably due to cellular dehydration induced by incorporated inorganic phosphate into erythrocytes [14]).

Intracellular pH (pH_i). With increasing 2,3-DPG content in erythrocytes, pH_i decreased, due to the alteration of Donnan equilibrium induced by the increment of impermeable 2,3-DPG in erythrocytes [15]. A linear relation between pH_i and 2,3-DPG content (mM/l cells), $pH_i = -0.014$ [2,3-DPG] + 7.312 ($7.10 < pH_i < 7.30$; $r = -0.880$), was obtained.

ATP content. With increasing the concentration of inosine in the medium, intracellular ATP concentration slightly increased, but the concentration of ADP and AMP was not altered so much.

Inosine monophosphate (IMP) and inosine triphosphate (ITP). The concentration of IMP and ITP increased to 0.7 and 0.5 mM/l cells, respectively but that of inosine diphosphate (IDP) was less than 0.03 mM/l cells (the content of these purine nucleotides in fresh erythrocytes was negligible). The concentration of inosine nucleotides reached plateau at 1 mM inosine under the present experimental conditions.

In order to decrease 2,3-DPG content, erythrocytes were incubated in isotonic phosphate-buffered saline without glucose at pH 6.5. 2,3-DPG decreased to 70% and 40% of original level during incubation time of 90 and 180 min, respectively (a further decrease of 2,3-DPG induced some echinocytosis). During incubation, level of purine nucleotides was not significantly altered, but MCHC slightly decreased.

Deformability of 2,3-DPG-altered erythrocytes

With increasing 2,3-DPG concentration in erythrocytes, the deformability of erythrocytes was remarkably impaired, and undeformable erythrocytes appeared even under shear stress of 82 dyn/cm^2. Most of erythrocytes containing 15 mM 2,3-DPG/l cells were undeformable, as shown in Fig. 2. The decreased deformability of 2,3-DPG-enriched erythrocytes were analyzed on the basis of hematological and biochemical alterations described above: MCHC, pH_i, ATP and IMP (and ITP). Echinocytosis, a determinant factor of erythrocyte deformability [10], was not induced under the present experimental conditions (all experiments were performed within 6 h after collection of blood).

MCHC. Erythrocyte deformability is greatly affected by the internal viscosity of erythrocytes. MCHC is a major determinant of internal viscosity in normal erythrocytes.

In order to eliminate the effect of MCHC, 2,3-DPG-enriched erythrocytes were suspended in hypotonic solution and the MCHC was adjusted to that of control erythrocytes by introducing water into erythrocytes. The deformability of 2,3-DPG-enriched erythrocytes was remarkably improved, and the erythrocytes became deformable. Therefore, increased MCHC was a major cause of decreased deformability of 2,3-DPG-enriched erythrocytes.

The deformability of 2,3-DPG-enriched erythrocytes and control erythrocytes was compared at same MCHC. A representative result is shown in Fig. 3. With increasing shear stress, the deformability of erythrocytes in-

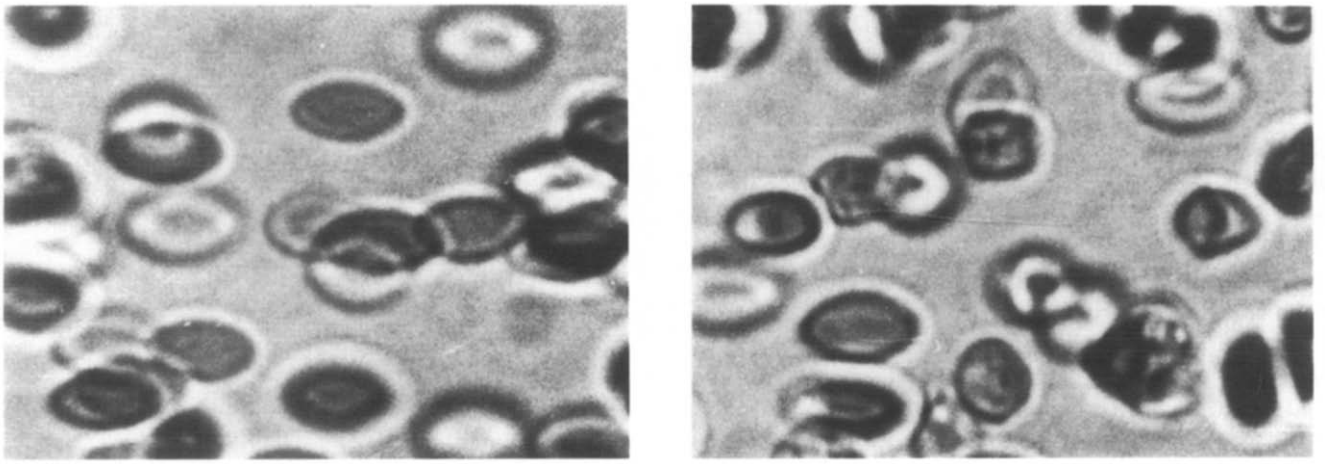

Fig. 2. Flash microphotographs of erythrocytes under high shear stress observed with a rheoscope. Erythrocytes were suspended in isotonic phosphate-buffered saline containing 16.5 g/dl Dextran T-40 (9.3 cP). A shear stress of 33 dyn/cm^2 was applied at 24°C. (Left) Deformation of control erythrocytes (2,3-DPG concentration, 5.5 mM/l cells). (Right) Deformation of 2,3-DPG-enriched erythrocytes (2,3-DPG concentration, 15 mM/l cells).

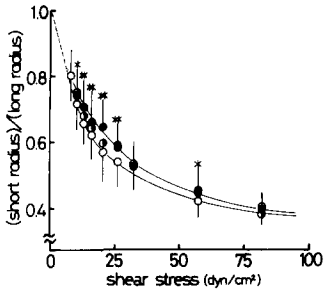

Fig. 3. Effect of 2,3-DPG on the erythrocyte deformability. Deformability of 2,3-DPG-enriched cells (15 mM 2,3-DPG/l cells, ●; 10 mM 2,3-DPG/l cells, ◑) and control cells (5 mM 2,3-DPG/l cells, ○) was determined with a rheoscope in osmotically controlled medium containing 16.5 g/dl Dextran T-40 (9.3 cP) at 24°C. Prior to the measurement, the increased MCHC of 2,3-DPG-enriched cells in isotonic medium was osmotically normalized by suspending in appropriate hypotonic medium (the MCHC was reduced from 40.2 to 36.3 g/dl in 15 mM 2,3-DPG-cells and from 38.8 to 36.6 g/dl in 10 mM 2,3-DPG cells. The MCHC in control cells was 36.2 g/dl). The deformability was expressed by mean of (short radius)/(long radius) ratio measured for more than 50 ellipsoidally deformed cells (symbol) and the standard deviation (bar). A representative result obtained from one blood donor is shown in the figure. Statistical significance between ● and ○: *, $P < 0.05$; **, $P < 0.01$. Before normalization of MCHC, undeformable cells on the photographs were more than 95% in 15 mM 2,3-DPG-cells, about 50% in 10 mM 2,3-DPG-cells and less than 5% in control cells, at a shear stress of 21 dyn/cm^2 ($\approx$ 80%, $\approx$10% and < 2%, respectively, at 82 dyn/cm^2).

creased, but the deformability of 2,3-DPG-enriched erythrocytes was clearly reduced, as compared with that of control erythrocytes, especially under low shear stress. The decreased deformability of 2,3-DPG-enriched erythrocytes at normalized MCHC was qualitatively reproducible on separate experiment, though the degree of deformability of 2,3-DPG-enriched and control erythrocytes was different among different individuals. In this connection, the decreased deformability of erythrocytes exposed to hypertonic medium (thus, with high MCHC) was completely reversed by exposing the erythrocytes to isotonic medium again. The deformability of 2,3-DPG-reduced erythrocytes was indistinguishable from that of control erythrocytes.

Intracellular pH (pH_i). The pH_i of control erythrocytes was altered by suspending in isotonic phosphate-buffered saline of various pH (pH_e). Since the shape of erythrocytes and the MCHC are dependent on pH [16,17], the deformability was compared under same MCHC by suspending erythrocytes in medium of different osmolality. Variation in pH_i between 6.8 and 7.6 did not alter the deformability of erythrocytes.

ATP. The effect of ATP on the erythrocyte deformability is still controversial [3,4] and it is not well understood. In order to examine the contribution of ATP on the deformability of 2,3-DPG-enriched and/or -decreased erythrocytes, ATP concentration in erythrocytes was varied by a modified method of Bartlett and Bucolo [18]: ATP content was increased up to 2 mM/l cells by

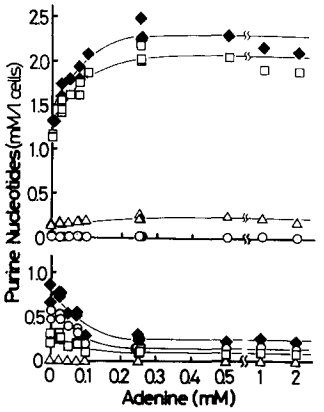

Fig. 4. Changes of purine nucleotides in erythrocytes in adenine-inosine-phosphate medium. Erythrocytes (hematocrit, 20%) were incubated with 0–2 mM adenine and 1 mM inosine in isotonic phosphate-buffered saline at 37°C for 90 min. (Upper) ATP (□), ADP (△), AMP (○) and ATP+ADP+AMP (◆); (lower) ITP (□), IDP (△), IMP (○) and ITP+IDP+IMP (◆).

incubating erythrocytes (the hematocrit, 20%) with various concentrations of adenine (0.025–2 mM), 1 mM inosine and 5.5 mM glucose in phosphate-buffered saline, at 37°C for 90 min (simultaneously, IMP and ITP increased at low concentration of adenine and IDP was scarcely detected, but the increment of IMP and ITP was slight at high concentration of adenine) (Fig. 4). On the other hand, the ATP content was decreased up to 0.5 mM/l cells by incubating erythrocytes (the hematocrit, 20%) in isotonic phosphate-buffered saline of alkaline pH (pH 7.4 or 8) at 37°C for 90 min.

The deformability of erythrocytes was not affected by the ATP concentration between 0.5–2.2 mM/l cells at all shear stresses examined here.

IMP (and ITP). Inosine nucleotides were increased in 2,3-DPG-enriched and ATP-enriched erythrocytes under above procedures. In order to examine the effect

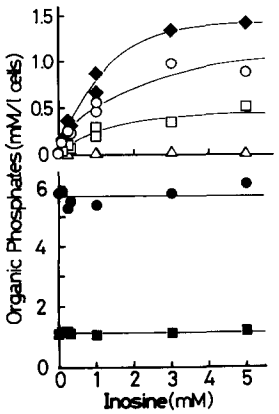

Fig. 5. Changes of purine nucleotides in erythrocytes in inosine-phosphate medium. Erythrocytes (hematocrit, 20%) were incubated with 0–5 mM inosine in isotonic phosphate-buffered saline at 37°C for 90 min. (Upper) ITP (□), IDP (△), IMP (○) and ITP+IDP+IMP (◆); (lower) 2,3-DPG (●) and ATP (■).

of these nucleotides on the erythrocyte deformability, erythrocytes (the hematocrit, 20%) were incubated with various concentrations of inosine (0.25–5 mM) and 5.5 mM glucose in isotonic phosphate-buffered saline at 37°C for 90 min.

IMP and ITP were selectively increased up to 0.9 and 0.5 mM/l cells, respectively, without altering the concentration of 2,3-DPG and ATP (Fig. 5). However, the deformability of these erythrocytes was not affected by the variation of these nucleotides.

Discussion

Physiologically, 2,3-DPG is one of the most important organic phosphates in erythrocytes. As 2,3-DPG lowers the affinity of hemoglobin toward oxygen, oxygen transfer from blood to tissues in microcirculation is increased. Thus much effort has been expended in preserving and/or augmenting 2,3-DPG in erythrocytes in blood preservation. However, the rheological consequences of such treatment in increasing 2,3-DPG in erythrocytes are not well understood.

Interaction of 2,3-DPG with membrane protein, spectrin, has been shown by Shaklai et al. [19]. Some studies have been reported on the interaction between 2,3-DPG and erythrocyte ghosts. Sheetz and Casaly [20,21] have observed that high concentration of 2,3-DPG dissociates spectrin-actin-band 4.1 complex in cytoskeletal meshwork, and they have suggested that 2,3-DPG controls lability in the meshwork, and thus cell deformability [21]. Schindler et al. [22] have also shown that high concentrations of 2,3-DPG increase lateral mobility of membrane glycoproteins through modifications of interactions among cytoskeletal proteins in ghosts. The effect of 2,3-DPG on the membrane dynamics may be related to the metabolism of triphosphoinositide [23].

Effect of 2,3-DPG on the mechanical properties of erythrocyte membrane has been also investigated by using some rheological techniques. Chasis and Mohandas [3] have observed with an ektacytometer that resealing erythrocyte membranes with increasing concentrations of entrapped 2,3-DPG result in a dose-dependent decrease in their deformability accompanying decreased membrane stability. On the other hand, Waugh [4] has reported no effect of 2,3-DPG on the membrane viscoelastic properties in both intact erythrocytes and the ghosts under physiological conditions using micropipette aspiration and flow channel techniques. However, in specified medium of low ionic strength he has detected the reduction of membrane shear modulus in 2,3-DPG-enriched erythrocytes (prepared by incubation in inosine-pyruvate-phosphate, as we did). These controversial effect of 2,3-DPG on the erythrocyte membrane must be resulted mainly from the methodological differences (as also discussed below). On the rheological characteristics of ghosts, Kon et al. [11] have demonstrated that the deformability of resealed ghosts measured with a rheoscope is greatly impaired due to the alteration of cytoskeletal structure of erythrocyte membrane. Therefore, the rheological characteristics of ghosts should be considered separately from that of intact erythrocytes.

The deformability of erythrocytes is affected by (i) internal viscosity of erythrocytes, mainly intracellular hemoglobin concentration (MCHC), (ii) shape, physiologically surface area to volume ratio of erythrocytes, (iii) viscoelasticity of erythrocyte membrane. In the present study, it was demonstrated with a rheoscope that decreased deformability of 2,3-DPG-enriched erythrocytes was derived from both increased internal viscosity and increased membrane viscoelastic properties.

(i) Internal viscosity. Increased intracellular hemoglobin concentration greatly contributed to the decreased deformability of 2,3-DPG-enriched erythrocytes, as shown in the present experiment. The phenomenon is due to concomitant release of chloride and water from erythrocytes induced by the uptake of inorganic phosphate for the synthesis of 2,3-DPG [14,15,24].

(ii) Shape. Dehydration of erythrocytes due to elevation of 2,3-DPG decreases the cell volume, thus surface area to volume ratio increases. This shape change rather increases the erythrocyte deformability, and is ruled out from a cause for the decreased deformability of 2,3-DPG-enriched erythrocytes. Echionocytosis, which also decreases the deformability, was not induced in the present experimental conditions.

(iii) Membrane viscoelasticity. In the present experiment, the MCHC of 2,3-DPG-enriched erythrocytes was adjusted to that of control cells by suspending in hypotonic solution (i.e., by hydrating). Thus, the shape (or the geometry) and the internal viscosity of 2,3-DPG-enriched erythrocytes was equalized to those of control erythrocytes. In spite of this procedure, the deformability of 2,3-DPG-enriched erythrocytes was still decreased. This phenomenon clearly demonstrates that the membrane viscoelasticity in 2,3-DPG-enriched erythrocytes is increased. Furthermore, it was shown that the increased membrane viscoelasticity did not result from the decreased intracellular pH and the increase of some purine nucleotides (ATP, IMP and ITP) in 2,3-DPG-enriched erythrocytes. Altered interactions among cytoskeletal proteins by 2,3-DPG [20,22] may contribute to the increased membrane viscoelasticity, in part.

Increased membrane viscoelasticity of 2,3-DPG-enriched erythrocytes in our experiment is in disagreement with the findings of Waugh [4] performed for intact cells. This discrepancy seems to come from the difference of methodology and/or medium suspending

erythrocytes. The rheoscope detects whole-cell deformability, including factors of morphology, membrane viscoelasticity and internal viscosity, while the micropipette technique detects mainly membrane properties. In our measurement, as the effect of shape and internal viscosity could be excluded (see above), the phenomena observed with the rheoscope reflect any impairment of membrane viscoelasticity in 2,3-DPG-enriched erythrocytes. The discrepancy may really reveal the methodological difference: e.g., the rheoscope may detect the interaction between cytoplasmic components (such as 2,3-DPG, hemoglobin) and membrane, while the micropipette technique hardly detects such interaction.

In the rheoscopic analysis, erythrocytes are suspended in non-ionic polysaccharide, Dextran T-40. Therefore, the results obtained with the rheoscope may partly reflect Waugh's results [4], i.e., 2,3-DPG reduces the membrane shear modulus in low ionic strength medium containing sucrose.

ATP contributes to the maintenance of the shape and the erythrocyte deformability [25–27], but the detailed mechanism is unknown. Sheetz and Casaly [20] have reported that high concentration of ATP (about 8 mM) induces conformational changes of membrane proteins in cytoskeletal meshwork, using erythrocyte ghosts. On the other hand, Card et al. [28] and Meiselman and Baker [29] have observed that the depletion of ATP does not impair the erythrocyte deformability. Further, Meiselman et al. [30] have shown that membrane mechanical properties are not altered by ATP depletion using micropipete and flow channel techniques. In agreement with Meiselman et al. [30], we observed that the variation of ATP in 0.5 to 2.3 mM/l cells did not affect the erythrocyte deformability and the cell shape. Therefore, direct effect of ATP on the membrane deformability (including cellular deformability) is ruled out in this range of ATP variation. The role of ATP on the erythrocyte deformability may be important through the interaction with calcium ion, then with cytoskeletal proteins [27,31–34] and through phosphorylation of membrane proteins [34].

In conclusion, augmentation of 2,3-DPG over physiological concentration may further accelerate oxygen transfer to tissues, but blood flow to the tissues may be reduced through decreased deformability of the erythrocytes for the sake of increased internal viscosity and increased membrane viscoelastic property.

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References

1 Chien, S. (1987) Annu. Rev. Physiol. 49, 177–192.
2 Kon, K., Maeda, N. and Shiga, T. (1983) J. Physiol. 339,573–584.
3 Chasis, J.A. and Mohandas, N. (1986) J. Cell Biol. 103, 343–350.
4 Waugh, R.E. (1986) Blood 68, 231–238.
5 Deuticke, B., Duhm, J. and Dierkesmann, R. (1971) Pflügers Arch. 326, 15–34.
6 Maeda, N., Chang, H., Benesch, R. and Benesch, R.E. (1971) N. Engl. J. Med. 284, 1239–1242.
7 Bartlett, G.R. (1968) Biochim. Biophys. Acta 156, 221–230.
8 Maeda, N., Kon, K., Sekiya, M. and Shiga, T. (1986) Biochem. Pharmacol. 35, 625–629.
9 Schmid-Schönbein, H., Gaehtgens, P. and Hirsch, H. (1967) Pflügers Arch. 297, 107–114.
10 Suda, T., Maeda, N., Shimizu, D., Kamitsubo, E. and Shiga, T. (1982) Biorheology 19, 555–565.
11 Kon, K., Maeda, N. and Shiga, T. (1987) Blood 69, 727–734.
12 Van Kampen, E.J. and Zijlstra, W.G. (1961) Clin. Chim. Acta 6, 538–544.
13 Enoki, Y., Tomita, S., Maeda, N., Kawase, M. and Okuda, T. (1972) J. Physiol. Soc. Japan 34, 761–762.
14 Bookchin, R.M., Lew, D.J., Balazs, T., Ueda, Y. and Lew, V.L. (1984) J. Lab. Clin. Med. 104, 855–866.
15 Duhm, J. (1971) Pflügers Arch. 326, 341–356.
16 Murphy, J.R. (1967) J. Lab. Clin. Med. 69, 758–775.
17 Maeda, N., Seike, M., Suzuki, Y. and Shiga, T. (1988) Biorheology 25, 25–30.
18 Bartlett, G.R. and Bucolo, G. (1968) Biochim. Biophys. Acta 156, 240–253.
19 Shaklai, N., Nenitez, L. and Ranney, H.M. (1978) Am. J. Physiol. 234, C36–C40.
20 Sheetz, M.P. and Casaly, J. (1980) J. Biol. Chem. 255, 9955–9960.
21 Sheetz, M.P. and Casaly, J. (1981) Scand. J. Clin. Lab. Invest. 41, 117–122.
22 Schindler, M., Koppel, D.E. and Sheetz, M.P. (1980) Proc. Natl. Acad. Sci. USA 77, 1457–1461.
23 Sheetz, M.P., Febbroriello, P. and Koppel, D.E. (1982) Nature 296, 91–93.
24 Duhm, J. (1975) Biochim. Biophys. Acta 385, 68–80.
25 Nakao, M., Nakao, T. and Yamazoe, S. (1960) Nature 187, 945–946.
26 LaCelle, P.L. (1970) Semin. Hematol. 7, 355–371.
27 Clark, M.R., Mahandas, N., Feo, C., Jacobs, M. and Shohet, S.B. (1981) J. Clin. Invest. 67, 531–539.
28 Card, R.T., Mahandas, N., Perkins, H.A. and Shohet, S.B. (1982) Transfusion 22, 96–101.
29 Meiselman, H.J. and Baker, R.F. (1977) Biorheology 14, 111–126.
30 Meiselman, H.J., Evans, E.A. and Hochmuth, R.M. (1978) Blood 52, 499–504.
31 Murakami, J., Maeda, N., Kon, K. and Shiga, T. (1986) Biochim. Biophys. Acta 863, 23–32.
32 Weed, R.I., LaCelle, P.L. and Merrill, E.W. (1969) J. Clin. Invest. 48, 795–809.
33 Shiga, T., Sekiya, M., Maeda, N., Kon, K. and Okazaki, M. (1985) Biochim. Biophys. Acta 814, 289–299.
34 Bennett, V. (1989) Biochim. Biophys. Acta 988, 107–121.