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Contribution of glycoproteins to fibrinogen-induced aggregation of erythrocytes

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The contribution of membrane glycoproteins to the velocity of fibrinogen-induced erythrocyte aggregation was examined using a rheoscope combined with a video camera, an image analyzer and a computer. The structure of glycoproteins was modified with proteolytic enzymes, trypsin or α-chymotrypsin. (1) Mild enzymatic treatment of erythrocytes decreased the velocity of erythrocyte aggregation, but more intense treatment increased the velocity remarkably. (2) The erythrocyte aggregation was affected not only by the density of surface negative charge of erythrocytes, but also by the structural changes of glycoproteins. (3) Erythrocyte deformability and the morphological characteristics were not altered by these enzymatic treatments. The physiological significance of glycoproteins of erythrocyte surface for the survival of erythrocytes and for the suspension stability of blood was discussed.

Introduction

Physiological and clinical implications of erythrocyte aggregation in circulation are recently discussed [1,2]. Erythrocytes in circulation aggregate at low shear rates, but the aggregates disintegrate at high shear rates. Erythrocyte aggregates reduce the blood flow at low shear regions by increasing blood viscosity and affect the capillary flow through sludge formation. Factors participating the erythrocyte aggregation are (i) shear stress under flow, (ii) properties of erythrocytes (cell number, deformability, surface charge, shape) and (iii) properties of macromolecules (such as fibrinogen and immunoglobulins in plasma) bridging between adjacent cells.

On the surface structure of erythrocytes, the role of sialic acid in the erythrocyte aggregation has been extensively studied [3,4]. Negative charge of erythrocyte surface, mainly due to the carboxyl group of sialic acid ($pK \approx 2.6$) and partly due to that of acidic amino acid residues ($pK \approx 3.4$) in sialoglycoproteins [5,6], is inhibitory for the erythrocyte aggregation, because of the electrostatic repulsive force between adjacent cells. However, the role of glycoproteins, which is inseparably related to sialic acid, on the erythrocyte aggregation is unknown. A lot of glycoproteins, band 3 and sialoglycoproteins, is present in erythrocyte surface; (i) Band 3, anion transporter (further carrying ABO-blood group antigen), is contained in about 1 000 000 copies per cell and is connected to cytoskeletal meshwork via ankyrin [7]. (ii) Among sialoglycoproteins, α and δ (600 000 and 80 000 copies per cell, respectively [8]) are most abundant and carry blood groups MN and Ss antigen activities, respectively [9]. Sialoglycoprotein β (about 50 000 copies per cell [10]) is associated with band 4.1, a cytoskeletal protein [11].

In the present report, the contribution of glycoproteins on the fibrinogen-induced erythrocyte aggregation was investigated in relation to (i) negative charge density of erythrocyte surface, (ii) structure of glycoproteins and (iii) erythrocyte deformability, by treating erythrocytes with proteolytic enzymes, trypsin or α-chymotrypsin.

Materials and Methods

Erythrocytes and plasma. Fresh blood from a healthy adult male (red cell type, O^+) was heparinized (10 units heparin/ml blood), and was centrifuged at 3000 rpm

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for 5 min at 4°C. After removing the buffy coat, erythrocytes were washed twice with isotonic phosphate-buffered saline (42.6 mM Na_2HPO_4, 7.4 mM NaH_2PO_4, 90 mM NaCl, 5 mM KCl, 5 mM glucose (pH 7.4); 285 mosM). Erythrocytes were used within 5 h after the collection of blood (during this period, the shape of erythrocytes did not change and the velocity of erythrocyte aggregation was not altered significantly).

Proteins. Human serum albumin (fatty acid free, fraction V) was purchased from Miles Lab. Inc. (Naperville, IL). Human fibrinogen (Grade L; 90% clottable) was purchased from AB Kabi (Stockholm, Sweden), and was used after passing through a lysine-Sepharose 4B column to remove contaminated plasminogen and/or plasmin [4,12]. The concentration of fibrinogen was determined spectrophotometrically by using an absorption coefficient of 15.1 [13] for 1 g/dl solution at 280 nm.

Enzymatic treatment of erythrocytes. Trypsin (from bovine pancreas; two times crystallized, dialyzed and lyophilized, type III), α-chymotrypsin (from bovine pancreas; three times crystallized and lyophilized, type II) and neuraminidase (from *Clostridium perfringens*) were purchased from Sigma Chem. Co. (St. Louis, MO).

Erythrocytes (hematocrit, 40%) were treated with trypsin (0.04–30 mg/dl), α-chymotrypsin (0.04–100 mg/dl) or neuraminidase (0.5–50 mg/dl) in isotonic phosphate-buffered saline (pH 7.4) containing 0.1 g/dl glucose at 37°C for 60 min. After enzymatic treatment, an aliquot of erythrocytes was washed with cold isotonic phosphate-buffered saline four times for the measurement of the velocity of erythrocyte aggregation. The others were washed with above saline without glucose in preparing ghosts for the electrophoretic analysis of membrane proteins and for the determination of sialic acid and sugar in the membrane proteins.

Measurement of the velocity of erythrocyte aggregation. A rheoscope apparatus [14] (composed of a transparent cone-plate viscometer and an inverted microscope) combined with a video camera (Sony, AVC 1150, Tokyo, Japan), an image analyzer (Toyo Ink Co., Luzex 450, Tokyo, Japan) and a computer (Hewlett Packard, HP-85, Palo Alto, CA) was used for the measurement of the velocity of erythrocyte aggregation [15] under a constant temperature [16].

Erythrocytes were suspended in isotonic phosphate-buffered saline (pH 7.4) containing 0.5 g/dl fibrinogen and 5 g/dl human serum albumin (albumin was used for preventing the adhesion of erythrocytes and/or aggregates on glass plate of the rheoscope [17]). The suspension was immediately applied to the rheoscope. With respect to the sensitivity and the reproducibility, the measurement was carried out at a hematocrit of 0.26%, at a shear rate of 7.5 s^{-1} and at a temperature of 25°C. The count of particles (i.e., single erythrocytes, one-dimensional aggregates (rouleaux) or three-dimensional aggregates) and the total area projected by particles in a frame of the video image (actual frame size, 200 μm × 150 μm) were consecutively encoded by the image analyzer at an interval of approx. 1.3 s, and transferred to the computer. The velocity of erythrocyte aggregation was expressed by the increment of area/count per unit time (v, μm^2/min) [15–18].

Measurement of erythrocyte deformability. Erythrocyte deformation under constant shear stress was observed at 25°C with a rheoscope [14] combining an inverted microscope (Olympus Optics Co., Model IMT, Tokyo, Japan), a transparent 0.8° cone-plate viscometer (Tokyo Keiki, Model B, Tokyo, Japan) and a flash light (Sugawara, Model MS-230, Tokyo, Japan) [19–21]. Erythrocytes (in a hematocrit of 0.26%) were suspended in isotonic solution containing 18 g/dl Dextran T-40 (11.0 cP at 25°C; Dextran T-40 was purchased from Pharmacia Fine Chem., Uppsala, Sweden). The shear rate was varied in the range of 90–890 s^{-1} (thus the shear stress, in the range of 10–100 dyn/cm^2). The degree of deformation of stably oriented and deformed cells was expressed by the deformation index, defined by $(L-S)/(L+S)$, where L and S are lengths of long and short axes of ellipsoidally deformed cells.

Electrophoretic analysis of erythrocytes. The electrophoretic mobility of erythrocytes was measured by free-flow electrophoresis (C. Desaga, GmbH; model FF 48, Heidelberg, F.R.G.) according to the method of Hannig [22].

10 mM triethanolamine-acetate buffer containing 0.26 M sucrose and 5 mM glucose (pH 7.4, 300 mosM) was used for the separation buffer, and 100 mM triethanolamine-acetate buffer (pH 7.4) for the electrode buffer [4]. The electrophoresis was performed at 4°C under a voltage gradient of 800 V for the migration distance of 7 cm (the electric current in this condition was 45 mA). The residence time [23] of erythrocytes in the electric field was 127 s. The electrophoretic mobility ($\bar{u}$) was expressed by $\mu m \cdot s^{-1}/volt \cdot cm^{-1}$.

Electrophoretic analysis of membrane proteins. Ghost was prepared from a known number of erythrocytes by hypotonic hemolysis, according to the method of Dodge et al. [24]. The polyacrylamide gel electrophoresis of membrane proteins was performed on a slab gel (2 mm in thickness) containing 7.5 (or 10) % polyacrylamide, 0.20 (or 0.27) % N,N'-methylene bisacrylamide and 0.1% sodium dodecyl sulfate, 0.025% tetramethylethylenediamine and 0.04% ammonium persulfate, essentially according to the method of Laemmli [25]. After staining with Coomassie brilliant blue R-250 or with periodic acid-Schiff reagent according to the method of Fairbanks et al. [26], the gels were scanned with a densitometer (Shimadzu Manuf. Co., Dual-Wavelength TLC Scanner CS-900, Kyoto, Japan).

Nomenclature of sialoglycoproteins by Anstee et al. [10] was adopted for gel stained with periodic acid-Schiff

reagent. The content of intact sialoglycoproteins (α, β and δ; γ, not determined) in erythrocytes treated with proteolytic enzymes was roughly estimated from the peak area of densitogram.

Determination of sialic acid and sugar in erythrocyte membrane. (i) Sialic acid: Ghost from a known amount of erythrocytes was treated with 0.05 M H_2SO_4 at 80 °C for 60 min to release sialic acid, and then the thiobarbituric acid method [27] was adopted for the determination of sialic acid. *N*-Acetylneuraminic acid was used as a standard. (ii) Sugar: Total sugar in ghost was determined by the phenol-sulfuric acid colorimetric method of Kushwaha and Kates [28]. Glucose was used as a standard and the sugar content was expressed by the glucose equivalent.

Hematological and morphological examination. Hematological indices (mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) were calculated from (i) cell number counted with an automatic counter (Toa Medical Electronic Co., model CC-110, Kobe, Japan), (ii) hematocrit measured with a microhematocrit centrifuge (Kubota Manuf. Co., model KH-120, Tokyo, Japan) and (iii) hemoglobin concentration determined by cyanmethemoglobin method.

Cell shape was observed by using a scanning electron microscope (Hitachi Manuf. Co., model S-500, Hitachi, Japan), after fixation in 1% glutaraldehyde, then in 1% OsO_4 (both fixatives adjusted to 285 mosM with buffer).

Results

The change of the velocity of erythrocyte aggregation by the treatment of erythrocytes with proteolytic enzymes was examined with respect to (1) negative charge density on the erythrocyte surface, (2) structure of membrane glycoproteins of erythrocytes, (3) shape of erythrocytes and (4) deformability of erythrocytes. Neuraminidase-treated erythrocytes were used as a reference.

(1) Negative charge density on the erythrocyte surface

The velocity of erythrocyte aggregation greatly depends on the negative charge of erythrocyte surface [4], which produces the electrostatic repulsion among erythrocytes [3]. The density of negative charge on the erythrocyte surface correlates to the electrophoretic mobility of erythrocytes. The relation between the velocity of erythrocyte aggregation and the electrophoretic mobility of erythrocytes is shown in Fig. 1.

(i) Trypsin-treated erythrocytes. As the electrophoretic mobility decreased, the velocity of erythrocyte aggregation increased. However, two states in the change of the velocity of erythrocyte aggregation were observed: (a) Upon mild treatment with trypsin, the electrophoretic mobility increased from ≈ 2.8 to ≈ 2.9 $\mu m \cdot s^{-1}/volt \cdot cm^{-1}$, and the velocity slightly decreased from ≈ 23 to ≈ 19 $\mu m^2/min$. (b) Successive digestion markedly increased the velocity, and the velocity was much greater than that of neuraminidase-treated erythrocytes at the same electrophoretic mobility. The velocity for exhaustively trypsin-treated erythrocytes (≈ 45 $\mu m^2/min$; $\bar{u} = \approx 2.6$ $\mu m \cdot s^{-1}/volt \cdot cm^{-1}$) was even greater than that for desialylated erythrocytes (≈ 35 $\mu m^2/min$; $\bar{u} = \approx 1.6$ $\mu m \cdot s^{-1}/volt \cdot cm^{-1}$). In this connection, a linear relation between velocity of erythrocyte aggregation (v $\mu m^2/min$, in 0.5 g/dl fibrinogen containing 5 g/dl albumin) and electrophoretic mobility of erythrocytes ($\bar{u}$ $\mu m \cdot s^{-1}/volt \cdot cm^{-1}$), $v = -9.5\ \bar{u} + 51$, was obtained for neuraminidase-treated erythrocytes, as previously reported [4].

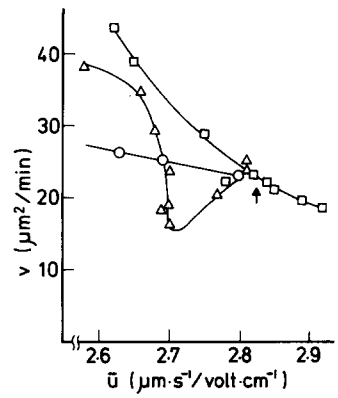

Fig. 1. Relationship between the velocity of erythrocyte aggregation and the electrophoretic mobility of erythrocytes. Erythrocytes were treated with trypsin (□), α-chymotrypsin (△) or neuraminidase (○) in their different concentrations at 37 °C for 60 min (see Fig. 3). The velocity of erythrocyte aggregation (v) was measured in 0.5 g/dl fibrinogen + 5 g/dl albumin (pH 7.4), at a shear rate of 7.5 s^{-1} at 25 °C. The electrophoretic mobility ($\bar{u}$) was measured by free-flow electrophoresis at pH 7.4. The electrophoretic mobility of untreated erythrocytes (marked with arrow) was about 2.8 $\mu m \cdot s^{-1}/volt \cdot cm^{-1}$ at pH 7.4.

(ii) α-Chymotrypsin-treated erythrocytes. Two states in the change of the velocity of erythrocyte aggregation were also observed. (a) With mild α-chymotryptic digestion of erythrocytes, the velocity decreased up to about 60% of that of untreated erythrocytes, in spite of the reduction of electrophoretic mobility from 2.8 to 2.7 $\mu m \cdot s^{-1}/volt \cdot cm^{-1}$ (i.e., the reduction of negative charge density). (b) Successive enzymatic treatment increased the velocity up to 160% of that of untreated erythrocytes. The velocity for exhaustively α-chymotrypsin-treated erythrocytes was also much greater than that for desialylated erythrocytes, though the negative charge in the former was denser than that in the latter.

In short, mild treatment of erythrocytes with trypsin or α-chymotrypsin was inhibitory for erythrocyte aggregation, but successive (or exhaustive) treatment was accelerative. It is clear that the erythrocyte aggregation does not always depend on the electrophoretic mobility

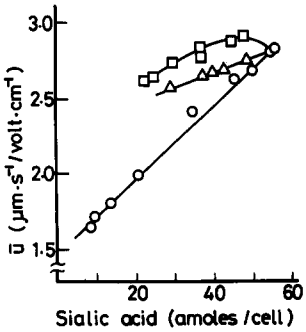

Fig. 2. Relationship between electrophoretic mobility ($\bar{u}$) and sialic acid content of erythrocytes. Treated with trypsin (□), α-chymotrypsin (△) or neuraminidase (○).

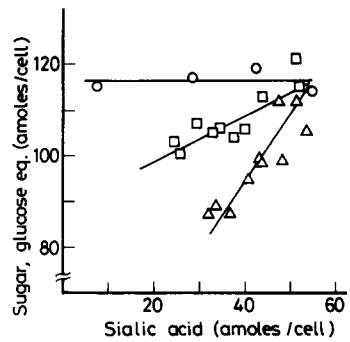

Fig. 4. Relationship between sugar content and sialic content in erythrocytes. Treated with trypsin (□), α-chymotrypsin (△) or neuraminidase (○). Sugar content is represented in glucose equivalent unit.

of erythrocytes, namely the charge density on the erythrocyte surface.

(2) Structure of membrane glycoproteins of erythrocytes

In order to understand the change of the velocity of erythrocyte aggregation upon the treatment with proteolytic enzymes, the structural changes of glycoproteins of erythrocyte membrane were examined in terms of sialic acid content, sugar content and cleavage of glycoproteins.

(i) Sialic acid content. Sialic acid accounts for a major portion of the surface negative charge of erythrocytes. The relationship between electrophoretic mobility and sialic acid content in erythrocytes treated with various enzymes is shown in Fig. 2. The degree of degradation of glycoproteins in various concentrations of enzymes was properly presented as a function of sialic acid content as shown in Fig. 3.

In neuraminidase-treated erythrocytes, the electrophoretic mobility ($\bar{u}$ $\mu m \cdot s^{-1}/volt \cdot cm^{-1}$) was linear to the sialic acid content (S amol/cell), as previously reported [5]: $\bar{u} = 0.023\ S + 1.47$. The electrophoretic mobility of trypsin- or α-chymotrypsin-treated erythrocytes was greater than that of neuraminidase-treated cells, in the same sialic acid content. Similar observation has been reported by Seaman and Uhlenbruck [29] with a microelectrophoresis apparatus.

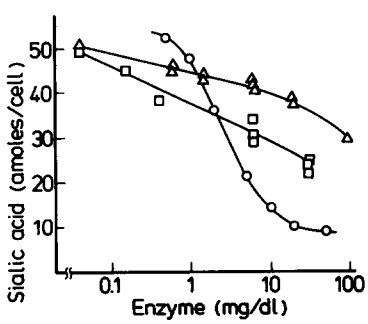

Fig. 3. Change of sialic acid content of erythrocytes by enzymatic treatment. Incubated with various concentration of trypsin (□), α-chymotrypsin (△) or neuraminidase (○) at 37 °C for 60 min.

In trypsin-treated erythrocytes, with decreasing the sialic acid content, the electrophoretic mobility was decreased with a transient increase. About 40% of sialic acid were still retained after the exhaustive tryptic digestion. On the other hand, in α-chymotrypsin-treated erythrocytes, a linear relation between the electrophoretic mobility and the sialic acid content was observed, $\bar{u} = 0.0094\ S + 2.27$. Only 40% of total sialic acid could be removed by α-chymotryptic digestion.

These different relations between electrophoretic mobility and sialic acid content reflect the structural change in different glycoproteins with various enzymes. Thus a linear relation of the velocity of erythrocyte aggregation to sialic acid content was not obtained.

(ii) Sugar content. Change of sugar content in erythrocyte membrane by enzymatic treatment is shown in Fig. 4, as a function of sialic acid content. Exhaustive digestion with trypsin and α-chymotrypsin released about 15% and 25% of total sugar from erythrocyte membrane, respectively (neuraminidase specifically released sialic acid from sialoglycoproteins, but did not alter the sugar content). The loss of sugar was proportional to the loss of sialic acid, but the degree was smaller in tryptic digestion than in α-chymotryptic digestion. These results also show the different actions of proteolytic enzymes on different membrane proteins.

(iii) Cleavage of glycoproteins. Changes of band 3 or sialoglycoproteins by enzymatic cleavage were examined by polyacrylamide gel electrophoresis. Typical densitograms after staining with Coomassie brilliant blue and periodic acid-Schiff reagent are shown in Fig. 5.

On the Coomassie brilliant blue-stained gel (Fig. 5, upper), no significant changes of membrane proteins were observed in erythrocytes treated with trypsin. However, in exhaustively α-chymotrypsin-treated erythrocytes, (a) disappearance of band 3, (b) appearance of a new peak of 61 kDa and (c) augmentation of band 4.1 peak (probably due to overlap of any degradation products with similar molecular weight as

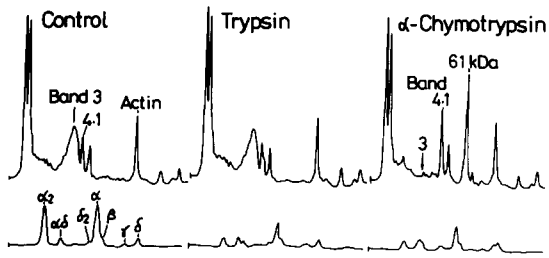

Fig. 5. Electrophoretic patterns of membrane proteins of erythrocytes. (Upper) Stained with Coomassie brilliant blue R-250. Electrophoresis was performed in 7.5% polyacrylamide gel with 0.1% sodium dodecyl sulfate by the method of Laemmli [25]. Erythrocytes treated with 30 mg/dl trypsin (middle) or 100 mg/dl α-chymotrypsin (right) are compared with those untreated (left). (Lower) Stained with periodic acid-Schiff reagent. Electrophoresis, performed in 10% polyacrylamide gel. Erythrocytes treated with 1 mg/dl trypsin (middle) or 0.1 mg/dl α-chymotrypsin (right) (these correspond to mildly treated erythrocytes in Table I).

protein 4.1) were clearly observed. The increase of 61 kDa peak well corresponded to the decrease of band 3. On the periodic acid-Schiff-stained gel (Fig. 5, lower), changes of all sialoglycoproteins, especially sialoglycoprotein α, were observed in both trypsin- and α-chymotrypsin-treated erythrocytes.

Typical data on the velocity of erythrocyte aggregation are summarized in Table I, in relation to the changes of membrane glycoproteins and erythrocyte deformability. (a) Upon tryptic digestion, the degradation of sialoglycoprotein α proceeded initially, followed by the degradation of β (with the continuous degradation of α). Sialoglycoprotein δ was relatively resistant to the tryptic digestion. Selective digestion of sialoglycoproteins α and β has been observed upon tryptic treatment [30]. (b) Upon α-chymotryptic digestion, on the other hand, the degradation of sialoglycoprotein α proceeded, followed by the degradation of δ. Sialoglycoprotein β was resistant to the α-chymotryptic digestion.

In short, the changes of the velocity of erythrocyte aggregation (Fig. 1) is associated to the complex degradation process of both band 3 and sialoglycoproteins.

(3) Shape of erythrocytes and hematological indices

The shape of erythrocytes, an important factor for erythrocyte aggregation [15], was not altered by exhaustive treatment with trypsin, α-chymotrypsin or neuraminidase, as far as examined by the scanning electron microscopy. The mean corpuscular volume, the mean corpuscular hemoglobin and the mean corpuscular hemoglobin concentration were not significantly altered by these enzymatic treatments.

(4) Deformability of erythrocytes

Sufficient contact area between adjacent erythrocytes in rouleaux is formed by the erythrocyte deformation [31]. The erythrocyte deformability was determined at shear stresses of 12.7 and 39.0 dyn/cm^2 by the rheoscopy. As shown in Table I, the enzymatic modification of surface glycoproteins in erythrocytes did not significantly affect the deformation of erythrocytes under high shear stress.

Discussion

Erythrocyte aggregation is induced by the bridging of macromolecules between adjacent erythrocyte surfaces.

TABLE I

Membrane glycoproteins in erythrocytes treated with various enzymes: relation to aggregation and deformability of the erythrocytes

Enzymatic treatment	Sialic acid (amol/cell)	Band 3 [a]	Sialoglycoproteins [a]			Aggregation [b]	Deformation index [c] at	
			α	β	δ	v (μm^2/min)	13 dyn/cm^2	39 dyn/cm^2
Control	59.4	100	100	100	100	25	0.211 ± 0.052 (58)	0.353 ± 0.049 (61)
Trypsin	39.0	–	34	78	80	22	–	–
	26.4	95	5	24	82	39	0.230 ± 0.054 (77)	0.344 ± 0.058 (74)
α-Chymotrypsin	45.4	89	48	104	106	18	–	–
	36.2	14	18	83	46	39	0.225 ± 0.052 (66)	0.348 ± 0.050 (72)
Neuraminidase	26.4	–	–	–	–	31	–	–
	7.0	–	–	–	–	36	0.227 ± 0.044 (54)	0.331 ± 0.048 (43)

[a] The content of the glycoproteins was calculated on the basis of the peak area of the densitogram, and those in control erythrocytes are expressed by 100, which correspond to 1000×10^3 copies/cell for band 3 [7], 600×10^3 copies/cell for α [8], 50×10^3 copies/cell for β [10] and 80×10^3 copies/cell for δ [8], respectively. Sialoglycoprotein γ, not determined.

[b] Velocity of erythrocyte aggregation measured in a hematocrit of 0.26% at a constant shear flow of 7.5 s^{-1}, at 25 °C.

[c] Measured by the rheoscopy at 25 °C. Values are presented by mean ± S.D. (number of cells measured on microphotographs).

Therefore, the surface structure of erythrocytes has an important role in the erythrocyte aggregation. In the present study, the contribution of glycoproteins on the velocity of erythrocyte aggregation was examined by treating the erythrocytes with various enzymes. The erythrocyte aggregation is affected by various properties of erythrocytes: (i) shape, (ii) deformability and (iii) surface structure of membrane.

(i) Shape of erythrocytes. The shape of erythrocytes is important for making appropriate contact between adjacent cells. The shape change to echinocytes greatly decreases the velocity of erythrocyte aggregation [15], and alters the electrophoretic mobility of erythrocytes [32] or the two-dimensional topography of negative charge sites [33]. The surface area to volume ratio also affects the velocity of erythrocyte aggregation [34,35].

It has been suggested that sialoglycoproteins β and γ have a role in maintaining the normal shape of erythrocytes [10]. In the present study, the shape and the hematological indices of erythrocytes were not altered, though sialoglycoprotein β was clearly degraded in trypsin-treated cells (Table I). Durocher et al. [36] have also observed no changes in hematological indices by neuraminidase treatment. Seaman and Heard [37] have observed the maintenance of biconcave disc shape after trypsin treatment.

(ii) Deformability of erythrocytes. Membrane deformability is important to get sufficient contact area between adjacent erythrocytes in rouleau formation [31]. Decreased deformability due to oxidative crosslinking of cytoskeletal proteins remarkably reduces the velocity of erythrocyte aggregation [20]. Chasis et al. [38] have recently shown that the specific binding of antibody to sialoglycoprotein α decreases the membrane deformability. However, the partial digestion of glycoproteins with neuraminidase or proteolytic enzymes did not affect the erythrocyte deformability by high shear force (Table I). Durocher et al. [36] have observed no alteration in the filterability of neuraminidase-treated erythrocytes.

(iii) Surface structure of erythrocyte membrane. The negative charge on the surface of erythrocyte membrane, mainly of sialic acid, provides the electrostatic repulsion among erythrocytes. Thus the removal of sialic acid by neuraminidase accelerates the erythrocyte aggregation (Fig. 1: also see Refs. 3 and 4).

The modification of erythrocyte surface with proteolytic enzymes reduces the sialic acid content, but it results complex relation between the electrophoretic mobility of erythrocytes (i.e., the negative charge density) and the sialic acid content (Fig. 2). The similar relation has been observed by Cook et al. [39] and Luner et al. [40]. Furthermore, a simple relation between the velocity of erythrocyte aggregation and the electrophoretic mobility was not obtained (Fig. 1). These phenomena are essentially due to the structural change of surface glycoproteins, which may result from the cleavage of peptide bonds and/or the removal of some (poly)peptides: changes of surface negative and positive charges, redistribution of charged groups, exposure of new structural moiety of glycoproteins, changes of interaction between proteins and proteins and/or lipids, and so on. A remarkable degradation of sialoglycoprotein α (one of the most abundant glycoproteins) common to both trypsin- and α-chymotrypsin-treated erythrocytes may contribute to the accelerated aggregation of these cells. These structural changes of membrane surface glycoproteins may alter the accessibility of bridging fibrinogen molecules. The present results clearly demonstrate (a) that the electrostatic repulsive force between erythrocytes (i.e., disaggregating force) is dependent on the content of sialic acid, but (b) that the bridging force of macromolecules between adjacent erythrocytes (i.e., aggregating force) is greatly altered by some structural change of surface glycoproteins, independently of the content of sialic acid.

Significance of glycoproteins on erythrocyte aggregation

Physiologically, the structural change of glycoproteins may be important for regulating the erythrocyte aggregation. The structural modification of glycoproteins on erythrocyte surface, e.g., the exposure of a new antigenic site [41], may be related to the removal of aged cells in the reticuloendothelial system. Henrich and Aminoff [42] have observed the differences in peptides released from surfaces of young and aged erythrocytes with trypsin. Very recently, we found that aged erythrocytes (fractionated by density gradient centrifugation on Percoll) have greater velocity of erythrocyte aggregation than young cells [43]. The present study demonstrates that the native structure of glycoproteins on the erythrocyte surface limits normal erythrocyte aggregation. The alteration of surface structure during ageing may not only expose new antigenic sites, but also may produce additional and/or strong binding sites to macromolecules leading to increased erythrocyte aggregation. Therefore, the structure of glycoproteins on the erythrocyte surface may be important for the survival of erythrocytes in circulation and the maintenance of the suspension stability of blood.

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