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# Opposite effect of albumin on the erythrocyte aggregation induced by immunoglobulin G and fibrinogen

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**The effect of albumin on the immunoglobulin G (IgG)-induced and fibrinogen-induced aggregation of human erythrocytes was quantitatively examined by using a rheoscope combined with a television image analyzer and a computer. (i) As albumin concentration in the medium was increased, the IgG-induced erythrocyte aggregation was inhibited, while the fibrinogen-induced erythrocyte aggregation was accelerated (albumin itself was not able to aggregate erythrocytes). These relations were empirically expressed by the equations, $v = aG^{1.8}/A$ and $v = a'F^{1.5}(A + b')$, respectively ($v$, the velocity of erythrocyte aggregation; $A$, $G$ and $F$, the concentrations of albumin, IgG and fibrinogen, respectively; $a$, $a'$ and $b'$, constant). (ii) The IgG-induced erythrocyte aggregation was remarkably inhibited by the addition of poly(glutamic acid), but the fibrinogen-induced erythrocyte aggregation was not. (iii) A mechanism for the interaction of immunoglobulin G and fibrinogen with the surface of erythrocytes was proposed.**

## Introduction

The aggregation of human erythrocytes is influenced by (i) the shearing force (shear stress), (ii) the properties of erythrocytes (concentration, deformability, surface charge, shape) and (iii) the bridging force of high-molecular-weight plasma proteins [1,2]. Pathophysiologically, the quantitative relation among these factors on the erythrocyte aggregation is very important for interpreting the control of blood flow in the low-shear region. In healthy man, fibrinogen is the most important factor accelerating erythrocyte aggregation. Thus, extensive studies have been performed in the relation between fibrinogen concentration and erythrocyte aggregation (as well as the blood viscosity and the erythrocyte sedimentation rate) [1,3–9]. On the other hand, the influence of various immunoglobulins on the erythrocyte aggregation has a great meaning in various pathological conditions (infectious diseases, multiple myeloma, macroglobulinemia and so on) [1,10]. Especially, it is well-known in clinical medicine that the increase of abnormal IgG and macroglobulins induces the hyperviscosity syndrome [11,12]. In the previous report [13], we have proposed an empirical equation expressed the velocity of aggregate formation of erythrocytes by the concentration of fibrinogen and the sialic acid content of erythrocytes, such that the aggregation of erythrocytes is accelerated by increasing fibrinogen concentration (due to the increased bridging force among erythrocytes) or by decreasing the sialic acid content (due to the reduction of the electrostatic repulsive force among erythrocytes). These experiments have been performed under a limited concentration of albumin. Chien et al. [5] have shown that serum albumin has very little effect on the aggregation of canine erythrocytes in Ringer solution.

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Abbreviation: IgG, immunoglobulin G.

Recently, we found the importance of albumin in the aggregation of human erythrocytes: i.e., (i) albumin itself, even if in high concentrations, did not induce the aggregation of human erythrocytes, but (ii) the fibrinogen-induced aggregation was accelerated by increasing albumin concentration, in contrast to (iii) the IgG-induced aggregation which was inhibited by albumin, in an artificial medium (isotonic phosphate-buffered saline). In the present paper, we report these opposite effects of albumin on the erythrocyte aggregation induced by IgG and fibrinogen. Furthermore, empirical equations are deduced for the relationship between the velocity of erythrocyte aggregation, IgG or fibrinogen concentration and albumin concentration. The mechanism of the interaction of IgG and fibrinogen (in the presence of albumin) with the surface of erythrocytes is discussed. The results will provide further information on the interpretation of erythrocyte aggregation in the low-shear blood stream.

## Materials and Methods

*Preparation of erythrocytes and plasma.* Fresh blood was obtained from a healthy adult male (red cell type $A^+$), and heparinized (10 units heparin/ml blood were used). Blood was centrifuged at 3000 rpm for 5 min at 4°C. Plasma was collected and recentrifuged at 15000 rpm for 15 min at 4°C to remove platelets. 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, pH 7.4) plus 5 mM glucose. Erythrocytes were used within 4 h after collection of blood (during this period, the erythrocyte shape was not altered, and the velocity of aggregation in the same medium scarcely changed).

*Immunoglobulin G, fibrinogen and albumin.* Human IgG (Lot No. 0045) was purchased from Miles Lab., Naperville, IL, U.S.A. In order to remove anti-$A^+$ in IgG (if any), the solution of IgG was incubated with the donor's washed erythrocytes (for 20 min at 37°C; hematocrit, 20%) and centrifuged. IgG in the supernatant was used for the experiment, thus the erythrocyte aggregation with IgG was a non-specific (or non-immunological) phenomenon. Human fibrinogen (Grade L; 90% clottable) from AB Kabi, Stockholm was used after passing through a lysine-Sepharose 4B column to remove contaminated plasminogen and plasmin, as described in the previous report [13]. Human serum albumin (fatty acid-free, Fraction V) from Miles Lab. was dissolved in isotonic phosphate-buffered saline, and centrifuged at 15000 rpm for 15 min at 4°C prior to use. Poly(L-glutamic acid) of 20 kDa (Lot No. 92F-5036; degree of polymerization, 130) and 8 kDa (Lot No. 101F-5085; degree of polymerization, 50) were purchased from Sigma, St. Louis, MO, U.S.A.

*Measurement of the velocity of erythrocyte aggregation.* The apparatus combined a rheoscope [14] (composed of a transparent cone-plate viscometer and an inverted microscope [15]) with a television image analyzer (Luzex 450, Toyo Ink Co., Tokyo, Japan), and a computer (Hewlett Packerd, HP-85) was used for the measurement of the velocity of aggregate formation of erythrocytes, as described previously [2,13,16].

The washed erythrocytes were resuspended in an artificial medium composed of various concentrations of IgG or fibrinogen in isotonic phosphate-buffered saline (pH 7.4) containing various concentrations of albumin (the minimum concentration of albumin for preventing the adhesion of erythrocytes and/or aggregates on the glass plate of the rheoscope was $7 \cdot 10^{-5}$ M). The final hematocrit was adjusted to 0.26% (in our apparatus, the erythrocyte concentration of 0.26% hematocrit was suitable for measuring the erythrocyte aggregation by the image analyzer) [2]. The mixture was immediately applied to the rheoscope, and the aggregate formation was observed at shear rate of 7.5–22.5 $s^{-1}$ at 25°C. The number of particles (i.e., erythrocytes and aggregates) and the total projected area of particles in the television image (actual frame size, 130 $\mu m \times 190$ $\mu m$) were consecutively encoded by the analyzer at an interval of about 1.3 s, and transferred to the computer. The velocity of aggregate formation was represented by the increment of averaged area projected by one particle per unit time in the stage of rouleau formation, and the velocity was converted to the averaged number of erythrocytes in an aggregate/min (cells/min) by assuming 1.5 $\mu m$ (averaged thickness) $\times$ 8 $\mu m$ (averaged diameter) as the area of one erythrocyte in aggregate, i.e., 12 $\mu m^2$ [2,13].

*Correction of the velocity of erythrocyte aggregation for different shear stresses.* The velocity of erythrocyte aggregation is dependent on the shear stress [1,2]: increased shear stress due to increased protein concentration disintegrates erythrocyte aggregates. Thus, the velocity of erythrocyte aggregation has to be corrected and compared under the same shear stress ($\tau = 0.1$ dyn/cm$^2$).

The viscosity of various artificial media was measured at various shear rates ($\dot{\gamma} = 7.5$–$750$ s$^{-1}$) at 25°C by a cone-plate viscometer (Tokyo Keiki Co., model E, mounted 0.8° cone). The apparent viscosity of the media ($\eta$) was independent of shear rate, i.e., all were Newtonian fluid, as has been already known [3]. On the other hand, the velocity of erythrocyte aggregation in various media was measured at a shear rate of 7.5–22.5 s$^{-1}$. Since $\tau = \eta \times \dot{\gamma}$ (approx. 0.1 dyn/cm$^2$ in this experiment), the velocity of aggregation in the medium with the increased viscosity could be corrected to the velocity at a defined shear stress by decreasing the shear rate.

## Results

*Relation between the velocity of erythrocyte aggregation and IgG concentration in various albumin concentrations*

The effect of IgG on the erythrocyte aggregation in various concentrations of albumin was examined in the artificial medium, as shown in Fig. 1. Clearly, as IgG concentration ($G$, in M) was increased, the velocity of aggregation ($v$, in cells/min) increased (Fig. 1a). Contrarily, as albumin concentration ($A$, in M) in the medium was increased, the velocity decreased in all IgG concentrations, i.e., IgG-induced erythrocyte aggregation was inhibited by albumin (Fig. 1b). It must be noted that erythrocytes in albumin solution never aggregate in the absence of IgG (or fibrinogen, as shown later), as has been already pointed out [5]. The linear relation was obtained between log $v$ and log $G$ in all albumin concentrations, as shown in Fig. 2a, and it was shown that the slope ($\alpha$) was constant, but the intercept ($\beta$) was dependent on albumin concentration. Thus, an empirical equation between the velocity of aggregation ($v$, in cells/min) and IgG concentration ($G$, in M) under various concentrations of albumin ($0.91$–$6.1 \cdot 10^{-4}$

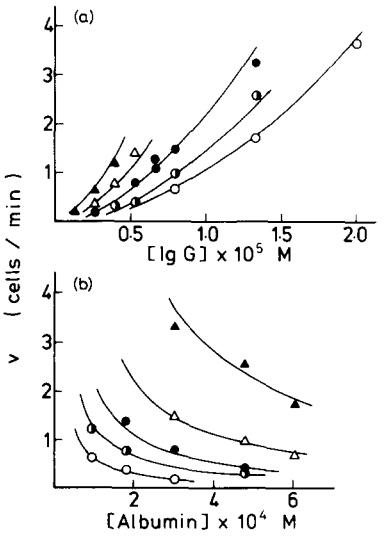


Fig. 1. Effect of IgG on erythrocyte aggregation under various concentrations of albumin. Measured at a shear stress of 0.1 dyn/cm$^2$ at 25°C. The solid lines in the figures are calculated according to the empirical equation as shown in the text. (a) Relation between the velocity of aggregation ($v$, in cells/min) and IgG concentration (in M). Albumin concentrations ($10^{-4}$ M): 0.91 (▲), 1.82 (△), 3.03 (●), 4.85 (◑), 6.06 (○). (b) Relation between $v$ and albumin concentration (in M). IgG concentrations ($10^{-5}$ M): 0.27 (○), 0.40 (◑), 0.53 (●), 0.80 (△), 1.33 (▲).

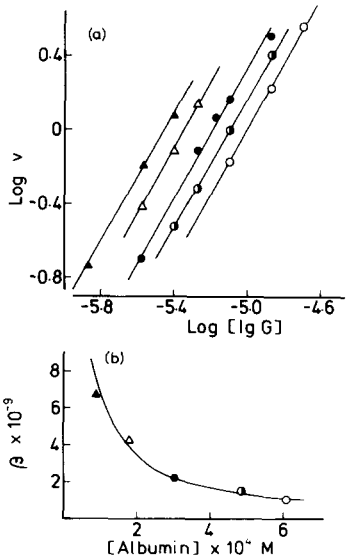


Fig. 2. (a) Logarithmic plot between the velocity of aggregation ($v$) and IgG concentration. Conditions as in Fig. 1a. (b) Dependence of $\beta$ on albumin concentration (see text). Symbols correspond to those in (a). The solid lines in both figures are calculated according to the empirical equation in the text.

M) is deduced:

$$\log v = \alpha \log G + \log \beta \quad (1)$$

The value of $\alpha$ calculated by the method of least squares was $1.8 \pm 0.1$ (for five different concentrations of albumin; at a shear stress of 0.1 dyn/cm$^2$). On the other hand, the $\beta$ values obtained from the regression lines in Fig. 2a were plotted against albumin concentration, as shown in Fig. 2b. A relation between $\beta$ and albumin concentration ($A$, in M), $\beta \times A = (6.6 \pm 1.3) \times 10^5$, was obtained. Thus, the empirical equation for IgG-induced erythrocyte aggregation becomes as follows:

$$\log v = 1.8 \log G - \log A + \log(6.6 \times 10^5) \quad (2)$$

or

$$v = 6.6 \times 10^5 \times G^{1.8}/A \quad (3)$$

*Effect of poly(glutamic acid) on the IgG-induced erythrocyte aggregation*

To obtain some information on the mechanism of IgG-induced erythrocyte aggregation, the effect of anionic polypeptide (poly(glutamic acid) of 20 and 8 kDa) was examined as shown in Table I.

Addition of poly(glutamic acid) (20 kDa; poly(glutamic acid) itself did not aggregate erythrocytes) drastically inhibited the IgG-induced erythrocyte aggregation in the presence of albumin (expts. 1–3). The drastic inhibition of erythrocyte aggregation was also observed by poly(glutamic acid) of 8 kDa (not shown in the table).

TABLE I

EFFECT OF POLY(GLUTAMIC ACID) ON THE ERYTHROCYTE AGGREGATION INDUCED BY IgG OR FIBRINOGEN

Poly(glutamic acid) of 20 kDa (PG-20) and 8 kDa (PG-8); A, albumin; G, IgG; F, fibrinogen.

| Expt. No. | Composition (M) | $v$ (cells/min) |
|---|---|---|
| 1 | $G(1.33 \cdot 10^{-5}) + A(3.03 \cdot 10^{-4})$ | 3.25 |
| 2 | $G(1.33 \cdot 10^{-5}) + A(3.03 \cdot 10^{-4}) + PG\text{-}20(2 \cdot 10^{-7})$ | 1.13 |
| 3 | $G(1.33 \cdot 10^{-5}) + A(3.03 \cdot 10^{-4}) + PG\text{-}20(2 \cdot 10^{-6})$ | 0 |
| 4 | $F(0.88 \cdot 10^{-5}) + A(6.06 \cdot 10^{-4})$ | 1.81 |
| 5 | $F(0.88 \cdot 10^{-5}) + A(6.06 \cdot 10^{-4}) + PG\text{-}20(1 \cdot 10^{-3})$ | 1.81 |
| 6 | $F(0.88 \cdot 10^{-5}) + A(6.06 \cdot 10^{-4}) + PG\text{-}8(1.3 \cdot 10^{-4})$ | 1.80 |

*Relation between the velocity of erythrocyte aggregation and fibrinogen concentration in various albumin concentration*

The effect of fibrinogen on the erythrocyte aggregation was also examined under various concentrations of albumin. The experimental results obtained in two different occasions, with 16 months in between are summarized in Fig. 3. It is clear that the results obtained in the artificial medium are very reproducible. The velocity of erythrocyte aggregation ($v$, in cells/min) increased, as fibrinogen concentration ($F$, in M) was increased (Fig. 3a). In contrast to IgG, when albumin concentration ($A$, in M) was increased, the velocity increased linearly in all fibrinogen concentrations (Fig. 3b). The effect of fibrinogen without albumin on erythrocyte aggregation, though was not mea-

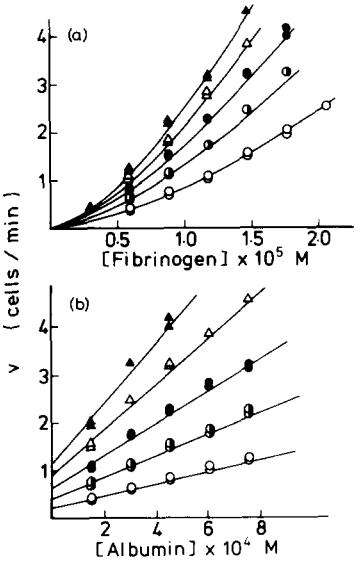


Fig. 3. Effect of fibrinogen on the erythrocyte aggregation under various concentrations of albumin. Measured at a shear stress of 0.1 dyn/cm$^2$ at 25°C. The solid lines in the figures are calculated according to the empirical equation in the text. (a) Relation between the velocity of aggregation ($v$, in cells/min) and fibrinogen concentration (in M). Albumin concentrations ($10^{-4}$ M): 1.52 (○), 3.03 (◑), 4.55 (●), 6.06 (△), 7.58 (▲). (b) Relation between $v$ and albumin concentration. Fibrinogen concentrations ($10^{-5}$ M): 0.59 (○), 0.88 (◑), 1.18 (●). 1.47 (△), 1.76 (▲). In some cases, experiments were performed on two different occasions, depicted with double symbols.

sured due to the methodological difficulty, can be deduced by extrapolating albumin concentration to 0. The dependence of the extrapolated velocity on fibrinogen concentration qualitatively agrees with the results of Wells et al. [3].

The linear relation was also obtained between log $v$ and log $F$ in all albumin concentrations (Fig. 4a). The slope ($\alpha'$) was constant, but the intercept ($\beta'$) was dependent on albumin concentration. Thus, another empirical equation between the velocity of aggregation and the concentrations of fibrinogen and albumin is deduced:

$$\log v = \alpha' \log F + \log \beta' \tag{4}$$

The value of $\alpha'$ calculated by the method of least squares was $1.5 \pm 0.1$ (for five different concentrations of albumin; at a shear stress of 0.1 dyn/cm$^2$). The $\beta'$ values obtained from the regression lines in Fig. 4a were plotted against albumin concentration, as shown in Fig. 4b. A linear relation between $\beta'$ and albumin concentration ($A$, in M), $\beta' = 8.3 \times 10^{10} A + 1.6 \times 10^7$, was obtained by the method of least squares. Thus, the empirical equation for fibrinogen-induced erythrocyte aggregation becomes as follows:

$$\log v = 1.5 \log F + \log(8.3 \times 10^{10} A + 1.6 \times 10^7) \tag{5}$$

or

$$v = 8.3 \times 10^{10} \times F^{1.5}(A + 1.9 \times 10^{-4}) \tag{6}$$

As shown in Figs. 3 and 4, good fitting of the empirical equation to the results was obtained.

The deviations of fibrinogen and albumin concentrations by 10% from the physiological range (about 0.3 g/dl ($8.8 \cdot 10^{-6}$ M) and 5 g/dl ($7.6 \cdot 10^{-4}$ M), respectively) change the velocity of aggregation in the present experimental conditions by 16 and 8%, respectively. Therefore, it is concluded that a change of fibrinogen concentration has a strong effect on the aggregation of erythrocytes, while a change in albumin concentration has a moderate effect on the aggregation.

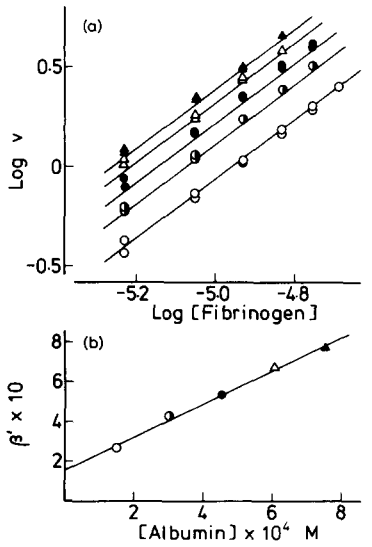


Fig. 4. (a) Logarithmic plot between the velocity of aggregation ($v$) and fibrinogen concentration. Conditions as in Fig. 3a. (b) Dependence of $\beta'$ on albumin concentration (see text). Symbols correspond to those in (a). The solid lines in both figures are calculated according to the empirical equation in the text.

*Effect of poly(glutamic acid) on the fibrinogen-induced erythrocyte aggregation*

The effect of poly(glutamic acid) on the erythrocyte aggregation induced by fibrinogen was different from those on the IgG-induced erythrocyte aggregation. The addition of poly(glutamic acid) of 20 and 8 kDa to the medium containing fibrinogen and albumin did not affect the velocity of erythrocyte aggregation (expts. 4–6 in Table I).

## Discussion

The aggregation of erythrocytes is influenced by (i) the mechanical shearing force, (ii) the macromolecular bridging force among erythrocytes, (iii) the electrostatic repulsive force among erythrocytes, (iv) the bending force of erythrocyte membrane, (v) the shape of erythrocytes, and (vi) the concentration of erythrocytes [2,17]. Among these factors, the effect of macromolecules, especially IgG and fibrinogen in plasma, is important for the erythrocyte aggregation induced at low shear rate and the sludging in the blood veins. In clinical medicine, the hyperviscosity syndrome due to the accelerated aggregation of erythrocytes is strongly dependent on the level of these macromolecules [11,12,18]. It is well recognized that IgG increases in various infectious diseases, and that abnormal

immunoglobulins are produced in multiple myeloma and macroglobulinemia. Fibrinogen also increases in various diseases, such as diabetes mellitus [19–21], myocardial infarction [6,22,23], nephrosis [6], reumatoid arthritis [6], and so on. The increase of these proteins in plasma accelerates the aggregation of erythrocytes, increases the rate of erythrocyte sedimentation, and decreases the filtrability of blood [1,11,12,18–24].

The present experiment pointed out the importance of albumin on the erythrocyte aggregation, i.e., the opposite effect of albumin on the IgG- and fibrinogen-induced erythrocyte aggregation: albumin inhibited the IgG-induced erythrocyte aggregation, but accelerated the fibrinogen-induced one, though albumin itself did not aggregate erythrocytes.

*Effect of albumin on erythrocyte aggregation: pathophysiological significance*

The systematic experiments resulted in two empirical equations for the effect of albumin on the erythrocyte aggregation induced by IgG and fibrinogen, as shown in Eqns 2 (or 3) and 5 (or 6). Strictly speaking, our equations may be valid within the experimental ranges of IgG, fibrinogen and albumin concentrations and of erythrocyte concentration. However, these equations are the first quantitative approach to the striking influence of both IgG and fibrinogen on the erythrocyte aggregation. It has been well recognized in clinical medicine that the suspension stability of blood is determined by the relation between IgG and albumin concentrations, well known as the $A/G$ ratio; IgG increases the blood viscosity at low shear rate remarkably, and accelerates the erythrocyte sedimentation [1,10]. Fibrinogen also increases the blood viscosity at low shear rate remarkably, as has been pointed out previously [1,3,5]. The present findings showed that the contribution of IgG and fibrinogen to erythrocyte aggregation was dependent on albumin concentration in the medium. As clearly understood from Figs. 1b and 3b, the opposite effect of albumin on the IgG- and fibrinogen-induced erythrocyte aggregation may be important for interpreting the increased erythrocyte sedimentation rate in various diseases: at high albumin concentration in plasma, the increase of fibrinogen is more effective for the acceleration of erythrocyte sedimentation than the increase of IgG, while at low albumin concentration in plasma, the increase of IgG has a serious effect.

*Molecular mechanism of erythrocyte aggregation induced by IgG and fibrinogen*

The present experiment gives some valuable informations on the mechanism of erythrocyte aggregation induced by IgG and fibrinogen. The different interaction of IgG and fibrinogen with erythrocytes in the presence of albumin will be explained by the following terms.

*Structure and charge of macromolecules*

The bridging force of macromolecules intercalated among erythrocytes depends on the molecular weight, the three-dimensional structure and the electric charge [1,13,20,25–27]. Human serum albumin is ellipsoidal protein (the dimension, 38 Å × 38 Å × 150 Å) of 66 kDa [28]. In considering the molecular size and the shape, the albumin molecule is too small to bridge between erythrocytes, though albumin is absorbed on the surface of erythrocytes [29–31]. Albumin molecule will interact with the negatively charged erythrocyte surface weakly, being most negatively charged in plasma proteins (the isoelectric point of albumin, 4.7 [28]). The dimension of IgG molecule (150 kDa) depends on the angle of Y-shaped two arms: the maximum length (in the fully extended state of molecule) is about 200 Å, and the dimension of the cylindrical Fab and Fc portions are 35 Å × 35 Å × 60 Å and 40 Å × 40 Å × 45 Å, respectively [32]. On the other hand, fibrinogen is fibrous protein (the dimension, 65 Å × 65 Å × 475 Å) of 340 kDa [33]. Furthermore, the isoelectric points of IgG and fibrinogen are 6.6 and 5.5, respectively [24,34]. In both molecular length and electric charge, IgG and fibrinogen bridge easily between erythrocytes compared with albumin.

*Binding of macromolecules to erythrocytes*

The erythrocyte aggregation induced by IgG was inhibited by albumin, while that induced by fibrinogen was accelerated by albumin. Furthermore, IgG-induced erythrocyte aggregation was

drastically inhibited by the addition of anionic polypeptide (poly(glutamic acid)), but the fibrinogen-induced one was not. These opposite results clearly suggested the different interaction of proteins with erythrocytes.

*Number of molecules bound to erythrocytes.* On the binding of IgG to erythrocytes, Pirofsky et al. [35] have shown that there are at least two types of non-immunological (non-specific) binding of IgG to erythrocytes: a loosely fixed and a firmly fixed one. Recently, Wegner et al. [36] have shown that the number of IgG molecules bound to high affinity (firmer binding sites) is about 15 per erythrocyte at low concentrations of free IgG. On the other hand, at higher concentrations of IgG (> 2000 molecules of free IgG per erythrocyte), erythrocytes are weakly coated by IgG [35–37]. According to Grob et al. [37], about $4 \cdot 10^5$ molecules of IgG are calculated to bind weakly to single erythrocyte at IgG concentration of $1 \cdot 10^{-5}$ M. On the other hand, according to Rampling [38], about $2 \cdot 10^4$ molecules of fibrinogen are calculated to bind to single erythrocyte at fibrinogen concentration of $1 \cdot 10^{-5}$ M. It is clear that IgG binds more (about 20-times) than fibrinogen in the same concentration of IgG and fibrinogen. On the other hand, the average number of albumin molecules adsorbed on single erythrocyte in the concentration of $7.6 \cdot 10^{-4}$ M is about $5 \cdot 10^6$ [29–31].

Now, when albumin is adsorbed at the end of the molecule on an erythrocyte surface, the occupied area of molecules is about 70 $\mu m^2$, as far as simply calculated by using the molecular dimension (as described before). This value shows that albumin molecules cover about half of erythrocyte surface, since the surface area of erythrocyte is given as 135 $\mu m^2$ by Evans and Fung [39]. On the other hand, when IgG attaches at one end of Fab portions on the surface of erythrocyte, the occupied area is about 8 $\mu m^2$ at the IgG concentration of $1 \cdot 10^{-5}$ M. In the same manner, fibrinogen attached at the end of molecule on the erythrocyte surface occupies only 0.8 $\mu m^2$ even at the fibrinogen concentration of $1 \cdot 10^{-5}$ M. Therefore, supposing the non-specific binding of these proteins to erythrocyte surface, the binding of IgG will probably compete with those of albumin, i.e., the increase of albumin inhibits the binding of IgG, thus erythrocyte aggregation by IgG is inhibited by albumin. Grob et al. [37] have shown that the high concentration of albumin inhibits the red cell uptake of IgG. However, the occupied area of fibrinogen is very small, thus albumin molecules cannot prevent further binding of fibrinogen. Rampling [38] has shown that the binding of fibrinogen is not affected by another plasma protein.

In addition, if the erythrocyte surface is fully covered with albumin molecules (150 Å in molecular length), the distance between the surface of two adjacent erythrocytes becomes 300 Å. Geometrically, the IgG molecule (200 Å in length even in the fully extended state) cannot bridge between adjacent cells, while the fibrinogen molecule (475 Å in length) can do enough. These geometric problems of macromolecules may be fundamental for interpreting the inhibitory effect of albumin on the IgG-induced erythrocyte aggregation and the accelerating effect of albumin on the fibrinogen-induced erythrocyte aggregation.

*Chemical nature of macromolecular binding.* The chemical nature of the binding of macromolecules to erythrocytes has not be clarified yet, perhaps all kinds of interactions (i.e., Van der Waals force, hydrogen bonding and electrostatic bonding) may contribute [1]. Until now, some evidences are presented for differences in the binding mode of IgG and fibrinogen: (i) the binding of IgG on the erythrocyte surface increases markedly, when the ionic strength is reduced [37,40]. On the other hand, the binding of fibrinogen increases in the medium of high ionic strength [38]. (ii) The stability of IgG fixation on erythrocyte surface is decreased by the divalent cation, $Ca^{2+}$ [40], while the binding of fibrinogen is not affected by $Ca^{2+}$ [38].

In the present study on the erythrocyte aggregation, distinct differences between IgG and fibrinogen are presented through the interaction with albumin and poly(glutamic acid). Puskás et al. [41] have shown that the non-specific interaction between IgG molecules and erythrocytes is Fc-dependent, and that the addition of the Fc fragment prevents the binding of IgG. Imaizumi et al. [27] have shown that IgG-induced aggregate formation is inhibited by the Fc fragment. The Fc portion in IgG molecule is more negatively charged

than the Fab portion [42]. The inhibitory effect of poly(glutamic acid) on the IgG-induced erythrocyte aggregation may be due to the similar mechanism to that of the Fc fragment, but the effect is stronger than Fc, because of more negative charges per molecule. Albumin, the most negatively charged protein in plasma, also inhibits the IgG-induced erythrocyte aggregation with a similar mechanism.

On the binding of fibrinogen to erythrocyte, Rampling [38] has shown that two sites in the fibrinogen molecule, which reside in fragment D (one of the major fibrinogen degradation products by plasmin), are necessary for bridging between two erythrocytes. Though albumin may not prevent the binding of fibrinogen (as described above), albumin might stabilize the binding of fibrinogen on the surface of erythrocyte by altering (i) the hydration shell of proteins, (ii) the net negative charge of erythrocyte surface, (iii) the effective ionic strength around erythrocyte surface and (iv) the holding (or the fixation) of fibrinogen molecules among adsorbed albumin molecules, through the interaction among fibrinogen, albumin and/or surface proteins of erythrocyte. The interaction of macromolecules with erythrocyte surface to induce erythrocyte aggregation is clearly dependent on the stereochemical structure of macromolecules and the structure of erythrocyte surface: e.g., when sialic acid on erythrocyte surface is removed, the erythrocyte aggregation by fibrinogen [13] and high molecular dextrans [1,17] is accelerated, but the aggregation by polylysine is inhibited [43].

*A simple model for the interaction of IgG, fibrinogen and albumin with the surface of erythrocyte*

We tentatively propose a simple model for the erythrocyte aggregation induced by IgG and fibrinogen in the presence of albumin, as shown in Fig. 5. In the low concentrations of albumin, a lot of IgG molecules can easily bridge between adjacent erythrocytes. But in the high concentrations of albumin, the binding of IgG is prevented geometrically and chemically by albumin occupying the surface of erythrocytes. On the other hand, fibrinogen (the number of bound molecules is much less than IgG, in the present experimental condition) can easily bind to the surface of erythrocytes. The binding of fibrinogen is not prevented by albumin geometrically and may be rather stabilized by albumin chemically.

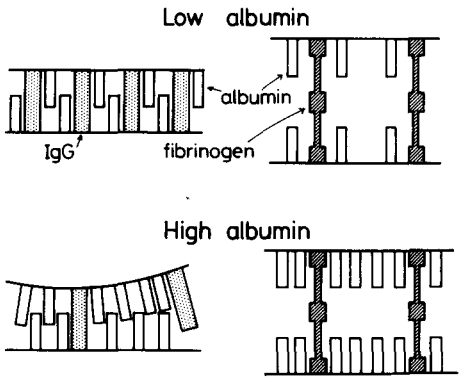


Fig. 5. A model for the mechanism of erythrocyte aggregation induced by IgG and fibrinogen in the presence of albumin.

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