Agglutination of like-charged red blood cells induced by binding of β_{2}-glycoprotein I to outer cell surface

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Abstract

Plasma protein-mediated attractive interaction between membranes of red blood cells (RBCs) and phospholipid vesicles was studied. It is shown that β_{2}-glycoprotein I (β_{2}-GPI) may induce RBC discocyte–echinocyte shape transformation and subsequent agglutination of RBCs. Based on the observed β_{2}-GPI-induced RBC cell shape transformation it is proposed that the hydrophobic portion of β_{2}-GPI molecule protrudes into the outer lipid layer of the RBC membrane and increases the area of this layer. It is also suggested that the observed agglutination of RBCs is at least partially driven by an attractive force which is of electrostatic origin and depends on the specific molecular shape and internal charge distribution of membrane-bound β_{2}-GPI molecules. The suggested β_{2}-GPI-induced attractive electrostatic interaction between like-charged RBC membrane surfaces is qualitatively explained by using a simple mathematical model within the functional density theory of the electric double layer, where the electrostatic attraction between the positively charged part of the first domains of bound β_{2}-GPI molecules and negatively charged glycolglycol of the adjacent RBC membrane is taken into account.

1. Introduction

Certain proteins that are present in plasma and are able to attach to the membrane surface were found to mediate interactions between phospholipid-containing structures such as cell membranes, microvesicles and lipoproteins [1,2]. It was shown that β_{2}-glycoprotein I (β_{2}-GPI) that is the major antigen for antiphospholipid antibodies in patients with antiphospholipid syndrome (APS) [3] can attach to the cell membrane surface [4,5] and induce adhesion between negatively charged giant phospholipid vesicles [6,7].

β_{2}-GPI is a 50 kD plasma protein (plasma concentration is 200 mg/ml) that circulates either as a free protein or associated to lipoproteins [8]. It is a single-chain protein with 326 amino acids and four N-linked glycosylation sites [9]. It is composed of four complement control protein modules and distinctly folding C terminal fifth domain [8]. Although the exact physiological role of β_{2}-GPI remains unclear, it was suggested that it may have an important function in blood coagulation and in the clearance of apoptotic bodies from the circulation [10]. It is indicated that β_{2}-GPI is an autoantigen for the production of antiphospholipid antibodies and may play an important role in many diseases including APS. The interactions of β_{2}-GPI with phospholipids are considered crucial for its physiological and pathogenic roles [8].

In most cases the binding of proteins to the membrane surface is driven by electrostatic forces and/or by penetration of the hydrophobic protrusions of the proteins into the lipid bilayer [12,13]. Experiments have demonstrated that β_{2}-GPI interacts with anionic phospholipids [8], such as cardiolipin and phosphatidylserine. While both the first and the fifth domains of β_{2}-GPI exhibit lipid binding properties [11,8], the fifth domain is the principal phospholipid binding domain of β_{2}-GPI [11]. It contains a sequence of positively charged (lysine) residues, which enable an electrostatic interaction with anionic phospholipids [8]. The hydrophobic protrusion of the fifth domain embedded in the outer membrane lipid layer additionally contributes to the binding of β_{2}-GPI to the outer membrane surface [3].

In this work we study the β_{2}-GPI-induced interactions between the negatively charged membranes of red blood cells (RBCs). Since β_{2}-GPI induces the discocyte–echinocyte RBC shape transformation we assume that the hydrophobic protrusion of membrane-bound β_{2}-GPI protrudes in the outer layer of the RBC membrane, similarly as in the case of the binding of β_{2}-GPI to giant phospholipid vesicles. The observed β_{2}-GPI-induced attractive interaction between membranes of RBCs is qualitatively explained by using a simple mathematical model within the functional density theory of the electric double layer, where the specific molecular shape and the internal charge...
distribution of membrane-bound $\beta_2$-GPI molecules is taken into account.

2. Experimental methods

2.1. $\beta_2$-GPI

$\beta_2$-GPI (Hyphen BioMed, France) was aliquoted and stored at $\sim -70^\circ C$. In all experiments, the final concentration of $\beta_2$-GPI was 180 mg/l, which is within the physiological range of concentration of $\beta_2$-GPI in human plasma.

2.2. Isolation of red blood cells

5 ml of blood was drawn from the authors by venipuncture into heparinized tubes and left at room temperature for 10 min. RBCs were isolated as previously described [14]. In detail, RBCs were washed three times in ice cold wash buffer (145 mM NaCl, 5 mM KCl, 4 mM Na$_2$HPO$_4$, 1 mM NaH$_2$PO$_4$, 10 mM glucose, 1 mM MgSO$_4$·7H$_2$O, 0.1 mM CaCl$_2$·2H$_2$O) by subsequent centrifugations at 1500 $\times$g, 750 $\times$g and 250 $\times$g (Centrifuge DR15, B. Braun Biotech International) at $4^\circ C$ for 10 min. After the first centrifugation plasma and buffy coat were removed and the pellet was resuspended in 10 ml of wash buffer. After the last centrifugation RBCs were resuspended in the wash buffer of the initial blood volume and finally diluted to the cell density of 1.65·10$^9$ cells/ml. RBCs were stored at $+4^\circ C$ and used within 10 h.

2.3. Treatment of red blood cells with $\beta_2$-GPI

RBCs were diluted 200 times in wash buffer. 5 $\mu$l of RBC aliquot was then mixed with 45 $\mu$l of the aqueous sugar solution (125 mM glucose, 75 mM sucrose) and placed in the observation chamber (final concentration 8.25·10$^5$ RBCs/ml). After approximately 15 min, when RBCs settled down onto the objective microscope glass slide, 5 $\mu$l of $\beta_2$-GPI (ionic strength $\approx$ 102 mmol/l) was added to the solution. For control 5 $\mu$l of wash buffer or 5 $\mu$l of phosphate buffered saline (PBS) (ionic strength $\approx$ 102 mmol/l) was added to the suspension of RBCs. In another experiment 5 $\mu$l of $\beta_2$-GPI (ionic strength $\approx$ 102 mmol/l) was added to the suspension containing 5 $\mu$l of RBCs and 45 $\mu$l of giant phospholipid vesicles. The experiments were performed at room temperature, the final ionic strength was 24 mmol/l, pH of experimental system was 7.4.

2.4. Observation of red blood cells

The observation chamber was placed onto microscope stage of Olympus GWB BH-2 microscope (magnification 2000×) with inter-

Fig. 1. RBC shape transformation and agglutination induced by progressive attachment (binding) of $\beta_2$-GPI to the outer surface of RBC membrane (A to D; A1 to D1 are magnified regions of A to D) as observed by Olympus GWB BH-2 microscope at high volume density of RBCs in the suspension. Black arrow in figure A points to a giant phospholipid vesicle, white arrow in figure D points to a large complex of adhered RBCs. White arrow in figure B points to echinocyte type I–II. Echinocyte type III in figure C is also marked by white arrow. Black arrows in figures C and D mark spherocytic RBCs.

Fig. 2. Agglutination of $\beta_2$-GPI-induced spherocytic RBCs at low volume density of RBCs in the suspension. White arrows point to complexes of RBCs, i.e. chains of self-assembled spherocytic RBCs. Dotted arrow points to RBCs which are not fully agglutinated but are close together. Black arrows point to the negatively charged phospholipid vesicles. Some spherocytic RBCs are attached to giant phospholipid vesicle surface (figure C). Olympus GWB BH-2 microscope was used in observations.
spherocytic shapes [19,27] (Fig. 3).

Fig. 3. Schematic figure of RBC shape transformations due to the preferential intercalation of surface-active molecules (detergents, proteins) into the outer layer of the membrane bilayer. At high concentrations of echinocytogenic molecules budding (exovesiculation) and release of spherical or tubular vesicles starts (see also [19,27] and references therein). As a result the RBCs are transformed into spherocytes.

ference contrast optics. Images were taken with Olympus Camedia C-3040ZOOM digital camera.

2.5. Giant phospholipid vesicles

Giant phospholipid vesicles were prepared at room temperature (23 °C) by the modified electroformation method originally proposed by Angelova et al. [15] as described in [7]. The synthetic lipids cardiolipin(1,1’2,2’-tetraoleyl cardiolipin), POPC(1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), and cholesterol were purchased from Avanti Polar Lipids, Inc.

3. Results and discussion

Fig. 1 shows the RBC shape transformation induced by the addition of β2-GPI to RBC suspension. After the addition of β2-GPI in RBC suspension the natural discocyte RBCs (marked by white arrows in Fig. 1A) gradually start to change to the echinocyte RBCs type I–II (marked by white arrow in Fig. 1B) and the echinocyte RBCs type III (marked by white arrow in Fig. 1C).

The echinocytes type I–II have a discoid shape with spikes slowly protruding out of RBC membrane. When more and more molecules of β2-GPI diffuse into the region of observed RBCs the RBCs change from discoid to spherical shape of echinocyte type III. Finally the echinocyte shape of RBC is transformed into a sphere (marked by black arrows in Fig. 1C, D), when the membrane microexovesicles are released from RBC membrane [16,17]. As shown in Figs. 1 and 2 β2-GPI induces RBC spherocytes agglutination if they come close enough together. RBCs may also adhere to the bottom of the observation chamber.

It has been shown that the stability of the echinocyte shape is primarily determined by the competition between the membrane bilayer Helfrich–Evans bending energy and the membrane skeleton shear energy [18,19]. The echinocyte shape is additionally modulated by nonhomogeneous lateral stretching of the membrane skeleton [20–22] and by nonhomogeneous lateral distribution of the membrane constituents (lipids and proteins) and membrane nano domains [24,23]. In the early experiments it was already shown that the normal discoid shape of RBCs may be transformed into spikulated echinocyte shape [25] by the addition of substances which intercalated preferentially into the outer membrane leaflet [26]. At high concentration of echinocytogenic molecules budding and microexovesicle (spherical or tubular) release from membrane surface starts [23,27–29], leading to spherocytic shapes [19,27] (Fig. 3).

Based on the observed β2-GPI-induced discocyte–echinocyte–spherocyte RBC shape transformation we propose that the hydrophobic protrusions of membrane-bound β2-GPI molecules [3] are embedded in the outer membrane layer of RBC membrane (Fig. 4) and thereby increase the relaxed area difference between the areas of membrane lipid layers ΔAΩ [30,31,26,32]. Consequently, the discocyte–echinocyte–spherocyte shape transformation is induced (Fig. 3) [18,19,22].

As it can be seen in Fig. 1, the RBCs in the suspension do not change their shape all at once and/or completely. This is probably because of slow partition of β2-GPI in RBC membranes due to time-dependent local concentration of unbound β2-GPI which increases gradually because of β2-GPI diffusion. Therefore, it is possible to observe all the stages of discocyte–echinocyte shape transformation in the same small region of RBC suspension (see for example Fig. 1C): effect on morphology of RBC (data not shown). The agglutination of β2-GPI treated RBCs probably depends on the proximity factor. Two RBCs will agglutinate if they come close enough together. In this case the formation of complexes consisting of many RBCs will occur (white arrow in Fig. 1D). In highly diluted RBC suspension RBCs seldom come close together. As a consequence the RBC complexes consist of only small number of RBCs (see Fig. 2).

Fig. 2 also shows the agglutination of RBCs to the negatively charged surface of POPC–cardiolipin–cholesterol vesicles (black arrows in Fig. 2C points to the phospholipid vesicles). In the lower left corner of Fig. 2C we can see the complex of phospholipid vesicles tightly attached to each other. Similarly, as in the case of RBCs, the agglutination between negatively charged surfaces of vesicles was also induced by β2-GPI (Fig. 2C).

β2-GPI is a J-shaped molecule composed of five domains (Fig. 4) [3,33]. The part of the first domain and the part of the fifth domain of β2-GPI are predominantly positively charged. Both of them bind to negatively charged lipid surfaces [11,8]. It was indicated that in liposome systems the β2-GPI binds to phospholipid membranes in two phases [34]. The initial fast phase when β2-GPI binds to membrane surface was assumed to be primarily driven by the electrostatic interactions between positively charged fifth domain of β2-GPI and negatively charged head groups of membrane lipids. The first phase thus strongly depends on the content of negatively charged membrane.
lipids, while the second slower phase does not substantially depend on the percentage of the negatively charged lipids in the membrane and is accompanied by clustering of $\beta_2$-GPI and rigidification of the membrane [34].

In RBC membrane negatively charged lipid molecules are almost entirely localised in the inner lipid layer of cell membrane. Therefore the negative charge of the outer surface of RBC membrane is mainly attributed to negatively charged glyocalyx (see [35,36] and references therein).

Based on the analogy to the above proposed mechanism of $\beta_2$-GPI binding to the negatively charged surface of liposomes we assume that also in the case of RBCs the binding of $\beta_2$-GPI to RBC membrane is at least partially mediated by the electrostatic attractive interaction between the positively charged part of the fifth domain of $\beta_2$-GPI and the negatively charged groups of glyocalyx. The binding of $\beta_2$-GPI to the outer surface of RBC membrane may be additionally promoted by the hydrophobic loop on the fifth domain of $\beta_2$-GPI which protrudes in membrane lipid bilayer.

On the basis of our experimental results we suggest that the observed $\beta_2$-GPI-induced adhesion of negatively charged membranes of RBCs is mediated by the electrostatic attractive interaction between the positively charged part of the first domains of bound $\beta_2$-GPI molecules and the negatively charged glyocalyx of the adjacent RBC membrane. Our hypothesis is additionally supported by the experimental results which showed that the first domain of $\beta_2$-GPI binds to negatively charged lipid surfaces [11,8].

**Fig. 5.** Schematic presentation shows two different regimes regarding the distance between the two RBC membrane lipid bilayer surfaces with attached $\beta_2$-GPI molecules: $h \geq 2D$ (left) and $h = 2D$ (right).

**Fig. 6.** Free energy $\Delta F(h) = F(h) - F(\lim_{h \to \infty})$ as a function of the distance $h$ for different values of $\sigma_1$: 0.01 As/m$^2$ (a), 0.008 As/m$^2$ (b), 0.006 As/m$^2$ (c), 0.004 As/m$^2$ (d) and 0.002 As/m$^2$ (e). The length of the $\beta_2$-GPI molecule $D = 10$ nm (see also Fig. 5). Values of the effective surface charge density at the outer lipid surface $\sigma = 0.01$ As/m$^2$. Salt concentration in the bulk solution $n_0/N_A = 25$ mmol/l, where $N_A$ is Avogadro’s number.
However, it should be stressed that the proposed attractive Coulomb (charge–charge) interactions between positively charged part of the first domain of membrane-bound β2-GPI molecules and the negatively charged glyocalyx groups of the adjacent RBC membrane are not the only possible electrostatic interactions responsible for β2-GPI-mediated agglutination between like-charged RBC membrane surfaces. In general, also the charge–dipole and dipole–dipole electrostatic interactions [37] between lipids and β2-GPI may contribute to β2-GPI-induced adhesion between like-charged RBC membrane surfaces.

In the following we shall describe the possible mechanism of electrostatic interaction between the positively charged part of the first domains of membrane-bound β2-GPI molecules and the negatively charged glyocalyx of the adjacent RBC membrane more in detail. In our simple theoretical model the volume charge distribution of the positively charged first domain of membrane-bound β2-GPI is in the first approximation described by a positively charged surface (with the surface charge density \( \sigma_1 \)) at a distance \( D \) away from the outer bilayer surface (see also Figs. 4 and 5). In the model the distance \( D \) is approximately equal to the length of β2-GPI molecule \( \approx 10 \) nm [3,33].

In glyocalyx the carboxyl groups of sialic acid molecules are mainly responsible for its negative charge. The mean number of sialic acid molecules per RBC varies from 1 to 4 \times 10^7 [38,35,39]. Using the value \( A = 150 \) m² for the surface area of RBC the value \( \sigma \approx 1 - 4 \times 10^{-2} \) As/m² is obtained for the effective surface charge density of outer RBC membrane surface. However, because of the volume charge distribution of glyocalyx the magnitude of the corresponding outer membrane surface potential is smaller as it would be in the case of planar charge distribution of glyocalyx [40,35,36]. Therefore and because of the positive charge of membrane-bound β2-GPI’s fifth domains we take in the model that the effective surface charge density of outer RBC membrane surface \( \sigma \) is at the lower limit of the above estimated range of the values for the effective membrane surface charge density \( \sigma \approx 1 - 4 \times 10^{-2} \) As/m².

In accordance with above assumptions and simplifications we describe the electrostatic interaction between the membranes of RBCs by two interacting electric double layers generated by two planar charged surfaces. In the model the space between the charged surfaces is filled with electrolyte (salt) solution. The space charge distribution due to phospholipid molecules and glyocalyx is described by the effective surface charge density of the two interacting charged surfaces \( \sigma \). The positive charge of the tips of β2-GPI molecules bound to both surfaces is represented by two additional charged surfaces (with the surface charge densities \( \sigma_1 \)) at distance \( D \) from each of the membrane bilayer surfaces (Fig. 5).

If the distance between the membrane bilayer surfaces \( h \) is larger than 2D, the space between both membrane bilayer surfaces can be divided into three different regions \( 0 \leq x < a \) and \( h-a \leq x < h \) (see Fig. 5). If \( h \geq 2D \) the value of \( a \) is equal to \( D \), while in the case of \( h < 2D \) the value of \( a \) depends on \( h \) and is always smaller than \( D \) (Fig. 5).

The linearized Poisson–Boltzmann (PB) theory overestimates the electrostatic free energy of the electric double layer [41]. Nevertheless, to keep our model traceable we adopt the result of the linear PB theory, i.e. the electrostatic potential \( \Psi(x) \) in the system was calculated from the linearized PB (LPB) equation: \( \nabla^2 \Psi = \kappa_0^2 \Psi \), where \( \kappa_0^2 = e \Psi/kT \) is dimensionless electrostatic potential and \( \kappa_0^2 = (\sqrt{\varepsilon_0 \varepsilon R}/(2\eta D^2)) \) is the Debye length. Here \( \varepsilon_0 \) is the dielectric constant of the aqueous solution, \( e \) is the permittivity of free space, \( \eta \) is the number density of cations and of the anions in the bulk solution (assuming a 1:1 salt such as NaCl), and \( \varepsilon_0 \) is the unit charge.

Due to the symmetry of the system we are searching for the solution of LPB equation only in the region \( 0 \leq x < h/2 \) (see Fig. 5). Thus, the solution of LPB equation can be written as:

\[
\phi = A \exp(-\kappa_0 x) + B \exp(\kappa_0 x), \quad 0 \leq x \leq \alpha,
\]

(1)

\[
\phi = C \exp(-\kappa_0 x) + D \exp(\kappa_0 x), \quad \alpha \leq x \leq h/2,
\]

(2)

where the constants \( A, B, C \) and \( D \) were determined analytically from the boundary conditions: \( \frac{d\phi}{dx} (x = 0) = -a \sigma_0/\varepsilon_0 \kappa_0^2 \kappa_0 \), \( \phi(x = a) = \phi(x = a) \), \( \frac{d\phi}{dx} (x = a) = \frac{d\phi}{dx} (x = a) + \sigma_1 \varepsilon_0/\varepsilon_0 \kappa_0 \kappa_0 \), and \( \frac{d\phi}{dx} (x = h/2) = 0 \). Including also the configurational entropy of the anions \( j = 1 \) and cations \( j = 2 \) of the salt dissolved in the water between both planar lipid surfaces, we can write the free energy of the system in the form [42,41,43]:

\[
F/A = \int_0^h \left( \frac{1}{\varepsilon_0 \varepsilon R} \frac{d\phi}{dx} \frac{d\phi}{dx} \right) + C_{18} \sum_{j=1}^{\pi_j} \left( \eta_j \ln \left( \frac{\eta_j}{\eta_0} \right) - (\eta_j - \eta_0) \right) dx,
\]

(3)

where \( n_1 = \eta_0 \exp(\phi(x)) \) and \( n_2 = \eta_0 \exp(-\phi(x)) \) are the number densities of anions \( j = 1 \) and cations \( j = 2 \) in the salt solution, \( \eta_0 \) is the number density of the anions and cations in the bulk solution (i.e. outside the space between the planar lipid surfaces). The bulk solution (outside the space between the membrane) provides a suitable reference also for electric potential \( \phi_{\text{bulk}} = 0 \) (see also [43]).

Fig. 6 shows the free energy \( \Delta F = F(h) - F(h = \infty) \) of the system as a function of the distance \( h \) between the two adjacent membrane surfaces with attached β2-GPI molecules. As it can be seen in Fig. 6 the free energy \( \Delta F \) first increases with decreasing intermembrane distance \( h \) until \( h = 2D \). For \( h < 2D \) the value of \( \Delta F \) decreases with decreasing \( h \) until the absolute minimum of \( \Delta F \) at \( h = D \) is reached. The results presented in Fig. 6 reflect the fact that the two adjacent membranes with bound β2-GPI molecules repel each other for \( h > 2D \). However, when the energy barrier at \( h = 2D \) is crossed, the force between two membranes becomes strongly attractive leading to the equilibrium distance at \( h = D \) where the positively charged first domains of bound β2-GPI molecules and negatively charged glyocalyx of the opposite membrane of the adjacent RBC are in close contact (Fig. 6).

Fig. 7 shows the strength of the β2-GPI-mediated electrostatic adhesion between negatively charged RBC membrane surfaces \( \Delta F(h = D)/A \) (see also Fig. 6) as a function of the bulk salt concentration \( [\eta_0]/[N_A] \). It can be seen in Fig. 7 that the absolute value of the adhesion strength \( \Delta F(h = D)/A \) strongly decreases with increasing ionic strength. This is in accordance with our experimental observations (data not shown) which indicate that capability of β2-GPI to induce agglutination of RBCs decreases with increasing ionic strength. The observed decreased degree of β2-GPI-induced agglutination of RBCs at higher ionic strength may be partially also the consequence of altered (reduced) β2-GPI binding to negatively charged RBCs at higher ionic strength (see also [49]).
In our experiments the bulk ionic strength in final RBC suspension was around 24 mmol/l corresponding to Debye length 2 nm. The number of bound β2-GPI molecules for echinocytes of type III can be roughly estimated from theoretically predicted value of the relaxed area difference between the two membrane monolayers (ΔA0). By taking into account ΔA0 = 3 μm² [19,22,44] and that the area of the cross-section of β2-GPI hydrophobic protrusion is around 1 nm² [34] it follows that the number of the bound β2-GPI is of the order of 10³. This means that the average distance between the membrane-bound β2-GPI for echinocytes of type III is around 5 times larger than the Debye length (~2 nm). In the case of spherocytes the average distance between the membrane-bound β2-GPI molecules is smaller since the area density of membrane-bound β2-GPI molecules must be higher (see [19,45]). However, also in this case the average distance between bound β2-GPI molecules is still larger than Debye length. Despite relatively large average distance between bound β2-GPI molecules we believe that the main predictions of our electrostatic model are still qualitatively correct since the average distance between negative electric charges of the RBC membrane(in glyocalyx) is much smaller (see also [40]).

Our experimental system substantially differs from the blood in vitro conditions. Plasma was removed in repeated washing procedures and the experimental solution was added. Therefore in experimental solution the conditions are different than in plasma. Plasma contains also other constituents such as proteins, lipoproteins and small ions. Some of the plasma components as for example LDL nanoparticles which bear net negative electric charge [46] may bind β2-GPI molecules [47]. Therefore the effect of β2-GPI on RBC membrane may be abolished or reduced by other plasma constituents. Also, the effects of other plasma constituents (beside β2-GPI) on RBC adhesion are not known. In blood (in in vivo conditions) the adhesion of discocytic RBCs is also prevented by blood flow. If the velocity of blood flow is relatively high, RBCs elongate and move with the flow, while if the velocity of blood flow is low (such as in the vicinity of vessel bifurcations or geometry changes) RBCs adhere to each other and may form rouleaux (see for example [48]).

4. Conclusions

In the presented work we studied β2-GPI-mediated attractive interaction between RBCs and the resulting agglutination of RBCs. It was suggested that the observed β2-GPI-induced attractive interaction between the two adjacent RBC membranes is at least partially of electrostatic origin where the specific molecular shape and the internal charge distribution of membrane-bound β2-GPI molecules may play a decisive role. The proposed β2-GPI-mediated attractive interactions between two adjacent like-charged RBC membranes was qualitatively explained by using the functional density theory of the electric double layer.

The β2-GPI-induced attractive interactions between negatively charged membranes were studied also in mixed suspensions of RBCs and POPC–cardiolipin–cholesterol vesicles, where we observed the adhesion of RBCs to the negatively charged surface of POPC–cardiolipin–cholesterol vesicles (Fig. 2B, C). These results are in accordance with the previous experimental results which showed that the first domain of β2-GPI binds to negatively charged lipid surfaces [11,8] and support our hypothesis that the observed β2-GPI-induced adhesion between negatively charged membranes of RBCs is at least partially mediated by electrostatic attraction between the positively charged part of the first domains of bound β2-GPI molecules and the negatively charged glyocalyx of the adjacent RBC membrane. A similar mathematical model of membrane electrostatics that was used to theoretically explain β2-GPI-induced agglutination of RBCs can also be applied in the case of β2-GPI-induced agglutination of RBCs to the lipid vesicles.

Beside the direct influence of membrane-bound proteins on the membrane electrostatics and attraction forces between equally charged adjacent membranes, the membrane-bound proteins can also modify the membrane bending rigidity [34,50]. Changed bending rigidity may affect fluctuations of the membrane and in this way also the agglutination process between the adjacent membranes [51].

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References


