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β_2-Glycoprotein I-dependent alterations in membrane properties

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Abstract

β_2-Glycoprotein I (β_2GP1), a 50 kDa serum glycoprotein, binds anionic phospholipids and plays a role in phosphatidylserine (PS)-dependent coagulation and apoptotic processes. To characterize the molecular consequences that occur to target membranes upon binding of β_2GP1, the interaction between β_2GP1 and PS-containing vesicles was investigated by fluorescent spectroscopy. Membranes containing pyrene-labeled lipid showed that binding of β_2GP1 induced a decrease in excimer/monomor ratios (E/M) of the target membrane. Although these membrane alterations occurred in isotonic buffer, the effects were greater in low ionic strength buffer and were coincident to membrane precipitation. In contrast, increases in membrane polarization were only seen in low ionic strength buffer. Analysis of β_2GP1 binding kinetics by resonance energy transfer between fluorescein-labeled β_2GP1 and rhodamine-containing PS vesicles revealed a two-component process: (1) a primary and rapid binding via the C-terminus that occurred <2 s in both isotonic and low ionic strength buffers, and (2) a sequential binding of the N-terminus that was $\sim$100-fold slower in low ionic strength solution. Taken together, these data suggest that β_2GP1 alters the fluidity and membrane polarization of its target membrane, which in low ionic strength buffer is of sufficient magnitude to induce precipitation.

Keywords: β_2-Glycoprotein 1; Apolipoprotein H; Phosphatidylserine; Small unilamellar vesicle

1. Introduction

β_2-Glycoprotein I (β_2GP1), a 50 kDa glycoprotein, is the major lipid binding protein in plasma and plays a role in autoimmunity [1–3], coagulation [4] and the clearance of apoptotic and senescent cells [5–8]. It is composed of 326 amino acids and is a member of the short consensus repeat (SCR) superfamily containing four typical repeating domains and an aberrant fifth domain constructed onto a SCR-like core at the C-terminus [9–11]. While both domains I and V exhibit lipid binding properties, the fifth domain is the principal lipid binding domain [12–14]. Lipid binding in domain V occurs through a series of

Abbreviations: β_2GP1, β_2-glycoprotein I; PC, phosphatidylcholine; PS, phosphatidylserine; Pyr-PC, 1-hexadecanoyl-2-(1-pyrenehexanoyl)-*sn*-glycero-3-phosphocholine; N-NBD-PE, *N*-(7-nitrobenz-2-oxa-1,3 diazo-4-yl) 1,2 diacyl-L-α-phosphatidylethanolamine; N-Rho-PE, *N*-(lissamine rhodamine B sulfonyl) 1,2 diacyl-L-α-phosphatidylethanolamine; DPH, 1,6-diphenyl-1,3,5-hexatriene; FITC, fluorescein-5-isothiocyanate; Tris–EDTA, 10 mM Tris, 0.1 mM EDTA, pH 7.0

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positively-charged lysine residues [15,16] that likely results in the insertion of a partially hydrophobic loop into the lipid bilayer [17]. From its crystal structure, Bouma et al. [18] suggested that domains I through IV remain in solution leaving the N-terminus available for other membrane interactions. Conceivably, this could occur through other negatively charged lipids or specific cell surface receptors.

Various studies have indicated that the binding of β_2GP1 with target membranes bearing negatively-charged lipids induce agglutination and precipitation [19,20]. However, the mechanism responsible for the precipitation of membranes upon binding of β_2GP1 is unknown. Conceivably, precipitation could be due to simple aggregation as a result of bivalent (or multivalent) protein–lipid interactions, or because of direct alterations in the properties of the target membrane. To characterize the interaction between β_2GP1 and phospholipid membranes, a series of fluorescent measurements, light-scattering intensity measurements and precipitation experiments were carried out. Our results suggest that precipitation of membranes with β_2GP1 involves at least two distinct binding interactions. Critical primary binding induces local membrane changes, which in turn, can propagate to sequential secondary interactions that result in global membrane alterations leading to precipitation.

2. Materials and methods

2.1. Materials and routine procedures

Dioleoylphosphatidylcholine (PC), bovine brain PS, N-Rho-PE and N-NBD-PE were from Avanti Polar Lipids (Alabaster, AL). Pyr-PC and DPH were purchased from Molecular Probes (Eugene, OR). Lipid concentrations were determined by phosphate assay and tested for purity by thin layer chromatography in $CHCl_3$/MEOH/H_2O (65:25:4). Fluorescence was measured with a Perkin–Elmer LS-50B spectrophotometer. Small unilamellar vesicles (SUV) were prepared by drying lipids under N_2 and sonicating in Tris–EDTA (10 mM Tris/0.1 mM EDTA; pH 7.0) at 4°C. Large vesicles and titanium fragments were removed by centrifugation at $15\,000 \times g$ for 15 min. G_{274}–C_{288} (GDKVSFFCKNKEKKC), a peptide corresponding to the domain V lipid binding site of β_2GP1, was synthesized by the synthetic antigen core facility at M.D. Anderson Cancer Center, Houston, TX. β_2GP1 antibodies were produced in rabbits as previously described [6].

2.2. Purification of β_2GP1

β_2GP1 was purified from pooled human plasma (Gulf Coast Regional Blood Center, Houston, TX) by perchloric acid (2.2% v/v) precipitation. The acid-soluble fraction was further purified by ion exchange chromatography on DEAE–cellulose and heparin-affinity chromatography [19–21]. Protein concentration was determined with the Pierce bicinchoninic acid assay [22]. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis revealed a single silver stained band at 50 kDa [6]. Western blotting confirmed the band to be β_2GP1 and lipoblotting revealed that the protein specifically bound PS-containing vesicles [23].

2.3. Fluorescent labeling of β_2GP1

Human β_2GP1 contains a free sulfhydryl at CYS 102 and/or CYS 169 [9,11] located in domains II and III, respectively. These sites were labeled with FITC–maleimide. Briefly, 20 nmol of β_2GP1 in Tris–EDTA were labeled with 2 μmol of FITC–maleimide (100 mM stock in DMSO) for 1 h at 20°C. Unreacted reagent was removed by overnight dialysis against Tris–EDTA followed by column chromatography on Biogel P-6 equilibrated in the same buffer. The FITC/protein ratio was estimated to be $\sim$2.1:1 from the absorbance at 493 and 280 nm [24].

2.4. Fluorescence measurements

Preliminary analysis of the kinetics of interaction between β_2GP1 and vesicles using the spectroscopic methods described below indicated no changes in fluorescence beyond $\sim$4 min. For this reason all fluorescent analysis were measured after 5 min of incubation.

2.4.1. Pyrene-labeled PC

The influence of β_2GP1 on membranes was monitored by the ratio of the fluorescence of the excimer

(E) and monomer (M) of Pyr-PC at 477 and 399 nm, respectively (λ_{ex} 345 nm) [25,26]. Briefly, PC/PS vesicles (4 μM) labeled with Pyr-PC (10 mol%) in Tris–EDTA with or without NaCl (150 mM) were incubated with increasing concentrations of β_2GP1. The percent change in E/M was calculated as described by Junker and Creutz [27]:

$$[[(E/M)_{final}-(E/M)_{initial}]/(E/M)_{initial}]\times 100 \quad (1)$$

where $(E/M)_{initial}$ was the fluorescence ratio of vesicles alone and $(E/M)_{final}$ was the fluorescence ratio after the addition of β_2GP1.

2.4.2. DPH Polarization

DPH was utilized to measure membrane polarization (P). Briefly, vesicles (4 μM) containing increasing concentrations of PS in PC were prepared in Tris–EDTA with or without NaCl (150 mM). The vesicles were then incubated with DPH (1 μM) at 30°C for 1 h. P was monitored (λ_{ex} 356 nm, λ_{em} 430 nm) at 25°C with increasing concentrations of β_2GP1. P was calculated by the following equation [28,29]:

$$P = (I_{hh}-GI_{hv})/(I_{hh}+GI_{hv}) \quad (2)$$

where I_{hv} and I_{hh} are the fluorescent intensities when the polarizers are perpendicular and parallel, respectively, and G is the grating factor.

2.4.3. Resonance energy transfer (RET)

RET experiments were carried out to monitor β_2GP1-dependent vesicle–vesicle interactions and protein–vesicle interactions. Vesicle–vesicle interactions were determined by incubating equimolar concentrations of donor and acceptor vesicles for 5 min at 20°C. Donor vesicles consisted of PC/PS vesicles containing N-NBD-PE (1 mol%); acceptor vesicles consisted of PC or PC/PS vesicles labeled with N-Rho-PE (2 mol%) [30]. Emission spectra were recorded between 500 and 600 nm (λ_{ex} 450 nm) before and after the addition of β_2GP1. RET was measured as a function of the decrease in NBD emission intensity at 528 nm. Vesicles fused with Ca^{2+} (5 mM) served as positive controls [31–33]. The interaction of β_2GP1 with vesicles was monitored as described above except that FITC-labeled β_2GP1 and N-Rho-PE labeled PC/PS vesicles were used as the fluorescent donor and acceptor, respectively.

2.5. Precipitation of vesicles with β_2GP1

β_2GP1-dependent precipitation curves were generated using vesicles labeled with N-NBD-PE and/or N-Rho-PE. Briefly, vesicles were incubated with β_2GP1 for 1 h at 20°C. Aliquots were removed before and after centrifugation (15 000 × g for 15 min). The fluorescence was measured at their respective wavelengths and the fraction of precipitated vesicles was calculated from the difference in intensities of the supernatant before and after centrifugation.

2.6. Light-scattering measurements

The association of β_2GP1 to PS-containing vesicles was measured by light-scattering intensity as described by Nelsestuen and Lim [34]. Briefly, 90° light-scattering intensity was monitored at 320 nm in Tris–EDTA with or without NaCl (150 mM). β_2GP1 was added to the cuvette and light-scattering intensity of the protein–vesicle complex (I_2) was compared to the light-scattering intensity of the vesicles alone (I_1). M_2/M_1 was calculated according to the following equation as described previously [34]:

$$I_2/I_1 = (M_2/M_1)^2 \quad (3)$$

where M_2 is the relative molecular mass of the complex and M_1 is the relative molecular mass of vesicles alone. I_2 and I_1 were corrected for dilution and light-scattering intensity of the free protein. Positive controls for the induction of large aggregates were generated from vesicle/β_2GP1 complexes that were treated with β_2GP1 antibodies (20 μg/ml) for 10 min at 20°C.

2.7. Statistical analysis

Results are expressed as mean ± S.E.M. for at least four separate experiments performed with different reagents.

3. Results

3.1. β_2GP1-Induced precipitation of PS vesicles

To determine the propensity of β_2GP1 to precipitate PS-containing membranes, N-Rho-PE-labeled

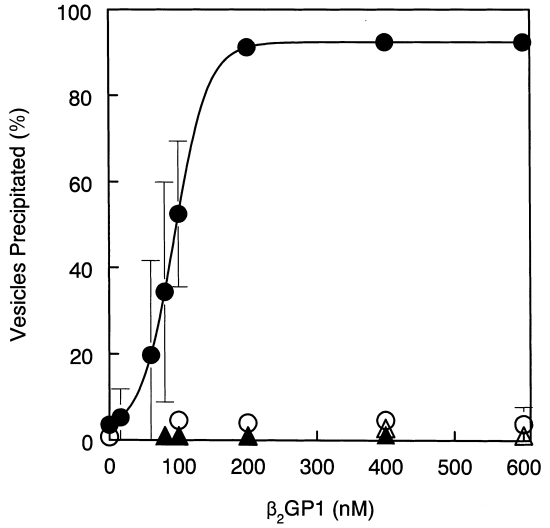

Fig. 1. Precipitation of N-Rho-PE/PC/PS (1:49:50) vesicles with β_2GP1 or G_{274}–C_{288}. Vesicles (5 μM) were incubated with increasing concentrations of β_2GP1 (○, ●) or peptide (△, ▲) for 1 h at 20°C. After centrifugation, the fraction of lipid precipitated was determined by fluorescence. Tris–EDTA (closed symbols), Tris–EDTA/150 mM NaCl (open symbols).

vesicles were incubated with increasing concentrations of β_2GP1 for 1 h at 20°C in the presence and absence of 150 mM NaCl. The data presented in Fig. 1 shows that maximum precipitation occurred at ~200 nM β_2GP1 in the absence of salt. The vesicles did not precipitate when the incubation mixture contained 150 mM NaCl or when the vesicles were incubated with G_{274}–C_{288}, a peptide corresponding to the β_2GP1 lipid-binding site. Pure PC vesicles did not precipitate (not shown).

3.2. *β_2GP1 induces decreased E/M ratio and increased membrane polarization*

The addition of β_2GP1 to PS containing vesicles resulted in a decrease in the excimer to monomer ratio (E/M), suggesting a decrease in the lateral mobility as well as a change in the local concentration of the fluorophore in the membrane [25,26] (Fig. 2). The changes in E/M were significantly greater when the experiment was carried out in the absence of salt. Consistent with the data obtained in the precipitation experiment described above, alterations in E/M with G_{274}–C_{288} were essentially identical to those obtained with the intact protein in the presence of 150 mM NaCl. The decrease in E/M could be due to alterations in the lateral dispersion of the probe or to a decrease in the collisional frequency as a result of enhanced membrane rigidity. To distinguish between these possibilities, global changes in membrane polarization as a function of both the concentration of PS and β_2GP1 were measured in DPH labeled vesicles. As can be seen in Fig. 3A, P increased as a function of both the concentration of β_2GP1 and the fraction of PS in the membrane. Unlike the results obtained with pyrene-labeled vesicles, however, the inclusion of 150 mM NaCl in the incubation medium resulted in ~60% reduction in β_2GP1-dependent changes in membrane polarization (Fig. 3B) that was coincident to the inhibition of vesicle precipitation (Fig. 1).

3.3. *Influence of β_2GP1 on vesicle size*

β_2GP1-Dependent precipitation of vesicles results in leakage of vesicle contents [35]. This raises the possibility that precipitation could be due to the formation of large fused vesicles. To determine whether β_2GP1 induced fusion, pyrene-labeled membranes were incubated with a 5-fold excess of unlabeled

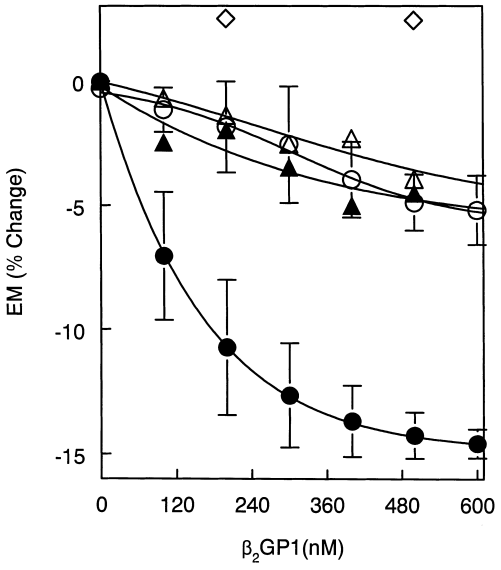

Fig. 2. β_2GP1-Dependent E/M changes in PS vesicles. Vesicles containing Pyr-PC/PC/PS (10:40:50) were incubated with increasing concentrations of β_2GP1 (○, ●) or G_{274}–C_{288} (△, ▲) for 5 min at 20°C. Vesicles were incubated in Tris–EDTA (closed symbols), Tris–EDTA/150 mM NaCl (open symbols). Vesicles containing only Pyr-PC/PC (10:90) were used as negative controls (◇).

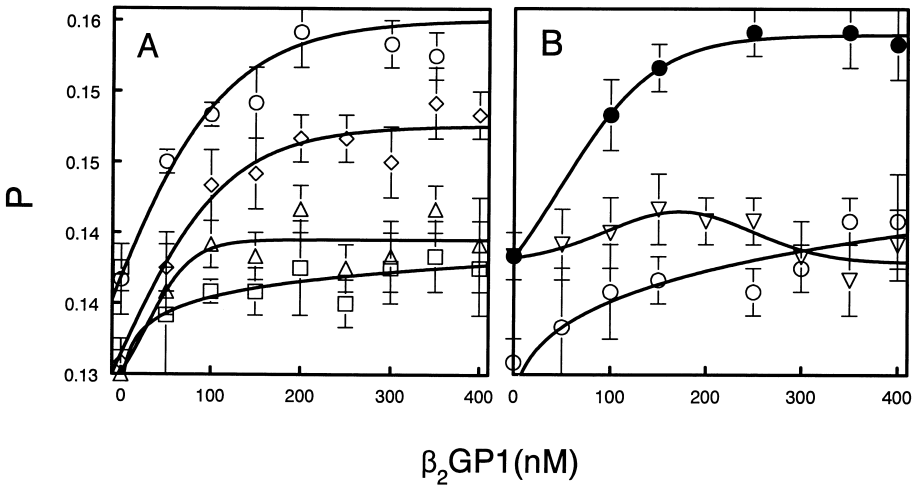

Fig. 3. DPH polarization of PS vesicles. (A) Vesicles (4 μM) of the indicated compositions were labeled with DPH (1 μM) and incubated with β_2GP1 in Tris–EDTA. (□) 0 mol% PS; (△), 20 mol% PS; (◇), 50 mol% PS; (○), 70 mol% PS. (B) The effect of ionic strength on *P* was determined with PC/PS (50:50) vesicles. Vesicles incubated with β_2GP1 in the absence (●) and presence of 150 mM NaCl (○). Vesicles incubated with ovalbumin (▽).

vesicles, and probe dilution was monitored as a function of E/M. In contrast to the dramatic ~65% decrease in E/M observed upon the addition of Ca^{2+}, the addition of β_2GP1 to PS-containing vesicles resulted in only a ~18% decrease in E/M (Fig. 4A). In addition, β_2GP1 did not promote RET between N-NBD-PE and N-Rho-PE labeled membranes (Fig. 4B). These independent results indicate that β_2GP1 did not induce vesicle fusion and raise the possibility that precipitation might be due to vesicle cross-linking. Light-scattering experiments indicated that particle size was dependent on both the concen-

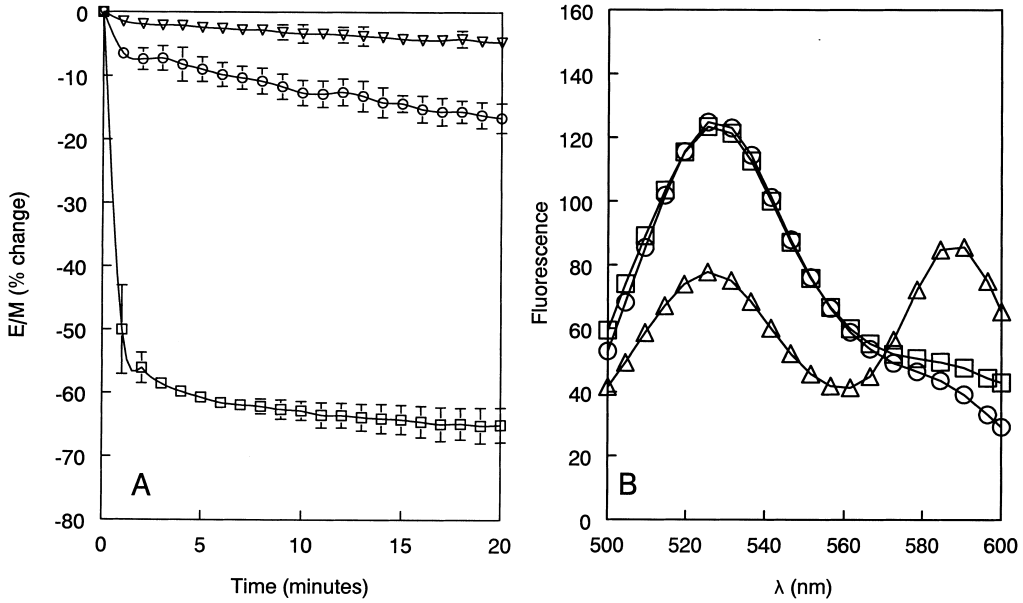

Fig. 4. β_2GP1 does not induce vesicle–vesicle fusion. (A) Pyr-PC/PC/PS (10:40:50) vesicles (4 μM) were mixed with unlabeled vesicles (20 μM) and incubated with 300 nM β_2GP1 (○), 300 nM ovalbumin (▽) or 5 mM Ca^{2+} (□). (B) RET between N-NBD-PE/PC/PS (1:49:50) and N-Rho-PE/PC/PS (2:48:50) vesicles. Vesicles (5 μM of each population) were mixed and emission spectra (λ_{ex} 450 nm) were recorded before (□), and after the addition of 300 nM β_2GP1 (○) or 5 mM Ca^{2+} (△).

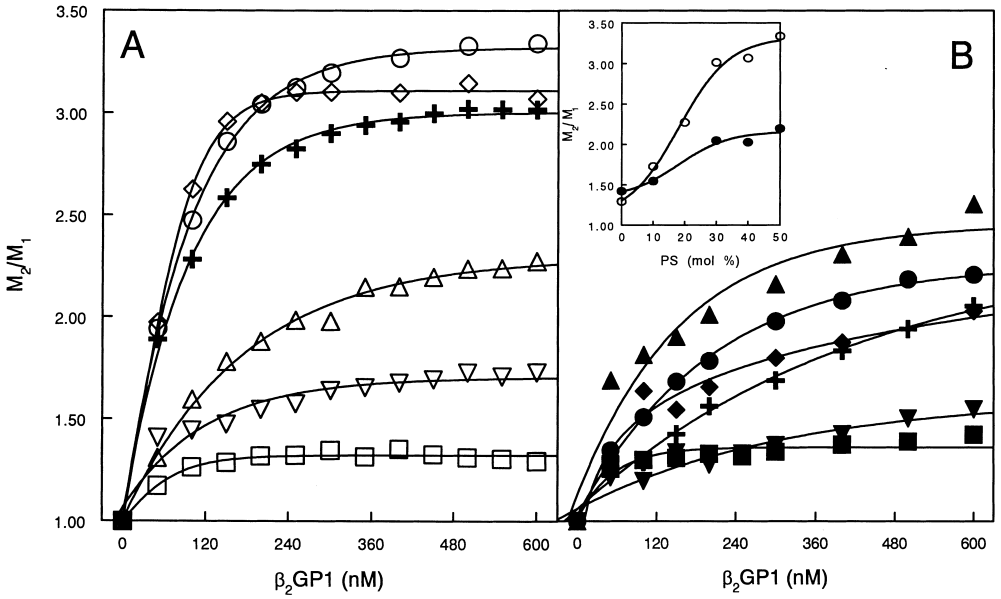

Fig. 5. Relative molecular mass of β_2GP1/vesicle complexes. M_2/M_1 was calculated for vesicles incubated for 10 min with increasing concentrations of β_2GP1 at different PC/PS ratios in the absence (A) and presence (B) of 150 mM NaCl by monitoring 90° light scattering at 320 nm. 0 mol% (□, ■), 10 mol% (▽, ▼), 20 mol% (△, ▲), 30 mol% (+), 40 mol% (◇, ◆), and 50 mol% PS (○, ●). Inset: M_2/M_1 for vesicles containing increasing fractions of PS incubated with β_2GP1 (600 nM) in the absence (open symbols) and presence of 150 mM NaCl (closed symbols).

tration of β_2GP1 and the fraction of PS in the membrane (Fig. 5). Although particle sizes were influenced by salt (M_2/M_1 salt ~2.4; M_2/M_1 no salt 3.2), precipitation occurred only in the absence of salt (see Fig. 1). These data suggest that β_2GP1 did not induce extensive vesicle–vesicle cross-linking. The addition of β_2GP1 antibodies (20 μg/ml), on the other hand, aggregated the vesicles and resulted in $M_2/M_1 > 20$.

3.4. Resonance energy transfer between β_2GP1 and target membranes

While the fifth domain of β_2GP1 is principally responsible for its binding specificity [12,14], it has recently been suggested that domains I through IV adsorb parallel to the bilayer surface in low ionic strength buffers [17]. To determine if binding through these domains are concurrent or sequential, the association of domain II/III by RET between FITC–maleimide-labeled β_2GP1 and N-Rho-PE containing vesicles was monitored in hypotonic and isotonic buffers. Sequential emission scans taken at 12 s intervals showed a rapid decrease in fluorescent intensity at 520 nm for vesicles in both the absence and presence of 150 mM NaCl. However, in contrast to the progressive decrease observed in low salt solutions, no decrease beyond the second scan was observed in the presence of 150 mM NaCl. To analyze the kinetics of these processes in greater detail, fluorescence intensities were monitored in real-time at 520 nM and RET efficiencies were calculated. Fig.

Table 1
Kinetics of RET[a]

	Fast component (%/s)	Slow component (%/s)
PC/PS 10 mM Tris	17.4	0.17
PC/PS 150 mM salt	17.3	< 0.0005
PC vesicles 10 mM Tris	–	< 0.0005

[a]Resonance energy transfer efficiency was calculated from the data presented in Fig. 6D. The initial rates were calculated from the linear components of each curve.

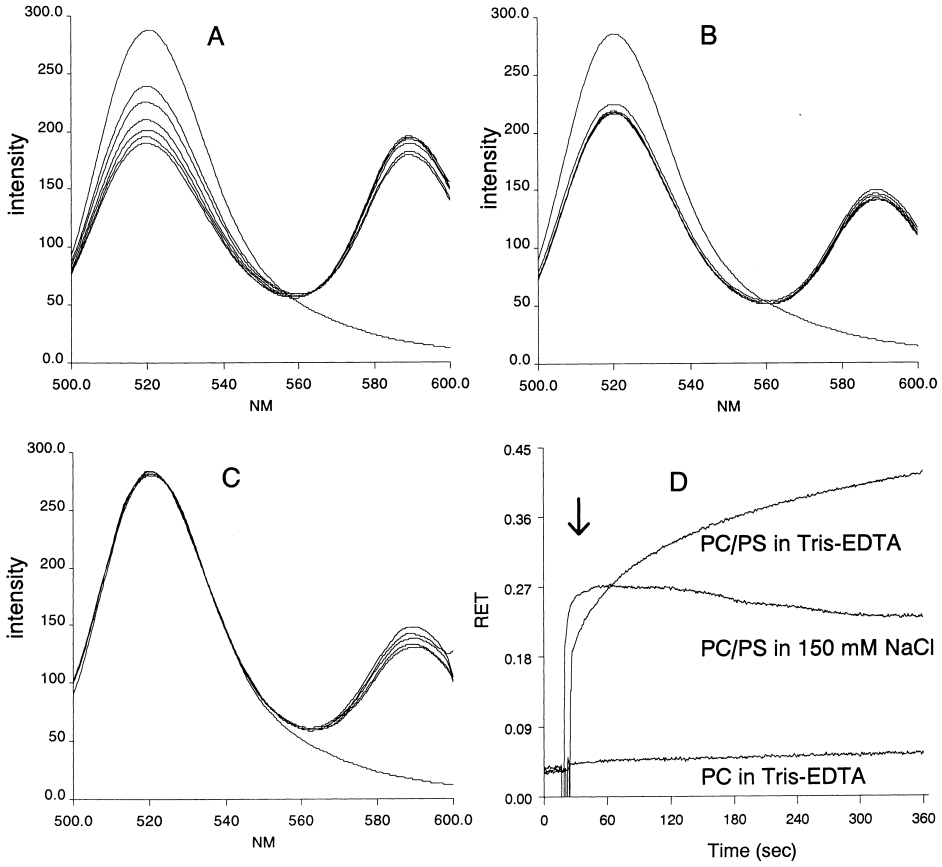

Fig. 6. Extrinsic (fluorescein-labeled) fluorescence of β_2GP1 incubated with PS vesicles. The change in the fluorescence intensity of β_2GP1 (15 nM) was monitored upon the addition of vesicles (40 μM). Panels A–C show sequential emission scans (λ_{ex} 450 nm) at ~12 s intervals for vesicles containing 2% N-Rho-PE. (A) PC/PS vesicles in Tris–EDTA; (B) PC/PS vesicles in Tris–EDTA/150 mM NaCl; (C) PC vesicles in Tris–EDTA. To calculate the rate of β_2GP1 association with the target vesicles, the intensity of FITC fluorescence was monitored at 520 nm (λ_{ex} 450 nm) before and upon the addition of vesicles (arrow) in real-time (intensity was recorded at 0.1 s intervals). The calculated RET is shown in panel D. RET was calculated using $1-I_2/I_1$ where I_1 and I_2 are the intensities of FITC-β_2GP1 in the absence and presence of acceptor vesicles, respectively.

6D shows that the initial rates of RET were similar in both buffer systems. However, while the reaction appeared to be complete within several seconds in isotonic buffer, the reaction progressed albeit at a lower rate in low salt (Table 1). Analysis of the primary domain V interaction monitored by tryptophan fluorescence [17] showed that the emission peak shift was complete in <4 s and was not influenced by the ionic strength of the buffer (Fig. 7). These data suggest that the rapid increase in RET and tryptophan wavelength shift report on a primary interaction between β_2GP1 and the target vesicle. In low ionic strength buffer, RET continues to increase at a much slower rate suggesting that a secondary interaction occurs at a site distinct from domain V. Since the initial rates in both systems were identical, we assume that the initial β_2GP1 interactions are the same and that the secondary interaction is sequential to the primary. Taken together, these data suggest that the binding of β_2GP1 to membranes can be separated into two distinct interactions: (1) primary binding to domain V that is insensitive to ionic strength, and (2) secondary binding involving domains II/III that are sensitive to ionic strength. It should be noted that while these binding activities are distinct, both are likely PS-dependent since differentially labeled PC/PS and PC vesicles did not co-precipitate (Table 2).

4. Discussion

Several studies have shown that binding of β_2GP1 with target membranes bearing negatively-charged lipids induce agglutination and precipitation [19,20]. While this might be due to differences in the dissociation constants at low and high ionic strength ($\sim 10^{-9}$ and $\sim 10^{-6}$ M, respectively [20,36]), the mechanism responsible for the precipitation is not known. Interestingly, Hagihara et al. [12] have shown that in addition to the relatively high affinity binding of domain V to lipids, domain I also exhibits lipid binding properties, albeit at relatively low affinities. These data raise the possibility that precipitation of membranes in low ionic strength buffers is the result of multiple protein/membrane interactions [17]. Conceivably, these interactions could occur within the same membrane through global membrane alterations, or between adjacent membranes by cross-linking.

The effect of β_2GP1 on target membranes was monitored in sonicated vesicles containing pyrene-labeled PC and DPH. The ability of β_2GP1 to alter the fluidity of membranes can be seen from the data presented in Figs. 2 and 3 where the addition of the protein decreased E/M with a concomitant increase in membrane polarization. These data suggest that since E/M depends on the rate of intermolecular collisions between pyrene moieties [25,26], the enhancement of membrane rigidity is responsible for the decreased collisional frequency. Since β_2GP1 binds multiple lipid residues [12], it is possible that it could induce intervesicular aggregation thereby juxtaposing the membranes that generate conditions conducive to vesicle–vesicle fusion. Pyrene-labeled probe dilution and RET experiments ruled out the possibility of vesicle–vesicle fusion (Fig. 4). Moreover, 90° light-scattering experiments indicated that large vesicle aggregates were not formed ($M_2/M_1 < 3$; Fig. 5).

Table 2
Differential precipitation of PC and PC/PS vesicles by β_2GP1[a]

	Fraction of lipid precipitated	
	NBD-(PC/PS)+ Rho-(PC/PS)	NBD-(PC/PS)+ Rho-(PC)
NBD	0.92	0.84
Rho	0.94	0.02

[a]Vesicles (10 μM) composed of PC/PS or PC containing N-NBD-PE or N-Rho-PE (~1 mol%) were incubated with β_2GP1 (600 nM) for 10 min at 20°C. Aliquots were removed before and after centrifugation at 15000×*g* for 15 min. The samples were diluted in 1% Triton X-100, and NBD and Rho fluorescence was measured.

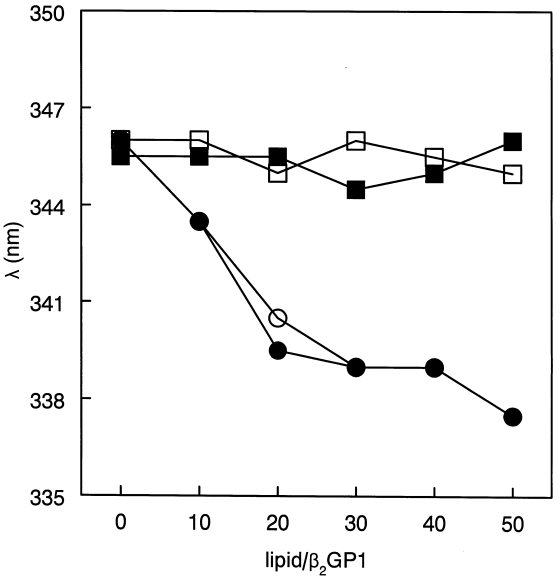

Fig. 7. Intrinsic (tryptophan) fluorescence emission peaks of β_2GP1 incubated with PS vesicles. β_2GP1 was incubated with PC (squares) and PC/PS (circles) vesicles in the presence (closed symbols) and absence (open symbols) of 150 mM NaCl for 5 min at 20°C. Tryptophan fluorescence emission spectra were monitored from 320–360 nm (λ_{ex} 280 nm).

In contrast to the data obtained in low ionic strength buffers, the inclusion of 150 mM NaCl decreased pyrene E/M ratios by ~50% but diminished the alterations in membrane polarization. Collectively, these data raise the possibility that the assays report on different binding activities. Conceivably, the primary interaction is seen as a reduction in pyrene E/M ratios, followed by a secondary interaction that is seen as a cumulative decrease in E/M as well as changes in membrane polarization. These data suggest that a β_2GP1 lipid-binding domain, distinct from the primary domain V site, is critical to membrane precipitation. Indeed, vesicles incubated with G_{274}–C_{288}, a domain V lipid binding sequence, produced E/M changes similar to β_2GP1 in the presence of 150 mM NaCl (Fig. 2) and did not induce precipitation (Fig. 1).

To distinguish between primary and secondary site

binding, the sequential deposition of both the C- (domain V) and N- (domain I) terminal regions of the protein were monitored by intrinsic tryptophan wavelength shifts [17] and RET between fluorescein-labeled β_2GP1 and rhodamine-labeled vesicles, respectively. Figs. 6 and 7 show an increase in RET between the protein and the target membrane and a $\sim$10 nm blue shift in the tryptophan emission spectra of β_2GP1 in both hypotonic and isotonic buffers. Although further time-dependent alterations in tryptophan fluorescence were not observed, RET continued to develop in low ionic strength buffer (Fig. 6). Since the fluorescent probe is localized to domains II/III, the progressive increase in RET most likely reflects ionic strength-dependent adsorption of the N-terminus on the membrane surface. Although these results cannot unequivocally rule out the possibility of intervesicle cross-links, the efficiency of energy transfer ($\sim$50%) would seem to favor minimal distances between fluorophores, arguing towards an intravesicle interaction. This conclusion is further supported by the light-scattering experiments (Fig. 5).

These data support the hypothesis that β_2GP1 can adopt at least two conformations upon binding to PS-containing targets [17,18]. In low ionic strength buffers, the protein assumes a conformation in which both the C- and N-termini are oriented along the plane of the lipid bilayer, facilitating binding to the membrane at two distinct sites. In isotonic buffers, however, only the C-terminus is bound while the N-terminus remains in solution because of its low affinity to lipids. Alternatively, the primary domain V interaction could induce a single conformational change independent of ionic strength that only makes binding to the newly accessible domain II/III binding site possible. Taken together, these data indicate that multivalent interactions within the same target membrane occur in hypotonic buffers that induce membrane alterations of sufficient magnitude to result in precipitation. In isotonic buffer, however, the conformational change would still occur, but association of the N-terminus to the target membrane would be precluded. Such a situation would provide a conformationally altered N-terminus available for other functionally relevant interactions.

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References

[1] E. Matsuura, Y. Igarashi, M. Fujimoto, K. Ichikawa, T. Koike, Lancet 336 (1990) 177–178.
[2] L.W. Chamley, E.J. McKay, N.S. Pattison, Thromb. Res. 61 (1991) 291–299.
[3] R.A. Roubey, Blood 84 (1994) 2854–2867.
[4] T.A. Brighton, P.J. Hogg, Y.P. Dai, B.H. Murray, B.H. Chong, C.N. Chesterman, Br. J. Haematol. 93 (1996) 185–194.
[5] B.E. Price, J. Rauch, M.A. Shia, M.T. Walsh, W. Lieberthal, H.M. Gilligan, T. O'Laughlin, J.S. Koh, J.S. Levine, J. Immunol. 157 (1996) 2201–2208.
[6] K. Balasubramanian, J. Chandra, A.J. Schroit, J. Biol. Chem. 272 (1997) 31113–31117.
[7] A.A. Manfredi, P. Rovere, G. Galati, S. Heltai, E. Bozzolo, L. Soldini, J. Davoust, G. Balestrieri, A. Tincani, M.G. Sabbadini, Arthritis Rheum. 41 (1998) 205–214.
[8] P. Thiagarajan, A. Le, C.R. Benedict, Arterioscler. Thromb. Vasc. Biol. 19 (1999) 2807–2811.
[9] J. Lozier, N. Takahashi, F.W. Putnam, Proc. Natl. Acad. Sci. USA 81 (1984) 3640–3644.
[10] T. Kristensen, I. Schousboe, E. Boel, E.M. Mulvihill, R.R. Hansen, K.B. Moller, N.P. Moller, L. Sottrup-Jensen, FEBS Lett. 289 (1991) 183–186.
[11] A. Steinkasserer, C. Estaller, E.H. Weiss, R.B. Sim, A.J. Day, Biochem. J. 277 (1991) 387–391.
[12] Y. Hagihara, Y. Goto, H. Kato, T. Yoshimura, J. Biochem. 118 (1995) 129–136.
[13] Y. Hagihara, K. Enjyoji, T. Omasa, Y. Katakura, K. Suga, M. Igarashi, E. Matsuura, H. Kato, T. Yoshimura, Y. Goto, J. Biochem. 121 (1997) 128–137.
[14] J. Hunt, S. Krilis, J. Immunol. 152 (1994) 653–659.
[15] H. Mehdi, X. Yang, M.E. Peeples, Virology 217 (1996) 58–66.
[16] Y. Sheng, S.A. Krilis, A. Sali, Ann. N. Y. Acad. Sci. 815 (1997) 331–333.
[17] S.X. Wang, G.P. Cai, S.F. Sui, Biochemistry 38 (1999) 9477–9484.
[18] B. Bouma, P.G. de Groot, J.H. van den Elsen, R.G. Ravelli, A. Schouten, M.A. Simmelink, R.M. Derksen, J. Kroon, P. Gros, EMBO J. 18 (1999) 5166–5174.
[19] I. Schousboe, Biochim. Biophys. Acta 579 (1979) 396–408.

[20] H. Wurm, Int. J. Biochem. 16 (1984) 511–515.
[21] E. Polz, H. Wurm, G.M. Kostner, Int. J. Biochem. 11 (1980) 265–270.
[22] P.K. Smith, R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, D.C. Klenk, Anal. Biochem. 150 (1985) 76–85.
[23] K. Balasubramanian, A.J. Schroit, J. Biol. Chem. 273 (1998) 29272–29277.
[24] G.P. Der-Balian, N. Kameda, G.L. Rowley, Anal. Biochem. 173 (1988) 59–63.
[25] H.J. Galla, E. Sackmann, Biochim. Biophys. Acta 339 (1974) 103–115.
[26] H.J. Galla, E. Sackmann, J. Am. Chem. Soc. 97 (1975) 4114–4120.
[27] M. Junker, C.E. Creutz, Biochemistry 32 (1993) 9968–9974.
[28] J.R. Lakowicz, F.G. Prendergast, D. Hogen, Biochemistry 18 (1979) 508–519.
[29] J.R. Lakowicz, F.G. Prendergast, D. Hogen, Biochemistry 18 (1979) 520–527.
[30] R.E. Pagano, O.C. Martin, A.J. Schroit, D.K. Struck, Biochemistry 20 (1981) 4920–4927.
[31] S. Nir, N. Duzgunes, J. Bentz, Biochim. Biophys. Acta 735 (1983) 160–172.
[32] S. Nir, J. Wilschut, J. Bentz, Biochim. Biophys. Acta 688 (1982) 275–278.
[33] R.A. Parente, B.R. Lentz, Biochemistry 25 (1986) 1021–1026.
[34] G.L. Nelsestuen, T.K. Lim, Biochemistry 16 (1977) 4164–4171.
[35] C.M. Celli, A.E. Gharavi, H. Chaimovich, Biochim. Biophys. Acta 1416 (1999) 225–238.
[36] G.M. Willems, M.P. Janssen, M.M. Pelsers, P. Comfurius, M. Galli, R.F. Zwaal, E.M. Bevers, Biochemistry 35 (1996) 13833–13842.