Short communication

Interaction of Erythrocyte Spectrin with Some Nonbilayer Phospholipids

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Abstract. Bovine erythrocyte spectrin was found to interact with lysophosphatidylcholine and lysophospatidylserine what was detected by small changes of the intrinsic fluorescence of spectrin. Lysophosphatidylethanolamine in contrast to its diacyl, natural counterpart did not affect the intrinsic fluorescence of spectrin at all. Dioleoylphosphatidylethanolamine induced distinct changes in the intrinsic fluorescence from these induced by natural phosphatidylethanolamine suspensions. Our data may indicate an importance of the presence of both fatty acyl chains in phosphatidylethanolamine molecule and perhaps, its bilayer structure for the interaction of this phospholipid aggregates with spectrin.

Key words: Erythrocyte spectrin — Lysophospholipids — Dioleoylphosphatidylethanolamine — Intrinsic fluorescence quenching — Erythrocyte membrane

Introduction

Spectrin, which accounts for 75% of the mass of the erythrocyte membrane skeleton is composed of two nonidentical subunits α and β of $M_r \sim 280\,000$ and 247 000 respectively (Sahr et al. 1990; Winkelman et al. 1990). Physiologically relevant unit of spectrin is $(\alpha\beta)_2$ tetramer (contour length 200 nm) that is formed by head-to-head association of two $\alpha\beta$ heterodimers.

Spectrin binds to erythrocyte membrane hydrophobic domain (membrane bilayer formed by lipids and intrinsic proteins) by interactions with ankyrin-anion transporter protein complex (for a review see e.g. Goodman et al. 1988 and Bennett 1990). The ends of the tetramers are engaged in so called junctional complexes

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composed of short actin filament, protein 4.1, adducin and protein 4.9 (Bennett 1990; Derick et al. 1992). Interaction with membrane lipid is a well known property of red blood cell spectrin that was demonstrated in model systems (Juliano et al. 1971; Mombers et al. 1980; Bitbol et al. 1989) as well as in the natural membrane (Haest et al. 1978; Sikorski and Kuczek 1985). Spectrin was also found to bind many amphipatic compounds as fatty acids and detergents (Isenberg et al. 1981; Sikorski et al. 1987b). Suggested in early studies specificity of this interaction towards phosphatidylserine was not confirmed in our and other's studies (Sikorski et al. 1987a; Bitbol et al. 1989). It seems that spectrin exhibits higher affinity and lower capacity towards PS, while lower affinity and much higher capacity for PE suspensions (Sikorski et al. 1987a; Michalak et al. 1993). Among several amphipatic compounds whose interaction with spectrin were studied (Isenberg et al. 1981; Sikorski et al. 1987a; Kahana et al. 1992) lysophospholipids have not been included. The fact that lyso-PE did not affect the intrinsic fluorescence of spectrin indicated the importance of the presence of both fatty acyl chains and perhaps, bilayer structure in the interaction of ethanolamine phospholipids with spectrin. The results of the experiments of the effect of DOPE on the intrinsic fluorescence of spectrin seem to confirm this suggestion.

Abbreviations used: DOPE – dioleoyl-phosphatidylethanolamine, lyso-PC – lysophosphatidylcholine, lyso-PE – lysophosphatidylethanolamine, lyso-PS – lysophosphatidylserine, PC – phosphatidylcholine, PE – phosphatidylethanolamine, PS – phosphatidylserine.

Materials and Methods

Bovine erythrocyte spectrin dimer was isolated as described previously (Michalak et al. 1993). Sepharose CL 4B column (2 × 60 cm) was equilibrated with the buffer: 20 mmol/l Na_2HPO_4, 50 mmol/l NaCl, 0.1 mmol/l EDTA, 1 mmol/l NaN_3, 0.1 mmol/l 2-mercaptoethanol, pH 7.4. Lipids: phosphatidylethanolamine (bovine brain) was from Koch Light, Colnbrook, England, dioleoylphosphatidylethanolamine, lysophosphatidylcholine (egg yolk), lysophosphatidylethanolamine (egg yolk) and lysophosphatidylserine (bovine brain) were purchased from Sigma Chem. Co. St. Louis, MO. USA. Lysophospholipids were dissolved in the above buffer. Phosphatidylethanolamine suspensions were prepared as described previously (Sikorski et al. 1987a and Michalak et al. 1993). Protein was determined according to Bradford (1976) and phospholipid phosphorus according to Bartlett (1959). Fluorescence measurements were performed at 20 °C with the use of a Perkin-Elmer MPF-3L spectrofluorimeter as described previously (Sikorski et al. 1987a; Michalak et al. 1993). For each experimental point a control measurements for the light scattering were performed. The control sample contained an appropriate volume of the suspension of phospholipid in the sample buffer. Samples at and below lipid concentrations indicated in Results were clear and displayed very low light scattering effect (no more than 10% of maximal fluorescence). The average values of triplicate measurements differing by no more 10% are presented.

Results and Discussion

The effect of lyso-PC on the intrinsic fluorescence of isolated spectrin dimer is shown in Fig. 1*A*. The quenching of the fluorescence was in the range of 10% what is similar to the values obtained for phosphatidylcholine vesicles at the same lipid to protein ratios (Sikorski et al. 1987a). It should be noted that the maximal lipid:protein ratio in Fig. 1*A* was 900; at higher lipid concentrations the solution became turbid. Lyso-PS caused ~ 10% increase of fluorescence at lower and ~ 10% decrease at higher concentrations of this phospholipid (Fig. 1*B*). Described above data might suggest that the effect of these two lysophospholipids, particularly lyso-PC more or less resembled the effect of their diacyl counterparts on isolated spectrin (see: Sikorski et al. 1987a, Fig. 1). On the other hand, the effect of lyso-PE did not resemble the effect of PE on isolated spectrin (Fig. 1*C*). PE suspension was found to quench up to 50% of tryptophan fluorescence of spectrin at pH 6.0 – 8.0 and up to 75% fluorescence at pH 5.5 (Sikorski et al. 1987a). In the wide range of lyso-PE concentrations (up to 78 μmol/l, lipid to protein ratio of ~ 1600) practically

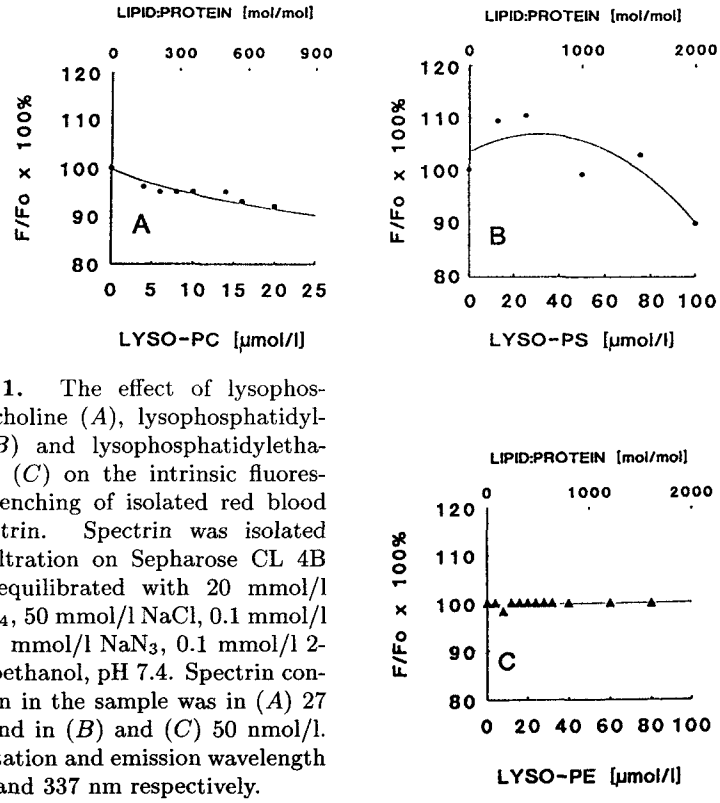

Figure 1. The effect of lysophosphatidylcholine (*A*), lysophosphatidylserine (*B*) and lysophosphatidylethanolamine (*C*) on the intrinsic fluorescence quenching of isolated red blood cell spectrin. Spectrin was isolated by gel filtration on Sepharose CL 4B column equilibrated with 20 mmol/l Na_2HPO_4, 50 mmol/l NaCl, 0.1 mmol/l EDTA, 1 mmol/l NaN_3, 0.1 mmol/l 2-mercaptoethanol, pH 7.4. Spectrin concentration in the sample was in (*A*) 27 nmol/l and in (*B*) and (*C*) 50 nmol/l. The excitation and emission wavelength was 290 and 337 nm respectively.

no changes of the tryptophan fluorescence of spectrin were observed (Fig. 1*C*). At lipid: protein ratio of 1400 more than 35% of initial spectrin fluorescence was quenched by PE suspensions (Sikorski et al. 1987a).

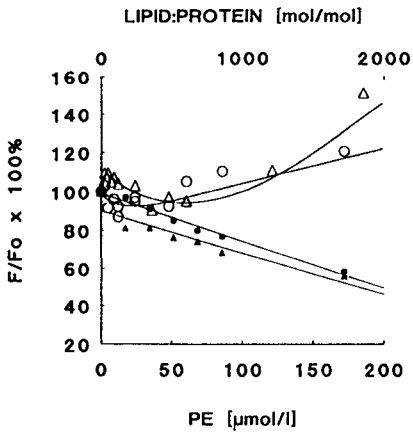

Figure 2. The effect of sonicated dioleoyl-phosphatidylethanolamine on the intrinsic fluorescence of spectrin. Experiment was carried out in the column buffer (Fig. 1 legend) –o– or with 0.15 mol/l NaCl in this buffer –Δ–. For comparison, the effect of natural PE (filled symbols and straight lines) in the same buffers is shown.

The effect of lyso-PE on PE suspension-spectrin interaction was rather small exhibiting a tendency to increase at low (up to about 4% at 50 μmol/l) and to decrease (up to about 3% at 160 μmol/l) at higher concentrations of lyso-PE of the PE effect on the fluorescence of spectrin (results not shown). In the case of lyso-PS, no effect could be detected of the fluorescence of PE-spectrin complex (data not shown).

The above data might suggest a role of the presence of both fatty acid chains and consequently micelle/bilayer/HII phase structure (Cullis and de Kruijff, 1978) for the interaction of PE with spectrin. To test this hypothesis the effect of sonicated dioleoyl-PE suspension on the intrinsic fluorescence of spectrin was analyzed. The results are shown in Fig. 2. At low ionic strength, at low DOPE concentrations quenching of the intrinsic fluorescence of spectrin by DOPE was about 10% and was similar to the effect induced by natural PE (Fig. 2). At higher DOPE concentrations, no quenching and even an increase of the fluorescence could be observed. At 0.2 mol/l NaCl, at low (up 30 μmol/l) DOPE concentrations ~ 10% increase and then (30–100 μmol/l DOPE) 10% quenching and at higher DOPE concentrations up to 50% increase of fluorescence was observed. This increase was not due to the light scattering effect since the fluorescence of each sample was corrected for light scattering (see Materials and Methods). For comparison the data on the effect of natural PE suspension at the same concentrations and the same ionic conditions are shown (Fig. 2). In spite small differences between the effects at low and moderate ionic strength, presented above data may indicate a distinct effect of DOPE, HII phase forming lipid [bilayer – HII phase transition temperature

$\sim$ 12°C (Ellens et al. 1986)] on isolated spectrin intrinsic fluorescence from that of natural PE suspensions. In conclusion the results both of lyso-PE and DOPE interaction with spectrin might indicate an importance of bilayer structure of PE aggregates for this interaction. The polymorphic state of lipid has not been taken into the consideration in the description of physiological significance of membrane skeleton – membrane bilayer interaction (Mombers et al. 1980; Maksymiv et al. 1987; Sikorski et al. 1987a; Bitbol et al. 1989). It should be noted again that above conclusion concerns only PE species, since no essential changes in the effect of lyso-PC and lyso-PS on intrisic fluorescence of spectrin compare to those of PC and PS suspensions were observed. It was also found previously that erythrocyte spectrin bound many amphipatic, micelle-forming compounds as fatty acids, brominated fatty acids and cationic and anionic detergents (Isenberg et al. 1981; Sikorski et al. 1987b; Kahana et al. 1992). Further studies on the physiological significance of PE – spectrin interaction and in particular, the effect of polymorphic state of this phospholipid suspension are to be conducted.

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