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Binding of the dystrophin second repeat to membrane di-oleyl phospholipids is dependent upon lipid packing

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Abstract

Dystrophin is the genetically deficient protein in Duchenne Muscular Dystrophy. Its C- and N-terminal ends interact with cytoskeletal and membrane proteins, establishing a link between the cytoskeleton and the extracellular matrix. In a previous study, we showed that there is an interaction between the second repeat of the rod domain and membrane phospholipids, which places tryptophan residues in close contact with the membrane. Here, we examine the binding of the dystrophin repeat-2 to small unilamellar vesicles with varying composition. We find that the protein binds predominantly to di-oleyl-phosphatidylserine. The binding as a function of increasing mol% of DOPS appears to be cooperative due to reduction of dimensionality, greatly enhanced in the absence of salts, and partly modulated by pH. Substituting small by large unilamellar vesicles induces a 30-fold lower affinity of the protein for the membrane phospholipids. However, modifying the packing of the acyl chains by introducing lipids such as phosphatidylethanolamine and cholesterol to the vesicle leads to an approximately 7-fold increase in affinity. Taken together, these results show that the binding involves electrostatic forces in addition to hydrophobic ones.

Keywords: Dystrophin; Spectrin repeat; Protein–lipid interaction; Membrane packing

1. Introduction

Dystrophin is the predominant protein of the normal skeletal muscle sarcolemma [1], which is lacking in Duchenne muscular dystrophy (DMD) [2]. The molecule of 427 kDa, is composed of four domains: an actin-binding amino-terminal domain, a rod domain comprising 24 spectrin-like repeats and a carboxy-terminal end made up of cysteine-rich and dystroglycan-interacting domains anchoring the dystrophin molecule to the sarcolemma [3]. The amino-terminal end and a cluster of basic repeats clearly associates with F-actin, suggesting an *in-vivo* association between actin and dystrophin [4]. All these protein–protein associations point to a model of dystrophin providing a structural and flexible link between the cytoskeleton and the extracellular matrix. After the first work of DeWolf [5] showing that the properties of a monolayer of lipids are modified in presence of the second repeat of dystrophin, we reported by fluorescence analysis that this repeat is able to associate with the anionic phospholipid phosphatildyserine, indicating a model where dystrophin rod domain lies along the plasma membrane [6]. This protein–lipid interaction may target the dystrophin molecule onto the sarcolemma, a process that could explain why the truncated dystrophin is nevertheless well localized at the sarcolemma in the muscle of patients expressing genetic variants of dystrophin lacking the carboxy-terminal end [7]. Such a targeting could also explain why the presence of several repeats and hinges is indispensable for a complete rescue of the dystrophic phenotype in *mdx* mouse [8].

However, after our first study revealing an interaction that places tryptophan residues in close contact with membrane phospholipids, we needed a quantitative description of the

Abbreviations: DOPC, dioleyl-phosphatidylcholine; DOPS, dioleyl-phosphatidylserine; DOPG, dioleyl-phosphatidylglycerol; DOPA, dioleyl-phosphatidic acid; DOPE, dioleyl-phosphatidylethanolamine; SUVs, small unilamellar vesicles; LUVs, large unilamellar vesicles; DYSR2, repeat 2 of dystrophin rod domain

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thermodynamics of binding and the nature of the attraction contributing to the formation of the protein–lipid complex. Therefore, the aim of the present investigation is to determine the specificity and affinity of the 2nd repeat of the dystrophin rod domain binding with membrane phospholipid vesicles. To address this issue, we used an ultrafiltration binding assay to examine the phospholipid binding properties of a 125-residues-long recombinant protein encoding for the 2nd repeat of the rod domain. We find that the binding is highly specific to phosphatidylserine, while there is a high cooperativity and the apparent association constant increases with decreasing salt content. In addition, the affinity is greatly reduced in the weakly curved LUV compared with the highly curved SUV, but is partially restored when cholesterol and phosphatidylethanolamine are included in the LUV composition, modifying the packing of the lipids. This study could serve as a model for the interaction with lipid membranes of all spectrin repeats of dystrophin.

2. Material and methods

2.1. Materials

All lipids were obtained from Avanti Polar Lipids (Alabama, US), and were used without further purification.

2.2. Preparation of phospholipid vesicles

Multilamellar vesicles (MLV) were first prepared. Mixtures containing variable molar ratios of the lipids DOPC, DOPS, DOPE, DOPG or DOPA in chloroform were dried overnight under vacuum and suspended in solution A containing: EDTA 0.1 mM buffered with TRIS–HCl 100 mM pH 7.6 with or without NaCl 150 mM. Small unilamellar vesicles (SUV) were prepared extemporaneously from MLV diluted at 25 mg/ml and subjected to sonication at room temperature with the micro-tip of a sonicator (U200S, UKA Labortechnic) for 1 min with half-duty cycles. The SUV were then centrifuged to eliminate titanium impurities. Large unilamellar vesicles (LUV) were obtained by extrusion 10 times through 100-nm pore membranes (Avanti Polar lipids). The homogeneity of the SUV and LUV sizes was controlled by dynamic light scattering (4700/PCS100 Malvern) and observation of individual the ^{31}P line widths of the NMR resonances below 150 Hz for SUV and around 2500 Hz for LUV (Avance 500, Bruker). As sonication could induce oxidation of the unsaturated acyl chains of the di-oleyl phospholipids, lipids from MLV and SUV were extracted, saponified and the fatty acyl methyl esters analysed by gas chromatography [9]. No oxidation products were detected in the various vesicle preparations obtained by sonication, including DOPC, DOPS, DOPE and cholesterol.

2.3. Protein preparation

The 125-residue protein of the 2nd repeat of human dystrophin rod domain (DYSR2) was prepared by expression in *Escherichia coli* (strain BL21/DE3) of plasmid kindly provided by W.B. Gratzer [10]. The N-terminus and C-terminus ends were taken at the 439th and 564th residues of human dystrophin (NCBI Protein Database NP_003997), respectively. Expressed protein was recovered as inclusion bodies from the *E. Coli* expression strain and purified as described below [6,11]. The protein was recovered by dispersion in 6 M guanidinium chloride with 10 mM dithiothreitol, and then purified by gel chromatography in the same solvent on Sephacryl-S100. Fractions were screened by UV absorption at 280 nm and SDS-gel electrophoresis. The purified protein was kept in guanidinium chloride and renatured by dialysis when required against the desired buffer, typically 150 mM NaCl and 50 mM sodium phosphate at pH 7.6 (buffer A). Protein concentration was determined spectrophotometrically at 280 nm using a molar extinction coefficient of 28570 cm^{-1} M^{-1} [12]. Renaturation of the protein was monitored by ^{1}H NMR and circular dichroism as previously described [6].

2.4. Binding assays

Several series were carried out using different mixtures of lipids. In all cases, DYSR2 was incubated with SUV or LUV for 3 h at 25 °C. The vesicles and protein–vesicle complexes so formed were separated from the unbound protein by ultrafiltration on membranes with a cut-off of 100 kDa (either Centricon YM 100 or Microcon YM 100) centrifuged for 2 h at 25 °C. The vesicles and protein–vesicle complexes were blocked by the membrane while the free protein was recovered in the filtrate. The protein content of the filtrate was either analysed by SDS-PAGE electrophoresis on a 14% acrylamide gel and visualized by Coomassie blue, or determined by a BIORAD microassay using a calibration curve established with bovine serum albumin (minimum detection is at 1 μg of protein in 0.8 ml). For each series, a control assay was performed without vesicles. This control assay showed that about 10% of the free protein is retained on the ultrafiltration membrane. Similar extents of free protein were found when SUV of non binding lipids were added to the protein. The calculation of the bound/total ratio was as follows:

$$\text{DYSR2 bound/total} = P_{\text{total}} - P_{\text{suvx}}/P_{\text{total}}$$

where P_{total} is the concentration of free protein in the filtrate for the control assay without vesicle, P_{suvx} is the concentration of the free protein for the respective assays performed in presence of vesicles.

2.5. Data analysis

To a first approximation, the binding equilibrium of a protein onto a lipid bilayer can be described according to a partition process:

$$[P] + [L] \overset{K}{\rightleftarrows} [PL]$$

The molar partition coefficient is defined as $K=[PL]/[P][L]$.

According to Ben Tal et al. [13], in case of limiting conditions, K may be regarded as an apparent association constant. However, in the case of protein binding to lipids in membranes, the binding involves several identical sites. There is often a positive apparent cooperativity in the binding that is well described by the Hill equation [14,15]

$$\text{With } K_a = [PL_n]/([P][L]^n) \quad (1)$$

$$P_b/P_{\text{total}} = K_a[L]^n/(1 + K_a[L]^n) \quad (2)$$

where n is the Hill coefficient, which increases with the degree of cooperativity of the binding, and K_a is the apparent association constant.

Experimental curves were fitted to Eq. (2) using Sigma-Plot software (Jandel Scientific).

3. Results

3.1. Specificity of binding to phospholipids

The aim of this study was to determine the apparent association constant of the binding of the protein to membrane phospholipids. We monitored the binding of the protein to SUV by ultrafiltration on membranes, and studied the effect of the phospholipid percentage in SUV of varying composition on the DYSR2 binding. For this purpose, 100 μM DYSR2 was mixed with increasing amounts of several lipid mixtures in the form of SUV. Either the lipids were made up of DOPC alone, or DOPC mixed in 2:1 mole ratio with the zwitterrionic DOPE, the anionic DOPG , DOPA or DOPS. After 3 h of incubation at

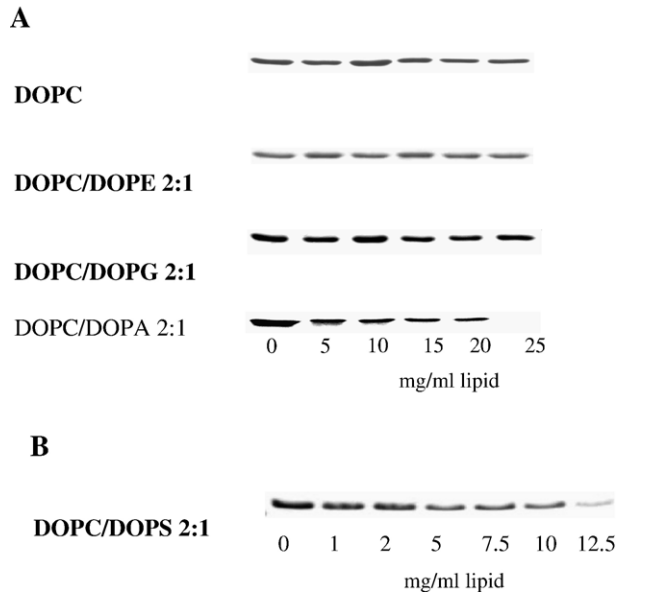

Fig. 1. Coomassie blue staining of SDS-PAGE electrophoresis of the DYSR2 unbound fraction obtained by ultrafiltration of incubation mixtures containing 100 μM DYSR2 with increasing concentration of lipids from SUV of various composition. (A) DOPC, DOPC/DOPE, DOPC/DOPG and DOPC/DOPA 2:1 (M:M) were used in total lipid concentrations of 0, 5, 10, 15, 20 and 25 mg/ml, respectively. (B) DOPC/DOPS 2:1 (M:M) were used in total lipid concentrations of 0, 1, 2, 5, 7.5, 10, 12.5 and 15 mg/ml, respectively.

room temperature, the SUV and bound protein were separated from the unbound protein by ultrafiltration. SDS PAGE electrophoresis (Fig. 1A) showed that the protein was not retained by SUV composed of DOPC alone, DOPC/DOPE or DOPC/DOPG. The DOPC/DOPA and DOPC/DOPS mixtures progressively retained increasing amounts of protein as illustrated by the decreasing amounts of protein present in the filtrate. The protein was completely retained at a concentration of 25 mg/ml of total lipids with DOPC/DOPA (Fig. 1A) and at a concentration of 15 mg/ml of total lipids with DOPC/DOPS (Fig. 1B). This first series of experiments demonstrate that this repeat of the rod domain does not bind to the zwitterrionic phospholipids PC and PE. The results show that the protein binds to PS or to a slightly lesser extent to PA, but not to the anionic PG at the concentrations used here. Therefore, all the following data were obtained using PS.

3.2. Binding of DYSR2 to SUV

SUV were prepared with six mixtures containing varying percentages of DOPS from 10 to 75% with the remaining phospholipid being DOPC. Since the 2.5 μM concentration of DYSR2 is far below the concentration levels of lipid, we present the titration curves as the fraction of DYSR2 bound to lipids vs. the total lipid concentration.

Fig. 2A shows the family of curves obtained. The apparent association constants, K_a, determined for each curve (Table 1) are in the range 0.2 to 4 mM^{-1} with Hill coefficients greater than 1.5. The progressive leftward shift of the curves implies that complex formation is highly dependent upon the anionic PS concentration and is well illustrated by the progressive increase

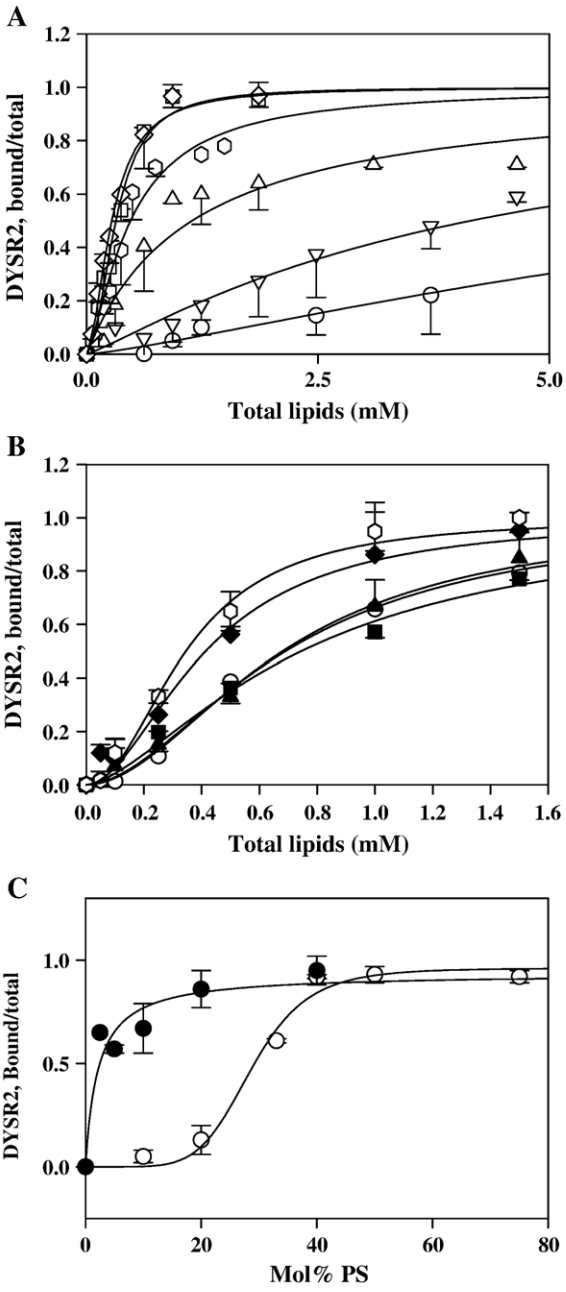

Fig. 2. Binding isotherms measured for DYSR2 binding to DOPC/DOPS vesicles. (A, B) For each isotherm, DYSR2 is 2.5 μM, % PS is held constant but total lipid concentration is increased from 0.1 to 5 mM (A) or 0.1 to 1.5 mM (B). With these conditions, series of binding isotherms were performed with variable mol% PS percentages as follows: 10% (circles), 20% (downward triangles), 33% (upward triangles), 40% (squares), 50% (hexagons), 75% (diamonds). Binding assays were performed in presence (A) or absence (B) of NaCl 150 mM. (C) The fraction of DYSR2 bound to 1 mM total lipid was calculated from the different isotherms of (A) and (B) and plotted against the mol% of PS. This corresponds to the fraction bound when the lipid concentration was held constant at 1 mM while the mol% of PS in increased. Bars show standard deviation from at least three analysis. Assays with salt (open symbols) or without salt (closed symbols). Lines represented the best fit for the Hill Eq. (2): for each curve, the R^2 (correlation coefficient) of the fit for the Hill Eq. (2) is better than 0.98.

Table 1
Binding constant (K_a, mM^{-1}) for binding of DYSR2 to SUV containing increasing percentages of PS, with or without salt (150 mM of NaCl)

PS in SUV (%)	K_a (mM^{-1}) with salt	K_a (mM^{-1}) without salt
2.5	nd	1.4
5	nd	1.3±0.03
10	0.2±0.08	1.8±0.06
20	0.3±0.1	2.4±0.07
33	1.35±0.02	nd
40	3.1±0.3	2.9±0.3
50	4.3±0.9	nd
75	3.2±0.8	nd

Mean±SD for at least three independent assays. Values from Fig. 2.

of the apparent K_a with the percentage of PS in the SUV. Therefore, the affinity of DYSR2 for SUV appears to be dependent upon the density of the PS molecules on the SUV surface, with a maximum binding being observed for a value of about 50% of PS in SUV. Hence, electrostatic attraction is likely to be involved in the binding. Such electrostatic interactions can be distinguished by their high sensitivity to ionic strength. Therefore, to address this issue, we performed binding assays of DYSR2 to SUV in the absence of salt. The family of curves obtained (Fig. 2B) shows also a progressive leftward shift of the curves obtained with SUV containing increasing percentages of PS. For low percentages of PS, in the range of 2.5 to 20%, K_a appears 3- to 4-fold higher than when the assay is performed using 150 mM NaCl (Table 1). Hill coefficients were all higher than 1.5. The affinity of DYSR2 towards SUV prepared with PS percentages higher than 20% is not different from the affinity determined in the presence of salt (Table 1).

Another way to examine PS dependence of the binding is to measure binding as a function of mol% of PS in the vesicles, a measure of the lipid surface density in the membrane while keeping total lipid concentration constant at 1 mM (Fig. 2C). The sigmoidal binding curve is indicative of an apparent cooperative binding and the curve performed with NaCl 150 mM can be fitted to an apparent Hill coefficient (n) of 6. The binding performed without NaCl does not show such an apparent cooperativity but the curve is left shifted to low values of mol% of PS with high levels of binding, indicative of a very high affinity to PS without salt for mol% of PS lower than 40%.

3.3. Effect of pH on the binding of DYSR2 to SUV

Since the formation of DYSR2-SUV complexes appears due to electrostatic attraction, this likely involves the amine groups of basic amino acid residues and the carboxylic group of PS. Therefore, increasing the pH will modify the equilibrium and the fraction of DYSR2 bound to SUV. Binding assays were thus performed with 1 mM SUV composed of DOPC/DOPS 2:1 and 2.5 μM DYSR2 without salt and under different pH conditions. In a first series, the incubation was performed at pH varying from 6.5 to 11.5. In a second series, incubation was performed at pH 7.5 and, after 2 h, the pH was increased to 11.5 using 1 M NaOH in half of the assays and further 2 h incubation was allowed. We separated the unbound DYSR2 by ultrafiltration as previously described. The data of the first series shows that the bound fraction decreases with pH following a sigmoidal curve exhibiting an inflection point at pH 8.5. At pH 11.5, the maximum amount of bound protein is 43±0.01%. In the second series, 50±7% (n=3) of DYSR2 is released from the vesicles when pH is increased to 11.5, which contrasts with the high percentage of bound fraction when pH is maintained at 7.5. The binding efficiency is not due to a conformational sensitivity of the protein to pH because we failed to detect any unfolding variations in the ^{1}H NMR and circular dichroism spectra of DYSR2 acquired at the different pH values (not shown).

3.4. Binding of DYSR2 to LUV compared to SUV of different compositions

It is known that the binding of protein to liposomes can be strongly influenced by the curvature stress of the liposome bilayer [16]. The curvature stress is high in the SUV; by contrast, the curvature stress is low in large unilamellar vesicles (LUV). Two binding assays were carried out in a paired experiment with SUV obtained as above and with LUV obtained by extrusion through a 100-nm pore diameter membrane. LUV and SUV were composed of the same mixture of PC/PS 2:1, with 150 mM NaCl. Even though the maximum of binding was observed for 40–50% of PS, we used vesicles with 33% of PS as it is close to the internal layer PS content of plasma membranes[17]. Fig. 3 clearly shows that the affinity is greatly reduced for LUV compared with SUV, with K_a values of 0.045 mM^{-1} and 1.35 mM^{-1}, respectively. This result strongly suggests that the affinity of the binding is dependent on the curvature or the packing.

As phosphatidylethanolamine (PE) and cholesterol are known to modify the packing of the acyl chain in liposomes, two other binding assays were performed with LUV and SUV composed of PC/PS/PE 1:1:1 with and without 30% cholesterol. The affinity of the binding to LUV increases from 0.045 mM^{-1} for the PC/PS LUV, to 0.20 mM^{-1} for the PC/PS/PE LUV and finally to 0.33 mM^{-1} for the PC/PS/PE LUV containing 30%

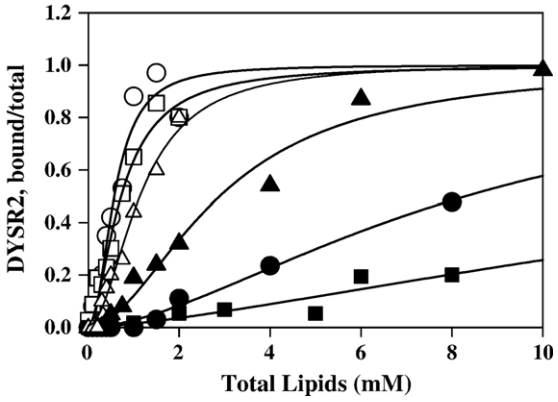

Fig. 3. Binding isotherm measured for DYSR2 binding to SUV and LUV of different compositions. The SUV (open symbols) or LUV (filled symbols) were composed of DOPC/DOPS 2:1 (squares), or DOPC/DOPS/DOPE 1:1:1 (circles), or DOPC/DOPS/DOPE 1:1:1 with 30 mol% of cholesterol (triangles). Lines represent the best fit for the Hill Eq. (2).

cholesterol (Fig. 3, Table 2). The binding to SUV made up of PC/PS shows an affinity of about 1.35 mM^{-1}, a value that does not change significantly when PE is present. The hydrodynamic diameter of LUV and SUV was not modified by the introduction of PE in the initial PC/PS mixture (Fig. 4) as previously reported [18] and was not modified by the binding of DYSR2. This is also the case for the LUV composed of PC/PS/PE (Fig. 4). However, in the case of vesicles containing PE and cholesterol, SUV were slightly larger than the other SUV and rather unstable in presence of the protein; they tended to slightly aggregate and caution is needed in the interpretation of these data. In addition, the corresponding LUV were slightly enlarged in presence of the protein. In summary, with the PC/PS mixture, the affinity for SUV is 30 times higher than for LUV, while with the mixture containing PE and cholesterol, the affinity for the LUV is increased and only 4 times lower than for PC/PS SUV.

3.5. Titration of 2:1 DOPC/DOPS SUV by DYSR2

We used a reverse assay to titrate 1 mM of SUV made up of DOPC/DOPS in a molar ratio of 2:1, without NaCl and at pH 7.5, against increasing concentrations of DYSR2 from 1.25 to 15 μM. Incubation and ultrafiltration were performed as usual and protein was measured in the filtrate. Fitting these data with the Michaelis–Menten equation shows that DYSR2 is bound to PS with a K_a of 800 mM^{-1} and a B_{max} of 5.5 μmol of DYSR2.

The stoichiometry therefore corresponds to 5.5 μmol of protein for 1000 μmol of lipid. Since vesicles are made up of a bilayer and the protein is interacting with the external layer, it could be assumed that one protein is in contact with about 100 lipid molecules. A dystrophin repeat is about 5 nm long and a phospholipid head group occupies about 0.7 nm^2, which means that the 100 lipid molecules occupy an area of 70 nm^2. This corresponds to a circle of diameter 9.5 nm. This is compatible with the area occupied by the DYSR2 molecule. Therefore, if we assume that the diameter of SUV is about 50 nm, about 100 DYSR2 molecules could be bound on the 7500 nm^2 total surface-area of each vesicle.

4. Discussion

It has long been known that spectrin repeats bind membrane phospholipids [19–21]. Recent studies have shown that dystrophin repeat-2 (DYSR2) is able to bind to membrane phospholipids [5] by a mechanism that places the protein along the membrane surface, thus allowing Trp residues to be in close contact with the glycerol backbone of the phospholipids [6]. The aim of the present study is to determine the specificity and affinity of the binding of DYSR2 to phospholipids.

Table 2
Association constant (K_a, mM^{-1}) for binding of DYSR2 to LUV and SUV of different composition, with salt (150 mM NaCl)

Vesicle composition	K_a (mM^{-1})	
	LUV	SUV
PC/PS 2:1	0.045±0.007	1.35±0.02
PC/PS/PE 1:1:1	0.20±0.12	1.39±0.50
PC/PS/PE 1:1:1, 30%Chol	0.33 *	1.30 *

Mean±SD for at least three independent assays. Values are from Fig. 3.
* Average from duplicated data.

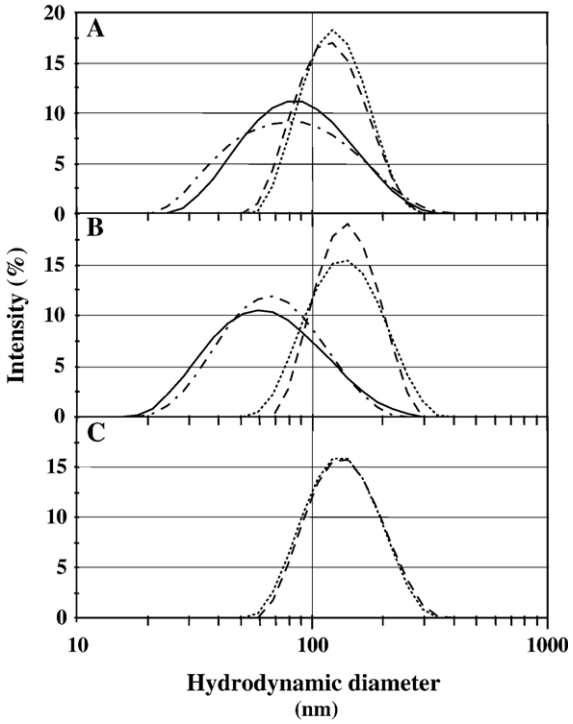

Fig. 4. Dynamic light scattering data of SUV and LUV for the determination of the hydrodynamic diameters. SUV 1 mM lipids in absence (solid line) or in presence of 1 μM DYSR2 (dot-dashed line), LUV in absence (dotted line) or in presence of 1 μM DYSR2 dashed line); vesicles made of (A) DOPC/DOPS 2:1, (B) DOPC/DOPS/DOPE 1:1:1, (C) DOPC/DOPS/DOPE 1:1:1 and cholesterol 30%.

For this purpose, we used an ultrafiltration binding assay that has the special advantage of physically separating the bound and unbound protein. However, this assay involves a centrifugation step of several minutes that could modify the apparent equilibrium. To obtain information about such an effect, we compared the values of lipid concentration observed at half saturation using fluorescence spectroscopy [6] with the results obtained here by ultrafiltration assay. Using DYSR2 at 1 μM and 2.5 μM in the respective methods, and increasing the concentration of total lipids from 1 to 6 mM in the presence of 0.15 M NaCl, we observed half saturation of DYSR2 by SUV composed of 2:1 DOPC/DOPS (M:M) at 0.55 mM (previous study) and 0.76 mM [6], respectively. Since these values can be considered very close, we therefore used the ultrafiltration method to separate the DYSR2–lipid complex from the free DYSR2.

Clearly, the binding is rather specific to PS as no DYSR2 was retained by PG and only a small part is retained by PA, largely lower than the amount retained by PS. The proportion of bound DYSR2 increases with the PS content of the SUV, as shown by the determination of the apparent dissociation constants. This effect is reinforced when salt is absent from the buffer, showing

that electrostatic attraction could drive the binding. Moreover, as pH increases, the percentage of bound DYSR2 decreases, while increasing the pH after the binding leads to a partial release of the protein. Taken together, these data indicate that electrostatic attraction plays an important role in the first step of the binding.

In addition, a Hill coefficient of 6 was calculated when examining the % PS dependence of the binding. This Hill coefficient is higher than observed for the MARCKS basic effector domain [22] or a fragment of N-WASP [15] and first indicates that DYSR2 binds to multiple PS molecules [23]. In the case of interacting binding sites, it could reflect the increase of the intrinsic association constants of the protein for PS as binding sites are progressively occupied. However, in our case as in the case of other interfacial proteins [24], it is likely that the sites are not interacting and cooperativity is apparent and explained simply by a reduction of dimensionality [13], i.e. by the increase of the local two-dimension concentration at the membrane surface. This increase is predominantly driven by the surface charges, according to the Gouy–Chapmann theory [14,25,26] and due to the presence of the anionic PS molecules. Thus, in addition to our first results showing that Trp residues are involved in the interaction [6], there appear to be two types of forces involved in the binding of DYSR2 to membrane lipids. One of these forces is electrostatic and likely involves the Lys and Arg charged residues in an interaction with the phosphatidylserine carboxylate negative charge, thus explaining the data obtained at different pH values and without salt. This electrostatic attraction could drive the interaction. The second force is hydrophobic and involves hydrophobic residues of the protein such as Trp or possibly Leu or Val, which could interact with the glycerol backbone or the lipid core of the membrane [27,28].

Our experiments show that DYSR2 binding to membranes is sensitive to the lipid packing, a physical parameter that depends on the shape of the phospholipid molecules and the curvature of the vesicle membrane. At a constant large diameter of liposome in LUV, the curvature is very low and the protein has a very low affinity when PC and PS make up the bilayer. The affinity increases when PE is introduced at the expense of cylindrical PC and in the presence of cholesterol. PE is a conical-shaped phospholipid with a polar head occupying a smaller volume than its acyl chains. Therefore, it occupies a large hydrophobic volume and causes the polar heads of PC and PS to move apart, thus modifying the packing. Cholesterol is accommodated in the hydrophobic core of the membrane, increasing the packing of the acyl chains. Similarly to PE, its presence spreads apart the large polar heads of PC and PS. Therefore, in both cases, Trp lateral chains are able to penetrate deeply into the glycerol backbone, a situation that is highly favourable for this residue [27,28]. At constant vesicle composition, the affinity of the protein for the membrane is increased with the curvature, which produces a large space between the head groups of the external layer of the SUV. This space allows the deep penetration of the lateral chains of Trp residues, even when PE or cholesterol are absent from the vesicle. This deep penetration could be prevented by the large polar heads of PC and PS when curvature is absent, as in the case of LUV. On the whole, these modifications of packing (curvature or composition) facilitate the anchoring of the Trp lateral chains in the glycerol backbone of the external layer of the membrane. Such a facilitation of binding by packing has been already observed for a number of proteins such as α-synuclein [29], Protein kinase C [23], or the small G protein ADP-ribosylation factor 1 (ArfGAP1) [30].

Nevertheless, this is an unexpected result that opens up interesting hypotheses about the role of dystrophin in the muscle cell. The rod domain of dystrophin is made up of 24 spectrin-like repeats separated by four hinges, which are supposed to lead to flexibility of the molecule [31]. In the absence of dystrophin, the fibres exhibit an increased susceptibility to eccentric contraction-induced sarcolemmal rupture [32]. In the case of the red cell membrane, it has been elegantly demonstrated that the modulation of membrane mechanical stability involves an interaction between aminophospholipids in the inner membrane leaflet and cytoskeletal proteins such as spectrin, a protein of the same family as dystrophin [33]. This interaction is mediated by several sites, and is restricted to a small number of repeats [21]. Further experiments are needed to know if dystrophin has a similar role in skeletal muscle.

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