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Biochimica et Biophysica Acta 1611 (2003) 115–122
BIOCHIMICA ET BIOPHYSICA ACTA
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Occurrence of lipid receptors inferred from brain and erythrocyte spectrins binding NaOH-extracted and protease-treated neuronal and erythrocyte membranes

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Received 7 August 2001; received in revised form 9 January 2003; accepted 16 January 2003

Abstract

It was previously shown in model systems that brain spectrin binds membrane phospholipids. In the present study, we analysed binding of isolated brain spectrin and red blood cell spectrin to red blood or neuronal membranes which had been treated as follows: (1) extracted with low ionic-strength solution, (2) the above membranes extracted with 0.1 M NaOH, and (3) membranes treated as above, followed by protease treatment and re-extraction with 0.1 M NaOH. It was found that isolated, NaOH-extracted, protease-treated neuronal and red blood cell membranes bind brain and red blood cell spectrin with moderate affinities similar to those obtained in model phospholipid membrane–spectrin interaction experiments. Moreover, this binding was competitively inhibited by liposomes prepared from membrane lipids. The presented results indicate the occurrence of receptor sites for spectrins that are extraction- and protease-resistant, therefore most probably of lipidic nature, in native membranes.

Keywords: Brain spectrin (fodrin); Red blood cell (erythrocyte) spectrin; Phospholipid; Neuronal membrane; Red blood cell (erythrocyte) membrane

1. Introduction

In red blood cells, spectrin is a major component of the membrane skeleton, whereas in nonerythroid cells, spectrin can be found not only in the plasma membrane, but also in many other membranes, as well as in the cytosolic compartment. This localization suggests that spectrin may be involved in many cellular processes (for a review, see e.g. Ref. [1]). For example, neuronal spectrin is thought to play an essential role in Ca^{2+}-regulated exocytosis in the nerve terminal and in neurotransmitter release [2].

Spectrin is usually a tetramer formed by head-to-head interaction of two identical heterodimers formed by parallel interactions of nonindentical α and β subunits. The spectrin α-subunit is a polypeptide of a molecular weight of 280 kDa [3], while the β subunit's molecular weight is 274 kDa (nonerythroid) [4] or 246 kDa (erythroid) [5].

It was previously shown by several laboratories [6–11] that erythroid spectrin binds directly to liposomes, in particular to these containing aminophospholipids: phosphatidylethanolamine (PE) and phosphatidylserine (PS). The capability of interaction of red blood cell spectrin with phospholipids was shown using numerous methods [8–13].

We also demonstrated that nonerythroid (brain) spectrin exhibited an ability to bind to liposomes made from PC and its mixture with PE and PS, as well as to liposomes prepared from total brain membrane lipids. The affinities of brain spectrin for liposomes prepared from these lipids were rather high—the obtained equilibrium dissociation constant (K_d) values were in the submicromolar range [14].

In spite of more than 25 years of studies of spectrin–membrane lipid interactions, the question whether protein-independent sites for spectrins could be demonstrated in natural membranes still remained to be answered. One possible experimental approach to explain this problem is presented in this work. Namely, we show that brain and erythrocyte spectrins are able to interact with natural mem-

Abbreviations: B_{max}, maximal binding capacity; DTT, dithiothreitol; K_d, equilibrium dissociation constant; PMSF, phenylmethanesulfonyl fluoride; PE, phosphatidylethanolamine

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doi:10.1016/S0005-2736(03)00032-4

branes (synaptic plasma membrane and red blood cell membrane) in which membrane protein receptors have been extracted or destroyed by proteolysis. Extraction and protease treatment of membrane preparations was performed according to Steiner and Bennett [15]. As a result, we have observed a specific, saturable, high to moderate affinity binding of brain and erythrocyte spectrins to membranes deprived of proteins by the extraction with 0.1 M NaOH and digestion with α-chymotrypsin and re-extraction with 0.1 M NaOH, indicating that these kinds of receptor sites are present in natural membranes. Lipidic nature of these sites is confirmed by competitive inhibition of binding by artificial vesicles prepared from membrane lipids.

2. Materials and methods

2.1. Materials

Sephacryl S-500 HR, DEAE-Sephacel, Sepharose 4B, EGTA, phenylmethanesulfonyl fluoride (PMSF), and α-chymotrypsin were from Sigma Chemical (St. Louis, MO, USA). Dithiothreitol (DTT), coomassie brilliant blue R-250, and EDTA were from Serva Feinbiochemica (Heidelberg, Germany). SDS was from BDH Laboratory Supplies (Poole, England). Dimethyl sulfoxide (DMSO) was from Merck (Darmstadt, Germany). Tetramethylrhodamine-5-maleimide single isomer (T-6027) was from Molecular Probes (Eugene, OR, USA).

2.2. Methods

Brain spectrin was isolated from bovine brains according to Bennett at al. [16]. Final preparations contained only spectrin bands, of apparent molecular weights of 280 and 274 kDa, with traces of a proteolytic ~ 150 kDa fragment of the α-spectrin subunit, as determined by SDS-(7%)-polyacrylamide gel electrophoresis in the Laemmli [17] system.

Erythrocyte spectrin was isolated from bovine red blood cells by extraction of red blood cell ghosts with low ionic-strength buffer at 37 °C, as was described previously [8]. Protein purified by Sepharose 4B column chromatography contained only erythrocyte spectrin bands of apparent molecular weights of 280 and 246 kDa, as analysed by SDS-polyacrylamide gel electrophoresis.

Three types of neuronal membrane and red blood cell membrane preparations were used in the study: the first were neuronal membranes and erythrocyte membranes remaining after extraction of spectrins with low ionic-strength buffer (here referred to as untreated membranes). The second type were the above membranes extracted with 10 volumes of 0.1 M NaOH for 30 min at 4 °C, as was described by Steiner at al. [18]. The NaOH-extracted membranes were layered over 10% sucrose in a 10-mM HEPES buffer containing 1 mM EGTA, 1 mM NaN_3, 1 mM DTT, 50 μg/ml PMSF, pH 7.5, and centrifuged at 17 000 × *g* for 40 min. The resulting pellet of NaOH-extracted membranes was washed with the HEPES buffer without sucrose (17 000 × *g* for 30 min) and membranes were resuspended in the same buffer without sucrose and PMSF. The third type of membrane preparations was the above membranes (NaOH treated) digested with TPCK-treated α-chymotrypsin (100 μg/ml), as described by Steiner et al. [18], and then re-extracted with 0.1 M NaOH and washed as described above.

Brain and red blood cell membranes were analysed by SDS-polyacrylamide gel electrophoresis performed on 3.5–15% gradient gel with the buffers of the Laemmli [17] system and were tested by Western blotting by using anti-brain spectrin and anti-ankyrin antibodies obtained in our laboratory. All the membrane preparations were finally washed with an assay buffer consisting of 5 mM Tris, 0.5 mM EDTA, 150 mM NaCl, 0.5 mM DTT, 1 mM NaN_3, at pH 7.5. Purified bovine brain and erythrocyte spectrin preparations were dialysed against the same assay buffer. Two hundred and twenty microlitres of the incubation mixture contained 22 μg membrane protein and 0–100 nM purified spectrin (final concentration). After 30 min incubation at room temperature (20 °C), 180 μl of the sample was layered on a 300-μl cushion of 0.5% Dextran T-40 in the assay buffer and centrifuged at 17 000 × *g* for 6 min. Resulting pellets were subjected to SDS-(7%)-polyacrylamide gel electrophoresis. Quantitation of bound spectrin in the pellets was performed by elution of coomassie brilliant blue stain from the excised polyacrylamide gel zones with DMSO, and measuring the absorbance at 595 nm, as described previously [14]. Spectrin for the experiments showing the effect of heat treatment was incubated at 67 °C (brain spectrin) and at 58 °C (erythrocyte spectrin) for 15 min before the assay, as was described by Steiner and Bennett [15].

Liposomes were prepared from lipids extracted from bovine brain synaptic plasma membranes [19,20] (for the composition of the prepared "natural lipids"—see Ref. [14]). Hand-shaken liposomes were calibrated using 100 nm polycarbonate membranes (Nucleopore Track-Etch Membrane PC MB 25 mm 1 UM, Whatman Nucleopore) in a Mini-Extruder (PPH Marker, Poland). For sedimentation tests (described above), liposomes of a diameter of 100–160 nm (79%) according to the measurements taken with ZetaSizer (Malvern Instruments, Malvern, UK) were used. They did not produce sediment upon centrifugation at 17 000 × *g*.

For fluorescence experiments, purified brain and erythrocyte spectrins were labelled with tetramethylrhodmine-5-maleimide. Proteins were dialysed against the buffer: 5 mM Na_2HPO_4, 50 mM NaCl, and 0.1 mM EGTA, pH 7.6, and 100 μl ethanolic solution of the tetramethyl-rhodamine-5-maleimide (20 mg/ml) was added. After incubation for 2 h in the dark, at room temperature, DTT was added to the mixture (final concentration of 0.5 mM). Mixtures were

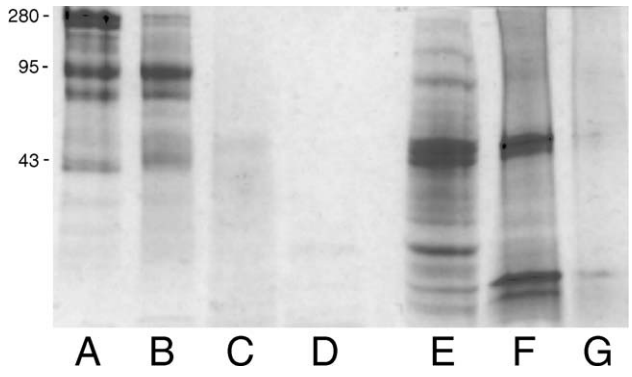

Fig. 1. Coomassie blue-stained SDS-(3.5–15%)-polyacrylamide gradient gel electrophoretogram of the membrane preparations. Lanes A–D: erythrocyte membranes; lanes E–G: synaptic plasma membranes; lane A: red blood cell ghosts; lanes B and E: red blood cell and synaptic plasma membranes, respectively, extracted with low ionic-strength solution at 37 °C (untreated membranes); lanes C and F: erythrocyte and synaptic plasma membranes extracted with 100 mM NaOH for 30 min at 4 °C; lanes D and G: the same membranes digested with TPCK-treated α-chymotrypsin according to Steiner et al. [18]. Other details in Materials and methods. Fifteen micrograms of membrane protein was applied on each lane.

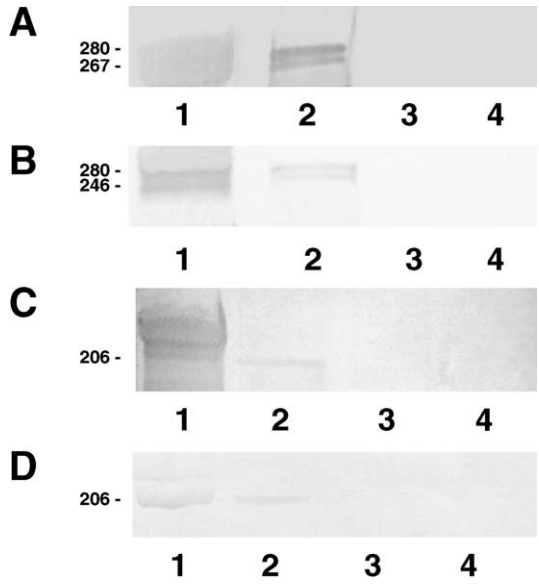

Fig. 3. Western blot analysis of brain (A, C) and erythrocyte (B, D) membranes. Membrane proteins separated in SDS-gel electrophoresis were transferred onto nitrocellulose membrane and probed with rabbit anti-spectrin (A, B) and anti-ankyrin (C, D) serum. (1) Red blood cell ghosts; (2) low ionic-strength extracted membranes; (3) the same, extracted with NaOH; (4) α-chymotrypsin-digested and NaOH-re-extracted membranes.

dialysed overnight against the buffer described above for 2 days (three buffer changes).

For the competition, the assays were as follows: labelled spectrins were incubated with α-chymotrypsin-digested and NaOH-re-extracted synaptic plasma membranes in the presence of unlabelled spectrins at concentrations ranging from 0- to 30-fold excess over labelled proteins. Obtained pellets were dissolved in 2 ml 1% SDS. Fluorescence measurements were taken after 15 min at 545 nm (excitation wavelength) and 567 nm (emission wavelength) in a Kontron spectrofluorimeter at room temperature. In control experiments, BSA instead of unlabelled spectrin was used.

3. Results

To find out whether natural membranes retain spectrin-binding activity after removal of proteins by extraction with NaOH and proteolysis, brain and erythrocyte membrane preparations treated as in Refs. [15,18] were used. After the extraction of neuronal membranes and erythrocyte ghosts with 0.1 M NaOH, we observed the presence of only traces of peripheral membrane proteins, mainly spectrin. The digestion of NaOH-extracted membranes with α-chymotrypsin and the re-extraction with 0.1 M NaOH removed the remaining spectrin, as well as other peripheral proteins, and reduced the molecular masses of integral membrane proteins [17] (Fig. 1). An example of the results of binding assay of brain and erythrocyte spectrins to the α-chymotrypsin-digested and NaOH re-extracted brain membranes is shown in Fig. 2. In Western blot analysis, no traces of spectrin and ankyrin were detected in α-chymotrypsin-digested and NaOH-re-extracted brain and red blood cell membranes by using anti-spectrin and anti-ankyrin antibodies (Fig. 3).

Fig. 4A shows the binding isotherms of purified brain spectrin to spectrin-depleted synaptic plasma membranes at pH 7.5. Increasing concentrations of purified brain spectrin (6–42 nM) were incubated with synaptic membranes remaining after the extraction of brain spectrin by incubation in low ionic-strength buffer. The same procedure was repeated using NaOH-extracted and α-chymotrypsin-digested, NaOH-re-extracted synaptic plasma membranes (Fig. 4, Materials and methods). In all the cases, we observed saturable binding of spectrin. The K_d values and maximal binding capacities (B_{max}) resulting from these experiments are given in Table 1. The K_d values obtained are in the nanomolar range, meaning that the affinities of these interactions are rather high.

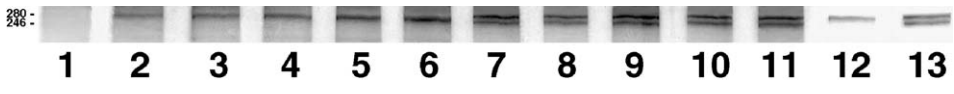

Fig. 2. An example of SDS-(7%)-polyacrylamide gel electrophoretogram of the assay of binding spectrin to the α-chymotrypsin-digested and NaOH re-extracted brain membranes. Lane 1: membranes only; lanes 2–6: brain spectrin at concentrations of 3.2, 4.8, 6.4, 8.0, and 9.6 μg/assay; lanes 7–11: erythrocyte spectrin at concentrations of 4.0, 6.0, 8.0, 10.0, and 12.0 μg/assay. Lanes 12 and 13: brain and erythrocyte spectrin reference.

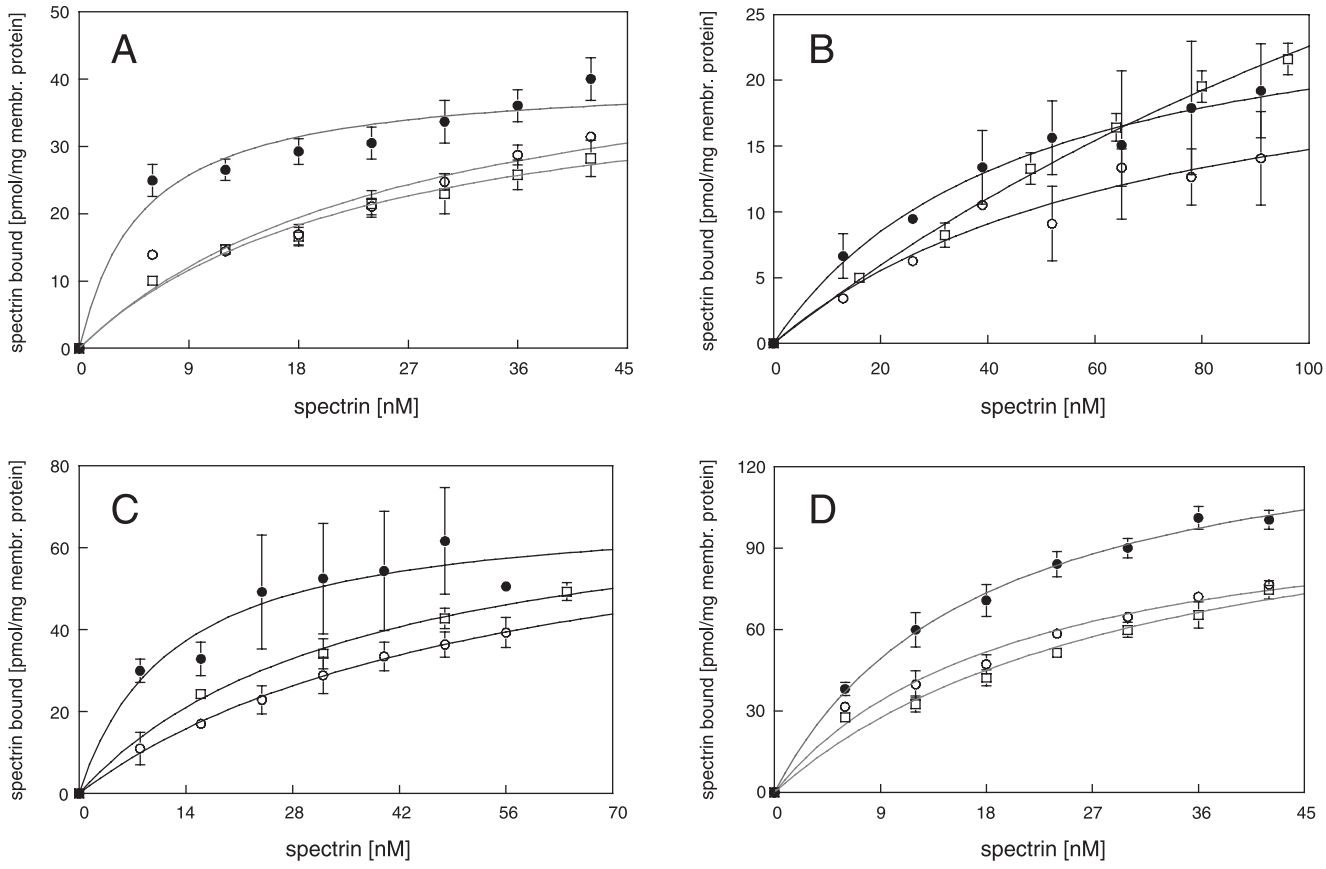

Fig. 4. Binding of the brain spectrin by synaptic plasma membranes (A), binding of the brain spectrin by red blood cell membranes (B), binding of the erythrocyte spectrin by red blood cell membranes (C), and binding of the erythrocyte spectrin by synaptic plasma membranes (D). Increasing concentrations of purified spectrin were incubated with membranes after extraction with low ionic-strength solution (●), NaOH-extracted membranes (○), and α-chymotrypsin-digested and NaOH-re-extracted membranes (□). Other details in Materials and methods. Error bar = standard error.

An interaction of brain spectrin with erythrocyte membranes treated the same way as neuronal membranes was also studied. Results shown in Fig. 4B indicate that isolated brain spectrin binds to untreated, extracted with 0.1 M NaOH as well as digested with α-chymotrypsin erythrocyte membranes under the conditions identical to those presented above, with regard to synaptic plasma membrane binding. The K_d values obtained are also in the nanomolar range (see Table 1), although 5- to 10-fold higher than in the case of brain spectrin binding to neuronal membranes.

The results of the analysis of the interaction of erythrocyte spectrin with erythrocyte membranes are shown in Fig. 4C. They indicate that isolated erythrocyte spectrin binds to untreated, extracted with 0.1 M NaOH, digested with α-chymotrypsin erythrocyte in a similar way to that shown for brain spectrin and neuronal membranes. These interactions are characterised by K_d values in the nanomolar range (see Table 1).

Our next goal was to examine whether erythrocyte spectrin binds to the membranes from the brain. The results of the experiments are shown in Fig. 4D and, as can be seen in Table 1, the parameters of the binding isotherms of erythrocyte spectrin attachment to neuronal membranes are similar to those for brain spectrin and brain membranes.

We posed a question whether heated spectrins bind to the membrane similarly to the native protein. We tested the interaction of brain spectrin with α-chymotrypsin-digested and NaOH-re-extracted synaptic membranes. The results of the experiments are shown in Fig. 5. The obtained binding isotherms of brain spectrin to α-chymotrypsin-digested membranes are saturable, indicating affinities in the nanomolar range (39 nM). The binding isotherm of erythrocyte spectrin to α-chymotrypsin-digested red blood cell membranes is similar to those observed for brain spectrin interaction (Fig. 5). This interaction is characterised by a K_d of 22 nM.

Fig. 6 shows the results of inhibition of brain spectrin interaction with brain membranes by exogenous phospholipid liposomes. Increasing concentrations of small liposomes prepared from ‘‘natural lipids’’ extracted from synaptic membranes were incubated with untreated brain membranes and constant concentrations of brain spectrin. The same procedure was repeated using NaOH-extracted and α-chymotrypsin-digested neuronal membranes. Dixon [21] plot of the results of inhibition curves indicated that liposomes

Table 1
Equilibrium dissociation constants (K_d) and maximal binding capacities (B_{max}) of the interaction of purified brain and erythrocyte spectrin with "natural" membranes from neural and erythrocyte cells

Interaction	Membranes	$K_d \pm$ S.E. [nM]	$B_{max} \pm$ S.E. [pmol/mg membrane protein]
Brain spectrin: synaptic plasma membranes	Untreated	5.1 ± 1.8	40.4 ± 2.9
	NaOH extracted	30.0 ± 11.6	51.6 ± 10.4
	Digested by α-chymotrypsin	31.3 ± 12.1	49.4 ± 9.8
Brain spectrin: erythrocyte membranes	Untreated	45.2 ± 36.3	28.1 ± 9.6
	NaOH extracted	76.1 ± 71.0	25.9 ± 12.0
	Digested by α-chymotrypsin	209.8 ± 87.4	69.4 ± 21.0
RBC spectrin: erythrocyte membranes	Untreated	17.7 ± 2.9	144.9 ± 9.8
	NaOH extracted	21.3 ± 3.7	112.5 ± 8.8
	Digested by α-chymotrypsin	34.1 ± 8.3	129.7 ± 17.2
RBC spectrin: synaptic plasma membranes	Untreated	14.3 ± 8.2	73.8 ± 14.7
	NaOH extracted	49.8 ± 24.0	74.3 ± 20.1
	Digested by α-chymotrypsin	58.4 ± 9.8	96.9 ± 7.2

Data presented here were derived from the results of experiments shown in Figs. 3–7 by using nonlinear regression. S.E. = standard error.

prepared from brain membranes competitively inhibited the binding of brain spectrin to NaOH-extracted (Fig. 6B) and α-chymotrypsin-digested (Fig. 6C) brain membranes. The effect of liposomes on interaction of these last membranes with spectrin was much larger when compared to that observed for the interaction between brain spectrin and untreated synaptic membranes (Fig. 6A).

Fig. 7 shows the results of inhibition of brain spectrin labelled with tetramethylrhodamine-5-maleimide interaction with α-chymotrypsin-digested and NaOH-re-extracted neuronal membranes by unlabelled brain spectrin (Fig. 7A). The molar ratio of the unlabelled to the labelled spectrin varied from 1:1 to 5:1. A distinct inhibition was observed. However, larger ratios at which plateau occurs were difficult to obtain, because of the aggregation of brain spectrin at concentrations higher than 200 μg/ml.

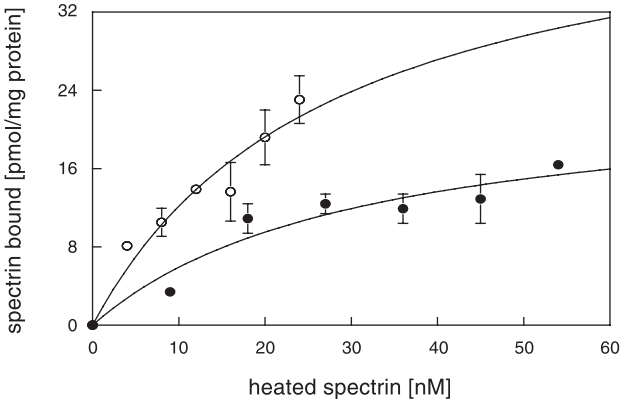

Fig. 5. Binding of heated brain spectrin to α-chymotrypsin-treated synaptic plasma membranes (○) and of heated erythrocyte spectrin to α-chymotrypsin-treated red blood cell membranes (●). Increasing concentrations of purified spectrins were incubated with α-chymotrypsin-digested and NaOH-re-extracted membranes. Other details in Materials and methods.

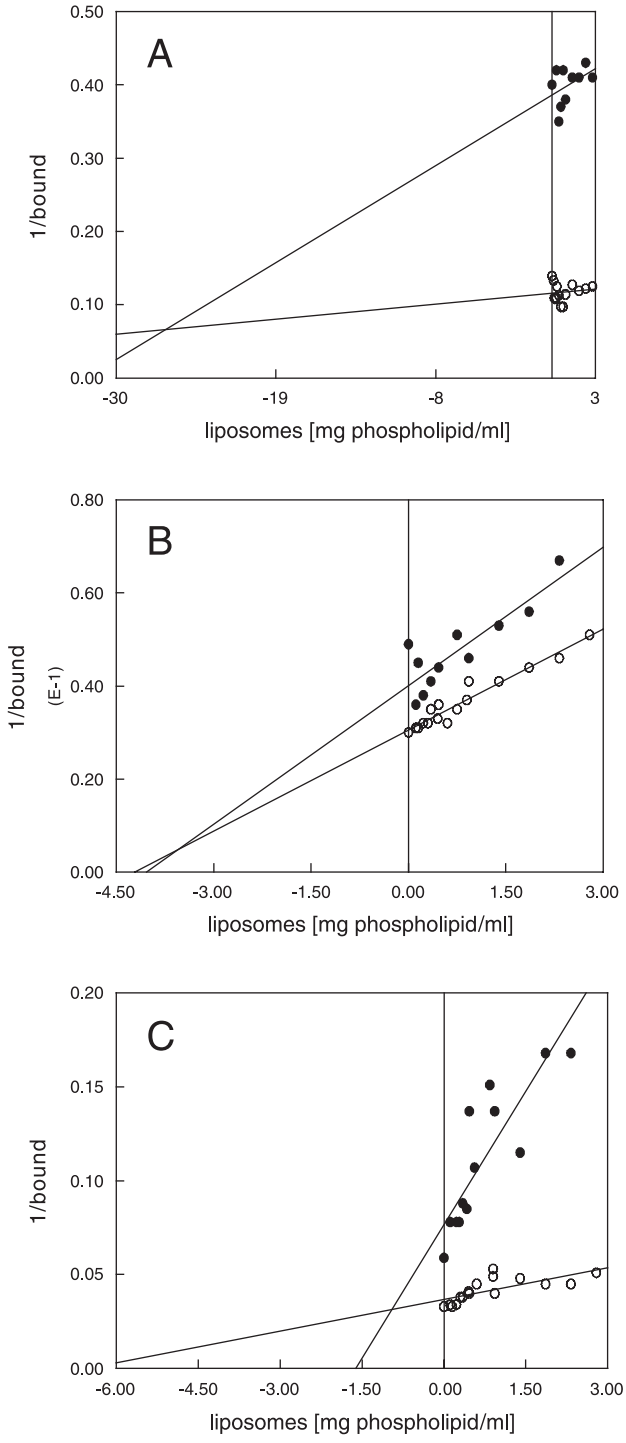

Fig. 6. Inhibition of brain spectrin interaction with synaptic membranes by liposomes prepared from natural membranes. Dixon [21] plot of the results obtained for interaction of purified brain spectrin with synaptic membranes after extraction with low ionic-strength solution (A), NaOH-extracted membranes (B), and α-chymotrypsin-digested and NaOH-re-extracted membranes (C). Brain spectrin concentration in the sample: 9 nM (●), 18 nM (○).

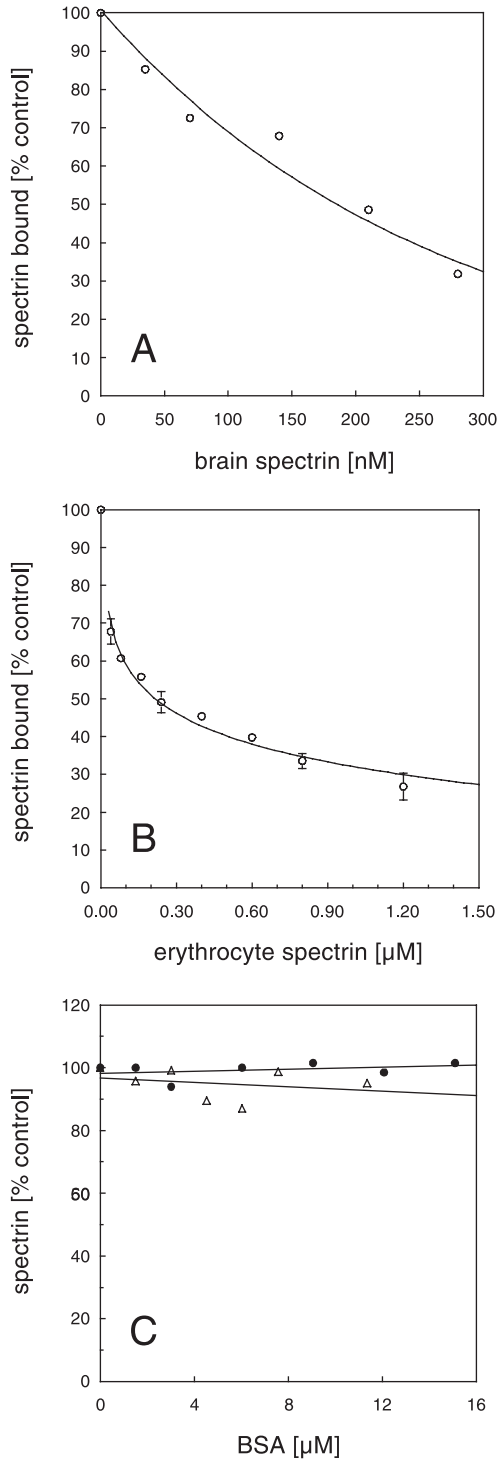

Fig. 7. Inhibition of tetramethylrhodamine-5-maleimide-labelled brain (A) and erythrocyte (B) spectrins (70 and 40 nM, respectively) interaction with α-chymotrypsin-digested and NaOH-re-extracted synaptic membranes by increasing concentrations of unlabelled brain spectrin (A) and erythrocyte spectrin (B). The effect of BSA on binding of labelled brain spectrin (△) and erythrocyte spectrin (●) is shown in C.

The same procedure was repeated using erythrocyte spectrin (Fig. 7B). The results were similar to those obtained for the inhibition of interaction of the brain spectrin. The molar ratio of the unlabelled to the labelled erythrocyte spectrin varied from 1:1 to 30:1. Already at the molar ratio of 1:1, a 20–30% inhibition of binding occurred. Maximal inhibition, of approximately 60–70%, was observed at a molar ratio of 10:1–30:1.

In control experiments, BSA instead of unlabelled spectrin was used (Fig. 7C). Even at a high weight ratio of 15:1 of BSA to spectrin, essentially no effect was observed.

4. Discussion

The aim of this study was to answer the question whether spectrin-binding sites of lipidic nature do exist in natural membranes. One of possible direct experimental approaches was to isolate the membranes and remove or destroy known protein receptors for spectrin by extraction and protease treatment. The characteristics of the membranes extracted with NaOH and digested with α-chymotrypsin were previously well documented by the studies of Steiner et al. [18]. Membranes prepared in this way were used by these and other authors in several studies [15,22,23]. There is a small probability that cannot be ruled out that some of integral protein loops are protease-resistant; therefore, we should talk about extraction and protease-resistant spectrin receptors in the membrane.

Our data suggest that brain and erythrocyte spectrins bind to "natural" membranes independently of the known protein receptors. The affinities of these interactions are rather high, characterised by K_d values in the nanomolar and submicromolar range. As was expected, the lowest K_d values were observed in the case of membranes containing ankyrin, that is, membranes extracted only with low ionic-strength solution. The second class of sites (also of proteinaceous nature) which were well characterised by others [15,22,23] are the so-called ankyrin-independent sites. These consist of two subclasses: calcium-calmodulin-dependent and calcium-independent spectrin-binding receptors located to the amino-terminal and carboxyl-terminal regions of the non-erythroid β-spectrin molecule, respectively [23]. These binding sites are formed by protein receptors not yet defined. The affinities of the third class of spectrin receptors, that is, protein-independent sites in the membrane, although the smallest, are also in the nanomolar range (Table 1). The B_{max} values should be taken with caution, keeping in mind that the amount of protein in the membrane is remarkably lower in the case of membranes extracted with NaOH than in the membranes extracted only with low ionic-strength solution, and the lowest concentration of protein was observed in the case of protease-treated membranes. Why were these lipidic sites not reported by others [22,23] studying the binding of brain and erythrocyte spectrins to natural membranes? One of the possible answers to this question seems to be: the binding of spectrins, particularly brain spectrin, to lipid domains of the membrane seems to be independent of intact conformation of this protein [14]. This heat treatment-

resistant binding was treated by these authors as nonspecific. Our data suggest that these sites are present in the native as well in the heated brain and erythrocyte spectrins (at temperatures defined by others as abolishing membrane proteins-binding capacity). The binding properties of the heated spectrin seem to indicate specific binding, that is, a relatively high affinity (K_d in the nanomolar range) and a relatively low B_{max}, comparable to those of the untreated membranes. This is in accordance with our previous data [14] for spectrin–phospholipid binding, in which brain spectrin was shown to bind liposomes after heat denaturation as well as after succinylation. The temperatures used for thermal treatment of red blood cell spectrin (58 °C) and for brain spectrin (67 °C) [15] were reasonably higher than the major transition temperatures (51 and 54 °C, respectively) [24]. It should be noted, however, that both in the case of the brain and of the erythrocyte spectrins, ~ 50% of the total residual ellipticity was observed at the end of the transition, suggesting the presence of an α-helical structure. The data of Brandts et al. [25] indicate that even after heating to above 70 °C, more than 30% of initial molar residue ellipticity was still observed, suggesting the presence of α-helical structure extremely resistant to thermal unfolding. Our spectrins preparations heated to 58 and 67 °C retained 80% (erythrocyte spectrin) and 70% (brain spectrin) of the initial molar ellipticity after cooling on ice. When heating time was increased to even 60 min, the residue ellipticity of the red cell spectrin did not change while brain spectrin's residue ellpticity dropped to near 50% of the initial value (data not shown). The differences with the data on the dependence of the ellipticity on the temperature obtained by others [24,25] are probably due to the fact that their spectra were taken upon heating and ours were taken after cooling as suggested by Steiner and Bennett [15]. Probably upon cooling, partial refolding took place. These facts would explain the lipid-binding activity of heat-treated spectrins and would be in agreement with the previously published data on folding-sensitive aminophospholipid vesicle binding by expressed dystrophin fragments [26].

The affinities obtained for protease-treated membranes are similar to those observed for the interaction of red blood cell spectrin [9] and brain spectrin [14] with liposomes FAT prepared from aminophospholipids and their mixtures. Moreover, small liposomes prepared from lipids extracted from demyelinated brain membranes seem to competitively inhibit spectrin–membrane interactions. The lowest apparent K_i value could be observed for the membrane preparation that had been extracted with NaOH and treated with α-chymotrypsin.

Specificity of these interactions is also confirmed by experiments in which fluorescently labelled erythroid and brain spectrins were displaced by unlabelled proteins, while high concentrations of bovine serum albumin, which was used as nonspecific competitor, remained without effect.

Available data suggest that the PH domain of nonerythroid spectrin could be involved in its interaction with the membrane [27–29]. A bacterially expressed N-terminal segment of the βIΣ2 spectrin PH domain binds to crude and extracted brain membranes, to protein-free brain lipids and to vesicles containing phosphatidylinositol-4,5-bisphosphate [28]. This, however, is probably not the case here, because recent structural studies [30,31] suggest that the specificity of the pleckstrin homology domain is the phosphorylated inositol ring. In addition, data presented here indicate that erythroid spectrin in which the PH domain is missing binds protein-independent sites as well.

Our previous data [14] suggest that brain spectrin interacts directly with membrane aminophospholipids which are located on the cytoplasmic leaflet of the synaptosomal membrane bilayer. In a monolayer study [32], it was shown that the site for this interaction is located in the β subunit of brain spectrin. It was previously found that erythrocyte ankyrin inhibits the interaction of red blood cell spectrin with phospholipid vesicles and monolayers [9]. This could mean that the binding site for ankyrin and phospholipids (mainly PE) may be, at least in part, overlapping. In the case of nonerythroid (brain) spectrin, we also observed strong inhibition of binding to a phospholipid monolayer by red blood cell ankyrin [32].

Available data indicate that in synaptic plasma membranes, there are at least several kinds of binding sites for brain spectrin: our data suggest a relationship between the ankyrin and the phospholipid (PE-containing) binding sites [9,10,32]. The possible relationship between the above-described protein-receptors-independent site(s) occurring in natural membranes and so-called ankyrin-independent binding of spectrin to the membranes remains to be elucidated, but it requires identification of these receptors.

Aminophospholipid binding to spectrin in the erythrocyte membrane seems to be physiologically important, as phospholipid asymmetry was shown to participate in stabilisation of the mechanical properties of this cell [33].

Acknowledgements

This work was supported by Grant nos. P04A 02010 and 6 P04A 052 18 from Komitet Badañ Naukowych. We thank Dr. Jerzy Gubernator for his assistance with the calibration of liposomes and Dr. M. Lisowski for his help in obtaining CD spectra of heated spectrins. We also thank Prof. Walter Gratzer for his gift of tetramethylrhodamine maleimide.

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