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Brain spectrin interacts with membrane phospholipids

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Spectrin is a major peripheral protein of the erythrocyte membrane forming, together with actin, ankyrin, protein 4.1, adducin and dematin, the membrane skeleton of the red blood cell (for review see [1]). Spectrins are abundant in most animal cells and tissues. One of the best studied analogue of the erythrocyte protein is brain spectrin. In the red blood cell membrane, spectrin is linked to the hydrophobic domain *via* ankyrin (high affinity binding site) bound to the cytoplasmic domain of the anion transporter, and *via* protein 4.1 bound to the actin protofilament or to glycophorin C. Apart from binding to the high affinity binding site(s), interaction of spectrin with membrane phospholipids has been observed by many authors either in natural membranes [2, 3] or in model systems [4–6]. In the literature there are no data on the interaction of brain spectrin with membrane phospholipids. In this communication, evidence is presented that isolated brain spectrin interacts with liposomes prepared from a mixture of phosphatidylethanolamine and phosphatidylcholine (60:40).

Brain spectrin was isolated from bovine brains as described elsewhere [7]. Liposomes filled with Dextran were prepared from a mixture of PE[1] and PC (60:40) (Sigma, St. Louis, MO, U.S.A.) by freezing and thawing [8] in the test buffer containing 20% Dextran T-40. The "test buffer" contained 5 mM MES, 0.5 mM EDTA, 150 mM NaCl and 1 mM NaN_3. At pH 7.5 MES was substituted by 5 mM Tris. Interaction of brain spectrin with liposomes was assayed as follows: 110 µl of the mixture containing 50 µl liposome suspension (250 µg phospholipid) and purified brain spectrin (10–250 nmol/l) was incubated at 20°C. After 30 min 90 µl of the mixture was overlayered on a 150 µl cushion of 0.5% dextran in the "test buffer" and centrifuged at 30000 × *g* for 6 min. Protein in the liposome pellet dissolved in 2% SDS was assayed according to Dulley & Grieve [9] using bovine serum albumin as a standard.

Previously we have studied [7] the interaction of erythrocyte spectrin with phospholipid vesicles. It was found that the mixtures containing phosphatidylethanolamine induced the largest changes in intrinsic fluorescence of this protein, therefore liposomes prepared from the (60:40) mixture of PE/PC were chosen for initial studies on the interaction of brain spectrin with phospholipids. In Fig. 1 the binding isotherm obtained in the presence of a constant concentration of spectrin and variable concentrations of liposomes at pH 6.0 is shown. To find the optimal binding conditions, the dependence on pH and salt (NaCl) concentration was tested. Two pH optima (6 and 7.5) were found (not shown). The optimal salt concentration for this interaction appeared to be 0.15 M NaCl (not shown). To obtain simple kinetic parameters the binding assays were carried out at constant liposome concentration (0.250 mg phospholipid). In Fig. 2 the binding isotherm resulting from the tests at pH 6.0 is presented. Using Scatchard [10] analysis a K_d (dissociation constant) of 27 nM and maximal binding ca-

[1]Abbreviations used: FAT, frozen and thawed; MES, 2(*N*-morpholino)ethane sulfonic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SDS, sodium dodecyl sulfate.

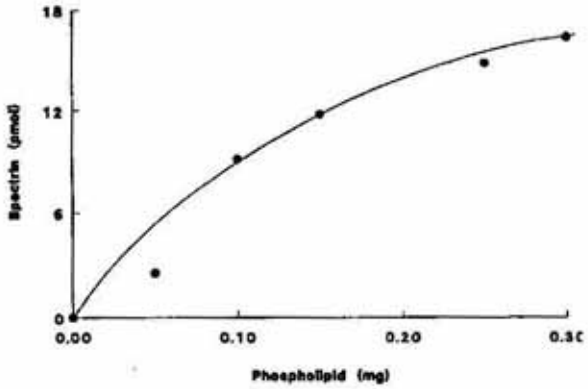

Fig. 1. Binding of isolated brain spectrin (constant concentration in the incubation mixture, 160 nM) by increasing concentrations of the PE/PC vesicle. The binding assay was carried out at pH 6.0. For further experimental details see text.

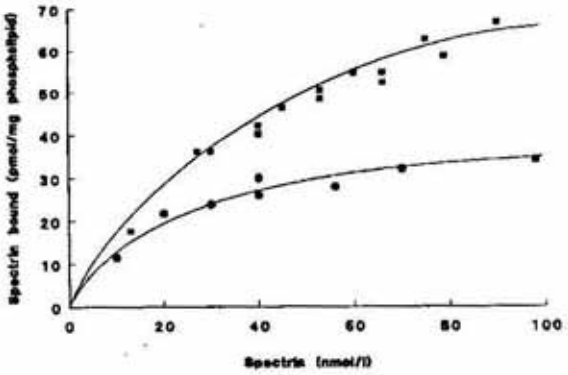

Fig. 2. Binding of increasing concentrations of isolated brain spectrin with constant concentrations (250 µg/sample) of phospholipid (PE/PC) vesicles at pH 6.0 (●) and pH 7.5 (■).

pacity (B_{max}) of 45 pmol spectrin/mg phospholipid were obtained. The same results for the reaction carried out at pH 7.5 are also presented in Fig. 2. A K_d of 68 nM and B_{max} of 114 pmol spectrin per milligram phospholipid were found.

The above presented data indicate that brain spectrin, similarly as its erythrocyte counterpart, exhibits the ability to bind phospholipid vesicles. This binding is saturable and displays two pH optima and a single ionic strength optimum which is close to physiological conditions. The reason for the double pH optimum may be related to: (1) ionisation of the protein (pH 6 is close to the isoelectric point of brain spectrin) and/or (2) "privileged" conformation at physiological pH with exposed hydrophobic domains. Simple calculations indicate that one brain spectrin tetramer molecule binds maximally 12–30 × 10^3 molecules of phospholipid, which would mean that several spectrin molecules should be bound by a single vesicle. Relatively low values of dissociation constants, 27 nM and 68 nM (at pH 6.0 and 7.5, respectively) indicate high affinity binding and possible physiological significance of this interaction in supporting the lipid bilayer in nonerythroid cells. Further studies should concern lipid specificity of this interaction and identification of lipid binding sites in the brain spectrin molecule.

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