Mapping of an ankyrin-sensitive, phosphatidylethanolamine/phosphatidylcholine mono- and bi-layer binding site in erythroid β-spectrin.

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It has been shown previously that binding of vesicles and monolayers containing PE (phosphatidylethanolamine) by either erythroid or non-erythroid spectrin proved sensitive to inhibition by purified erythrocyte ankyrin. We tested the lipid-binding affinities of the purified ankyrin-binding domain of β-spectrin and of its truncated mutants in four ways, by analysing: (1) penetration of 'loose' PE/PC (phosphatidylcholine) monolayers; (2) binding to liposomes in suspension; (3) competition with spectrin for liposomes; and (4) binding of a PE/PC monolayer in a surface plasmon resonance system. The results obtained indicated that the full-length ankyrin-binding domain bound PE/PC mono- and bi-layers with moderate affinity, penetrated monolayers and competed with spectrin for liposomes. Moreover, its truncated mutants that retained the N-terminal part, in contrast with those lacking eight or 38 N-terminal residues (which bound lipid mono- and bi-layers with lower affinity), bound PE/PC mono- and bi-layers with an affinity and capacity comparable with those of the full-length ankyrin-binding domain, and this activity was inhibited by purified erythrocyte ankyrin. The full-length domain, in contrast with the mutant lacking 38 N-terminal residues, induced a small increase in the fluidity of PE/PC membranes when probed with 5′-doxyl stearate, similar to the effect of purified spectrin. Therefore we conclude that the binding site for PE-rich lipids, which is sensitive to ankyrin inhibition, is located in a 38-residue N-terminal fragment of the β-spectrin ankyrin-binding domain, and that the first eight residues play a key role in this activity.

Słowa kluczowe

ankyrin-binding domain, membrane skeleton, phospholipid, β-spectrin, spectrin–phospholipid interaction