Four amino acid residues are responsible for ankyrin-sensitive PE-binding by βI–spectrin

Marcin Wolny[*], Michał Grzybek[†], Ewa Bok[‡], Anna Chorzalska[*§], Marc Lenoir[x], Aleksander Czogalla[*], Klaudia Adamczyk[*], Adam Kolondra[*], Witold Diakowski[*], Michael Overduin[x], Aleksander F. Sikorski[*$]

[*]University of Wrocław, Biotechnology Faculty, Laboratory of Cytobiochemistry, Przybyszewskiego 63-77, 51-148 Wrocław, Poland; [†]Max Planck Institute of Molecular Cell Biology and Genetics; Pfotenhauerstr. 108, 01307 Dresden, Germany; [‡] University of Zielona Góra, Department of Molecular Biology, Z. Szafrana 1, 65-516 Zielona Góra, Poland; [x] Henry Wellcome Building for Biomolecular NMR Spectroscopy, School of Cancer Sciences, University of Birmingham, Birmingham, B15 2TT, UK

It was shown previously that an ankyrin-sensitive, phosphatidylethanolamine/phosphatidyl-choline (PE/PC) binding site maps to the N-terminal part of the ankyrin-binding domain of β-spectrin (ankBDn). Here we have identified the amino acid residues within this domain which are responsible for recognizing monolayers and bilayers composed of PE/PC mixtures. *In vitro* binding studies revealed that a quadruple mutant with substituted hydrophobic residues W1771, L1775, M1778 and W1779 not only failed to effectively bind PE/PC, but its residual PE/PC binding activity was insensitive to inhibition with ankyrin. Structure prediction and analysis, supported by *in vitro* experiments, suggests that "opening" of coiled-coil structure underlies the mechanism of this interaction. Experiments on red blood cells and HeLa cells supported the conclusions derived from the model and *in vitro* lipid-protein interaction results, and showed potential physiological role of this binding. We postulate that direct interactions between spectrin ankBDn and PE-rich domains play an important role in stabilizing the structure of the spectrin-based membrane skeleton.