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The role of hydrophobic interactions in ankyrin–spectrin complex formation

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ABSTRACT

Spectrin and ankyrin are the key components of the erythrocyte cytoskeleton. The recently published crystal structure of the spectrin–ankyrin complex has indicated that their binding involves complementary charge interactions as well as hydrophobic interactions. However, only the former is supported by biochemical evidence. We now show that nonpolar interactions are important for high affinity complex formation, excluding the possibility that the binding is exclusively mediated by association of distinctly charged surfaces. Along these lines we report that substitution of a single hydrophobic residue, F917S in ankyrin, disrupts the structure of the binding site and leads to complete loss of spectrin affinity. Finally, we present data showing that minimal ankyrin binding site in spectrin is formed by helix 14C together with the loop between helices 15 B/C.

1. Introduction

During its 120-day life span, an erythrocyte circulates many times through the human body, often being squeezed when passing through capillaries, which may have a diameter one fourth that of the red cell. Key to maintaining the shape, structural integrity and remarkable mechanical stability is the fact that these cells possess a robust membrane skeleton of spectrin and actin. These proteins form a dense proteinaceous network, which is markedly different from the typical cytoskeletal systems [1–3]. Here the red cell skeleton is attached to the membrane bilayer via integral membrane proteins and direct interactions with lipids [4–6]. Erythrocytes with defects in the spectrin-based skeleton lose their shape and stability, as seen in hemolytic anemias in humans or mice [7–9]. One of the most studied erythrocyte spectrin–membrane interactions is that of spectrin–ankyrin binding [10,11]. Crystal structures with both spectrin/ankyrin binding domains and the complete complex have identified charged surfaces as being key to this interaction [12]. In a series of elegant biochemical studies, the binding surface of beta-spectrin was localized to helix 14C (1773–1788) and 15 B/C loop residues (1865–1867) [12–14]. Additionally, the ankyrin-binding site has a very important inter-repeat kink, that is required for complex formation [12,14]. The complementary surface on ankyrin is formed by the DAR sequence (920–922) [15], followed by residues 944–946 that interact directly with the 15 B/C loop [12]. Although it has been proposed that spectrin–ankyrin binding involves both complementary charge interactions as well as hydrophobic interactions, only the former is, thus far, supported by biochemical evidence.

Here we assessed the role of hydrophobic interactions in spectrin–ankyrin binding. By evaluating binding under various salt and pH conditions, we show that the interactions between the complementary surfaces of ankyrin and spectrin exclude the possibility that binding is exclusively mediated by interactions of distinctly charged surfaces. Interestingly, screening of single point mutations in the ZU5 domain of ankyrin revealed a crucial role of phenylalanine at position 917, as substitution with serine at this position abrogated the ability of GFP-AnkSBD (spectrin-binding domain of ankyrin) to bind spectrin. Finally, consistent with a recent proposal [13], we present evidence indicating that helices 14 A/B of beta spectrin are not involved in ankyrin binding.

2. Materials and methods

2.1. Cloning, expression and purification of His(6)GFP-AnkSBD fragment and its mutants

AnkSBD (909–1050) ankyrin fragment was previously identified as a minimal spectrin binding fragment [16]. It was cloned into pRSETC expression vector followed by Green Fluorescent Protein obtained from the pEGFP-C1. His(6)GFP–AnkSBD construct was amplified in XL1-Blue *E. coli* and positively screened plasmids were sequenced and transferred into expression BL21(DE3)pLysS *E. coli* strain using a standard protocol.

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Mutations were performed with QuickChange Site-Directed Mutagenesis Kit (Stratagene). The PCR products were stored in *E. coli* strain XL1-Blue. The resulting mutant cDNAs were sequenced to verify the mutations. Plasmids were then transferred into BL21(DE3) pLysS cells for expression of the proteins.

Overexpressed proteins were purified using Talon® resin as described previously [16]. Purity and concentration of His(6)GFP-AnkSBD proteins were determined using a Cary 1E spectrophotometer employing extinction coefficient parameters determined for each protein. Purity and concentration were also estimated from coomassie stained SDS/PAGE on 10% gels.

2.2. Labeling of proteins

Human erythrocyte spectrin was purified by extraction of erythrocyte ghosts with low-ionic-strength buffer at 37 °C and separated by gel filtration on a Sepharose 4B column (Sigma Aldrich), as described [17]. Purified erythrocyte spectrin, or spectrin fragments (ankyrin-binding domain of spectrin) were fluorescently labeled using tetramethylrhodamine-5-maleimide, which reacts with thiol groups on proteins to give thioether. Labeling was performed according to the manufacturer's directions. Labeling stoichiometry was determined using a molar extinction coefficient for rhodamine of 88430/55350 at 541/570 nm. Labeling efficiency was at least 80%. Purified erythrocyte spectrin SpAnkBDΔ15BC fragment was fluorescently labeled using rhodamine isothiocyanate (RITC), as described previously [18].

2.3. CD analysis

CD measurements were performed on a JASCO CD spectrometer, using a thermostat-controlled cell with 0.1 cm path length, from 20 to 80 °C in 0.1 °C increments, at 220 nm. Ellipticity (θ: in degrees) values from CD spectra were converted into molar residue ellipticity ($[\theta]_M$: in degrees cm^2 $dmol^{-1}$) value.

2.4. FRET assay

Either tetramethylrhodamine-5-maleimide or rhodamine isothiocyanate labeled spectrin fragments were used at a concentration of 0.5 μM with ascending concentrations of His(6)GFP-AnkSBD fragment or its mutants in a total volume of 1 ml (150 mM NaCl, 20 mM Tris–HCl, pH 7.4). After 20 min incubation at room temperature, rhodamine and GFP fluorescence were measured in a Cary Eclipse (Varian) Spectrofluorimeter with 488 nm excitation wavelength for GFP tag protein and 576 nm emission wavelength for rhodamine. Standard curves for GFP tagged ankyrin constructs and for rhodamine labeled spectrin fragments were made independently.

2.5. EIA (electrochemiluminescence immunoassay)

Spectrin fragments (25 ng/μl) were adsorbed on to a high bind carbon electrode surface (MesoScale Discovery) (1 h, 23 °C), the rest of the surface was blocked with 0.1% fat free BSA (1 h, 23 °C). The surface was then washed three times with 150 mM NaCl, 20 mM Tris–HCl pH 7.5, and subsequently, the defined concentrations of proteins were added to each well. Binding was allowed for 2 h at 23 °C (different conditions of binding are stated along the paper together with the results). The wells were again washed and primary anti-GFP antibody was applied (ROCHE, Rabbit anti-GFP, 1 μg/ml, 23 °C, 1 h). The wells were again washed and secondary antibody (goat anti-rabbit-STAG) was added (1 μg/ml, 23 °C, 1 h). The wells were washed and reading buffer was added (MSD surfactant free reading buffer). As a background, both primary and secondary antibodies binding to BSA were used. Data were acquired on a SECTOR Imager 6000.

2.6. Sedimentation assay

Spectrin-binding activity of AnkSBD polypeptide was determined in an experiment in which a metal affinity resin (Talon) saturated with a His-tagged AnkSBD, or its mutants, was incubated with increasing concentrations of RITC-labeled erythrocyte spectrin in the test buffer (20 mM Tris–HCl, 150 mM NaCl, pH 7.4) for 30 min at room temperature. The resin was then pulled down at 2000×*g* for 5 min, resuspended in 50 mM imidazole in the test buffer, and centrifuged again. Bound protein was solubilized with 0.1% SDS in the test buffer. The amount of bound, labeled spectrin in the SDS extract was determined using a Varian Cary Eclipse Spectrofluorimeter. Control experiments were performed using the Talon resin equilibrated in the test buffer and incubated with corresponding amounts of RITC-labeled spectrin. Bound values were obtained by subtracting control values from those for spectrin bound to the resin saturated with AnkSBD.

2.7. Data analysis

The recorded data were analyzed in GraphPad Prism 5.0 software using One site (total and nonspecific) binding algorithm.

2.8. Modeling of ankyrin (AnkSBD) and spectrin (SpAnkBD) constructs

Structural models corresponding to AnkSBD and SpAnkBD constructs were generated by homology modeling using MODELLERv9 [19]. For each construct, five models with hydrogen atoms were generated and ranked according their fit to the structure of human erythroid spectrin and ankyrin (PDB ID 3KBU). Multiple templates (3KBU and 3EDV) were used for SpAnkBD in order to generate the 15–16 repeat. AnkBD was calculated from a single template (3KBU). Protonation states for different pH values and electrostatic surface charges were calculated within PROPKA [20] and APBS [21], respectively, and visualized in PyMOL.

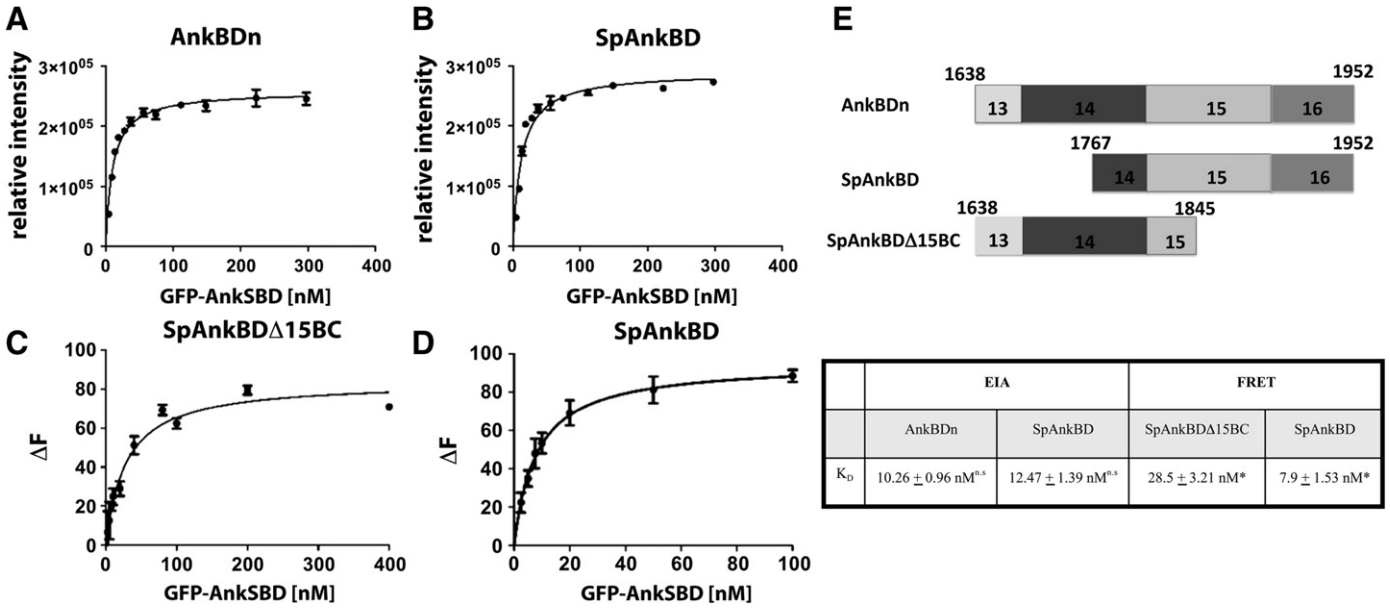
3. Results

3.1. Binding studies of ankyrin and spectrin

To determine the minimal spectrin binding surface required for full ankyrin binding activity, the binding affinities of various spectrin fragments were recorded, using two independent approaches. In an EIA assay (Fig. 1), using unlabeled ankyrin-binding domain of β spectrin (AnkBDn—for graphical representation of the fragments refer to Fig. 1) and its truncated form (SpAnkBD), we obtained similar K_D values for both fragments. These values are very similar to earlier data reported by us [16] and by others [22,23]. In a parallel assay, we measured fluorescence (Forster) resonance energy transfer (FRET) between the rhodamine-maleimide-labeled ankyrin-binding domain of SpAnkBD or SpAnkBDΔ15BC fragments and GFP-AnkSBD (Fig. 1). We observed significant differences ($p<0.05$) in binding affinities between the fragments. These results demonstrated that residues comprising helices 14A and 14B are irrelevant for the high-affinity complex formation—a finding, which is in agreement with the previously reported data [13,14]. Moreover, the results obtained from the SpAnkBDΔ15BC fragment encompassing residues 1638–1845, which lacks the 15 B/C-loop, further confirm the importance of loop residues in proper binding, as suggested previously [13–15].

3.2. Dependence on binding conditions

It has been suggested that the interface between spectrin and ankyrin is formed by ionic interactions between charged residues on the respective proteins [12,24]. If true, this would suggest that high salt conditions would be best for spectrin–ankyrin dissociation. In



	EIA		FRET	
	AnkBDn	SpAnkBD	SpAnkBDΔ15BC	SpAnkBD
K_D	10.26 ± 0.96 nM[n.s]	12.47 ± 1.39 nM[n.s]	28.5 ± 3.21 nM*	7.9 ± 1.53 nM*

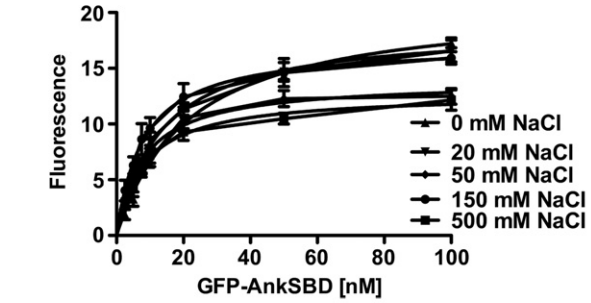
Fig. 1. Amino acid residues within loop 15B/C are important for proper binding, whereas residues of helices 14A/B are not. Binding of AnkSBD to full length ankyrin-binding domain of spectrin (AnkBDn) and its truncated mutants (SpAnkBD, SpAnkBDΔ15BC) was assessed. The EIA results revealed that SpAnkBD (B) binds to AnkSBD with similar characteristics as the full length ankyrin-binding domain (A) (K_D and B_{max}—details shown in table below the graphs). In a separate FRET assay, the observed K_D values for SpAnkBDΔ15B/C (C) were significantly higher ($p<0.05$) than for SpAnkBD (D). ΔF is the relative increase of fluorescence signal of rhodamine labeled spectrin fragments in comparison to control (fluorescence of spectrin fragment in assay buffer). The measured K_D for SpAnkBD was consistent between both assays. Statistical analysis was performed using paired *t*-test. (n.s., not significant, *p-value<0.05). (E) The colored bars depict the schematic representation of the various spectrin fragments used in the assays with respect to their length and helical repeats.

contrast, we observed a high consistency of K_D values for a wide range of higher NaCl concentrations (50–500 mM NaCl) (Fig. 2). In fact, the routine method of spectrin isolation, which has been known for more than three decades, uses low ionic strength solutions to extract spectrin from the erythrocyte membrane [25,26]. To determine the effect of ionic strength on spectrin–ankyrin binding affinity, a series of binding assays was performed. Measurements made in low salt buffers (0–20 mM NaCl) showed a moderate (three-fold) increase in the K_D value of this interaction when compared to the "control" buffer containing 150 mM NaCl (Fig. 2). These changes, although significant ($p<0.01$), were not dramatic, which was rather unexpected, given the established protocols for spectrin extraction from erythrocyte membranes. In spite of these results, it was clear that temperature was a key factor in dissociation of the complex, as the actual procedure included using low ionic strength solutions at temperature of 37 °C [25,26]. However, performing the binding assay at 37 °C in corresponding salt conditions gave ambiguous results due to protein instability in low salt conditions (Fig. 2 lower panel). The observed protein instability was clearly time dependent and rather irreversible (not shown). It remains unclear, therefore, whether similar domain instability occurs during spectrin isolation. The overall results suggest that non-ionic interactions are of substantial importance.

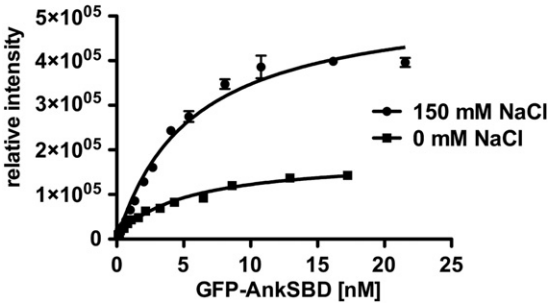
To further estimate the contribution of ionic interactions for the spectrin–ankyrin association, binding of GFP-AnkSBD to SpAnkBD was performed at various pHs using EIA. Increasing the pH to 9.0 did not significantly change the binding. On the other hand, a decrease in pH had a significant effect on the binding. At pH 5.0 the interactions were greatly decreased, $K_D>122$ nM, while at pH 6.0 far less dramatic changes in binding affinity were observed (Fig. 3). Calculations using PyMOL showed that the surface of SpAnkBD in the 15th triple helical repeat was essentially positively charged at pH 5.0, but was negatively charged at pH 9.0. However, the changes in surface charge did not correspond to the binding site region (Supplementary Figure 1). The charges of residues comprising the potential binding site of ankyrin were conserved at either pH (Supplementary Figure 1). A further investigation of the stability of the studied fragments revealed that SpAnkBD was unstable at low pH, which was used in the aforementioned experiments. Therefore the observed loss of binding is most probably caused by instability of the SpAnkBD fragment. Generally, the results presented above indicate that although charged residues of both spectrin and ankyrin are important components of complex formation, these residues do not explain all potential binding in the interface of the complex.

3.3. Point mutations in ZU5 domain

Based on our analysis of binding data and the binary complex crystal structure, we introduced point mutations within blocks of four hydrophobic amino acid residues within the ZU5 domain. All mutants were tested for SpAnkBD fragment binding affinity using both FRET and EIA. Mutations of phenylalanine 913 and 917 affected binding of SpAnkBD; substitution of F913S led to an increase in K_D, while in the case of F917S the binding to SpAnkBD fragment as well as to isolated and TRITC-labeled native αIβI spectrin was almost completely abrogated (Fig. 4), indicating that this residue was crucial for appropriate spectrin–ankyrin interactions. According to the crystal structure the side chain of F917 is buried inside the core of the domain. Circular dichroism (CD) analysis confirmed that the loss of binding could not be caused by the instability of the mutant. The actual T_m of the mutant was even higher than that for the WT. However, the recorded CD spectra showed that F917S mutation results in some changes in the secondary structure (not shown). It should be noted that the F917 residue is part of the same β-sheet as the 920DAR922 sequence previously reported as being crucial for spectrin–ankyrin interactions [15]. Therefore, though the two phenylalanines (913_{Ank}, 917_{Ank}) do not seem to be directly involved in formation of the binding interface, it is plausible that their side chains are important parts of the hydrophobic core of the domain and that they are involved in the proper orientation of the binding site. This would indicate that there is a strong need for a correct conformation of the corresponding binding sites in both ankyrin as well as in spectrin (as shown previously [12,14]).



NaCl [mM]	K_D [nM]
500	$8.12 \pm 1.36^{n.s.}$
150 (control)	8.04 ± 1.24
50	$8.04 \pm 1.04^{n.s.}$
20	$13.22 \pm 1.22^{n.s.}$
0	21.75 ± 2.71*

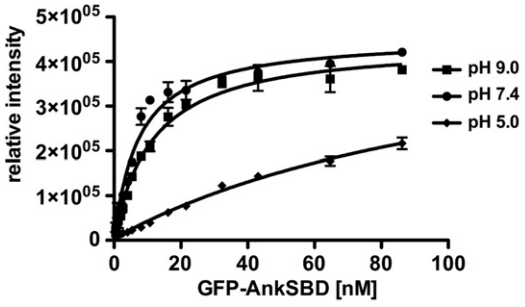


NaCl [mM]	K_D [nM]	Bmax
150	$5.53 \pm 0.86^{n.s.}$	496 760 ± 33 250
0	$5.35 \pm 0.55^{n.s}$	178 620 ± 8 635***

Fig. 2. Binding assay for GFP-AnkSBD and SpAnkBD performed at room temperature (top panel) or at 37 °C (bottom panel) at various NaCl concentrations. The binding of GFP-AnkSBD at 37 °C in no salt conditions showed strongly decreased binding capacity due to protein instability, as observed by a drop in B_{max}. Binding affinities were tested using FRET or EIA and statistical analyses were performed using paired *t*-test. ($^{n.s.}$not significant, $^{*}p$-value<0.05, $^{***}p$-value<0.001).

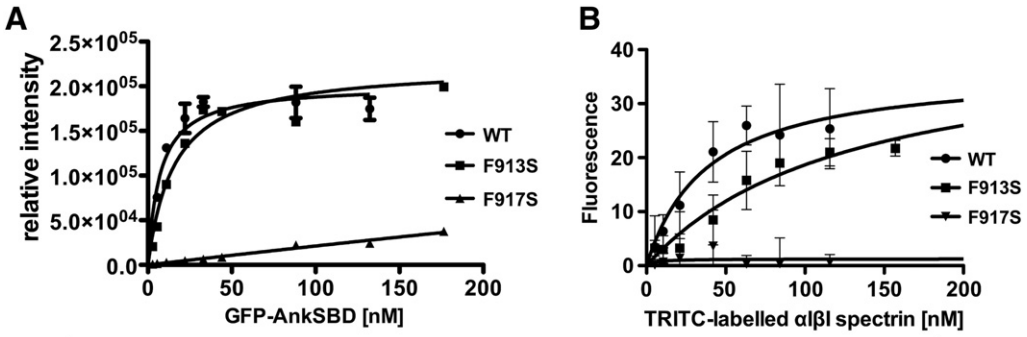
4. Discussion

It has been suggested that the major factor responsible for proper ankyrin–spectrin binding is the presence of complementary charged surfaces on respective proteins [24]. These suggestions come from known clinical cases of anemia, which were caused by various point mutations of hydrophilic residues within the ankyrin-binding domain of spectrin. The data were further supported by biochemical studies on point mutants of spectrin, where again, hydrophilic residues were mutated [13,14]. Not surprisingly, these results were confirmed by the crystal structure of the spectrin–ankyrin complex, which showed the presence of these residues in the actual binding interface [12]. However, as we show here in a series of experiments at different salt concentrations, the binding affinity decreases at low salt concentration (0 mM NaCl) and remains unchanged at higher salt concentration (500 mM NaCl) when compared to the control (150 mM NaCl) (Fig. 2). Ionic interactions are usually easily disrupted in high salt, which is not the case here. This finding, together with the fact that spectrin is actually extracted from the membrane at low ionic strength, indicates significant importance of nonpolar residues in the binding. On the basis of the ionic strength and pH effect on the interaction, we conclude that hydrophilic interactions play an important role, although they cannot be the only forces that maintain the spectrin–ankyrin complex, because at high ionic strength we do not observe prominent changes in binding affinity. To further investigate this issue, we analyzed point mutants of AnkSBD, where highly hydrophobic phenylalanines were substituted by serine residues. The obtained results indicate that a single point mutation, F917S, localized in the N terminal region of the ZU5 domain in a hydrophobic block (916SFMV919), abrogated binding of this domain to SpAnkBD or TRITC-labeled spectrin. According to the crystal structure, the side chain of F917 is buried inside the structure of AnkSBD and, as such, does not make direct contact with spectrin. Also, the substitution of F with S does not lead to increased protein instability (Fig. 5). Therefore, it is difficult to imagine how this particular residue could have such a crucial role for the proper binding. There are several hydrophobic residues in both ankyrin and spectrin that could be involved in direct interactions ($L914_{Ank}$, $V915_{Ank}$, $M918_{Ank}$, $T950_{Ank}$; $A1780_{Sp}$, $L1785_{Sp}$, $T1788_{Sp}$, $L1792_{Sp}$,). One possible explanation is that F917, which is localized at the center of a dense hydrophobic core, interacts with F913 such that the side chains of the F917 and F913 are involved in a T-shaped π–π interaction that stabilizes the protein shape in this region [27]. The replacement of the phenylalanine by a serine could disrupt the proper



pH	K_D [nM]
9.0	$12 \pm 1.16^{n.s}$
8.5	$12.71 \pm 1.42^{n.s}$
7.4 (control)	7.5 ± 0.96
6.0	19.63 ± 2.16*
5.0	$>122.1 \pm 16.98$***

Fig. 3. Spectrin–ankyrin interactions are susceptible to low pH. Equilibrium dissociation constants for GFP-AnkSBD and SpAnkBD at various pHs were determined. Binding affinities were tested using EIA and statistical analyses were performed using paired *t*-test. n.s.—not significant, $^{**}p$-value<0.01, $^{***}p$-value<0.001.



	SpAnkBD	F913S	F917S
EIA	7.2 ± 1.5 nM	40.51 ± 7.29**	>760***
FRET	38.7 ± 13 nM	129.3 ± 60.8**	x

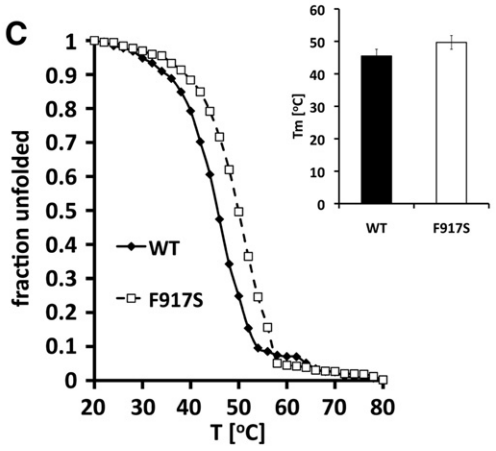


Fig. 4. Hydrophobic residues F913 and F917 are important for proper ankyrin–spectrin complex formation. Binding curves and equilibrium dissociation constants of SpAnkBD or its point mutants. The binding affinity was tested using EIA (A) or Sedimentation assay (B). The table shows the summary of the obtained data. Statistical analysis was performed using paired *t*-test: $^{**}p>0.01$: $^{***}p>0.001$. (C) CD thermal denaturation curves (fraction unfolded versus temperature, left) showed that the F917S mutation had a stabilizing effect on folding. Bar graph (inset) shows the average thermal stability of the proteins (the error bars correspond to the standard error).

conformation of this region, in which event the segment 910–920 that contains a beta-turn and a beta-sheet could adopt a different orientation, and, as a consequence, lose the ability to bind spectrin. This in fact would indicate that not only spectrin adapts a unique conformation in the ankyrin-binding site [12,14] but that also ankyrin has a very defined structure that is able to recognize the site of interaction.

The results presented here suggest that both hydrophilic and hydrophobic forces are engaged in binding between spectrin and ankyrin. We conclude that the major interaction site for ankyrin binding lies within helix 14C and that residues in the 15 B/C loop act as additional support. This conclusion is, in principle, in agreement with recently published data [12]. Our results provide also additional evidences on the role of proper fit between spectrin and ankyrin. In this respect, $F917_{Ank}$ is crucial for the proper orientation of the of the spectrin binding site in ankyrin as replacement of $F917_{Ank}$ with a serine residue abolished spectrin-binding activity.

Supplementary materials related to this article can be found online at doi:10.1016/j.bbamem.2010.07.024.

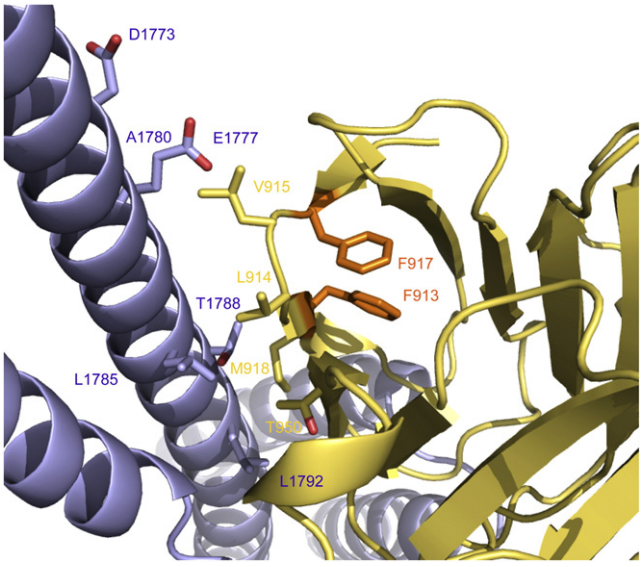


Fig. 5. Details of the ankyrin–spectrin binding interface. Ankyrin and spectrin are colored in yellow and blue, respectively. Residues $F913_{Ank}$ and $F917_{Ank}$ (orange) are buried within the hydrophobic core of the protein. Other hydrophobic residues at the interface ($A1780_{Sp}$, $T1788_{Sp}$, $T1785_{Sp}$, $L1792_{Sp}$ for spectrin and $L914_{Ank}$, $V915_{Ank}$, $M918_{Ank}$ and $T950_{Ank}$) are displayed with sticks. The figure was prepared using coordinates of the spectrin–ankyrin complex (PDB ID 3KBU) [12].

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References

[1] P.J. Mohler, A.O. Gramolini, V. Bennett, Ankyrins, J. Cell Sci. 115 (2002) 1565–1566.
[2] V. Bennett, A.J. Baines, Spectrin and ankyrin-based pathways: metazoan inventions for integrating cells into tissues, Physiol. Rev. 81 (2001) 1353–1392.
[3] V. Nicolas, I. Mouro-Chanteloup, C. Lopez, P. Gane, A. Gimm, N. Mohandas, J.P. Cartron, C. Le Van Kim, Y. Colin, Functional interaction between Rh proteins and the spectrin-based skeleton in erythroid and epithelial cells, Transfus. Clin. Biol. 13 (2006) 23–28.
[4] J.A. Gimm, X. An, W. Nunomura, N. Mohandas, Functional characterization of spectrin–actin-binding domains in 4.1 family of proteins, Biochemistry 41 (2002) 7275–7282.
[5] N. Mohandas, E. Evans, Mechanical properties of the red cell membrane in relation to molecular structure and genetic defects, Annu. Rev. Biophys. Biomol. Struct. 23 (1994) 787–818.
[6] M. Grzybek, A. Chorzalska, E. Bok, A. Hryniewicz-Jankowska, A. Czogalla, W. Diakowski, A.F. Sikorski, Spectrin–phospholipid interactions. Existence of multiple kinds of binding sites? Chem. Phys. Lipids 141 (2006) 133–141.
[7] W.T. Tse, S.E. Lux, Red blood cell membrane disorders, Br. J. Haematol. 104 (1999) 2–13.
[8] S. Perrotta, P.G. Gallagher, N. Mohandas, Hereditary spherocytosis, Lancet 372 (2008) 1411–1426.
[9] M.J. Broderick, S.J. Winder, Spectrin, alpha-actinin, and dystrophin, Adv. Protein Chem. 70 (2005) 203–246.
[10] V. Bennett, P.J. Stenbuck, The membrane attachment protein for spectrin is associated with band 3 in human erythrocyte membranes, Nature 280 (1979) 468–473.
[11] J. Yu, S.R. Goodman, Syndeins: the spectrin-binding protein(s) of the human erythrocyte membrane, Proc. Natl Acad. Sci. USA 76 (1979) 2340–2344.
[12] J.J. Ipsaro, A. Mondragon, Structural basis for spectrin recognition by ankyrin, Blood 115 (2010) 4093–4101.
[13] P.J. La-Borde, P.R. Stabach, I. Simonovic, J.S. Morrow, M. Simonovic, Ankyrin recognizes both surface character and shape of the 14–15 di-repeat of beta-spectrin, Biochem. Biophys. Res. Commun. 392 (2010) 490–494.
[14] P.R. Stabach, I. Simonovic, M.A. Ranieri, M.S. Aboodi, T.A. Steitz, M. Simonovic, J.S. Morrow, The structure of the ankyrin-binding site of beta-spectrin reveals how tandem spectrin-repeats generate unique ligand-binding properties, Blood 113 (2009) 5377–5384.
[15] P.J. Mohler, W. Yoon, V. Bennett, Ankyrin-B targets beta2-spectrin to an intracellular compartment in neonatal cardiomyocytes, J. Biol. Chem. 279 (2004) 40185–40193.
[16] A. Kolondra, M. Grzybek, A. Chorzalska, A.F. Sikorski, The 22.5 kDa spectrin-binding domain of ankyrinR binds spectrin with high affinity and changes the spectrin distribution in cells in vivo, Protein Expr. Purif. 60 (2008) 157–164.
[17] K. Michalak, M. Bobrowska, A.F. Sikorski, Interaction of bovine erythrocyte spectrin with aminophospholipid liposomes, Gen. Physiol. Biophys. 12 (1993) 163–170.
[18] A. Hryniewicz-Jankowska, E. Bok, P. Dubielecka, A. Chorzalska, W. Diakowski, A. Jezierski, M. Lisowski, A.F. Sikorski, Mapping of an ankyrin-sensitive, phosphatidylethanolamine/phosphatidylcholine mono- and bi-layer binding site in erythroid beta-spectrin, Biochem. J. 382 (2004) 677–685.
[19] N. Eswar, B. Webb, M.A. Marti-Renom, M.S. Madhusudhan, D. Eramian, M.Y. Shen, U. Pieper, A. Sali, Comparative protein structure modeling using MODELLER, Curr. Protoc. Protein Sci. Chapter 2 (2007) Unit 2 9.
[20] N.A. Baker, D. Sept, S. Joseph, M.J. Holst, J.A. McCammon, Electrostatics of nanosystems: application to microtubules and the ribosome, Proc. Natl Acad. Sci. USA 98 (2001) 10037–10041.
[21] H. Li, A.D. Robertson, J.H. Jensen, Very fast empirical prediction and rationalization of protein pKa values, Proteins 61 (2005) 704–721.
[22] J.J. Ipsaro, L. Huang, L. Gutierrez, R.I. MacDonald, Molecular epitopes of the ankyrin–spectrin interaction, Biochemistry 47 (2008) 7452–7464.
[23] S.P. Kennedy, S.L. Warren, B.G. Forget, J.S. Morrow, Ankyrin binds to the 15th repetitive unit of erythroid and nonerythroid beta-spectrin, J. Cell Biol. 115 (1991) 267–277.
[24] J.J. Ipsaro, L. Huang, A. Mondragon, Structures of the spectrin–ankyrin interaction binding domains, Blood 113 (2009) 5385–5393.
[25] V.T. Marchesi, Isolation of spectrin from erythrocyte membranes, Meth. Enzymol. 32 (1974) 275–277.
[26] W.B. Gratzer, G.H. Beaven, Properties of the high-molecular-weight protein (spectrin) from human-erythrocyte membranes, Eur. J. Biochem. 58 (1975) 403–409.
[27] C.H. Suresh, N. Mohan, K.P. Vijayalakshmi, R. George, J.M. Mathew, Typical aromatic noncovalent interactions in proteins: a theoretical study using phenylalanine, J. Comput. Chem. 30 (2009) 1392–1404.