Solution Structural Studies on Human Erythrocyte α-Spectrin Tetramerization Site*

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We have determined the solution NMR structure of a recombinant peptide that consists of the first 156 residues of erythroid α-spectrin. The first 20 residues preceding the first helix (helix C′) are in a disordered conformation. The subsequent three helices (helices A′, B′, and C′) form a triple helical bundle structural domain that is similar, but not identical, to previously published structures for spectrin from Drosophila and chicken brain. Paramagnetic spin label-induced NMR resonance broadening shows that helix C′, the partial domain involved in α- and β-spectrin association, exhibits little interaction with the structural domain. Surprisingly, helix C′ is connected to helix A′ of the structural domain by a segment of 7 residues (the junction region) that exhibits a flexible disordered conformation, in contrast to the predicted rigid helical structure. We suggest that the flexibility of this particular junction region may play an important role in modulating the association affinity of α- and β-spectrin at the tetramerization site of different isoforms, such as erythroid spectrin and brain spectrin. These findings may provide insight for explaining various physiological and pathological conditions that are a consequence of varying α- and β-subunit self-association affinities in their formation of the various spectrin tetramers.

Spectrin, a member of the spectrin superfamily and a major protein in the membrane (cyto)skeleton, is ubiquitous among vertebrate tissues, as well as in simple metazoans, implying that spectrin plays a fundamental role in cells (1–5). After first being identified in erythrocytes (4), many distinct spectrin isoforms have since been discovered. In humans, two α-spectrin subunits (αI and αII), four β-spectrin subunits (βI, βII, βIII, and βIV), and a β-II subunit have been sequenced (5). Alternative splicing (e.g. see Ref. 6) adds additional diversity among α- and β-spectrin isoforms. Many functions of different spectrin isoforms involve interactions with other molecules such as spectrin-actin interaction, spectrin-membrane interaction, spectrin-ion channel interaction, etc. (1). Yet some of the most fundamental functions of spectrin involve spectrin “self-association.” The activity of spectrin in stabilizing cell-cell contacts and in achieving normal columnar epithelial cell shape requires the formation of tetramers (1, 7, 8). Spectrin tetramers have been suggested to be cooperatively coupled to membrane assembly (9). Many hereditary hemolytic anemias involve single amino acid mutations in erythrocyte spectrin that destabilize its tetramers, resulting in low levels of spectrin tetramers and high levels of dimers (9–11). Thus, the tetramerization site is an important functional site for most spectrins.

In erythrocyte spectrin (αI/βI), α- and β-spectrin associate with relatively high affinity (μMKd values) at the N-terminal end of the β-spectrin and the C-terminal end of the α-spectrin (dimer nucleation site) to give αβ heterodimers (12, 13). Two dimers then associate with low affinity (μMKd values) to form spectrin tetramers. Two sets of identical interactions between the N-terminal region of α-spectrin (αN) of one αβ dimer and the C-terminal region of β-spectrin (βC) in another dimer give an αβ12 tetramer (14, 15).

Based on secondary structural prediction from sequence, it has been suggested that both α- and β-subunits of erythrocyte spectrin mostly consist of triple helical bundles (coiled coils) and that these triple helical bundle structural domains are linked sequentially (16, 17). Because α- and β-spectrin form very large, flexible oligomeric complexes, detailed experimental information on the structure of intact spectrin has been difficult to obtain. Structural studies of recombinant peptides of spectrin fragments, by both x-ray and NMR methods, support the general model of the triple helical bundle (helices A–C) for each structural domain (18–22). However, no experimental information is available for the partial domains involved in the tetramerization process. Unfortunately, peptides with just the partial domain alone do not seem to be suitable for structural studies due to their tendency to aggregate in solution at low concentrations. However, a recombinant peptide, consisting of the first 156 residues of αΣI spectrin (Spα1–156)† and thus of the proposed partial domain and the first full structural domain, is a suitable model for studying the α-tetramerization site by solution NMR methods.

† The abbreviations used are: Spα1–156, a peptide consisting of the first 156 residues of αΣI spectrin; MTSSL, 1-oxyl-2,2,5,5-tetramethyl pyrroline-3-methylmethane thiosulfonate; r.m.s.d., root mean square deviation; EPR, electron paramagnetic resonance; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; SW, sweep width.

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**MATERIALS AND METHODS**

NMR Samples—Spα1–156 peptide was prepared as before (21, 23, 24). NMR samples were prepared in 5 mM phosphate buffer at pH 6.5 containing 150 mM NaCl and 0.01% NaN₃ as well as 90% H₂O and 5% D₂O.

NMR Experiments— Backbone resonance assignments using triple resonance spectra were reported previously (24). Additional two-dimensional NOESY, H′,15-N-NOESY-HSQC, H″,13-C-NOESY-HSQC, HCH-TOCSY, and four-dimensional H′,13-C-HMQC-NOESY-HSQC spectra were acquired with either a Bruker DRX 500 or a DMX 750 (17.62 Tesla) spectrometer equipped with triple axis gradient triple resonance probes at 20 °C. Four-dimensional HMQC-NOESY-HSQC spectra were acquired using the aliasing scheme described previously (25). The directly detected proton domain was acquired with 512 complex points, tₚmax of 56.9 ms and sweep width (SW) of 89929.2 Hz; the t3 domain with 16 complex points, tₚmax of 3.7 ms and SW of 2136 Hz; the t2 domain with 36 complex points, tₚmax of 2.98 ms and SW of 6092 Hz, and the t1 domain with 72 complex points, tₚmax of 5.96 ms and SW of 6024 Hz. Side chain resonances were assigned using H′,15-N-NOESY-HSQC, H′′,13-C-TOCSY-HSQC, HCH-TOCSY, and four-dimensional H′,13-C-HMQC-NOESY-HSQC spectra. All raw data were processed and analyzed using Felix 97.0 software (MSI/Accelrys, San Diego, CA).

Structure Calculation—A published protocol for structure calculation (26) was followed. Briefly, the three-dimensional structure was calculated based on NOE-derived distance and angle constraints. The distance restraints were derived from NOE intensities on the two-dimensional NOESY (for an unlabeled sample in deuterium oxide), 13C-edited and 13C-edited three-dimensional (NOESY-HSQC) spectra, and 13C-edited four-dimensional (HMQC-NOESY-HSQC) spectra. The NOEs were classified as strong (<2.7 or 2.9 Å for amide protons), medium (3.0 or 3.5 Å for amide protons), weak (<5.0 Å), and very weak (<6.0 Å). A correction of 0.5 Å was added to proton distances involving methyl groups. Standard pseudo-atom corrections were applied as needed. The lower distance bounds were set to 1.8 Å in all intensity categories. The backbone θ and ψ angle constraints were obtained from 3-bond coupling constants (Jprog) and the chemical shift values using the program TALOS (27). For regular helical residues where those angle constraints are unavailable, Φ angles were constrained to −60° to 20° and Ψ to −40° to 20° (19).

Hydrogen bond constraints derived from amide proton-deuterium exchange experiments were used to constrain the NH-O distance to 1.5–2.8 Å and the N-O distance to 2.4–3.5 Å. The CNS program was used for the actual calculation (28), implementing the conformational data bases and the secondary chemical shifts data base described earlier (29–31). The side chain rotamers were determined by a combination of NOE and dihedral and data base terms. The data base term highly biased the dihedral angles to physiologically relevant angles (e.g. Ψ = −60, 180, or 460°) in the absence of NOE or dihedral terms. The calculation started from an extended conformation subjected to simulated annealing in torsion angle space followed by Cartesian space annealing (32). 150 simulated annealing runs with differing initial trajectories were performed. The resulting structures were subjected to conjugate gradient energy minimization. The 10 lowest energy conformers were used as final structures; the mean structure was generated by coordinate averaging of all structures, followed by restrained energy minimization.

The stereochemical geometries of the structures in the ensemble were compared and analyzed by PROCHECK (33). The electrostatic map and structural figures were generated by the molecular graphics program MMOLM (34).

**Paramagnetic Spin Labeling**—For paramagnetic spin labeling at residue 154, leucine was replaced with cysteine, using a mutagenesis protocol (QuickChange from Stratagene, La Jolla, CA) to give Spα1–156L154C. The primers had the following sequences: 5′-AGAAGGGTG-GACCGATCTCCGCGTAGAATTCC (sense) and 5′-GAATTCC-TACGCGGAGCAGCCTTTC (antisense). Protein purification steps used for Spα1–156 were followed except for the final step, where diithiothreitol was added (2 mg/ml final concentration) to the sample to reduce any potential intermolecular disulfide formation. The unreacted diithiothreitol was removed with a Sephadex G-25 gel filtration column (Amersham Biosciences). The spin label, 1-oxyl-2,2,5,5-tetramethyl pyrroline-3-methylmethane thiosulfonate (MTSSL), was then added to the sample to a molar ratio of 5:1, incubated for 3 h in the dark, followed by gel filtration to remove excess MTSSL. The number of spin labels bound to Spα1–156L154C was checked by EPR method (35).

**Model Building of Tetrimerization Region**—The three-dimensional structure of the C-terminal end of β-spectrin (βT1) is not yet available.

**RESULTS AND DISCUSSION**

**Resonance Assignment**—Resonance assignments and secondary structures were described previously (21, 24). Backbone resonances were all assigned with double and triple resonance spectra except for residues 36, 83, and 125. The side chain proton and carbon assignments were quite challenging, due to the relatively low experimental temperature (20 °C), the extensive overlap of α-proton and α-carbon chemical shifts from the mostly helical nature of the protein, and the presence of a very long and highly flexible region (residues 1–20) that gave intense truncation artifacts in the processed spectrum. Neverthe-
less, the proton and carbon resonances of most of the methyl-containing residues were assigned, which were essential for getting long range distance constraints. Aromatic protons were assigned based on NOESY and TOCSY experiments using samples dissolved in deuterium oxide. An example of NOE peak assignment on the four-dimensional NOESY spectrum for the methyl group of leucine 65 is shown in Fig. 1. Thirty eight residues scattered throughout the entire peptide have no assignments for the side chain carbons.

Structural Refinement and Structural Statistics—A total of 1473 structural constraints (1206 distance and 267 dihedral angle constraints) (Table I) were used to determine the structure of Sp1–156. The number of distance constraints is similar to that of earlier studies of chicken brain spectrin peptide (1035 distance constraints) (19) and is relatively small compared with globular proteins of a similar number of amino acid residues, due to the nature of helical bundling with fewer than usual inter-segmental contacts, as noted previously (19). The number of distance constraints as a function of residue number is shown in Fig. 2A.

About 150 initial structures were obtained. The 10 lowest energy structures were taken as our structural ensemble (Fig. 3). The bond angle and improper torsion angle analyses show that the structures have close to ideal geometries when compared with known values. There were no dihedral angle violations greater than 0.5°, except for one violation of 5.008°. In addition, none of the 10 structures showed NOE violations larger than 0.5 Å. The structural domain region (residues 52–156), when calculated alone, converges much better than the full-length protein (residues 1–156). The root mean square deviation (r.m.s.d.) values of each structure against the coordinate-averaged mean structure as a function of residue number for the structural domain is shown in Fig. 2B. For the regions with α-helical conformation, the r.m.s.d. value was 1.0 Å for the backbone atoms and 1.6 Å for all heavy atoms (Table I). The quality of the calculated structures in terms of the backbone Φ and Ψ angle distribution is represented by the Ramachandran plot for all of the ensemble structures (Fig. 4). The analysis shows that 99.6% of all of the residues in the 10 structures (1560 residues) are in the overall allowed regions (as defined by the PROCHECK program (33)) (Table I). The remaining residues (0.4% or 6 residues) in the disallowed regions were residues in loop or random coil regions, and none of the residues in the regular helical regions appeared in the disallowed region (Table I).

Structural Features—The calculated ensemble structures show that the first 20 residues in Sp1–156 are in random coil conformation, followed by a helix. This helix is connected to a triple helical bundle by a random coil segment of 7 residues. We refer to the first helix as helix C′, the 7 residue random coil segment as a junction region, and the three bundled helices in the structural domain as helices A1, B1, and C1.

Helical Length in the Structural Domain—Helices A1, B1, and C1 in the first structural domain of α-spectrin are slightly bent around each other, similar to helices in other coiled-coil systems and in non-erythroid spectrins (18–20). In comparison with those in chicken brain α-spectrin and in Drosophila α-spectrin (see Ref. 21 and see Table I in Ref. 40), these spectrin helices are generally similar in structure. However, small structural differences that may be critical to functional differences are noted in the curvature and the length of these helices, as well as in the random coil segments linking the helices. The differences in helical lengths may be due to specific locations of
different structural domains in spectrin. It has been suggested that structural domains may consist of helices with somewhat differing helical lengths, depending on the location of specific domains along the whole length of spectrin (42). Our structural domain is the 1st structural domain in \( \text{Sp/H9251} \)-spectrin; the one in \( \text{Drosophila} \) x-ray structure is the 14th structural domain (18) and that in the chicken brain NMR and x-ray structures is the 16th structural domain (19, 20). It is also quite possible that the differences in helical length are due to different spectrin isoforms and that the fine structural features in human erythrocyte spectrin differ from those in chicken brain and \( \text{Drosophila} \) spectrin. In chicken brain spectrin, there is a large difference in lengths between helices A and B (14 residue difference), leading to the formation of an apparent double helical bundle at the beginning of the structural domain, instead of a triple helical bundle (20). \( \text{Drosophila} \) spectrin does not appear to have an appreciable portion of double helical region. \( \text{Sp/H9251} \)–156 forms a triple helical bundle with the helical ends mostly matched since helices A, B, and C have about the same number of residues. Because chicken brain \( \alpha \)-spectrin helices are of different lengths, it has been suggested that conformational rearrangement of the helices may provide flexibility in erythroid spectrin (20). Our findings for erythroid spectrin do not support this speculation.

**Inter-helical Interactions**—Four groups of significant side chain interactions have been identified in bundling helices A–C.
in Drosophila α-spectrin (18). Interaction in the group 1 region involves hydrophobic interactions between the side chains of the 7th residue in helix A (A7), the 29th residue in helix B (B29), and the 4th residue in helix C (C4) (Table II). Group 2 region interactions involve Trp/His/Trp (A24/B28/C24) side chains, and group 3 region interactions involve hydrophobic side chains at the A24/B28 positions. Similar interactions in all four groups are also found in chicken brain α-spectrin (19) (Table II). Our structure revealed similar side chain interactions in all group 1–3 regions (Table II).

In Drosophila and chicken brain α-spectrin, interactions in the group 4 region involve three side chains with positive (Lys or Arg) and negative charges (Glu or Asp) providing salt bridge formation among the side chains of A28/B27/C25 residues (18, 19). However, the side chain of the residue in αI spectrin equivalent to the negatively charged Glu-1795 (at A28 position) in chicken brain spectrin, or Asp-1419 in Drosophila spectrin, is positively charged (Lys-29 at A27 position) (Table II). Thus, three lysine side chains (Lys-79, Lys-93, and Lys-150) are found in this region. In the energy-minimized mean structure that we obtained from NMR data, the lysine side chain terminal amino groups are bent away from the inter-helical interface for Lys-79 and Lys-93 and toward the C-terminal end of the helical bundle for Lys-150. Although side chain conformations, especially those for long aliphatic side chains like lysine, are not well defined in the NMR structure, an examination of other side chains around these three lysine residues indicates that there are no available negatively charged salt bridge partners for the lysine residues in our structure. Thus, helices A1, B1, and C1 in αI spectrin do not form salt bridges at the end of the helical bundle, as occurs in chicken brain and Drosophila α-spectrin.

Partial Domain and Junction Region—We believe that the most significant finding of this work is the determination of the solution conformation of the region prior to the first structural domain in α-spectrin, a region responsible for the association with β-spectrin to form spectrin tetramers. This region has been predicted to consist of a single helix (15, 37, 38), yet detailed structural features responsible for specific association affinity with its β-spectrin partner were not known. Our NMR data show that this region consists of a random coil segment, followed by a lone helix C′, as predicted. It is somewhat surprising to find that helix C′ is connected to helix A of the structural domain by a segment of 7 residues with a random coil conformation. Although a random coil junction region was predicted by some (17) but not by others (43), recent NMR and x-ray studies of Drosophila and chicken brain α-spectrin suggest that this region is helical (18–20) for their samples. Their findings were then used to predict the tetramerization mechanism of erythrocyte spectrin (18–20). However, our data indicate that the conformation of erythrocyte α-spectrin N-terminal junction region differs from those of Drosophila or chicken brain α-spectrin. This structural difference may have significant functional implications.

The associations of helical coils in several coiled-coil systems have been extensively studied. Despite their rather simple structural architecture, coiled coils represent a highly versatile protein folding motif (44). The Kd values for many coiled-coil systems are often in the nM range (e.g. Ref. 45), yet the coiled-coil association affinity of erythrocyte spectrin is in the μM and not the nM range, whereas the association affinity for human brain spectrin (αIIβII) is 10–20-fold higher than that of erythrocyte spectrin (αIβII) (46). Although the αIIβII spectrin tetramerization site structure has not been determined, it is assumed that tetramer formation also involves coiled coils association. The high degree of sequence homology among αIβII and αIIβII at the tetramerization sites (the sequences of helices A′, B′, and C′ between αIIβ and αIIβII are about 74% homologous and 66% identical) suggests similar coiled-coil helical bundling for αIIβII association and thus similar self-associa-
tion affinities ($K_d$ values). Yet tetramerization affinities of $\alpha_{I\beta I}$ and $\alpha_{II\beta II}$ are different. Thus, the differences in affinities are probably due to multiple small differences in conformation or to a large conformational difference in a small area. Assuming that human brain spectrin is more similar to chicken brain spectrin than to erythroid spectrin, we suggest that the difference in the junction region, with $\alpha_{I\beta I}$ as random coil and $\alpha_{II\beta II}$ as helix, contributes toward the difference in association affinity between erythroid and brain spectrin. Obviously, our suggestion that this particular junction region in human brain $\alpha_{II\beta II}$ is helical awaits experimental verification with systems featuring the tetramerization region.

The Orientation of Helix $C'$—Because the junction region is flexible, helix $C'$ may assume many orientations with respect to the triple helical structural domain. Thus, it is possible to have many structures, from a structure with a lone helix extending out from the triple helical bundle to a structure with a four helical bundle, and variations in between. Analysis of structural NMR data of Sp$\alpha_{1}$–156 does not unambiguously distinguish between these possibilities. However, NMR studies of the spin-labeled Sp$\alpha_{1}$–156L154C provide very useful information. Residue 154 is located at the C-terminal end of the structural domain. Helix C$_1$ ends at residue 153. Thus, residue 154 is in a random coil region and a site that will interact with residues at the N-terminal end of helix $C'$ if helix $C'$ were to bundle with the structural domain. Because the paramagnetic electron in

![Figure 5. A, HSQC spectrum of Sp$\alpha_{1}$–156 L154C labeled with spin label MTSSL at residue 154. Crossed boxes indicate the resonances that are observed in the HSQC spectrum of unlabeled sample but are missing in the spectrum of the labeled sample. Arrows indicate those in the N-terminal part of helix $C'$ that retain the original intensities of unlabeled sample. B, regions of Sp$\alpha_{1}$–156 that are affected and unaffected in their HSQC signals by the MTSSL spin label. Residues that are missing in the labeled sample are colored red. Residues in the N-terminal part of helix $C'$ from relatively non-overlapping regions of the spectrum that retain the original intensities are colored green. The position of Leu-154 is shown in red space-filling representation. One conformer from the calculated ensemble of structures is arbitrarily chosen.](image-url)
the spin label molecule relaxes extremely fast, it can induce very efficient relaxation of the spatially adjacent nuclei by a dipolar relaxation mechanism. A comparison of the HSQC spectra with and without the bound paramagnetic spin label allows us to identify those residues in close proximity to the spin label. In the spin-labeled sample, significant line broadening and some changes in chemical shifts were indeed observed (Fig. 5A). Peaks in the severely overlapped regions of the spectrum became difficult to correlate with those in the spectrum of non-labeled samples. Nevertheless, several resonances in the relatively well resolved regions of the HSQC spectrum were clearly broadened and disappeared in the spectrum of the spin-labeled sample. These resonances were all of residues around residue 154 in helices A, B, and C (Fig. 5B). Some residues with broadened resonances were as far as 25 Å away from the spin label, which is consistent with a result reported previously for spin label-induced nuclear signal attenuation (47). Most importantly, several resonances from the N-terminal region of helix C retained their intensities (Fig. 5A). These results unambiguously show that helix C does not bundle with the structural domain to form a four-helix bundle. Instead, helix C must be a lone helix, with all of its residues at least 25 Å away from residue 154, or the C-terminal end of the structural domain.

This observation of a structure with a lone helix (helix C) is consistent with published circular dichroism (36) results of the $\theta_{120}/\theta_{220}$ ratio being less than 1, indicating the presence of an unassociated helix, and with protease digestion results (23) in which chymotrypsin cleaves the Phe-Lys amide bond between residues Phe-38 and Lys-39 and not between Phe-58 and Lys-59, suggesting residues Phe-38 and Lys-39 are not protected, whereas Phe-58 and Lys-59 are protected.

**Modeling $\alpha$- and $\beta$-spectrin Partial Domain Association**—In the modeled structure, the hydrophobic faces of helices A', B', and C' containing "a" and "d" residues form the core of the helical bundle, while also including positively and negatively charged groups in these hydrophobic faces. Generally, the positively charged residues on helix C' of $\alpha$-spectrin interact with the negatively charged residues on the hydrophobic face of helices A' and B' of $\beta$-spectrin (Fig. 6).

We also examined inter-helical interactions at positions discussed above in the structural domain (helices A, B, and C). Residues corresponding to the group 1 region ($\beta$Phe-2041/$\beta$Thr-2072/$\alpha$Lle-24), the group 2 region ($\alpha$Tyr-35/$\beta$Trp-2024/$\beta$Trp-2061), and the group 3 region ($\beta$Tyr-2031/$\beta$Phe-2054) all exhibit side chain interactions similar to those in the structural domains (Table II). Residues in the group 4 region ($\beta$Gly-2035/$\beta$Arg-2050/$\alpha$Arg-45) differ from those of chicken brain and Dro sophila and do not form salt bridges. The lack of salt bridges at the C-terminal end of helix C may further reduce the association affinity in tetramer formation, when compared with brain spectrin, for example.

This model provides insight toward a molecular understanding of abnormal mutations at the N-terminal region of $\alpha$-spectrin, leading to a reduced level of tetramers and eventually hereditary hemolytic anemia. It has been suggested that, based on findings from the chicken brain $\alpha$-spectrin system, mutation of Residue 45 (R45S or R45T) generates a loss of electrostatic interaction normally found between Arg-45 and a residue in $\beta$-spectrin (an Asp or Glu residue), causing impaired binding of $\alpha$- and $\beta$-spectrin at the erythroid tetramerization site (19). However, our model of the erythroid tetramerization site indicates that residue 45 is not involved in salt bridges in normal erythroid spectrin tetramers (Table II).

In a previous NMR study, we introduced mutations at position 45 in Spa1–156 (R45S and R45T), and we obtained evidence for similar and very localized structural changes in R45S and R45T (48). Because patients with R45S mutation have different clinical severity than those patients with R45T mutation (11), and because we found that R45T and R45S exhibited different affinity toward $\beta$-spectrin peptide, we suggested that the previously proposed critical involvement of Arg-45 to interact with its partner residues in $\beta$-spectrin (19) does not apply to erythroid spectrin. High resolution structural information for the $\alpha$/$\beta$ complex will be required to confirm our suggestion.

**Tetramerization of Various Spectrin Isoforms**—The high degree of sequence homology among different spectrin isoforms at the tetramerization sites (the sequences of helices A', B', and C' between $\alpha$I/$\beta$ and $\alpha$II/$\beta$II are about 74% homologous and 66% identical) suggests similar structures, similar coiled-coil helical bundling, and thus similar self-association affinities. It has thus been suggested that the structure-function relationship in one isoform can be extrapolated to that of other isoforms (20). Yet at least two of the better studied isoforms, erythroid and brain spectrins, exhibit quite different affinities. The self-association affinity in the brain spectrin ($\alpha$II/$\beta$II) system is about 15-fold higher than the affinity in the erythroid spectrin ($\alpha$I/$\beta$I) system (46). We suggest that the random coil junction region between helix C' and helix A' in erythroid spectrin allows helix C' to assume multiple orientations with respect to helix A', resulting in a relatively low association affinity with its $\beta$-partner. Assuming the junction region between partial domain and first structural domain is similar to the helical segment linking the 16th and 17th structural domains (20), the brain spectrin junction region is then more rigid, and thus generates a higher association affinity. Thus, in general, we suggest that the more flexible the junction region, the lower the affinity, and conversely, the more rigid the junction region, the higher the affinity in $\alpha$- and $\beta$-spectrin association at the tetramerization site. An understanding of the structural and affinity studies of various spectrin isoforms may provide insight for explaining various physiological and pathological conditions that are a consequence of varying affinities of $\alpha$- and $\beta$-spectrin in self-association to form various functional spectrin tetramers.
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