Crystal Structure of the Nonerythroid α-Spectrin Tetramerization Site Reveals Differences between Erythroid and Nonerythroid Spectrin Tetramer Formation*

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We have solved the crystal structure of a segment of nonerythroid α-spectrin (αII) consisting of the first 147 residues to a resolution of 2.3 Å. We find that the structure of this segment is generally similar to a corresponding segment from erythroid α-spectrin (αI) but exhibits unique differences with functional significance. Specific features include the following: (i) an irregular and frayed first helix (Helix C’); (ii) a helical conformation in the junction region connecting Helix C’ with the first structural domain (D1); (iii) a long A1B1 loop in D1; and (iv) specific inter-helix hydrogen bonds/salt bridges that stabilize D1. Our findings suggest that the hydrogen bond networks contribute to structural domain stability, and thus rigidity, in αII, and the lack of such hydrogen bond networks in αI leads to flexibility in αI.

We have previously shown the junction region connecting Helix C’ to D1 to be unstructured in αI (Park, S., Caffrey, M. S., Johnson, M. E., and Fung, L. W. (2003) J. Biol. Chem. 278, 21837–21844) and now find it to be helical in αII, an important difference for α-spectrin association with β-spectrin in forming tetramers. Homology modeling and molecular dynamics simulation studies of the structure of the tetramerization site, a triple helical bundle of partial domain helices, show that mutations in α-spectrin will affect Helix C’ structural flexibility and/or the junction region conformation and may alter the equilibrium between spectrin dimers and tetramers in cells. Mutations leading to reduced levels of functional tetramers in cells may potentially lead to abnormal neuronal functions.

Spectrin isoforms are proteins associated with the cytoplasmic surface of plasma membranes of most cells. Spectrin associates with other cytoskeletal proteins, such as ankyny and pro-tein 4.1, to establish and maintain a diverse set of specialized plasma membrane domains (1). Nonerythroid, or brain, spectrin (spectrin II) exhibits high sequence homology with erythroid spectrin (spectrin I), despite their different cellular physiological functions (1). Because of their sequence homology, it is possible in theory to apply most of the molecular information obtained from the well studied spectrin I (both αI and βI subunits) to the less studied spectrin II (αII and βII) or from one domain to another domain (2). Yet, the two spectrin isoforms exhibit quite different functional properties as follows: (i) the ability of the spectrin I network to deform or the spectrin II network to remain rigid, and (ii) the ability αβ heterodimers to associate to form functional (αβ)2 tetramers with much higher affinity in spectrin II than in spectrin I. Tetramer formation in nonerythroid spectrin is essential in the regulatory step for neuritogenesis (3). αII-spectrin has recently been reported to be essential for stabilizing nascent sodium channel clusters (4), assembling the mature node of Ranvier (4), and regulating endothelial cell-cell contacts (5).

The C terminus of αI or αII and the N terminus of βI or βII associate to form heterodimers (αIβI or αIIβII) (6). Two αβ dimers then associate with a pair of interactions at the opposite ends, the N terminus of αI or αII and the C terminus of βI or βII, to form a functional (αIβI)2 or (αIIβII)2 tetramer (1). The N-terminal partial domain region in αII shares over 60% sequence similarity and more than 50% identity with the corresponding region in αI (7,8), and the C-terminal partial domain region in βII (residues 2010–2087) shares over 80% sequence similarity and over 70% identity with βI (residues 2002–2079). Yet spectrin II exhibits about 15-fold higher affinity than spectrin I in its αβ association at the tetramerization site (9). In

3 The abbreviations used are: αI, human erythroid α-spectrin; αII, human nonerythroid α-spectrin; D1, first structural domain; αI-N1, recombinant protein consisting of residues 1–147 of αI; βI, human erythroid β-spectrin; βI-C1, recombinant protein consisting of residues 1898–2083 of βI; βII, human nonerythroid β-spectrin; βII-C1, recombinant protein consisting of residues 1906–2093; MD, molecular dynamics simulation; NBC, nonbonded contacts; r.m.s.d., root mean square deviation; r.m.s.f., root mean square fluctuation; Helix A, the first helix of the first structural domain of αI-N1 or αII-N1; Helix B, the second helix of the first structural domain of αI-N1 or αII-N1; Helix C, the third helix of the first structural domain αI-N1 or αII-N1; Helix A’, the first helix of the partial domain of βI-C1; Helix B’, the second helix of the partial domain of βI-C1; Helix C’, the helix of the partial domain of αI-N1 or αII-N1; ITC, isothermal titration calorimetry; WT, wild type; PDB, Protein Data Bank.
model proteins (spectrin segments) used to study these regions of spectrins, the $K_d$ value is around 10 μM for the spectrin II systems and around 1 μM for the spectrin I systems (7, 10, 11). The molecular mechanism for this difference is not clear. Thus, insight into the association mechanism may provide important clues about the pathophysiology of various neurological disorders (12).

In previous work, we have provided indirect evidence that the conformation of the region connecting the first structural domain and the partial domain region of α-spectrin plays an important role in the interactions between α- and β-spectrin leading to the formation of spectrin tetramers (7, 11, 13). Specifically, our NMR (14), small angle x-ray scattering (7), and spin label EPR (13) studies of αII have shown that the junction region in αII is unstructured and that mutations in Helix C' produce decreased association that correlates with the severity of hereditary spherocytosis (15). More recently, our small angle x-ray scattering (7) and spin label EPR (8) studies of αII suggest that the junction region in αII is helical, whereas our spin label EPR studies show that the N-terminal end of Helix C' is frayed (8).

We have now solved the crystal structure of the segment consisting of the first 147 residues of αII, including the first triple helical structural domain (D1) and the important N-terminal partial domain that is responsible for association with the C-terminal partial domain of β-spectrin. Using the structure of Helix C', we developed a homology model of the αII Helix C' bundled with Helices A' and B' of β-spectrin to form a three helix-bundle (A'B'C') at the tetramerization site, formed by MD simulations. We compare the structures of A'B'C' spectrin I and II and find that the unstructured junction region in αII and the helical junction region in αII play an important role in the association of Helix C' of α-spectrin with Helices A' and B' of β-spectrin. We show that the analysis of inter-helix interactions in these predicted structures of A'B'C' of spectrin I and II is not productive.

The apparent stiffness or rigidity of spectrin molecules is correlated with its structural domain thermal stability (16). Using the x-ray structural information, together with our previous solution NMR structure of the corresponding N-terminal region of erythrocyte spectrin (14), we examine inter-helix interactions in the D1 of αII (x-ray structure) and of αII (NMR structure). We identify more hydrogen bonds and hydrogen bond networks in αII than in αI, suggesting that they contribute toward the higher domain thermal stability in αII than in αI. Thus, brain spectrin with higher rigidity indeed shows greater inter-helix interactions in structural domains, at least in D1, compared with those in erythrocyte spectrin, despite the similarity in their primary structures.

EXPERIMENTAL PROCEDURES

Recombinant Proteins

An N-terminal segment of brain αII-spectrin consisting of the first 147 residues (αII-N1), similar to a well studied erythroid α-spectrin segment consisting of the first 156 residues (αI-N1) (14), was prepared from a similar recombinant protein with 149 residues (7), following standard procedures (8, 13, 17). This segment is predicted to include a partial domain that is responsible for associating with α-spectrin to form tetramers, followed by the first full structural domain of brain α-spectrin (7, 8).

The procedures for preparing l-selenomethionine (seleno-Met)-labeled αII-N1 samples were similar to those used for NMR samples (11), except with methionine auxotrophic and protease-deficient competent cells B834(DE3) (Novagen-EMD Chemicals, Gibbstown, NJ) with our spectrin construct, and cells were grown in the M9 medium with seleno-Met (60 mg/liter, ACROS Organics, Thermo Fisher Scientific Inc.).

Three other recombinant model proteins, αI-N1, a C-terminal segment of erythroid βI consisting of residues 1898–2083 (βI-C1), and a nonerythroid βII segment consisting of residues 1906–2093 (βII-C1), were also prepared. The plasmids of αII-N1 with R37P mutation was prepared using site-directed mutagenesis methods with mutated primers to prepare the DNA construct and transformed into Escherichia coli cells for protein expression and purification. CD spectra at 20 °C for all proteins were obtained using a CD spectrometer (J-810, Jasco, Japan) with a 0.1-cm path length sample cell. Mean residue molar ellipticity values at 222 nm were used to calculate the helical content of the proteins using a value of −36,000 degrees cm² dmol⁻¹ for a 100% helical conformation (18). The actual helicity values are probably higher, as we discussed recently (11) in comparing the helicity values from CD and NMR studies.

Crystallography and X-ray Data Collection

αII-N1 and the seleno-Met-labeled αII-N1 in 10 mM Tris buffer at pH 7.4 were concentrated to 7.5 mg/ml and used for crystallization with hanging-drop vapor-diffusion methods at 20 °C. Each protein solution was mixed with an equal volume of reservoir solution to give a 2-μl droplet. The reservoir solution contained a mixture of PEG8000 (Hampton Research, Aliso Viejo, CA) and PEG10000 (1:2 molar ratio) for αII-N1 or of a PEG4000 and PEG1000 mixture for seleno-Met-labeled αII-N1, both with 4% butane-1,4-diol (Hampton Research). Crystals from solutions without butane-1,4-diol (Hampton Research). Crystals from solutions without butane-1,4-diol diffracted poorly. To reduce radiation damage, all crystals used for diffraction experiments were frozen at 100 K, with 20% (v/v) paratone-N (Hampton Research) added as a cryo-protectant.

Diffraction intensities for the native αII-N1 crystals were collected to a resolution of 1.95 Å on an R-AXIS IV++ detector equipped with confocal optics. CuKα x-rays were generated using a Rigaku RUH2 rotating-anode generator. Images were collected at a crystal-to-detector distance of 150 mm with an exposure time of 5 min per 0.5° oscillation. Images were processed with XDS (19).

Diffraction intensities for the seleno-Met-labeled αII-N1 crystals were collected at the Northeast Collaborative Access Team (NE-CAT) 24-ID beamline at the Advanced Photon Source, Argonne National Laboratory. X-ray data were processed to 3.0 Å resolution using HKL2000 (20).

Structure Solution and Refinement

The crystal structure of the seleno-Met-labeled αII-N1 was solved by a single-wavelength anomalous diffraction phasing using the automated search routine AUTOSOL from the
Crystal Structure of the N-terminal Region of all-Spectrin

Python-based Hierarchical Environment for Integrated Xtallography (PHENIX) program suite (21). The crystal structure of all-N1 was solved using the phase information from the crystal of the seleno-Met-labeled all-N1. The phases were applied to the native data, and extended to 2.3 Å. Refinement of coordinates was then performed using REFMAC5.5 and PHENIX refine. Ten percent of the total observed unique reflections were randomly selected and used as a test set to calculate the free R value. The model was manually built using the program COOT (22) and validated using MolProbity (23). The coordinates and structure factors for the final model have been deposited with the Protein Data Bank code 3F31.

Structural Analysis

The secondary structural elements (24), protein-protein interface areas, and molecular interactions were analyzed with PDBsum (25). For molecular interactions, PDBsum identifies nonbonded contacts (NBC), and within NBC, specific interactions were identified as hydrogen bonds using strict geometric criteria (26, 27). In addition, salt bridges (28) were identified separately.

Because PDBsum identifies protein-protein interactions as chain-chain interactions, we labeled each helix in our structure as a chain, including half of the loops connecting to the adjacent helices, in each PDB file to give inter-helical interactions. From these inter-helical interactions, we examined clusters of atoms involved in NBC, which consist of hydrogen bonds, and salt bridges. We also identified hydrophobic clusters from the NBC atom pairs, considering Phe, Ile, Leu, Met, Val, Trp, and Tyr residues as hydrophobic cluster members (29).

Protein Thermal Stability

Protein samples (10–15 μM) of all-N1 and seleno-Met-labeled all-N1 and all-N1 in 5 mM phosphate buffer with 150 mM NaCl at pH 7.4 were used for thermal stability studies. CD signal intensities at 222 nm of a sample were monitored as a function of temperature with a thermostated cell, from 20 to 100 °C at an interval of 1 °C. Fractions of thermal unfolding as a function of temperature were obtained from curve fitting of mean residue molar ellipticity values as before (30) to give T_m and ΔG values for each unfolding.

MD Simulation

GROMACS (version 4.01) (31) with the 43A1 version of GROMOS force field (32) was used for MD simulations of solvated protein molecules. We compared different force fields for MD simulations of the spectrin system and found similar results (17). The particle mesh Ewald method (33) was used for long range electrostatic interactions. Simulations were set up by solvating the protein molecule with water (“simple point charge”), followed by a 5000-step energy minimization with position restraint on proteins before simulation for 10 ns. Coordinates and velocities were saved every 2 ps. The time step for integration was 2 fs. Simulation trajectories/structures were analyzed using tools from the GROMACS package. r.m.s.f. values are the average values of root mean square fluctuation of Ca in each residue of the last 1,000 structures (the last 2 ns of the 10-ns simulation). The GROMACS “dominant structure,” defined as the most representative structure with the smallest root mean square deviation (r.m.s.d.) values, from the last 2 ns (among the last 1,000 snapshot structures), was used as the “MD structure.”

We note that when particle mesh Ewald mode was not used, the structures fell apart after a 2-ns simulation, indicating the importance of appropriate electrostatic simulation parameters to give stable and reliable structures. Structural images were produced using PyMOL (DeLano Scientific LLC, Palo Alto, CA).

Isothermal Titration Calorimetry (ITC)

ITC measurements were performed at 25 °C with an isothermal titration calorimeter (VP ITC, MicroCal, LLC, Northampton, MA). Protein titration pairs (all-N1 and βI-C1, all-N1 and βII-C1, all-N1 and βI-C1, all-II-N1 and βII-C1, and all-II-N1 R37P and βI-C1) were diazylated together in 5 mM phosphate buffer with 150 mm sodium chloride at pH 7.4 to ensure identical solution conditions of the titration pairs to avoid introducing heat of dilution in the titration experiments. Each α-spectrin protein (about 400 μM αII-N1 or 60 μM αII-N1) was titrated into the sample cell containing one of the β-spectrin proteins (about 40 μM in αI or 6 μM in αII titration). The titration isotherm was analyzed by a single binding site model, provided by MicroCal software, to obtain an association constant (K_a), which was converted to a dissociation constant (K_d), and the values of ΔH and ΔS of the association.

Homology Modeling

Triple Helical Bundle A’ B’ C’ Complex—The structure of the spectrin tetramer, (αβ)_2, at the partial domain association region was modeled by bundling the single α-spectrin partial domain helix (Helix C’) with the two helices of the β-spectrin partial domain (Helices A’ and B’). We built an A’ B’ C’ complex consisting of Helix C’ of αII and Helices A’ and B’ of βI or βII, using homology modeling methods similar to those used for the α and β complex (14, 17). In these models, the sequence of β at the region predicted to be Helices A’ and B’ (34) is aligned with that of α-spectrin Helices A_1 and B_1 to determine the boundaries of Helices A’ and B’. To assess the sensitivity of models to assumptions about structural details, we used two different templates to model the tetramer association region. The two templates, α1L and α2LII, were used to position Helices A’, B’, and C’ in the complex and to determine the length of each helix. α1L is based on the first structural domain of αI, consisting of Helices A_1, B_1, and C_1 of the NMR structure (PDB code 1OWA) (14). The NMR structure of αII consists of an ensemble of 10 structures. The average value of the Ca atom positional r.m.s.d. (Ca r.m.s.d.) values of these structures is about 2 Å, indicating all structures are similar. The coordinates of the structural domain of structure 1 were arbitrarily selected as α1L for homology modeling. α2LII was based on the crystal structure of the first structural domain of αII from this work. Note that Helix A_1 refers to Helix A in the structural domain 1, and Helix A’ refers to that in the partial domain.

Because the Helix C’ structure is now known for both αI and αII, we replaced Helix C_1 with Helix C’ of either αI (in αIβI
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complex, for example) or αII (in αIIβ1 complex) by overlaying Helix C’ over Helix C₁ (e.g. residues 12–31 in αII Helix C’ is overlayed with residues 117–136 of Helix C₁ in template tα₁), using operations in PyMOL. Helix C’ in M1 and M2 (see below) assumes slightly different structures in the crystal, and we selected the structure of M2 for αII Helix C’ because this structure, with the bend involving only one residue, is in better agreement with our EPR studies (8).

The lengths of Helices A’ and B’ of αI or αII in tα₁ and tβ₁ vary slightly due to different helix lengths in the first structural domains of αI and αII. Helix A’ is five residues longer and Helix B’ is four residues shorter, both at the C terminus, in tβ₁ than in tα₁. Details on helical boundaries of the three helices in the models are discussed under “Results.” Note that the numbering systems for residues in αI and αII and in β₁ and ββ₁ differ, with residue 21 in αI corresponding to residue 12 in αII. Residue 2044 in β₁ corresponds to residue 2052 in ββ₁.

We have previously published two different models of A’B’C’ of αIIβ with tα₁, with Helix C’ terminating either at residue 45, as in free Helix C’ (14), or at residue 52, as in bound Helix C’ (17). In this study, because we use the crystal structure of free (unbound) Helix C’ for αII, we used the unbound Helix C’ in the αIIβ-tα₁ model (14) for MD simulations (see below).

**αII Mutations**—We introduced a mutation in the model at position 37 of all Helix C’, from Arg to Pro, in both the free form and the bound form (MD structure of A’B’C’ of αIIβ-tα₁, see below). We also introduced αII mutations at positions 19 (R19C) and 40 (L40C) to the bound Helix C’ in A’B’C’, because mutations at these positions in recombinant model proteins show abnormal binding affinities (8).

**RESULTS**

Protein Characterization—The expected mass for αII-N1 is 17,662.9 Da, and the experimental mass from the high resolution mass spectrometry is 17,662.2 Da. The expected mass for seleno-Met-labeled αII-N1, with all four methionine residues labeled, is 17,850.6 Da, and the experimental mass is 17,850.3 Da. The αII mutant R37P mass is 17,604.1 Da, with an expected mass of 17,603.9 Da. The helical content from CD measurements is about 70% for αII-N1, 62% for the seleno-Met-labeled αII-N1, 48% for αII-N1-R37P, and 52% for αII-N1. The values for αII WT and αII-N1 are similar to our published values (34). Dynamic light scattering experiments indicate that αII-N1, at 2 mg/ml, is monomeric in solution, with a hydrodynamic radius (R₉) of about 30 Å (11).

Overall Structure of αII-N₁—X-ray diffraction data (Table 1) show that the asymmetric unit contains two crystallographically independent structures of αII-N₁, monomers 1 (M1) and 2 (M2). Each monomer consists of a short unstructured segment followed by an unpaired helix with either a bend or short β-turns plus a triple helical bundle (Fig. 1). M1 and M2 are antiparallel to each other, with the triple helical bundle of M1 facing that of M2 by a pseudo 2-fold axis. The interfacial area between the paired monomers is 1,530 Å² for M1 (about 14% of total area 10,747 Å²) and 1,450 Å² for M2 (about 13% of 10,966 Å²).

**N-terminal Partial Domain Helix C’**—M1 and M2 assume slightly differing conformations in the first 36 residues. This N-terminal region is critical for the association with the C-terminal partial domain of β-spectrin. In M1, we observe poor electron density for residues 1–10. Residues 11–13 form a β-turn (following the PDBsum secondary structure terminology) (Table 2). Residues 14–25 and 30–36 are helical segments, connected by a four-residue β-turn. In M2, residues 1–7 also exhibit poor electron density, and residues 8–11 form a β-turn. The helix starts at residue 12 and has a bend that involves only residue 33. To simplify the discussion when comparing structures with other spectrin isoforms (e.g. with the corresponding region in αII-spectrin), we designate residues 14–36 in M1, or 12–36 in M2, as the lone Helix C’, which bends at residues 26–29 in M1, or at residue 33 in M2 (Fig. 2A; Table 2). A rotation of the N-terminal end of the M1 Helix C’ at the bend by about 55° brings it largely into superposition with Helix C’ in M2 (Fig. 2A). After the bend, M1-Helix C’ is very similar to that of M2. Thus, in summary, the conformations of Helix C’ in M1 and M2 differ only slightly, with the start of the helix differing

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**TABLE 1**

Data collection and refinement statistics

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<tr>
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<td>Rfree (B = 0.85)</td>
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<td>Completeness</td>
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Refinement statistics

| Resolution range | 20.0 to 2.3 |
| No. of reflections in working set | 14,733 |
| No. of reflections in test set | 1,323 (~9%) |
| Rcryst | 22.0% |
| Rfree (B = 0.85) | 28.0% |
| Wilson B (Å²) (protein) | 30.0 |
| No. of structures in asymmetric unit | 2 |
| r.m.s.d. from ideal geometry |
| Bond lengths | 0.010 Å |
| Bond angles | 1.15° |
| Ramachandran plot |
| Allowed | 98.2% |
| Generous | 1.1% |
| Disallowed | 0% |
| No. of water molecules | 206 |
by two residues, and slightly differing bends at slightly different locations, but are otherwise quite similar.

The flexibility in Helix C’ is also shown by the values of the Ca B-factors, with M1 being less ordered than M2 (Fig. 3). Helix C’ is thus an irregular and frayed helix, capable of conformational mobility, at least going from that in M1 to that in M2. The flexible nature of Helix C’ implies that this region may undergo conformational change upon interacting with neighboring molecules/atoms, as in the unit cell of crystals, and potentially with its interactor proteins in cells.

FIGURE 3. B-factors of monomer 1 (A, solid line) and monomer 2 (B, solid line) are generally similar, except for some variation at the N termini, with values for Helix C’ in monomer 1 (residues 14–36) higher than those in monomer 2 (residues 12–36). The helical regions (marked by solid horizontal bars above the x axis) generally show relatively low B-factor values (<30 Å²), indicating relatively minor disorder in these parts of the molecule. The values for residues 14–28 in Helix C’ of monomer 1 (M1) are above 50 Å², indicating a large disorder for this particular section of the molecule. The r.m.s.f. values for residues from MD simulations of the monomer 1/2 pair are also shown, with the scale on the right x axis. The values were also converted to B-factor values for comparison, (left z axis), following published methods (36). The converted B-factor values of the A1B1 loop are higher than those from x-ray for monomer 1 but lower for monomer 2 (M2), suggesting that the flexibility of this loop in solvated structures is modulated by interactions between this loop in monomer 2 and Helix C’ in monomer 1.

Helical Junction Region—The most interesting feature of our crystal structures is that residues 37–43, downstream of Helix C’, are helical in both M1 and M2. Based on the secondary structural analysis and the homologous sequence alignment with αl, we assign this region to be the junction region. However, there are no clear structural boundaries for this junction region, with the end of Helix C’, the junction region, and the start of the first helix in the structural domain being a long continuous helical segment. We have previously shown that the corresponding region in α-erythroid spectrin (αl residues 46–52) is unstructured (14). Our previous hypothesis that the differences in the association affinity between erythroid and nonerythroid spectrin in forming tetramers can be associated with differences in the junction regions of αl and all (7) is thus shown to be correct.

First Structural Domain of all-Spectrin (D1–all) —The regions after residue 43 in M1 and M2 superimpose very well, with an average Ca r.m.s.d. value of 0.57 Å, indicating essentially identical structures for residues 44–147. We define this region as the first triple helical structural domain of all-spectrin (D1–all), with the three helices being A1 (residues 44–66), B1 (residues 78–111 or 112), which has a bend around residues 94–96, and C1 (residues 117–143 or 146) (Table 2). Helices A1 and B1 are connected by a long 11-residue A1B1 loop, whereas Helices B1 and C1 are connected by a short B1C1 loop (4–5 residues) (Fig. 1 and Fig. 2A; Table 2). The most flexible region in the structural domain is the long A1B1 loop, which exhibits B-factors more than 100 Å² for residues 73–74 (Fig. 3).
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**Thermal Stability and Inter-helix Interactions**—We observe similar thermal unfolding profiles for αl-N1 and seleno-Met-αl-N1. The $T_m$ values are 73.1 and 74.6°C, whereas $\Delta G$ (25°C) values are 9.1 and 8.5 kcal/mol, respectively (Fig. 4). The unfolding profile of the corresponding αl protein (residues 1–156; αl-N1) showed a similar unfolding pattern, except at lower temperatures, with a $T_m$ value of 56.0°C and a $\Delta G$ of 5.3 kcal/mol (Fig. 4). The difference in $\Delta G$ ($\Delta G_t$) at 25°C is 3.8 kcal/mol. Thus, αl-N1 is more stable than αl-N1 by about 4 kcal/mol, in good agreement with previous results on similar proteins (16, 35).

With an atomic resolution structure of D1-αl, interaction analysis of inter-helical interactions shows multiple hydrogen bonds in M1 and M2. These hydrogen bonds satisfy the published strict geometric criteria (see “Experimental Procedures”) (26, 27). The details of hydrogen bond atom pairs and their locations in the domain are shown in Fig. 5B. These hydrogen bonds spread throughout the interfaces of the three helices to form hydrogen bond networks that stabilize the triple helical structural domain D1-αl. No additional salt bridges are evident. Similar interaction analysis of D1-αl of four randomly selected NMR structures (PDB code 1OWA) shows only 1–2 inter-helix hydrogen bonds and no hydrogen bond networks (Fig. 5A). Also, no additional salt bridges were identified by the PDBsum program.

All structures of αl and αl show 17–19 residues to be involved in inter-helix hydrophobic clusters, with 4–8 of these residues having multiple close-distance partners. These hydrophobic clusters are distributed evenly in the hydrophobic core of the structural domain. In brief, D1-αl and D1-αl exhibit similar hydrophobic clusters, further suggesting that the higher stability in D1-αl than in D1-αl is due to specific inter-helix hydrogen bond interactions.
MD Structures of M1 and M2—MD simulations of the explicitly solvated M1/M2 crystal pair or separated monomers generally reach equilibrium after 2–3 ns, as indicated by the r.m.s.d. values (Fig. 6), with larger r.m.s.d. values in monomers of separated M1 and M2 than in the M1/M2 crystal pair. All four (each monomer in the pair and two from the separated monomers) MD structures (defined as the dominant structures of the last 2-ns simulations, see “Experimental Procedures”) are generally similar to each other and to the x-ray structures, with some differences at the N terminus of Helix C’. The differences between monomers in the crystal structures of Helix C’ (starting residue, bend position, and bend length) are also found in MD structures. Helix C’ starts at residue 11 in separated M2 but at residue 17 in separated M1 (Table 2), further demonstrating its flexibility (Figs. 2, 3, and 6). The \( \alpha \) r.m.s.f. values for residues and the corresponding B-factor values, converted from r.m.s.f. values following published methods (Fig. 3) (36), show that the N-terminal residues at the frayed end of Helix C’ exhibit different conformations, in good agreement with x-ray conformations, further suggesting that this region may undergo conformational exchange.

It is interesting to note that the converted MD B-factor values of the A, B, I loop are higher than those from x-ray in M1, but lower for M2, suggesting that the flexibility of this loop in solvated structures is modulated by interactions between this loop in M2 and Helix C’ in M1 (Fig. 1). We suggest that the long, flexible A, B, I loop is a special feature of this particular structural domain (D1-all), discussed further below.

\( \alpha \beta \) Association Affinity at the Tetramerization Site—Typical ITC results for the titration of \( \alpha \)-N1 or \( \alpha \)-N2 with \( \beta \)-I or \( \beta \)-II (Fig. 7) show that \( \beta \)-spectrin I and II do not contribute to the observed differences in dimer association to form trimers; the differences are mostly due to differences in \( \alpha \)-spectrin I and II. In fact, sequence alignment shows 54% sequence identity for \( \alpha \)-N1 and \( \alpha \)-N2 and 70% for \( \beta \)-I and \( \beta \)-II. Our previously published \( K_d \) values are about 1 \( \mu \)M for the association of \( \alpha \)-N1 with \( \beta \)-C (7, 11, 37), and about 10 nM (8, 11) for \( \alpha \)-N1 with \( \beta \)-C.

To better characterize the thermodynamic contributions to association, we examined the accuracy of the \( K_d \), \( \Delta H \), and \( \Delta S \) parameters obtained from ITC experiments. \( K_d \) values are obtained from fitting the amounts of heat evolved as various mole fractions of \( \alpha \)-N1 are added to \( \beta \)-C and can be obtained relatively accurately. \( \Delta H \) can also be obtained relatively accurately in well-designed experiments such that, at the start of the titration, most all of the titrating protein (\( \alpha \) proteins in our case) forms a complex with its partner (\( \beta \) proteins), giving a relatively flat plateau (for example, see \( \alpha \)II\( \beta \)I isotherm in Fig. 7). With the heat determined from the initial titration and an accurate determination of protein concentration, an accurate value (kcal/mol) for \( \Delta H \) can be obtained. The \( K_d \) and \( \Delta H \) values are obtained directly from regression of the ITC data. However, the \( \Delta S \) accuracy may be lower, because \( \Delta S \) is a secondary parameter calculated from \( K_d \) and \( \Delta H \), and it includes the uncertainties of both. Consequently, the \( \Delta G \) values obtained from summing the experimental values of \( \Delta H \) and \( -\Delta S \) (\( \Delta G \)-sum) are not necessarily equal to the value calculated directly from \( K_d \) values (\( \Delta G \)-\( K_d \)). As a consistency check, we examined 64 ITC data sets and found that the ratios of the \( \Delta G \)-sum/\( \Delta G \)-\( K_d \) can range from 0.6 to 1.4, although most are close to 1.0. Thus, we include only the results from experiments where the \( \Delta G \)-sum/\( \Delta G \)-\( K_d \) ratios were within a range of 1.0 ± 0.05, thus providing the most reliable \( \Delta S \) values from ITC results.

For the \( \alpha \)-N1 and \( \beta \)-C1 system at 298.15 K, the average value (\( n = 4 \)) for \( K_d \) is ~900 nM; \( \Delta H \) is ~27.7 kcal/mol, and \( -\Delta S \) is ~19.4 kcal/mol. For the \( \alpha \)-N1 and \( \beta \)-C1 system, the average value for \( K_d \) is 8.5 nM; \( -\Delta H \) is ~37.9 kcal/mol, and \( \Delta S \) is ~26.9 kcal/mol (Table 3). For \( \alpha \)-N1–R37P, the \( K_d \) is ~11,000 nM, with a \( \Delta H \) of ~16 kcal/mol and a \( \Delta S \) of ~9.4 kcal/mol. Most of our \( K_d \) values generally agree with surface plasmon resonance studies of similar \( \alpha \), \( \alpha \)-I, \( \beta \), and \( \beta \)-II model systems (10), although the \( \Delta H \) and \( \Delta S \) values for \( \alpha \)-II\( \beta \)I differ signifi-

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**FIGURE 6.** r.m.s.d. values of the trajectories from molecular dynamics simulations of the explicitly solvated M1/M2 pair (bottom tracing). Equilibrium conformations were reached after a 2–3-ns simulation. Monomer 1 (M1) and monomer 2 (M2) were separately solvated and simulated under the same conditions as monomers. Their r.m.s.d. values are much larger than the corresponding values for the M1/M2 pair. The solvated structure of monomer 1 appears to reach equilibrium at about 6 ns. These results indicate that monomers 1 and 2 stabilize each other in the paired form.
Crystal Structure of the N-terminal Region of αII-Spectrin

The three helices remain a stable triple helical bundle after MD simulations reach equilibrium. The most interesting structural feature of the A′B′C′ complex is that the bend in the free Helix C′ around residue 33 (Fig. 8, A and B, and Table 4) straightens to give a more regular Helix C′ in the complex (Fig. 8, C and D). The conformational change in Helix C′ upon binding Helices A′ and B′ to form the A′B′C′ complex provides an inter-helix hydrophobic cluster at the C-terminal end, downstream from the bend, that involves αII-L40 and V2044/2052-L2047/2055-L2048/2056 (with the first number for β and the second italic number for βII) (Fig. 8E). Note that hydrophobic residue clusters were identified from the NBC list in PDBsum (see “Experimental Procedures”). We also identify an N-terminal hydrophobic cluster that involves three residues in Helix C′ (Ile-15, Val-22, and Leu-23) (Fig. 8, F and G), two residues in Helix A′ (V2011/2019 and F2014/2022) (Fig. 8F), and one residue in Helix B′ (F2065/2073) (Fig. 8G).

In addition to hydrophobic clusters near residue 33, there are five charged residues downstream of residue 33 (Arg-36, Arg-37, Lys-39, Glu-41, and Asp-42) and seven charged residues at the N-terminal end of Helix B′ (E2045/2053, K2046/2054, K2049/2057, R2050/2058, E2052/2060, E2055/2063, and K2056/2064). These charged side chains might also be responsible for bringing the C-terminal end of Helix C′ (such as Arg-37 at the “e” position in the heptad) closer to the N-terminal end of Helix B′ (such as E2052/2060 at the “b” position and E2055/2063 at e position) close together (Fig. 8H).

In principle, the analysis of inter-helix hydrogen bonds in the A′B′C′ complex will allow us to examine the stability of this complex, as we show above for D1 in α and αII. However, the predicted (MD) structures of the complex lack the structural precision that would allow critical analysis of hydrogen bonds and their networks to predict stability with energy differences of only a few kilocalories/mol, in contrast to the analysis of either the x-ray or NMR structures. The numbers of inter-helix hydrogen bonds obtained from the analysis of A′B′C′ structures of αI and αII are quite different (data not shown). Thus, inter-helix interaction analysis of the complex awaits determination of its atomic resolution structure. However, we believe that the MD structures provide good prediction of the conformation for Helix C′ bound in the complex and identify potential hydrophobic clusters, which are not distance-dependent, although the van der Waals interactions of the atoms involved are distance-dependent.

We have previously shown, with yeast-two-hybrid methods, that the mutant R37P in αII has an impaired ability to form tetramers with βII (38). A proline residue mutation is, of course, likely to disrupt the helical conformation that we observed in the αII WT junction region. Thus, we prepared an αII-N1-R37P model protein. The CD measurements show that the helical content value is 48% for R37P, a value not only lower than that of αII-N1 but also lower than that of αII-N1. The ITC results show a $K_d$ value of about 10 μM (Fig. 7 and Table 3), a value much higher than that for WT, and even an order of magnitude higher than that for αI. The $\Delta H$ value is $-16.2$ kcal/mol and $\Delta T$ is $-9.4$ kcal/mol. For αII-N1 association with either template (tol or tolII) generally appear the same.
Crystal Structure of the N-terminal Region of αII-Spectrin

β1-C1, the ΔΔG is 4.2 kcal/mol between the WT and R37P systems.

Model Structure of Free R37P—With a helical conformation around R37P as the starting conformation (from homology modeling) (Fig. 9A), the MD structure shows that eight residues around residue 37 (residues 32–39) become unstructured (three β-turns) (Fig. 9B), consistent with the CD results. We also note that, for comparison, the unstructured junction region in α (residues 46–52) remains unstructured after MD simulation (structure not shown). As a consequence of the conformational change, we identify only two intra-helix hydrogen bonds between residues 30 and 43 of Helix C’ in R37P, as compared with nine in WT and four in α for the corresponding region (residues 39–52). Thus, these three α-spectrin models exhibit very different conformations at the C-terminal end of Helix C’ and the junction region, which are likely responsible, at least in part, for the differences in the affinity (ITC) results reported above.

Model Structure of the A’B’C’ Complex of R37P—When residue Arg-37 in the A’B’C’ homology model is changed to Pro-37, the neighboring residues (34–42), which are helical in the homology model, again become unstructured after MD simulation (Fig. 9C). In contrast to an unstructured to helical conformational change upon binding β-spectrin in the junction region in α, the unstructured region (residues 32–39) in the free form (Fig. 9B) remains unstructured. Furthermore, it lengthens (residues 32–43). Thus, despite the presence of potential inter-helix interactions provided by Helices A’ and B’ with residues in Helix C’, the mutation causes this region in the complex to unwind.

Model Structure of the A’B’C’ Complex of R19C and of L40C—We introduced two other mutations (R19C and L40C) to our A’B’C’ complex model, because the association affinities of these two mutants with β-spectrin are lower than that for the WT (8). The A’B’C’ model of the WT discussed above provides us with a possible mechanism for the effect of this mutation on the affinity. The MD structure of αββ A’B’C’ with the R19C mutation in Helix C’ shows that the general conformations of Helices C’ and A’ both remain similar to those of the WT. However, Helix B’ is considerably shortened, with the last residue reduced from Thr-2072 to Arg-2064, probably due to the inability

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**TABLE 4**

The boundaries of helices, and the number of helical residues (number of amino acids (aa)), in the models of a triple helical bundle (A’B’C’) of Helices A’ and B’ from β1 or βII-spectrin and Helix C’ from αII-spectrin, using either αD1 or αII-D1 structural domain as a template (tα or tII, respectively) in homology modeling (HM).

<table>
<thead>
<tr>
<th>A’B’C’</th>
<th>Template</th>
<th>Helix A’</th>
<th>Helix B’</th>
<th>Helix C’</th>
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<tr>
<td>αββ</td>
<td>tα</td>
<td>2009–36*</td>
<td>28</td>
<td>2010–38</td>
</tr>
<tr>
<td>αβII</td>
<td>tα</td>
<td>2017–45*</td>
<td>29</td>
<td>2018–45</td>
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<tr>
<td></td>
<td></td>
<td>2017–40</td>
<td>24</td>
<td>2018–40</td>
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<tr>
<td></td>
<td></td>
<td>2009–37</td>
<td>29</td>
<td>2012–36</td>
</tr>
</tbody>
</table>

* The numbering system for α and β differs from that of αII and βII. In the regions of interest, residue 12 in αII corresponds to residue 21 in α. Residue 2017 in βII corresponds to residue 2009 in β. Italic residue numbers are for αII or βII.

a MD simulation for 20 ns is shown.
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of residue Cys-19 to interact with the Glu-2069 residue downstream of the helix, as in the Arg-19 to Glu-2069 WT interaction (Figs. 8I and 10A). In the WT, this ion pair keeps the C-terminal end of Helix B’ close to the N-terminal ends of Helices A’ and C’, forming a stable triple helical bundle, whereas the C-terminal end of Helix B’ in A’B’C’ of R19C moves away from the N termini of Helices A’ and C’ (Fig. 10B).

For L40C, the general conformation of Helix C’ remains similar to that of the WT. However, Helix B’ exhibits a kink around residues Lys-2049 to His-2051. Residues Val-2044, Leu-2047, and Ile-2048 are still able to form a hydrophobic residue cluster. However, this hydrophobic residue cluster has one less member (Leu-40) than that in the WT (Leu-40, Val-2044, Leu-2047, and Ile-2048) (Figs. 8E and Fig. 10D). Again, the A’B’C’ model of the WT provides us a possible mechanism for the effect of this mutation on the affinity.

DISCUSSION

The perception that we largely understand spectrin structure-function relationship once the structural domains (often referred to as spectrin repeats) are found to be triple helical bundles far oversimplifies the details and may lead to inaccurate conclusions. The canonical triple helical bundle conformation does not reveal specific functions associated with either different domains or different isoforms. We suggest that many α- and β-spectrin structural domains include unique structural features, such as the BC loop region of domain 15 of β-spectrin, which is a unique ankyrin-binding site (39), and the different conformations in the junction regions of N-terminal α- and αII-spectrin which affect tetramerization affinity (8, 13).

The atomic structures of 12 different spectrin domains have been published. These domains include D14 of Drosophila spectrin (PDB code 2SPC (40)), human erythroid αL-D1 (PDB code 1OWA (14)), βL-D8-D9 (PDB code 1S35 (41)), βL-D14-D15 (PDB code 3EDU (42), and PDB code 3F57 (43)), nonerythroid αII-D14-D16 (PDB code 3EDV (39)), and chicken brain αII-D15-D17 (PDB codes 1U5P and 1U4Q (44); PDB code 1CUN (2); and PDB code 1AJ3 (45)). The structures of four different spectrin domains have already been compared, and the results show extensive variation as follows: helices are straight, curved, or bent; the lengths of the helices vary from 21 to 31 residues; and the length of the loops between helices also differs (46). The findings of structural domain variation are valid for all 12 published structures, as well as for the structure from this study. The unique structural features in each domain may lead to different contributions to the overall functional properties of spectrin, such as the rigidity/flexibility of spectrin in the cytoskeletal network of different cells.

The important specific features identified in the N terminus of αII-spectrin in this study include the following: (i) the irregular Helix C’ with a variable, flexible bending region; (ii) the helical conformation in the junction region connecting Helix C’ with the first structural domain; (iii) the long A,B, loop; and (iv) the specific inter-helix hydrogen bonds/salt bridges that stabilize structural domain 1. We discuss each of these features in detail below.

Irregular Helix C’—The irregular or frayed Helix C’ with a bend seen in the x-ray structures is in good agreement with the heptad sequence analysis of this region, as well as with the solution structure by spin label EPR studies (8). The sequence of this region shows one heptad residue deletion after residue 30, with Lys-30 at e and Glu-31 at g. This one-residue deletion in the heptad pattern in the sequence corresponds to two successive stammers in coiled coil helices (47) and is able to tighten up a coiled coil and shorten the local pitch length (48). The existence of a stammer is usually responsible for local flexibility of the helix (47). These structural features may enhance the interfacial
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interactions when associating with its binding partner (48) or may disrupt the interactions, and lead to diseases, such as an in-frame deletion in the desmin gene that leads to skeletal or cardioskeletal myopathy (49). Unwound, bent, or broken helices have been shown to be important components in understanding variations in protein-protein interactions (50). Thus, Helix C*, as an irregular and frayed helix, is capable of conformational mobility upon interacting with β-spectrin and/or an interactor protein in cells. It is possible that proteins other than β-spectrin may exhibit specific interaction with this irregular Helix C* to modulate/regulate the tight association between Helix C* of αII and Helices A' and B' of βII during specific cellular events. We have identified several proteins that interact with αII but do not interact with an αII Helix C* mutant V22D, which also does not interact with β-spectrin (51). Thus, these proteins may interact specifically with the flexible Helix C* to modulate the affinity of α-spectrin in its association with β-spectrin in this region.

It is interesting to note that Helix C* becomes more regular in the model structure of the αβ complex. We included the bend feature into the initial structure (homology model) of the complex, and yet Helix C* is observed without a significant bend in the structure resulting from MD simulation.

Helical Junction Region—We previously inferred a helical conformation for the junction region of αII from our low resolution small angle x-ray scattering (7) and spin label EPR (8) structural studies, and we have now confirmed its existence in this study. Because we have shown that the corresponding junction region in αI-spectrin is unstructured in the absence of its β-spectrin binding partner (7, 14), but is helical in the presence of β-spectrin (11, 13, 58), we now suggest that this helical junction plays an important role in the “on-rate” of the association. Indeed, it has been shown that in studies with αIβI and αIIβI model systems slightly different from ours, the on rate for αII is slower than that for αII (10). We have shown that when the junction region in αII spectrin is not able to undergo the unstructured to helical conformational change upon binding β-spectrin, as in the αII L49F mutant, the association affinity with β-spectrin is low (17).

Long A1B1 Loop—The D1-αII structure includes a long A1B1 loop, with 11 residues, whereas the corresponding loop in D1-αI consists of six residues (14). The other 11 known spectrin domain loops consist of 7–10 residues (we used PDBsum to analyze the published structures for this information), with an average of eight residues. We speculate that this long loop may provide binding sites for other, nonspectrin, proteins to recognize this specific site for interaction. The sequence of this loop is ASDENYKDPTN (residues 67–77), a combination of residues, many of which are polar, with differing side-chain properties, well suited for specific molecular recognition/interaction.

Specific Inter-helix Hydrogen Bonds/Salt Bridges—Generally speaking, hydrogen bonds (27, 28) and salt bridges stabilize protein structures (28). To use atom pairs for determining interactions quantitatively, salt bridges are two atoms of opposite charge within 4 Å but that do not qualify as hydrogen bonded (52). An increased temperature of stabilization is related to a structure with an increased number of hydrogen bonds/salt bridges and/or a better hydrophobic internal packing. Recently, it has been shown that the replacement of a single amino acid residue that affects hydrogen bonds as well as other steric strains could affect the $T_m$ by up to 16°C and $\Delta G$ by 4–5 kcal/mol (52, 53). Similarly, a specific interaction between two helices in partial domain association may lead to differences in $K_f$ values by 2 orders of magnitude, which corresponds to a $\Delta G$ value of 2–3 kcal/mol. Thus, it is clear that a single specific hydrogen bond or salt bridge is able to increase stability of specific domains in spectrin by 10–20°C in $T_m$ values. Various spectrin segments have been reported to have different $T_m$ values (16, 35). Examination of specific interactions for both D1-αI and D1-αII shows more specific hydrogen bonds in D1-αII than D1-αI, but with similar hydrophobic internal packing, and provides an atomic level of understanding of the different stabilities in these two structural domains.

Predicting Spectrin Tetramer Structure at the αβ Association Region—In the absence of either NMR or x-ray structures of the A′B′C′ complex structure in the spectrin tetramer, a general structure of the helical bundle A′B′C′ is relatively simple to predict, due to heptad repeats in sequences of the three helices involved in spectrin tetramer formation, with the residues at the “a” and “d” positions of each helix facing each other to form a hydrophobic core of the bundle (17). Furthermore, the sequences of the partial domain Helices A′, B′, and C′ are homologous to those in structural domains, it is reasonable to select a known spectrin structural domain structure as a template to model the structure of A′B′C′. This approach has been validated for helical membrane protein systems, due to the reduced degrees of freedom that a protein can adopt in a lipid bilayer (54), and our coiled coil helix system also has reduced degrees of freedom due to hydrophobic residues at heptad $a$ and $d$ positions forming hydrophobic clusters.

The structures from MD simulations of the homology models of the complex of αIIβI, and of αIIβII, show inter-helix hydrophobic clusters at both the N-terminal end and the C-terminal end of the helical bundle. Similar hydrophobic clusters are also found in the αIβI model structure (17). Thus, hydrophobic clustering does not appear to be responsible for the differing association affinities of αIIβI and αIIβII, although mutations eliminating members of the cluster (e.g. L49C in αII and L40C in αI) do lead to reduced association affinities (8, 13).

The conformation of the junction region of α-spectrin, a region connecting the partial domain that is responsible for the association with the first structural domain, differs uniquely among three complexes studied (αII-WT/βI, αII-R37P/βI, and αI/βI), ranging from a regular helical structure to completely unstructured. We showed that both in the free form and in the bound form, the C-terminal region of Helix C′ and the junction region exhibit different degrees of helicity in αII, αII-R37P, and αI. The junction region of αII is already in a helical conformation in the free form (with nine intra-helix hydrogen bonds around the junction region), whereas that of αI is unstructured in the free form (with four intra-helix hydrogen bonds), but becomes helical in the bound form, whereas the region in αII-R37P is unstructured (with two intra-helix hydrogen bonds) in the free form and remains unstructured in the bound form. Thus, we suggest that the conformational entropy changes of
the junction region play an important role in the differences observed between α-erythroid (αI) and nonerythroid (αII) spectrin association affinity of tetramer formation. We suggest that the helical conformation in the junction region controls the on-rate of the association. Indeed, it has been shown that, in slightly different αIβ and αIββ model system studies, the on-rate for αI is much slower than that for αII (10).

Strictly speaking, conformational entropy is the entropy associated with motions of the ligand and/or the protein, and the binding free energy includes a loss in conformational entropy due to the decrease in freedom of the ligand and the protein upon binding (55). Changes in conformational entropy on binding are often large, cancel much of the energy change that drives binding, and must be accounted for to obtain good correlations with measured affinities (56). In protein systems, the conformational entropy is introduced by local fluctuations in the neighborhood of a well defined structure as well as from the larger scale conformational variations that occur in less well defined structures (55). The complex conformational state is the larger scale conformational variations that occur in less well defined structures (55). The complex conformational state is determined by its associated free energy, and a binding event shifts the population of the unbound conformational state to a bound conformational state (57). The change at the binding site is determined dynamically via residue-residue interactions to release the created strain energy if there is a conformational change at an allosteric site. Because ITC measurements, in principle, provide information on entropy change of αβ spectrin association, we examined the ΔH and ΔS values of the association. We selected only the measurements that should provide the most reliable thermodynamic information for the association, and we found that the entropy change in αIββI association (with TΔS about −27 kcal/mol) is indeed more favorable than that of αIβI (−19 kcal/mol). However, the ΔH term in αIββI (−38 kcal/mol) is also more favorable than that of αIβI (−28 kcal/mol). Furthermore, it is impossible to separate the conformational entropy change from the solvation entropy change in the ΔS values obtained from ITC. Thus, at this time, we are not able to experimentally demonstrate the extent of conformational entropy change in spectrin αβ association. However, our experimental data are consistent with the importance of a conformational entropy contribution to the association.

In the absence of structural differences in the junction region, mutations R19C and L40C in αI (8) or mutations R28C and L49C in αI (13) exhibit differing affinities due to small variations in local conformation, with similar effects in tetramers of either αI or αII. Other mutations that exhibit abnormal affinity and perturb local interactions, rather than the conformation in the junction region, have been reported for αI-R45S and R45T (15).

In conclusion, the canonical triple helical bundle conformation of structural domains in spectrin isoforms does not reveal specific functions associated with either different domains or different isoforms. The unique structural features in each domain, and even each partial domain, lead to different contributions toward the overall functional properties of spectrin, such as the rigidity/flexibility of spectrin and the ability of dimers to associate to form tetramers, in the cytoskeletal network of different cells. In this study, we find that the N-terminal regions of αI and αII are generally similar but with each exhibiting specific unique features. One important common feature is the structural flexibility in the first helix, Helix C′, to allow association with the helices in β-spectrin to form spectrin tetramers. An important feature unique to each is the conformation of the junction region connecting the first lone helix to the triple helical structural domain, with an unstructured conformation in αI and a helical conformation in αII. These differences modulate the association affinity of αβ spectrin. αIββI tetramer formation is essential in the regulatory step for neuritogenesis (3). αII is essential for stabilizing nascent sodium channel clusters (4), assembling the mature node of Ranvier (4), and regulating endothelial cell-cell contacts (5). Mutations affecting the Helix C′ structural flexibility and the junction region conformation in α-spectrin, as well as inter-helix electrostatic interactions, will alter the equilibrium between spectrin dimers and spectrin tetramers. Consequently, mutations in this region will lead to substantially reduced levels of functional tetramers in cells and potentially abnormal functions of neurons, as similar mutations in erythroid spectrin lead to hematological disorders.

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Crystal Structure of the Nonerythroid α-Spectrin Tetramerization Site Reveals Differences between Erythroid and Nonerythroid Spectrin Tetramer Formation
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