Conformational Stabilities of the Structural Repeats of Erythroid Spectrin and Their Functional Implications*

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The two polypeptide chains of the erythroid spectrin heterodimer contain between them 36 structural repeating modules, which can function as independently folding units. We have expressed all 36 and determined their thermal stabilities. These repeats can function as independently folding units. We have expressed all 36 and determined their thermal stabilities. These repeats may be expected to permit adaptation of the membrane to the large distortions that the red cell experiences in the circulation.

Spectrin, an elongated flexible tetrameric molecule, defines the unusual shear resistance and elasticity of the red cell membrane (1, 2). The tetramers are made up of two αβ heterodimers, associated head-to-head (3). Both the α and the β chain are characterized by a series of homologous repeating units of about 106 amino acids, each with the structure of a loose triple-stranded α-helical coiled-coil (4, 5). Repeats of this nature also characterize the many proteins that comprise the spectrin superfamily (6–9).

Successive spectrin repeats are joined by a continuous α-helix, of which the N-terminal half forms the C-terminal helix (C-helix) of the three-helix bundle of one repeat and the other half forms the first helix (A-helix) of the adjoining repeat; 5 residues in the center of the helix are not part of the repeating structure and are referred to as a “linker” region (9–11). It has been suggested (10, 11) that the flexibility of the spectrin chain under physiological conditions may be governed by kinks resulting from a low conformational stability of some of the linkers. On the other hand, the folding of individual repeats is cooperative, which implies that thermal “melting” of a linker would cause the repeats on either side to unfold. It has indeed been found that some isolated repeats have only marginal stability and are partly or largely unfolded at 37 °C (12, 13). At the same time, the thermal, solvent-induced, or forced unfolding of constructs containing contiguous repeats has been found in some cases to display a conformational coupling between them (14).

To establish how repeats of low stability are distributed through the spectrin molecule, we have examined the thermal unfolding of all of the 36 repeats that make up the two chains of erythroid spectrin. Some of the repeats show exceptionally high stability, the structural origins of which we have tried to identify. We have additionally compared thermal unfolding profiles of single repeats with those of several two-repeat fragments and with two five-repeat constructs to investigate the extent of conformational coupling within the chains. Where discrete unfolding transitions of contiguous repeats can be observed, analysis of the propagation of conformational cooperativity along the polypeptide chain becomes possible. Implications for the mechanical function of spectrin in the cell are considered.

EXPERIMENTAL PROCEDURES

Materials—pGEX-4T-2 vector and glutathione-Sepharose 4B were purchased from Amersham Biosciences. pET-31b(+)-vector and nickel resin were from Novagen (Madison, WI). QuikChange site-directed mutagenesis kit and BL21 (DE3) bacteria were from Stratagene (La Jolla, CA). Restriction enzymes were from New England BioLabs (Beverly, MA). Proteinase inhibitor mixture set II was from Calbiochem. Reduced form glutathione, thrombin, isopropyl-1-thio-β-d-galactopyranoside, and bovine serum albumin were from Sigma. SDS-PAGE and electrophoresis reagents were from Bio-Rad, and GelCode staining reagent was from Pierce. All other chemicals were reagent grade from standard sources.

Design and Subcloning of Recombinant Spectrin Polypeptides—The boundaries of all repeats were defined by the SMART data base, with two exceptions; β-spectrin repeat 1 (here designated βR1+) starts 8 residues before the predicted repetitive segment (15), whereas α-spectrin fragment 9–10 contains a triple helix βR9 interrupted by an SH3 domain (segment 10) between helices B and C. We expressed these as a single polypeptide and did not attempt to make a separate construct of βR9 without the SH3 domain. To ensure proper folding, we extended the N and C termini of our single-repeat constructs by 5 residues. The residue numbers of all sequences are given in Table 1. αR14, αR18, and αR19 were subcloned into the pGEX-4T-2 vector, using BamHI and SalI. All other spectrin single repeats and two-repeat fragments were subcloned into the pGEX-4T-2 vector, using EcoRI and SalI restriction.
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enzymes upstream and downstream, respectively. aIR18 was also subcloned into the pET-31b (+) vector, using NsiI and XhoI upstream and downstream, respectively. One five-repeat fragment IR5-9 was subcloned as described previously (16), and another five-repeat fragment IR6-10 was subcloned into the pGEX-4T-2 vector, using EcoRI and Sall restriction enzymes. The template used to amplify α-spectrin single repeats and tandem-repeat fragments was full-length α-spectrin cDNA. Similarly, full-length β-spectrin cDNA was used to amplify β-spectrin single repeats and tandem-repeat fragments. Mutations found in aIR14, aIR15, βIR3, and βIR7 constructs were corrected by site-directed mutagenesis using the Stratagene QuikChange kit. The fidelity of all the constructs was confirmed by DNA sequencing. Mutations were incorporated into repeat sequences using the Stratagene QuikChange kit and verified by sequencing.

Preparation of Recombinant Spectrin Polypeptides—The cDNA encoding the desired polypeptide was transformed into Escherichia coli BL21. Because some of the polypeptides were insoluble when expressed at 37 °C, all the fragments were expressed at 16 °C. This greatly improved the yield of soluble product and ensured that all the polypeptides were folded under the same conditions. All the polypeptides were satisfactorily expressed and soluble, although the yields varied from 1 to 50 mg/500 ml bacterial culture. Expression of recombinant proteins was induced by 0.1 mM isopropyl-β-D-thiogalactopyranoside at 16 °C for 3–4 h. Glutathione S-transferase fusion polypeptides were purified on a glutathione-Sepharose 4B affinity column. Glutathione S-transferase was cleaved with thrombin. The exception was aIR18, which could not be recovered after thrombin cleavage and was therefore expressed in His-tagged form and purified on a nickel column. Concentrations of most polypeptides were determined spectrophotometrically, using extinction coefficients calculated from the tryptophan and tyrosine contents (17). IR9 does not contain tryptophan and IR11 does not contain tyrosine, and because of their low absorbances, an approximate concentration of these two repeats was obtained by the Bradford (18) method, with bovine serum albumin as standard. Proteins were dialyzed against phosphate-buffered saline and clarified before use by ultracentrifugation at 230,000 × g, for 30 min at 4 °C.

Mass Spectrometric Analyses—Mass spectrometric analyses were performed in an Applied Biosystems (Applied Biosystems, Foster City, CA) Voyager DE MALDI mass spectrometer. Samples at a concentration of 10 µl were mixed with an equal volume of sinapinic acid matrix solution in 50% acetonitrile, 0.1% trifluoroacetic acid (Sigma), and 1–2 µl of each mixture was spotted onto a sample plate and dried. The results were externally calibrated against aldolase (Sigma; [M + H]+ = 39, 212.28) and bovine serum albumin ([M + H]+ = 66,430.09).

Sedimentation Equilibrium Analysis—The molecular weights of proteins were measured by sedimentation equilibrium in a Beckman Coulter ProteomeLab™ XL-A analytical ultracentrifuge at a rotor speed of 20,000 rpm. Equilibrium distributions were scanned at 280 and 235 nm. Partial specific volumes were calculated from amino acid compositions.

Circular Dichroism Spectra and Thermal Unfolding—Far-UV CD spectra were recorded in a Jasco 700 spectropolarimeter, equipped with a thermostated cell housing, in cells of 1-mm path length. For determination of unfolding profiles, the temperature was increased from 4 to 90 °C at a rate of 0.4 °C/min, and the ellipticity was recorded at 2°C intervals. To test for reversibility, several of the samples were cooled back to 4 °C, and the CD spectrum was recorded. To estimate fractional unfolding from the ellipticity temperature profiles, corrections were made for the effectively linear changes in ellipticity in the temperature ranges above and below the sigmoidal transition region.

Molecular Modeling—To assign individual residues to the a, d, and e positions in the heptad hydrophobic repeats, we aligned all the sequences with Protein Data Bank entry 1S35 (tandem-repeat fragment βIR8-9), for which the heptad repeats have been defined (9, 10), with the aid of T-coffee (19). For generation of three-dimensional models of individual or tandem repeats, we used the automated Swiss-Model server (20). Models were displayed and analyzed in Pymol. Surface accessibility of side chains was estimated using STRIDE (21).

Forced Unfolding by AFM—AFM3 measurements were made on IR5-9 and IR6-10 using recently described methods and analyses (22). In particular, a Nanoscope IIIa Multimode AFM (Digital Instruments, Santa Barbara, CA) was equipped with a liquid cell and a heater to maintain hydration and control temperature. For any one temperature, 5,000 surface-to-tip contacts were collected and thoroughly analyzed with the aid of a semiautomated visual analysis program, custom-written in C++. Since protein unfolding events are stochastic and the experiment is intrinsically random in many ways, collecting and analyzing thousands of events is necessary to provide an accurate statistical survey of the unfolding possibilities.

RESULTS

Design and Characterization of Recombinant Spectrin Fragments—The spectrin fragments examined here are defined in Table 1. All contain an additional 6 residues from the vector at the N terminus. All products had the expected chain length and composition, as measured by mass spectrometry. Sedimentation equilibrium revealed each of the products to be monodisperse and monomeric. Circular dichroism spectra of all expressed fragments presented the typical appearance of proteins of high α-helicity, with the exception only of aIR9-10, which contains the SH3 domain and therefore has a lower α-helix content, estimated from the molar residue ellipticity at 222 nm (23) as 40%. As the cooperative thermal unfolding transitions also showed, all fragments had entered the folded state at low temperature.

Temperature-induced Unfolding of Recombinant Spectrin Single Repeats—Fig. 1A shows representative thermal transition profiles of single repeats of α-spectrin. In all cases, we observed a sigmoidal transition, suggestive of a two-state unfolding equilibrium, with no stable intermediates. Fig. 1B displays the Tm of all individual α-spectrin repeats, and Fig. 1, C and D, display those of the β-chain repeats. For all polypeptides examined, the transition was almost entirely (not less than 90%) reversible. There are, however, much smaller, more or less linear, changes in ellipticity below and above the cooperative region of unfolding, as also observed by others. As in the earlier work, we have applied a linear correction to obtain the presumptive fraction of tertiary structure unfolded at each temperature. Our results show that the thermal stabilities of the individual repeats from both spectrin chains differ widely, with transition mid-points (Tm) ranging from 21 and 72 °C (Fig. 1, B and D). Of the 36 repeats making up the two chains, 8 have Tm below 37 °C.

Basis of Differential Stability of Repeats—The accumulation of unfolding data for all 36 erythroid spectrin repeats allows us to search for structural features that determine the very wide range of observed stabilities. Earlier studies have pointed to interactions between the 2 tryptophan residues, conserved through most of the repeats (12, 24), as a stabilizing feature. In one instance, reduced stability has been linked to the loss of intrahelical hydrogen bonds in helix B of the three-helix bundle (12). We have focused on the exceptionally stable repeat, βIR16. Here the second of the tryptophans is replaced by arginine. This approach is not a stabilizing feature for it is found also in repeat aIR13.

3 The abbreviation used is: AFM, atomic force microscopy.
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TABLE 1
Information of erythrocyte spectrin single repeats
The boundaries of all repeats were basically defined by the SMART database with the exception that 5 amino acids are extended at both N-terminus and C-terminus to ensure proper folding. The N-terminal 6 amino acids are from pGEX-4T-2 vector.

| Name   | Amino acids | Nucleotides | Name   | Amino acids | Nucleotides |
|--------|-------------|-------------|--------|-------------|-------------|
| aIR1   | 45–163      | 133–489     | bIR1+  | 293–422     | 877–1266    |
| aIR2   | 154–269     | 460–807     | bIR2   | 426–532     | 1276–1596   |
| aIR3   | 260–375     | 778–1125    | bIR3   | 523–641     | 1567–1923   |
| aIR4   | 365–481     | 1093–1443   | bIR4   | 632–747     | 1894–2241   |
| aIR5   | 472–587     | 1414–1761   | bIR5   | 738–852     | 2212–2556   |
| aIR6   | 578–692     | 1732–2076   | bIR6   | 843–958     | 2527–2874   |
| aIR7   | 683–798     | 2047–2394   | bIR7   | 949–1065    | 2845–3195   |
| aIR8   | 789–902     | 2365–2706   | bIR8   | 1056–1171   | 3166–3513   |
| aIR9–10| 893–1087    | 2677–3261   | bIR9   | 1162–1278   | 3484–3834   |
| aIR11  | 1078–1188   | 3232–3564   | bIR10  | 1269–1382   | 3805–4149   |
| aIR12  | 1179–1294   | 3535–3882   | bIR11  | 1374–1472   | 4120–4446   |
| aIR13  | 1285–1400   | 3853–4200   | bIR12  | 1473–1588   | 4417–4764   |
| aIR14  | 1391–1505   | 4171–4515   | bIR13  | 1579–1694   | 4735–5082   |
| aIR15  | 1496–1611   | 4486–4833   | bIR14  | 1685–1801   | 5053–5403   |
| aIR16  | 1602–1717   | 4804–5151   | bIR15  | 1792–1907   | 5374–5721   |
| aIR17  | 1708–1822   | 5122–5466   | bIR16  | 1898–2013   | 5692–6039   |
| aIR18  | 1813–1932   | 5437–5796   |        |             |             |
| aIR19  | 1923–2047   | 5767–6141   |        |             |             |
| aIR20  | 2038–2155   | 6112–6465   |        |             |             |
| aIR21  | 2146–2264   | 6436–6792   |        |             |             |

FIGURE 1. Representative thermal melting profiles and bar representation of \( T_m \) of spectrin single repeats (A and B, \( \alpha \)-spectrin, C and D, \( \beta \)-spectrin). A and C show the normalized change in ellipticity at 222 nm with temperature. B and D show the \( T_m \) of the isolated repeats of the two spectrin chains.

(T\( _m \) 36 °C); neither could we discern any relation in our sequence alignments between stability and the hydrophobicity of the residues at positions \( a \) and \( d \) of the heptad hydrophobic repeat. On the other hand, there was a trend toward increasing hydrophobicity in stable repeats at other positions in the heptads; this was particularly evident when the data were combined with analyses (not shown) of all- and \( \beta \)-spectrin repeats. At the second and third \( e \) positions (\( e_2 \) and \( e_3 \)) in helix A, the most stable repeats typically have at least 1 hydrophobic residue. Overall, about half the helix A \( e_2 \) and \( e_3 \) positions have charged residues (17/36 are Glu, Asp, Lys, or Arg at \( e_2 \) and 19/36 at \( e_3 \)). In the highly stable \( \beta \)IR16, these positions are occupied by Leu and Ile (Fig. 2A). Molecular modeling of \( \beta \)IR16 indicates that helix A residues Leu-1916 (\( e_2 \)) and Ile-1923 (\( e_3 \)) are close to residues Val-1987 and Met-1994 in helix C and may thus form a hydrophobic cluster (Fig. 2B). In the unstable repeat \( \beta \)IR8 (12), glutamine residues at \( e_2 \) and \( e_3 \) in helix A are apoposed to glycines in helix C, presumably precluding significant inter-helix contacts. Mutations of the Leu and Ile at \( e_2 \) and \( e_3 \) in \( \beta \)IR16, such as would disrupt the hydrophobic cluster, are therefore predicted to reduce the stability. We accordingly prepared the mutants L1916A and I1923A and the double mutant L1916A,I1923A. At low temperatures, the helicities of the wild-type and mutant polypeptides were indistinguishable, indicating that the alanine side chains are compatible with folding of the triple helix. Fig. 2C shows the effects of these mutations on their thermal stabilities. The single mutations reduce the \( T_m \) of 72 °C in the wild type to 61 °C in L1916A and 56 °C in I1923A. The double mutant has \( T_m \) of 47 °C, the median \( T_m \) for all repeats. The corresponding free energies of destabilization (see below), due to the two single mutations and the double mutation, at the \( T_m \) of the wild-type polypeptide are about 2.8, 2.5, and 4.3 kcal mol\(^{-1}\). In considering whether alanine might be an especially unfavorable residue in the \( e_2 \) and \( e_3 \) positions, we noted that alanines do indeed appear at these locations in a few repeats. In preparing an additional mutant, L1916E,I1923E, we selected glutamic
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FIGURE 2. Hydrophobic residues controlling stability of IR16. A, the heptad hydrophobic repeat pattern of helix A of IR16; positions a and d are indicated, and also, the mutated residues at position e in heptads 2 and 3 are indicated. B, a molecular model of IR16 showing Leu-1916 (position e2) and Ile-1923 (position e3). Probable interactions with residues in helix C Val-1987 and Met-1994 are indicated. C, Tm center; N, N terminus. C, the effect on thermal stability of mutating Leu-1916 and/or Ile-1923 to Ala or Glu. Mutations are indicated in the figure. Note the strong reduction of thermal stability, especially by the double Ala mutation. WT, wild type.

FIGURE 3. Thermal melting profiles of fragments of two tandem repeats and of spectrin aβ dimer. Normalized thermal melting profiles were based on the ellipticity at 222 nm. A and B show observed (closed circles) and calculated (open circle) melting profiles of αIR7-8 and IR9-10, respectively. The calculated data represent the summed unfolding profiles of the isolated constituent repeats. Note the stabilization of the less stable by the more stable repeat. C, summed melting profiles of all single repeats (closed circles), and melting of intact spectrin aβ dimer (open circles). The corresponding first-order derivatives (solid line for intact dimer, broken line for summed individual repeats) are also shown. Note the absence of any substantial unfolding in the intact dimer at 37 °C or below.

Acid because this is the charged residue often seen in these positions and evidently compatible with high Tm (e.g. Tm 60 °C in αIR16). Again, as Fig. 2C shows, IR16 L1916E,1923E has Tm of 60 °C, with a corresponding free energy of destabilization at the wild-type Tm of about 2.1 kcal mol−1. Thus the double glutamic acid mutant is more stable than the double alanine mutant, but glutamic acid in both positions cannot reproduce the stability engendered by leucine and isoleucine in the wild type. We conclude that a high hydrophobicity of the side chains in these positions may account for the unusually high Tm of the repeat.

Temperature-induced Unfolding of Tandem-Repeat Fragments—To examine whether structural stabilities of contiguous repeats are cooperatively linked, we prepared three fragments, each of two tandem repeats, αIR3-4, αIR7-8, and IR9-10. Fig. 3, A and B, show the thermal melting profiles of αIR7-8 and IR9-10 together with the calculated profiles for the summed constituent repeats. It can be seen that in both cases, there is stabilization of the less stable by the more stable folding unit. Within the limits of error, the higher temperature transition is unaffected (as expected, since it is attached at this stage to an unfolded segment of polypeptide chain). The elevations of Tm (ΔTm), due to coupling with the attached repeat, of αIR4 in αIR3-4, αIR8 in αIR7-8, and IR9 in IR9-10 were, respectively, 6.6, 11, and 13 °C.

The corrected melting profiles allow the evaluation of the equilibrium constant for unfolding at each temperature within the transition range (K = α/(1 − α), where α is the fractional unfolding at the given temperature), and thus from a van’t Hoff plot, of the free energy change of a perturbed (conformationally coupled) repeat at the Tm of the unperturbed (isolated) repeat (where ΔG = 0), with the assumption that ΔCp remains unchanged. The difference in free energy of unfolding is as easily derived by the reasoning of Becktel and Schellman (25), as

\[ \Delta \langle \Delta G \rangle = \Delta S \times \Delta T \]

where ΔS is the entropy of unfolding and ΔT is the change in Tm. This assumes that ΔS = −d(ΔG)/dT is the same for both fragments and that within the limited temperature range in question, d(ΔG)/dT can be regarded as linear. We found both conditions to be fulfilled, and the two methods gave concordant results, namely that the free energies of stabilization, at the transition mid-points, of αIR4 by αIR3, of αIR8 by αIR7, and of IR9 by IR9-10 were, respectively, 1.7, 3.6, and 2.4 kcal mol−1.

The extensive nature of conformational coupling between the repeats along the spectrin chains is most graphically expressed by a comparison of the summed unfolding profiles of the single repeats with that of the intact chains. We show (Fig. 3C) only the result for the aβ-dimer; the data for the separated α- and β-chains are very similar to this, implying that no significant conformational stabilization derives from lateral interactions between the chains. It is at once apparent that there is little unfolding in any part of the unperturbed spectrin dimer at physiological temperature. The derivative of the dimer melting profile shows that unfolding predominantly occurs within a narrow temperature range centered at about 50 °C. This is in satisfactory agreement with the result of scanning calorimetry of spectrin, both in solution and on the membrane, which displays a maximum at 49 °C (26); this is also the temperature at which the red cell membrane vesicles (27).

Mechanism of Unfolding in a Multirepeat Construct—A fragment of five successive repeats, IR5-9, was also prepared. We took advantage of this larger fragment to examine in more detail the mechanism of unfold-
ing. Fig. 4 shows two resolved phases in the unfolding profile by circular dichroism. A relatively broad transition with a mid-point close to 40 °C and accounting for about 40% of the change in helicity presumably reflects the unfolding of βIR8-9. We also followed the unfolding by changes in forced unfolding extension curves, using AFM to probe the presence of folded units at different temperatures. At 23 °C, up to five unfolding peaks were observed in AFM as the construct was subjected to forced unfolding (see inset for representative sawtooth pattern). This indicates that up to five structural modules resist pulling at low temperature. At 30 °C, only four transitions were observed in AFM; thus only four modules resist pulling, which suggests loss of integrity of βIR9. Another module is lost between 30 and 33 °C, consistent with loss of βIR8 integrity. Between 33 and 42 °C (the highest temperature obtainable with our instrument), no further loss of modules was observed. To determine whether the unstable terminal repeat, βIR9, may be significantly perturbed by interaction with the adjacent repeat on the C-terminal side in the intact β-spectrin chain, we also prepared the overlapping construct βIR6-10. This proved to behave almost identically to βIR5-9 in tests of both thermal and forced unfolding (not shown).

**DISCUSSION**

We have defined the entire conformational stability profile along both the α-chain and the β-chain of the erythroid spectrin, revealing structurally weak regions. The apposition of unstable domains in the two laterally associated chains of the αβ-dimer, noted by MacDonald and Cummings (13), is not a general feature when the entire chains are compared. Nevertheless, the marginal stability of some repeats at points along the chains is presumably a functional characteristic of the protein. It can be expected to allow for the large membrane deformations induced by shear stresses.

As many other studies, based on thermal, solvent-induced, and mechanical unfolding (10, 14, 28, 29, 30), have shown, contiguous spectrin repeats may be conformationally coupled. We have quantified the coupling free energies between three pairs of tandem repeats, and we have shown that coupling prevails within a fragment of five successive repeats. It appears then that stabilization through interaction of successive repeats is widely propagated along the two chains and that the instability of some isolated repeats at physiological temperature is mitigated by this effect. However, it should also be remarked that for a more complete description of the stability profile of the spectrin chains, the effect of temperature on inter-repeat coupling must be taken into account. Baty et al. (29) found a breakdown of cooperativity in denaturant-induced unfolding with increasing temperature, but the opposite is the case in the forced unfolding transitions observed here.

Some insight into the factors governing the stability of the spectrin repeats results from an examination of βIR16. We have identified 2 hydrophobic residues, Leu-1916 and Ile-1923, at the e positions in the heptad repeats of helix A, that are evidently inseparable from its exceptional stability. Modeling suggests that they form a hydrophobic cluster with residues Val-1987 and Met-1994 in the opposing helix C. The hydrophobicity of these residues appears to be critical for function since in all βI-spectrin sequences from fish to human, the positions equivalent to Leu-1916 and Ile-1923 are occupied by Leu, Ile, Val, or Met. Indeed, Leu-1916 is conserved, or changed only to methionine, in all known β-spectrins from *Caenorhabditis elegans* to human. Repeat 16 strengthens interaction of the adjacent partial repeat 17 with the α-spectrin N terminus (31), and the stability of its conformation may therefore be demanded by the α-β interaction. As yet, no naturally occurring mutations are known in βIR16 that could test this inference.

The AFM measurements on βIR5-9 and βIR6-10 reveal that by about 33 °C, repeats 8 and 9 of β-spectrin are incapable of resisting mechanical pulling, yet CD indicates that most helical melting occurs above this temperature. In native spectrin *in situ*, we may infer that these repeats readily adopt an extended conformation under mechanical strain. The question remains whether the degree of unfolding of spectrin repeats in solution at physiological temperature, and their stability with respect to mechanical unfolding applies equally to tetramers in the very different environment of the red cell. We have not found any significant displacement of thermal melting profiles of phospholipid-binding repeats in the presence of phosphatidylserine vesicles. That the *Tm* of spectrin is practically the same in red cell ghosts as in solution (26) also argues for the relevance of solution data to physiological function.

In the cells of solid tissues such as muscle, spectrin is presumed to stabilize membrane structures against forces arising from animal movement and to control protein localization at membranes (32, 33). It seems likely that tissue spectrins (such as the abundant αβII-spectrin) will be more resistant to mechanical deformation, and this may manifest itself in high thermal stability of the repeats. Spectrin isolated from brain is markedly stiffer than erythrocyte spectrin (34). Brain spectrin is a mixture of different gene products but gives the impression of a structure generally more resistant to deformation than its erythrocyte counterpart. Further studies are needed to determine whether and how the rigidity of αβII-spectrin is related to the conformational stability of its constituent repeats.

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