

YEAST TWO-HYBRID AND ITC STUDIES OF ALPHA AND BETA SPECTRIN INTERACTION AT THE TETRAMERIZATION SITE

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ABSTRACT

Yeast two-hybrid (Y2H) and isothermal titration calorimetry (ITC) methods were used to further study the mutational effect of non-erythroid alpha spectrin (α II) at position 22 in tetramer formation with beta spectrin (β II). Four mutants, α II-V22D, V22F, V22M and V22W, were studied. For the Y2H system, we used plasmids pGBKT7, consisting of the cDNA of the first 359 residues at the N-terminal region of α II, and pGADT7, consisting of the cDNA of residues 1697 - 2145 at the C-terminal region of β II. Strain AH109 yeast cells were used for colony growth assays and strain Y187 was used for β -galactosidase activity assays. Y2H results showed that the C-terminal region of β II interacts with the N-terminal region of α II, either the wild type, or those with V22F, V22M or V22W mutations. The V22D mutant did not interact with β II. For ITC studies, we used recombinant proteins of the α II N-terminal fragment and of the erythroid beta spectrin (β I) C-terminal fragment; results showed that the K_d values for V22F were similar to those for the wild-type (about 7 nM), whereas the K_d values were about 35 nM for V22M and about 90 nM for V22W. We were not able to detect any binding for V22D with ITC methods. This study clearly demonstrates that the single mutation at position 22 of α II, a region critical to the function of non-erythroid α spectrin, may lead to a reduced level of spectrin tetramers and abnormal spectrin-based membrane skeleton. These abnormalities could cause abnormal neural activities in cells.

Key Words: Spectrin tetramerization subunit interactions, Yeast two-hybrid, Isothermal titration calorimetry

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List of the abbreviations: α II - non-erythroid alpha spectrin; α II-N - a recombinant protein consisting of the first 359 residues at the N-terminal region of α II; α II-N-V22 Δ - a recombinant protein with a single residue replacement at position 22 of α II-N; β I - erythroid beta spectrin; β I-C - a recombinant protein consisting of residues 1898-2083 at the C-terminal region of β I; β II - non-erythroid beta spectrin; β II-C - a recombinant protein consisting of residues 1697-2145 at the C-terminal region of β II; CD - circular dichroism; ITC - isothermal titration calorimetry; K_d - equilibrium dissociation constant; pAD - yeast plasmid pGADT7; pBD - yeast plasmid pGBKT7; Y2H - yeast two-hybrid.

INTRODUCTION

Spectrin, a prominent cytoskeletal protein, exerts its fundamental role in cells by forming a sub-membrane filamentous network. An essential aspect of the spectrin network formation is the tetramerization of spectrin $\alpha\beta$ heterodimers. We have previously used the yeast two-hybrid system and random mutagenesis to investigate the effects of amino acid mutations on the tetramerization of non-erythroid (brain) spectrin (fodrin) [1]. The Y2H techniques have been developed as convenient and useful methods to screen for protein interactors [2-4], particularly when libraries of vectors containing protein cDNAs are commercially available. We have used such methods to identify some interactors of non-erythroid alpha spectrin (α II) [5]. These studies are often qualitative in nature - a protein either interacts or does not interact with another protein. However, several studies report quantitative results from Y2H studies. For example, colonies of Y2H system with common polymorphisms of *BRCA1* from cancer predisposing mutations were considerably smaller than controls [6], colony growth rates (cell viability) correlate with the strengths of interactions [7, 8], the levels of transcription activation correlate with the strength of the binding interaction in a “small colony phenotype”, a growth phenotype discovered serendipitously [9], and β -galactosidase activities correlate with protein-protein interaction affinities [10, 11]. Yet, some authors indicate that, “our results emphasize the difficulty of attempting to quantitate differences in affinity from two-hybrid experiments alone” [7]. Others show that Y2H results do not correlate with protein affinities [12-14]. Since protein expression, structures and nature of interaction may vary from system to system in Y2H systems, many studies have focused on studying single mutation effects on protein-protein interactions [e.g., 9].

In our study, we used both the Y2H system and ITC methods to further study the mutational effect of α II at position 22 on tetramer formation. Previously we have used ITC methods to determine K_d values of $\alpha\beta$ heterodimer association to form tetramers in model systems [e.g., 15-19]. Recently, we found that mutation of α II at position 37 increases the K_d value from about 9 nM for α II with beta I spectrin (β I) to 10 μ M for the R37P mutation [15]. Residue 22 in α II corresponds to a “d” position in the heptad repeat and is in the interface of the triple helical bundle in $\alpha\beta$ tetramers [15]. In this study, we found that the mutation effect was most severe for V22D, followed by V22W and V22M, whereas little effect was observed for V22F.

METHODS

Yeast two-hybrid assays

The Y2H system with colony growth and β -galactosidase detection methods were used to determine the interaction between β II and α II, wild-type or its mutants, at the tetramerization region. The Matchmaker GAL4 Two-Hybrid System 3 (Clontech, Mountain View, CA) was used. The yeast strain Y187, which is auxotrophic for leucine and tryptophan with Gal4-inducible *lacZ* gene, or strain AH109, which is auxotrophic for adenine, histidine, leucine, lysine, tryptophan and uracil and with Gal4-inducible *lacZ* genes was used. Plasmids pGBKT7 (pBD) with the cDNA of the non-erythroid alpha spectrin (α II) consisting of the first 359 residues at the N-terminal region (α II-N) (pBD- α II-N) and pGADT7 (pAD) with the cDNA of beta-spectrin consisting of residues 1697-

2145 at the C-terminal region (β II-C) (pAD- β II-C) were previously prepared [1]. Plasmids of two mutations at position 22 of α II-N, V22W and V22M, prepared by standard methods [20] as well as two previously prepared mutants (V22D and V22F) [1] (pBD- α II-N-V22 Δ) were also used.

For the colony growth assay, AH109 cells with pAD- β II-C and pBD- α II-N, or pBD- α II-N-V22 Δ , were grown at 30 °C on agar plates with a growth medium containing all essential amino acids but tryptophan, leucine and histidine, and lacking adenine (SD/-W/-L/-H/-A with SD Minimal Agar Base and -Leu/-Trp/-His/-adenine DO Supplement, both from Clontech) for three days before photography. Under this high-stringency growth condition, cells with strongly interacting protein pairs grow and form colonies, whereas colonies with proteins with low-affinity interactions may be missed (Clontech user manual). We also prepared pAD- β I-C, with β I-C consisting of residues 1898-2083 of β I and performed colony growth assay with pBD- α II-N or pBD- α II-N-V22D.

For β -Galactosidase assay via colony lift method, strain Y187 cells with pAD- β II-C and pBD- α II-N, or pBD- α II-N-V22 Δ , were grown at 30 °C on agar plates with a growth medium containing all essential amino acids but leucine and tryptophan (SD/-Leu/-Trp with SD Minimal Agar Base and -Leu/-Trp DO Supplement; both from Clontech) for three days before colony lifting steps, as described in the manufacturer user manual. Cells with interacting protein pairs produce β -galactosidase to give a blue color on filter papers when soaked with a solution consisting of its substrate, 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal soaking solution, see Clontech user manual).

Isothermal titration calorimetry

Recombinant proteins α II-N, α II-N-V22 Δ (V22D, V22F, V22M and V22W) and β I-C were prepared, following standard laboratory techniques [15]. Briefly, protein expression vector pGEX-2T was used to express glutathione S-transferase fusion protein, and purified with affinity column chromatography, with thrombin cleavage of fusion protein. DNA sequence analysis and protein mass spectrometry analysis results were obtained (Research Resources Center, University of Illinois at Chicago). Protein purity was checked with gel electrophoresis, using 16% polyacrylamide gel with 0.1% SDS. Helical contents of the proteins were determined using circular dichroism spectra [20]. We have found that β I-C and β II-C proteins exhibit similar affinities for α II-N [15]. However, β II-C recombinant protein is more difficult to prepare than β I-C protein due to its low expression level. Thus, β I-C was used for ITC experiments.

ITC measurements were performed at 25 °C using an isothermal titration calorimeter (VP ITC, MicroCal, LLC, Northampton, MA) [15]. Protein pairs (β I-C with α II-N, or α II-N-V22 Δ) were dialyzed overnight in 5 mM phosphate buffer with 150 mM sodium chloride at pH 7.4 (PBS) to ensure identical solution conditions in titrating protein pairs. In addition, all samples were thoroughly degassed prior to calorimetry titration. Each α II-N or α II-N-V22 Δ sample (30 μ M) was titrated into the sample cell containing β I-C protein (3 μ M). Titrations of β I-C (30 - 100 μ M) into α II-N or α II-N-V22 Δ (3 μ M) were also performed. Titration isotherms were analyzed with a single binding site assumption, as before [15], to obtain dissociation constants, K_d .

RESULTS

Yeast two-hybrid assays

For colony growth assay, cells with either β I-C or β II-C and with α II-N or α II-N-V22F, -V22M, or -V22W formed well separated colonies with diameters of 2 - 5 mm after 3 days, with no specific colony size associated with cells of a particular mutant (Fig. 1). However, cells with α II-N-V22D, with either β I-C (data not shown) or β II-C (Fig. 1) did not show any growth after 3 days (Fig. 1).

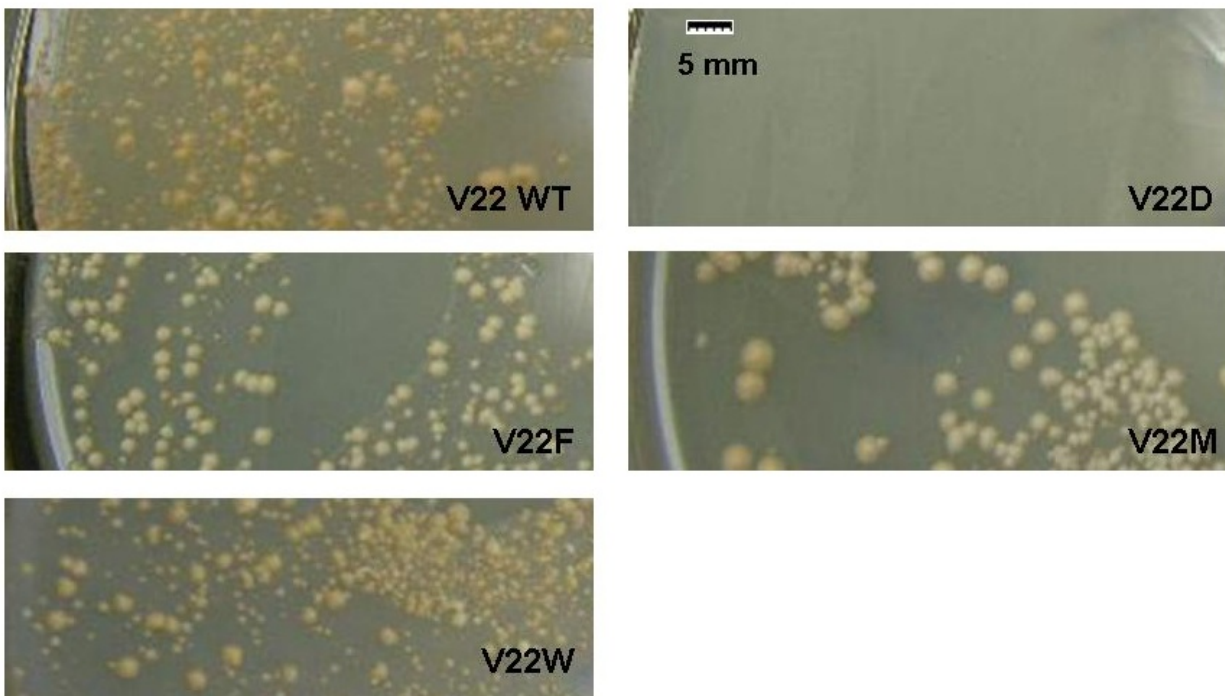


Fig. 1. Colony Growth Assay. AH109 cells co-transformed with pAD- β II-C and pBD- α II-N, or pBD- α II-N-V22D, -V22F, -V22M, or -V22W, were grown for 3 days at 30 °C, following procedures from the manufacturer (Clontech). Colonies, 2 - 5 mm in diameter, were found for cells expressing α II-N (marked as V22 WT above), α II-N-V22F (V22F), α II-N-V22M (V22M), or α II-N-V22W (V22W), whereas cells expressing α II-N-V22D (V22D) did not show any growth. The scale bar is shown in top right panel.

For the β -galactosidase activity (colony-lift) assay, Y187 cells with α II-N or α II-N-V22F, -V22M, or -V22W showed a distinct blue color, but without a consistent color variation associated with cells with a particular mutation (Fig. 2).

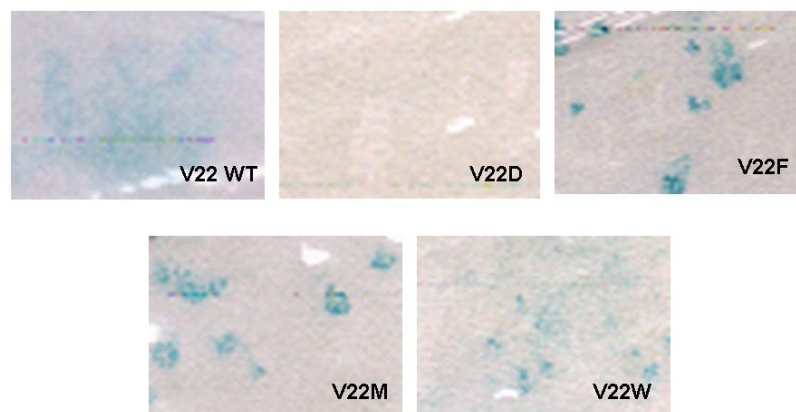


Fig. 2. β -Galactosidase Activity Assay via Colony Lift Method. Y187 cells co-transformed with pAD- β II-C and pBD- α II-N, - α II-N-V22D, - α II-N-V22F, - α II-N-V22M, or - α II-N-V22W were grown for 3 days at 30 °C following procedures from the manufacturer (Clontech). Colonies were transferred onto filter papers, subjected to freeze-thaw cycles, and incubated on a second set of filter papers pre-soaked with β -galactosidase substrate (X-gal) for 30 min. Filter papers for colonies with α II-N, α II-N-V22F, α II-N-V22M and α II-N-V22W all showed blue color, but those with α II-N-V22D did not show blue color.

Isothermal Titration Calorimetry Assay

Recombinant protein analysis

The SDS gel electrophoresis data showed that all α II-N (wild type and mutants) and β I-C proteins were ~90% pure. Electrophoretic masses were ~42 kDa for α II-N proteins and ~22 kDa for β I-C. Mass spectrometric results showed 42,241.0 Da for α II-N (expected mass is 42,242.5 Da), 42,258.6 Da for α II-N-V22D (expected mass is 42,258.5 Da), 42,289.0 Da for α II-N-V22F (expected mass is 42,290.6 Da), 42,274.8 Da for α II-N-V22M (expected mass is 42,274.6 Da), 42,329.8 Da for α II-N-V22W (expected mass is 42,329.6 Da) and 22,036.9 Da for β I-C (expected mass is 22,036.9 Da). The CD spectra of α II-N, α II-N-V22 Δ and β I-C exhibited characteristic features of similar spectrin recombinant proteins [21], with minima at 222 and 208 nm. Helical contents were ~75%, in good agreement with published results [21].

ITC results

The ITC isotherm of β I-C/ α II-N system at 25 °C showed that sufficient heat (-0.45 μ cal/sec) was released during titration of α II-N into β I-C (Fig. 3), with an average K_d value of 6.9 ± 0.5 nM ($n = 3$), in good agreement with previous findings of a similar system (with β I-C) [19], and the values are similar to that with β II-C [18]. The K_d value was 6.7 ± 0.3 nM for β I-C/ α II-N-V22F, 35 ± 4 nM for β I-C/ α II-N-V22M and 93 ± 28 nM for β I-C/ α II-N-V22W. However, for β I-C/ α II-N-V22D system, there was insufficient heat released either when α II-N-V22D (30 μ M) was titrated with β I-C (3 μ M) (Fig. 3, Tab. 1), or when β I (30 - 100 μ M) was titrated with α II-N-V22D (3 μ M), indicating that the K_d value for this system is larger than 100 μ M.

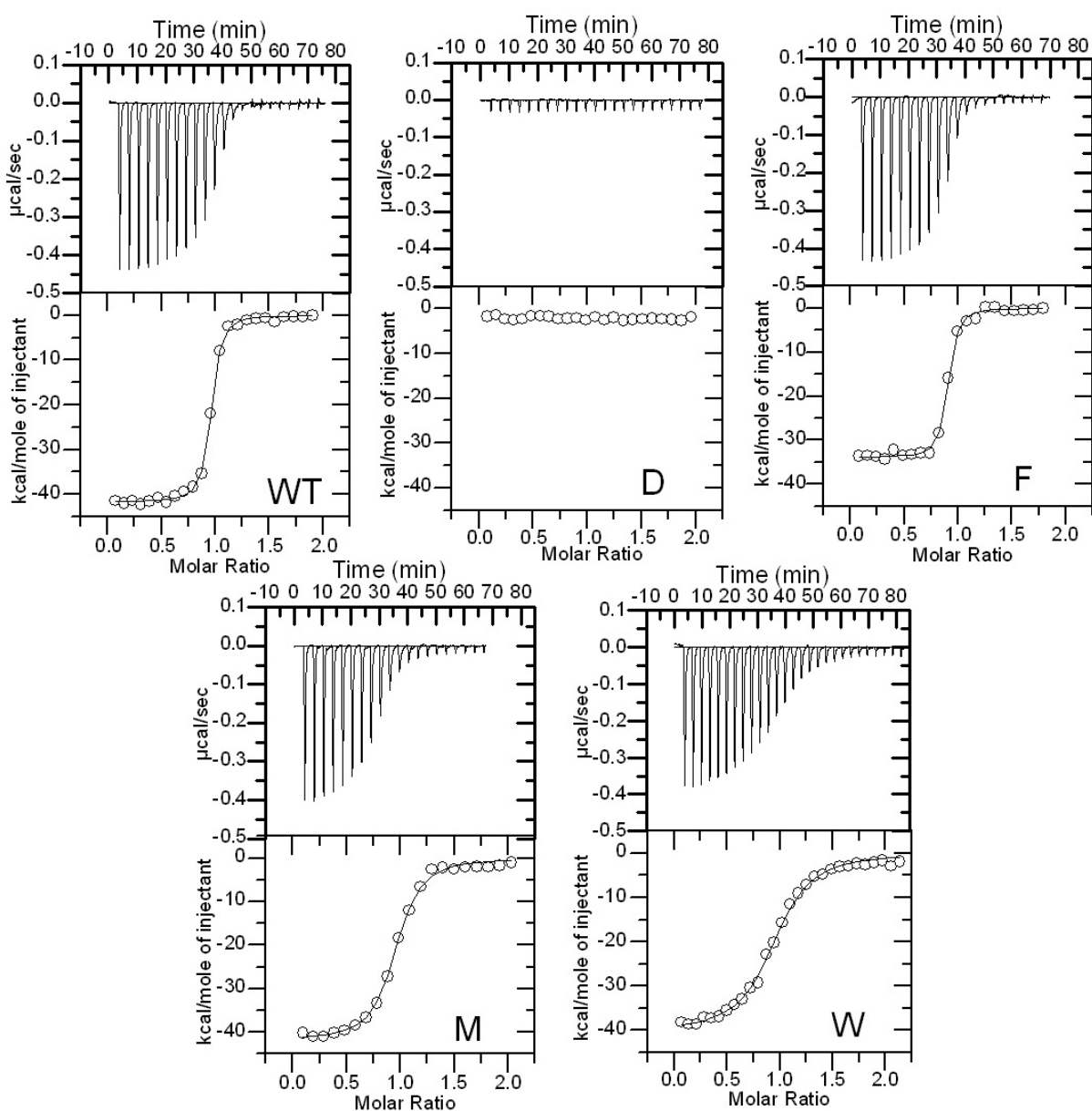


Fig. 3. ITC Measurements. Recombinant protein samples of β I-C, α II-N (marked as WT above), α II-N-V22D (D), α II-N-V22F (F), α II-N-V22M (M) and α II-N-V22W (W) were dialyzed together in 5 mM phosphate buffer with 150 mM sodium chloride at pH 7.4 and degassed thoroughly prior to ITC measurements. α II-N proteins (29 - 35 μ M) were each individually titrated into the sample cell containing β I-C protein (3 μ M). Typical ITC titration isotherms and fitted curves are shown. The average K_d values ($n = 3$), determined from the fitted curves using a single-binding site model of the manufacturer (MicroCal) software, were 6.9 nM for β I-C/ α II-N and 6.7 nM for β I-C/ α II-N-V22F, 35 nM for β I-C/ α II-N-V22M and 93 nM for β I-C/ α II-N-V22W. Little heat was released for β I-C/ α II-N-V22D titration and no K_d was obtained.

Tab. 1 Y2H and ITC results of alpha and beta spectrin model proteins interaction.

pBD Plasmid^a	Colony Growth^b	β-Galactosidase Activity^c	K_d (nM)^d
α II-N	Yes	Blue color	6.9
α II-N-V22D	No	No color	not detectable
α II-N-V22F	Yes	Blue color	6.7
α II-N-V22M	Yes	Blue color	35
α II-N-V22W	Yes	Blue color	93

^apAD- β II-C with different pBD- α II-N plasmids in the Y2H experiment; we also used pAD- β I-C with pBD- α II-N or pBD- α II-N-V22D, and the results were the same as those with pAD- β II-C;

^byeast AH109 cells were grown in a medium containing all essential amino acids but tryptophan, leucine and histidine, and lacking adenine; ^cyeast Y187 cells were grown in medium containing all essential amino acids but leucine and tryptophan for the colony lift assay; ^dITC experiments using recombinant proteins of α II-N and mutants listed and of β I-C were carried out at 25 °C in 5 mM phosphate buffer with 150 mM sodium chloride at pH 7.4.

DISCUSSION

The Y2H systems have been widely used to study protein-protein interactions. In this study, both colony growth and β -galactosidase activity detection results showed that α II spectrin with mutations V22F, V22M or V22W interacted with β II spectrin at the tetramerization site (N-terminal region of α II and C-terminal region of β II). However, α II-N-V22D did not interact with β II-C. With those α II mutants that interacted with β II-C, we were not able to detect any differences in interactions between V22F, V22M or V22W with β II-C. Both colony growth rate and colony size, as well as the blue color indication for β -galactosidase activity did not show detectable differences between V22, V22F, V22M and V22W.

The ITC methods require not only the preparation of recombinant proteins but also the characterization of these proteins for proper functional analysis. In our systems, we characterized the protein systems with high resolution mass spectrometry analysis as well as by circular dichroism analysis. We have found that, for both α and β spectrin recombinant proteins used for tetramerization studies, it is important to obtain their CD results to demonstrate that the proteins are folded properly before ITC experiments. The ITC results show that the K_d values for V22F and the wild type with β I-C were about the same, with a K_d of about 7 nM. However, V22M and V22W both exhibited lower affinity than the wild type, with K_d values of 35 nM and 93 nM, respectively. The ITC results of V22D titration with β I-C showed little interaction, with K_d values larger than 100 μ M. As indicated in **METHODS**, we have found that β I-C and β II-C proteins exhibit similar affinities for α II-N [15], and in this study we showed that results similar to those of β II-C were obtained when β I-C was used with α II-N wild type or with V22D.

Spectrin tetramer formation involves the bundling of three helices, one from α (Helix C') and two from β (Helix A' and Helix B'), forming a triple helical bundle [15, 21]. Mutations that affect the triple helical bundling lead to lower affinity. Previous studies reveal that the V22 position of α II is critical for its tetramerization with β II [1]. Sequence alignment shows that α II V22 corresponds to V31 in erythroid α spectrin (α I). α I V31 has

been identified as a hot spot that leads to severe clinical symptoms [22]. In triple helical bundling of α II and β II helices, an N-terminal hydrophobic cluster [18] involves three residues in the α II Helix C' (I15, V22, and L23) and two residues in the β II Helix A' (V2019 and F2022), and one residue in the β II Helix B' (F2073) [15]. Thus, it is not surprising that mutations at the V22 position may affect non-erythroid spectrin tetramer formation. Since V22 is involved in a hydrophobic cluster during helical bundling to form tetramers, a mutation from V to a charged residue D clearly weakens the hydrophobic cluster and thus severely reduces the ability of V22D to interact with Helices A' and B' in β II-C. Mutation of V22 to other hydrophobic residues such as V22F did not affect its interaction with β II-C. The mutations of V22M and V22W lowered the affinity by about 5 times and 10 times, respectively. Hydrophobicity of individual side chains, and the properties of the interacting clusters also affected the triple helical bundling. The K_d values determined by ITC represented a ΔG value of about -46.6 kJ/mol (-11.1 kcal/mol) for β I-C with either α II-N or α II-N-V22F, -42.6 kJ/mol (-10.1 kcal/mol) with α II-N-V22M, and -40.1 kJ/mol (-9.6 kcal/mol) with α II-N-V22W. Thus, the tetramers of these α II mutants and β spectrin exhibit slightly differing stabilities from each other. As discussed previously [18], α II spectrin has recently been reported to be essential for stabilizing nascent sodium channel clusters [23], assembling the mature node of Ranvier [23], and regulating endothelial cell-cell contacts [24]. The tetramer formation of α II- β II spectrin is also essential in the regulatory step for neuritogenesis [25]. Tetramerization is clearly important for spectrin function. At present, no clinical mutations in α II spectrin, including the tetramerization region, have been identified. A reduced level of spectrin tetramers and abnormal spectrin-based membrane skeleton could cause abnormal neural activities in cells.

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REFERENCES

1. Sumandea, C. A. and Fung, L. W.-M. Mutational effects at the tetramerization site of nonerythroid alpha spectrin. **Mol. Brain Res.** 136 (2005) 81-90.
2. Fields, S. and Song, O. A novel genetic system to detect protein-protein interactions. **Nature** 340 (1989) 245-246.
3. Fields, S. Interactive learning: Lessons from two hybrids over two decades. **Proteomics** 9 (2009) 5209-5213.
4. Hu, X., Kang, S., Chen, X., Shoemaker, C. B. and Jin, M. M. Yeast surface two-hybrid for quantitative in vivo detection of protein-protein interactions via the secretory pathway. **J. Biol. Chem.** 284 (2009) 16369-16376.
5. Oh, Y., and Fung, L. W.-M. Brain proteins interacting with the tetramerization region of non-erythroid alpha spectrin. **Cell. Mol. Biol. Lett.** 12 (2007) 604-620.

6. Humphrey, J. S., Salim, A., Erdos, M. R., Collins, F. S., Brody, L. C., and Klausner, R. D. Human *BRCA1* inhibits growth in yeast: Potential use in diagnostic testing. **Proc. Natl. Acad. Sci. U. S. A** 94 (1997) 5820-5825.
7. Estojak, J., Brent, R. and Golemis, E. A. Correlation of two-hybrid affinity data with in vitro measurements. **Mol. Cell. Biol.** 15 (1995) 5820-5829.
8. Jabbour, A. M., Puryer, M. A., Yu, J. Y., Lithgow, T., Riffkin, C. D., Ashley, D. M., Vaux, D. L., Ekert, P. G., and Hawkins, C. J. Human Bcl-2 cannot directly inhibit the *Caenorhabditis elegans* Apaf-1 homologue CED-4, but can interact with EGL-1. **J. Cell Sci.** 119 (2006) 2572-2582.
9. Coyne, R. S., McDonald, H. B., Edgemon, K., and Brody, L. C. Functional characterization of BRCA1 sequence variants using a yeast small colony phenotype assay. **Cancer Biol. Ther.** 3 (2004) 453-457.
10. Stavolone, L., Herzog, E., Leclerc, D. and Hohn, T. Tetramerization is a conserved feature of the virion-associated protein in plant pararetroviruses. **J. Virol.** 75 (2001) 7739-7743.
11. Ma, L.-Y., King, G., and Rothfield, L. Mapping the MinE site involved in interaction with the MinD division site selection protein of *Escherichia coli*. **J. Bacteriol.** 185 (2003) 4948-4955.
12. Larin, D., Mekios, C., Das, K., Ross, B., Yang, A.-S., and Gilliam, T. C. Characterization of the interaction between the Wilson and Menkes disease proteins and the cytoplasmic copper chaperone, HAH1p. **J. Biol. Chem.** 274 (1999) 28497-28504.
13. Grootjans, J. J., Reekmans, G., Ceulemans, H. and David, G. Syntenin-Syndecan binding requires syndecan-syntenin and the co-operation of both PDZ domain of syntenin. **J. Biol. Chem.** 275 (2000) 19933-19941.
14. Crowther, L. J., Yamagata, A., Craig, L., Tainer, J. A., and Donnenberg, M. S. The ATPase activity of BfpD is greatly enhanced by zinc and allosteric interactions with other Bfp proteins. **J. Biol. Chem.** 280 (2005) 24839-24848.
15. Mehboob, S., Song, Y., Witek, M., Long, F., Santarsiero, B.D., Johnson, M. E. and Fung, L. W.-M. Crystal structure of the nonerythroid α -spectrin tetramerization site reveals differences between erythroid and nonerythroid spectrin tetramer formation. **J. Biol. Chem.** 285 (2010) 14572-14587.
16. Kang, J., Song, Y., Sevinc, A., Fung, L.W.-M. Important residue (G46) in erythroid spectrin tetramer formation. **Cell Mol. Biol. Lett.** 15 (2010) 46-54.
17. Lam, V.Q., Antoniou, C., Rolius, R., Fung, L.W. Association studies of erythroid alpha-spectrin at the tetramerization site. **Br. J. Haematol.** 147 (2009) 392-395.
18. Li, Q. and Fung, L. W.-M. Structural and dynamic study of the tetramerization region of non-erythroid α -spectrin: a frayed helix revealed by site-directed spin labeling electron paramagnetic resonance. **Biochemistry** 48 (2009) 206-215.
19. Mehboob, S., Jacob, J., May, M., Kotula, L., Thiyagarajan, P., Johnson, M. E. and Fung, L. W.-M. Structural analysis of the α N-terminal region of erythroid and nonerythroid spectrins by small-angle X-ray scattering. **Biochemistry** 42 (2003) 14702-14710.
20. Mehboob, S., Luo, B.-H., Fu, W., Johnson, M. E. and Fung, L. W.-M. Conformational studies of the tetramerization site of human erythroid spectrin by

- cysteine-scanning spin-labeling EPR methods. **Biochemistry** 44 (2005) 15898-15905.
21. Mehboob, S., Luo, B.-H., Patel, B. M. and Fung, L. W.-M. $\alpha\beta$ spectrin coiled coil association at the tetramerization site. **Biochemistry** 40 (2001) 12457-12464.
 22. Lecomte, M.C., Garbarz, M., Gautero, H., Bournier, O., Galand, C., Boivin, P., Dhermy, D. Molecular basis of clinical and morphological heterogeneity in hereditary elliptocytosis (HE) with spectrin alpha I variant. **Br. J. Haematol.** 85 (1993) 584-595.
 23. Voas, M.G., Lyons, D.A., Naylor, S.G., Arana, N., Rasband, M.N., and Talbot, W.S. α II-Spectrin is essential for assembly of the nodes of Ranvier in myelinated axons. **Curr. Biol.** 17 (2007) 562-568.
 24. Benz, P.M., Blume, C., Moebius, J., Oschatz, C., Schuh, K., Sickmann, A., Walter, U., Feller, S.M., and Renne, T. Cytoskeleton assembly at endothelial cell-cell contacts is regulated by α II-spectrin-VASP complexes. **J. Cell Biol.** 180 (2008) 205-219.
 25. Bignone, P.A., King, M.D., Pinder, J.C., and Baines, A.J. Phosphorylation of a threonine unique to the short C-terminal isoform of β II-spectrin links regulation of α - β -spectrin interaction to neuritogenesis. **J. Biol. Chem.** 282 (2007) 888-896.