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Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C NMR backbone assignments of the N-terminal region of human erythrocyte alpha spectrin including one structural domain

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Biological context

Human erythrocyte spectrin is a major constituent of the red blood cell skeletal protein network. Spectrin is thought to be responsible for the remarkable elasticity and stability of the erythrocyte membrane, and is composed of α and β subunits. Each subunit is mainly composed of multiple homologous sequence motifs, each consisting of about 106 amino acid residues in a triple helical bundle conformation. α and β subunits interact laterally in an antiparallel manner with high affinity (K_D of ∼10 nM) to form an αβ heterodimer. The heterodimer associates with the N-terminal region of the α subunit (Nα region) and the C-terminal region of the β subunit (Cβ region), producing the physiologically relevant heterotetramer $(\alpha\beta)_2$. Sequence homology has shown that the Nα region consists of a partial domain of about 30 residues prior to the first complete domain, with a structure similar to helix C (the third helix) in the triple helical bundle domain, and that the Cβ region also has a partial domain that resembles helices A and B (the first and second helices). It has been suggested that, in the tetramerization process, these regions interact together to form a final structure resembling the triple helical bundle domain.

Mutations in these regions lead to altered spectrin tetramerization and eventual weakening of the erythrocyte membrane (Lux and Palek, 1995; Hassoun and Palek, 1996; Gallagher, 1998). Although the 3D structure of a single domain for *Drosophila* spectrin was reported using X-ray crystallography (Yan et al., 1993) and for chicken brain spectrin by NMR spectroscopy (Pascual et al., 1997), the structure of the Nα region or the domain junction region is not yet available, and our understanding of the properties of this key region in spectrin is still limited. Thus, we have undertaken a multidimensional heteronuclear NMR study of a recombinant α-spectrin peptide, consisting of the first 156 residues of human erythrocyte α-spectrin; we report here the assignment of backbone ^{1}H, ^{15}N and ^{13}C resonances of this peptide.

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Methods and results

An expression vector of an α-spectrin fragment containing the first 156 residues (Spα 1–156) was prepared as previously reported (Lusitani et al., 1994; Menhart et al., 1996). The protein was expressed as a glutathione S-transferase (GST) fusion protein in DH5α *E. coli* (Pharmacia Biotech) under *taq* promoter control. The transformed *E. coli* was grown in M9 based minimal media. ^{15}N-, ^{15}N/^{13}C- and ^{2}H/^{15}N/^{13}C-labeled protein samples were prepared using correspondingly labeled ammonium chloride (> 99% ^{15}N), glucose (> 99% U-^{13}C), and deuterium oxide (> 99.9% ^{2}H). The fusion protein was purified by glutathione affinity chromatography. The desired spectrin peptide was cleaved from the fusion protein using immobilized thrombin (CalBiochem). The NMR sample contained 5 mM phosphate buffer (pH 6.5), 150 mM NaCl, 0.01% NaN_3, 1 mM protein in 95% H_2O/5% D_2O. A relatively high salt concentration and low temperature (20 °C) were needed to

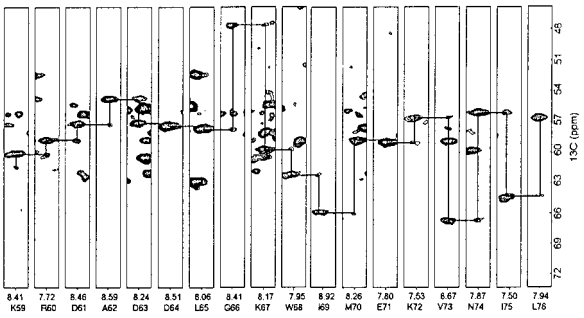

Figure 1. HNCA strip plot showing the sequential assignment from K59 to L76. Each strip corresponds to the ^{15}N plane of the indicated residues. All Cα(i−1) correlation peaks were confirmed using HN(CO)CA, and the assignments were verified with other spectra as stated in the text.

prevent sample precipitation over an extended period of time. NMR spectra were acquired on Bruker DRX-600 or DMX-600 spectrometers equipped with a triple resonance, triple axis gradient probe. All data were processed and analyzed using Felix 97.0 (MSI).

Resonance assignments were obtained by use of ^{1}H,^{15}N-HSQC, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HN(CA)CO, HNCO, HN(CA)H, HBHA(CO)NH, ^{1}H,^{15}N-NOESY-HSQC, ^{1}H,^{15}N-TOCSY-HSQC (Bax and Grzesiek, 1993; Cavanagh et al., 1996). Most of the triple resonance experiments were recorded using deuterated samples. Attempts to obtain the sequential assignment using the usual approaches based on Cα and Cβ resonance dispersion left many ambiguities due to the lack of β sheet structure. The high degeneracy in amino acid composition (e.g. 25 Glu, 11 Gln, 14 Lys, and 11 Arg) also made the approach rather unsuccessful. To resolve these problems, we acquired HN(CA)CO and ^{1}H,^{15}N-NOESY-HSQC with deuterated samples and found them very useful since CO chemical shifts were largely uncorrelated with Cα, and the apparent highly helical conformation gave d_{NN} peaks for many residues. The final assignments are demonstrated in Figure 1 as a strip plot for residues K59 to L76.

The CSI analysis for Cα and CO suggests a 20 residue-long random coil at the start of the peptide, and a helix spanning the A21–R45 region. The rest of the molecule has three helices corresponding to the triple helical bundle domain of *Drosophila* (Yan et al., 1993) or chicken brain spectrin (Pascual et al., 1997). However, our NMR data indicate that the connecting region between the partial domain and the first complete domain has a random coil conformation, as opposed to a long helix as reported for the two-domain peptide of chicken brain α spectrin using X-ray crystallography (Grum, et al., 1999). A detailed study on this region is being pursued and will be reported elsewhere.

Extent of assignments and data deposition

H_N and ^{15}N for 149 out of 153 (156 less 3 prolines) possible amide resonances were assigned (97.4%). ^{13}Cα and ^{13}CO resonances were assigned for all of the above residues and the 3 prolines using HNCA, HN(CO)CA, HN(CA)CO and HNCO. For ^{1}Hα, about 91% of all residues were assigned. The signals for the rest of the residues were either invisible or ambiguous in assignment. The chemical shift values of proton, carbon and nitrogen have been deposited in the BioMagResBank database (accession number: BMRB-4393).

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