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NMR assignments for acid-denatured cold shock protein A

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Biological context

Our group has been investigating the extent to which residual structure is conserved in the denatured forms of proteins that share similar native-state folds. We have focused on three non-homologous proteins from the OB-fold superfamily (Murzin, 1993): staphylococcal nuclease (SN) (Alexandrescu et al., 1996; Wang and Shortle, 1996); cold shock protein A (CspA) (Chatterjee et al., 1993); and the anticodon-binding domain of lysyl t-RNA synthetase (LysN) (Commans et al., 1995). Here we report NMR assignments for the acid-denatured form of CspA, a transcriptional regulator induced when *E. coli* are grown at low temperatures (Chatterjee et al., 1993). The NMR assignments provide a basis for characterizing the mechanism by which the acid-denatured protein forms fibrils.

Methods and results

^{15}N and ^{15}N/^{13}C labeled samples of *E. coli* CspA were prepared according to a published method (Chatterjee et al., 1993), with the modification that cells were grown in MOPS (Serpersu et al., 1986) rather than M9-Casamino acid medium. Protein samples were dissolved in 90% H_2O/10% D_2O, and contained no added buffers or salts. NMR data were collected at 20 °C on a Varian Unity+ 600 MHz spectrometer. Assignments for acid-denatured CspA were based on 3D TOCSY-HSQC (65 ms mixing time), 3D NOESY-HSQC (200 ms mixing time), and 3D HNCACB experiments (Kay, 1995). The 3D HNCACB experiment, which correlates ^{1}H-^{15}N spin pairs with the C^α and C^β chemical shifts of the same and of the preceding residue, is extremely powerful for assigning denatured proteins. In contrast to N resonances, C^α and C^β resonances of denatured proteins closely approximate the chemical shifts of free amino acid monomers. Residue types such as Ala, Gly, Ile, Leu, Ser, Thr and Val could be identified solely on the basis of C^α and C^β chemical shifts. Together, these types of amino acids account for 53% of the 70-residue protein. 3D ^{1}H-^{15}N TOCSY-HSQC data were used to extend ^{1}H-^{15}N assignments to H^α and side-chain protons. 3D ^{1}H-^{15}N NOESY-HSQC data provided additional (i,i+1) dNN, dαN, and dβN sequential NOE connectivity pathways.

Aqueous solutions of acid-denatured CspA become viscous with time, eventually forming clear gels. Electron microscopy demonstrates that the acid-denatured protein self-assembles into fibril-like polymers (A.T.A., M. Häner and U. Aebi, in preparation). Each NMR experiment was performed on a fresh sample of CspA. Provided that the time of incubation at pH 2 is short compared to that for polymerization, refolding of the acid-denatured protein is reversible based on HSQC spectra of refolded and freshly prepared protein at pH 6. The time required for polymerization is highly concentration dependent: 5 mM protein samples gel in about 8 h, 1 mM samples in about one month. 3D TOCSY-HSQC and NOESY-HSQC data sets were collected on 1 mM samples of the acid-denatured protein. At this concentration, peak heights decay to about 25% of initial values during the 35 to 40 h time period of data collection. In an attempt to optimize sensitivity, a 3D HNCACB experiment was collected on a 2 mM protein sample. Peak heights for the C-terminal half of the protein (residues 40 to 70) decayed to about 20% during the course of the 44 h experiment; this portion of the molecule was readily assigned. Signals from the first 40 residues decayed close to baseline noise by the end of the experiment.

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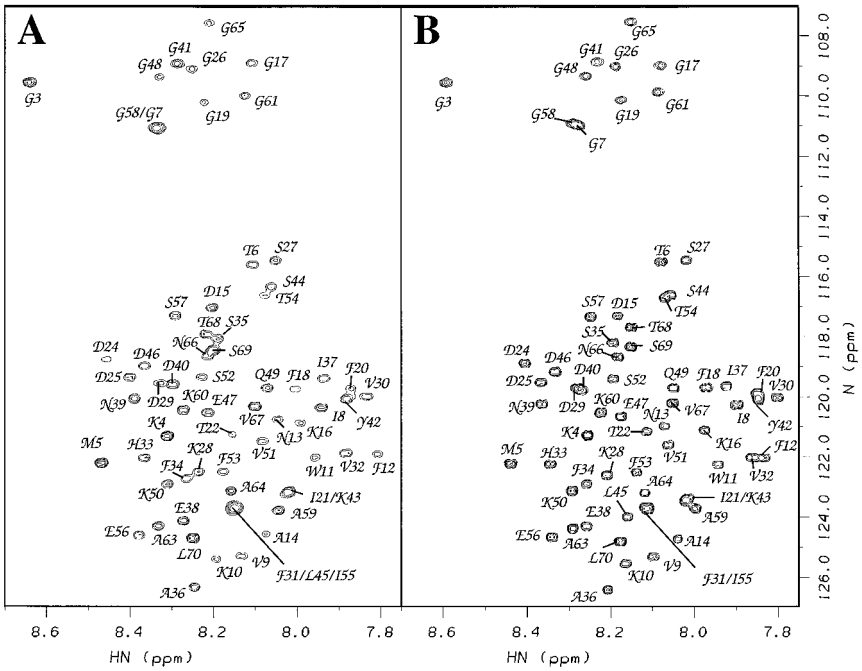

Figure 1. ^{1}H-^{15}N HSQC spectra (Kay, 1995) for fresh 1 mM samples of (A) acid-denatured (pH 2.0), and (B) acid/urea-denatured (pH 2.7, 6M urea) CspA at 20 °C. Residues 5–13, 18–23, 30–33, 50–56 and 63–69 which make up the five strands of β-sheet in the native protein (Schindelin et al., 1994), are selectively broadened with increasing protein concentrations. The first three β-strands show the largest effect. In the presence of urea (B), peaks are uniformly sharp as evidenced by the resolved $^{3}J_{HNH\alpha}$ splittings in the F2 dimension.

To facilitate NMR assignments we made avail of the fact that aggregation of the acid-denatured protein is suppressed in 6M urea. The ^{1}H-^{15}N resonances of the acid-, and acid/urea-denatured proteins are in fast exchange so that a titration in 1M urea increments enabled us to correlate chemical shifts between the two sets of conditions (Figure 1). We then took advantage of the excellent 3D HNCACB data for the protein under strongly denaturing conditions (pH 2.7, 6M urea), to aid in the interpretation of 3D HNCACB data obtained under milder denaturing conditions (pH 2.0). Similar strategies might be of general use in studies of denatured proteins, when NMR spectra are complicated by line broadening due to aggregation or chemical exchange.

Extent of assignments and data deposition

Backbone ^{1}HN, $^{1}H^{\alpha}$, ^{15}N assignments were obtained for all residues except Met[1], Ser[2], Pro[23], and Pro[62]. C^{α} assignments were obtained for 96% of residues; C^{β}, H^{β}, and other side chain proton assignments for 95%, 92%, 48% of residues, respectively. Assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession numbers 4107 and 4108.

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