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Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N backbone assignments and secondary structure for the 60.8 kD dimer of the NAD^{+} synthetase from *Bacillus subtilis*

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Biological context

Nicotinamide adenine dinucleotide (NAD^{+}) is essential in living systems. NAD^{+} synthetase catalyzes the final step in the synthesis of NAD^{+}, common to both the *de novo* and salvage pathways, the conversion of nicotinic acid adenine dinucleotide into NAD^{+}. Prokaryotic and eukaryotic NAD^{+} synthetases are strikingly different. The bacterial form requires ammonia as the nitrogen source while the human form can use glutamine or ammonia (Nessi et al., 1995). The human form has two distinct domains while the bacterial form has only one. In the conserved domain, there is only 17% sequence identity between the *Bacillus subtilis* and *Homo sapiens* forms. These differences suggest it may be possible to specifically target the bacterial enzyme to develop a new class of antibiotic compounds.

The structure of *B. subtilis* NAD^{+} synthetase has been solved by X-ray crystallography alone and in the presence of different ligands (Rizzi et al., 1996; Devedjiev et al., 2001). The protein chain contains 272 amino acids and forms a stable dimer. Two loops (containing 6 and 22 residues) do not give sufficient electron density to be placed in the structure of the protein alone but can be traced in the complex with ATP. Assignments of the enzyme have been undertaken to provide residue-specific probes for changes in the presence of substrates, product, and other ligands to better understand the interactions in solution.

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Methods and experiments

B. subtilis NAD^{+} synthetase was cloned into plasmid pET28a (Novagen) and expressed in *E. coli* strain BL21(λDE3). ^{2}H-, ^{13}C-, ^{15}N-labelled protein was produced in minimal medium with $^{15}NH_4Cl$, ^{13}C,^{2}H-glucose, and $^{2}H_2O$ (Cambridge Isotope Labs). Cells were lysed with a microfluidizer. Lysate was cleared by centrifugation then flowed through a POROS HS column. NAD^{+} synthetase was further purified by chromatography on successive columns including POROS HQ, POROS PI hydroxyapatite (BioRad) and a Toyopearl phenyl column followed by a G3000 silica (TosoHaas) sizing column. The sizing column and light scattering experiments confirm that the protein exists as a dimer in solution. The final yield was 18 mg per liter of cells. To exchange amide ^{2}H with ^{1}H, NAD^{+} synthetase was incubated at 40 °C for 24 h in 50 mM HEPES, 200 mM NaCl, pH 7.5. For NMR spectroscopy, the protein was transferred to 50 mM deuterated HEPES, 100 mM NaCl, 20 mM $MgCl_2$, pH 6.50, using an Ultrafree-4 unit with a NMWCO of 10 kD (Millipore). The NMR sample was 1 mM in NAD^{+} synthetase at 300 μl in a microcell (Shigemi, Inc.).

Spectra were recorded at 35 °C on a Bruker DRX 600 spectrometer, equipped with a triple resonance probe with x, y, and z gradients. TROSY versions of experiments, including HNCO, HN(CA)CO, HNCA, HN(CO)CA, HNCACB, and HN(CO)CACB were recorded (Salzmann et al., 1998, 1999) A 3D, ^{15}N separated TROSY-NOESY was also recorded with a mixing time of 150 ms. Spectra were processed us-

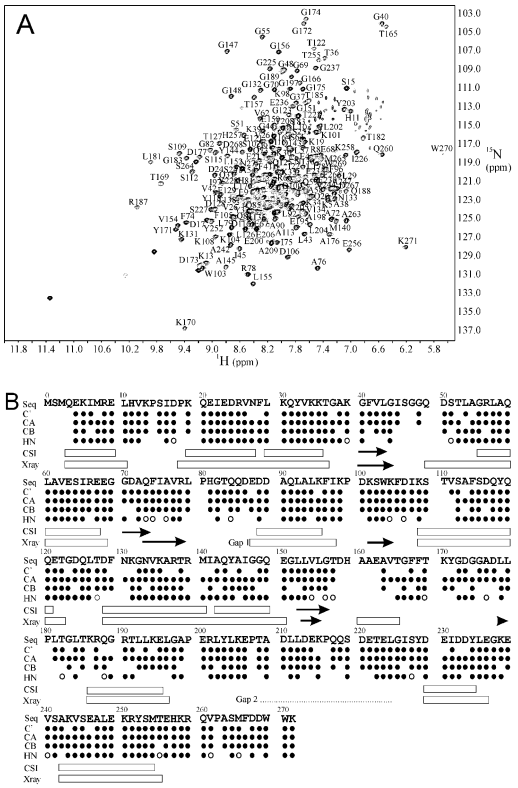

Figure 1. (A) 2D ^{1}H-^{15}N TROSY spectrum, recorded on ^{2}H ^{13}C ^{15}N NAD^{+} synthetase. (B) Summary of the assignments and secondary structure of NAD^{+} synthetase. In the rows labelled 'C', 'CA' and 'CB', a dark circle occurs if a sequential connection can be made through the C′, Cα, and Cβ carbon chemical shifts, respectively. In the row labelled 'HN', a circle appears when there is an NOE from the amide proton of the current residue to the amide proton of the preceding residue in the 3D ^{15}N separated TROSY-NOESY spectrum, with dark circles indicating strong NOE crosspeaks and open circles indicating weaker crosspeaks. The row labelled 'CSI' depicts the secondary structure based on the chemical shift index while the row labelled 'Xray' shows the secondary structure based on the crystal structure of NAD^{+} synthetase alone, determined using Quanta (MSI). In both rows, rectangles indicate helices and arrows indicate strands.

ing nmrPipe and nmrDraw (Delaglio et al., 1995) and analyzed using PIPP (Garrett et al., 1991).

Extent of assignments and data deposition

Bacillus subtilis NAD^{+} synthetase gives good quality spectra, as demonstrated by the 2D ^{15}N TROSY spectrum shown in Figure 1A. Assignments are more than 95% complete, with gaps for the first three residues (Met 0 to Met 2), Ser 46 to Gly 47, Gln 49, Thr 110, Asp 158 to Glu 162, and Lys 186, as summarized in Figure 1B, numbering with the initiator methionine as 0 to agree with the crystal structures.

Interestingly, the loops that do not give rise to clear electron density in the crystal structure of the protein alone (Rizzi et al., 1996) are readily assigned (residues 82–87 and 204–225, see Figure 1B). Furthermore, some of the residues in the longer loop give rise to two sets of peaks in the spectra. Thr 208-Lys 215 and Gln 217 all show two sets of peaks. Since there are two prolines nearby (at 207 and 216), conformational heterogeneity may be due to cis-trans isomerization of one or both of the peptide bonds preceding the proline residues, as seen for other proteins (Gitti et al., 1996; Markus et al., 1997).

From the carbon chemical shifts, the regions of regular secondary structure can be identified using the chemical shift index (Wishart and Sykes, 1994). The results are compared to the secondary structure as determined from the pdb file of NAD^{+}synthetase alone (Rizzi et al., 1996) in Figure 1B. In general, the secondary structure predicted from the carbon chemical shifts agrees well with that observed in the crystal structure.

The chemical shift assignments reported here have been deposited in the BioMagResBank with accession number BMRB-5854.

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