Journal of Biomolecular NMR **29:** 209–210, 2004.

Letter to the Editor: Backbone ^{1}H, ^{15}N and ^{13}C resonance assignments of α-ADT and β-ADT

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Received 16 September 2003; Accepted 28 November 2003

Key words: chaperonin, NMR-assignments, thermosome

Biological context

Chaperonins are large, barrel-like, double-ring assemblies, which play an essential role in the recognition and ATP-dependent refolding of misfolded substrates, e.g., upon heat shock. Whereas the mechanism of action of the bacterial GroEL/GroES complex as a representative of the so called Group I chaperonins is well studied, detailed information about the archaeal/eukaryotic Group II chaperonins has been gained only recently. The archaeal 930 kDa hexadecameric chaperonin – usually referred to as 'thermosome' – is composed of two homologous alternating subunits α and β, each forming three distinct domains connected by flexible hinges: An equatorial or ATPase domain, an intermediate and an apical or substrate binding domain. So far, the only information about the different mechanistic states in the reaction cycle of the thermosome was obtained from cryo-electron tomography and X-ray crystallography. These studies showed the thermosome in an open (Klumpp et al., 1997; Nitsch et al., 1998) and a closed conformation (Ditzel et al., 1998). Additionally, helical segments protruding from the apical domains were found to act as an eight-membered, central intersubunit β-barrel. This iris-type aperture was suggested to control access to and exit from the folding cavity. Recently, the crystal structures of the α- and the β- apical domain of the thermosome (ADT) were solved as isolated 17 kDa constructs consisting of 153 residues, of which 101 are conserved (Klumpp et al., 1997; Bosch et al., 2000). Large hydrophobic patches in the protrusions might play some role in substrate binding. However, no natural substrates have been identified yet. As in crystalline environment, these sections are strongly subject to intermolecular interactions, we have applied multi dimensional NMR techniques to study both proteins in solution. Here we report the backbone ^{1}H, ^{15}N and ^{13}C resonance assignments of α-ADT and β-ADT.

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Methods and experiments

Expression of ^{15}N or ^{15}N, ^{13}C isotopically enriched α-ADT and β-ADT protein was performed as described (Heller et al., *JMB*, in press). Briefly *E. coli* BL21-CodonPlus^{TM}(DE3)-RIL cells (Stratagene) containing the plasmid coding for either α-ADT or β-ADT were grown in Martek9-medium. Cells were cultured at 30 °C and pH 7.0 to OD_{600} of 0.7. This expression was induced by adding 1 mM IPTG. After harvesting, cell lysis and centrifugation, the clarified supernatant was purified on a NiNTA-affinity-column (Quiagen) at 4 °C. Protein containing fractions were desalted on a Sephadex G-25 column (Amersham Lifesciences) and applied to a HighQ-support-column (Bio-Rad) at pH 8.0. The desired proteins were further purified on a Superdex-75 column (Amersham Lifesciences) using 25 mM sodium phosphate, 100 mM NaCl with pH adjusted to 5.5 for α-ADT- and to 7.8 for β-ADT-preparations. Samples for NMR experiments contained 1 mM protein in the respective buffer in 90% H_2O/10% D_2O. TSP (3-(Trimethylsilyl)-propionic acid sodium salt) in the same solvent was

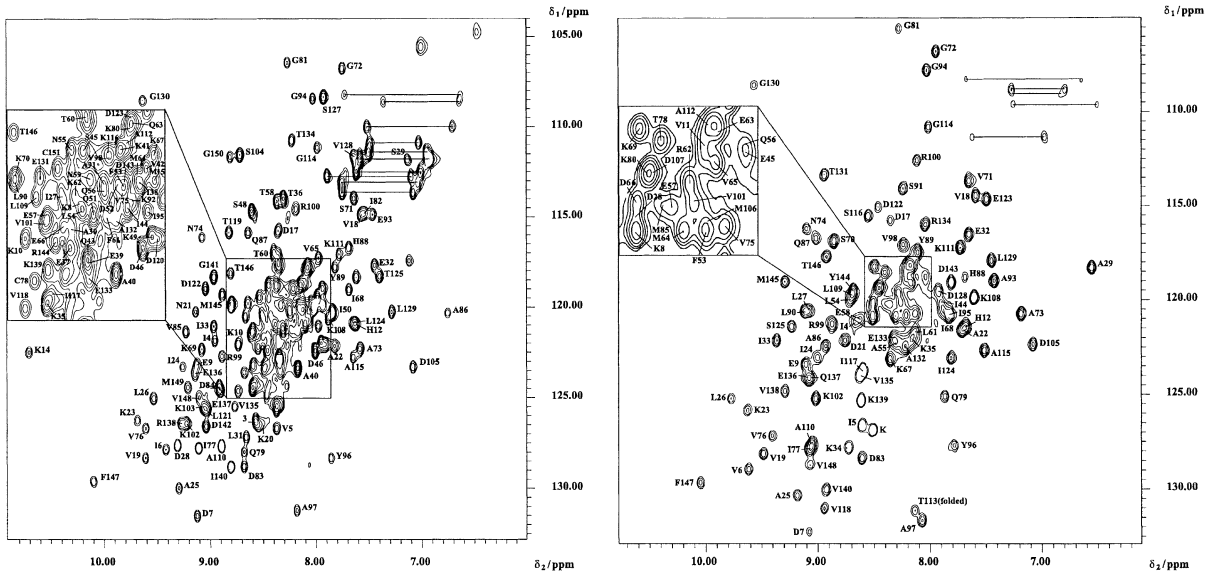

Figure 1. Annotated ^{1}H-^{15}N-HSQC spectra of α-ADT (left hand panel) and β-ADT (right hand panel), both recorded at 315 K and at a proton frequency of 600 MHz. The regions of strong overlap are expanded for the purpose of clarity. The amide side-chain resonances of asparagine and glutamine residues are connected by horizontal lines.

used as an external chemical shift standard (Wishart et al., 1995). All NMR experiments were performed at 315 K on Bruker AVANCE DMX-600 and DMX-750 spectrometers equipped with 5 mm TXI ($^{1}H/^{13}C/^{15}N$) triple-axis-gradient probes. Backbone sequential connectivities were obtained using the following standard 3D experiments (Sattler et al., 1999 and references cited therein): HNCO, H(CA)CO, HNCA, HNCACB, CBCA(CO)NH, HNHA, HNHB, HBHA(CBCACO)NH. The 3D (H)CCH-COSY and (H)CCH-TOCSY experiments helped to complete the C^{β} chemical shift information and amino acid identification. The spectra were processed and analyzed using XWINNMR 2.6, AURELIA 2.8.4 (both Bruker) and PASTA TOOLKIT V1.0 (Leutner et al., 1998).

Extent of assignments and data deposition

For α-ADT 142 of the 150 non-proline H^{N} and ^{15}N backbone resonances could be assigned as shown in Figure 1A. However, in the $^{1}H,^{15}N$-HSQC (Figure 1B) of the β-counterpart only 110 of 148 non-proline residues were observed due to fast exchange for highly solvent exposed H^{N} protons at pH 7.8 as it occurs in the termini, loops and at the beginnings of α-helices. H^{α}/C^{α}-resolved experiments to complete the assignment of the remaining residues failed due to strong signal overlap. The assignments have been deposited in the BioMag-ResBank (http://www.bmrb.wisc.edu) under BMRB accession numbers 5930 (α-ADT) and 5936 (β-ADT).

Acknowledgements

This work was supported by Deutsche Forschungsgemeinschaft (SFB 456). We thank Tammo Diercks for acquisition of spectra of β-ADT.

References

Bosch, G., Baumeister, W. and Essen, L.-O. (2000) *J. Mol. Biol.*, **301**, 19–25.

Ditzel, L., Loewe, J., Stock, D., Stetter, K.-O., Huber, H., Huber, R. and Steinbacher, S. (1998) *Cell*, **93**, 125–138.

Klumpp, M., Baumeister, W. and Essen, L.-O. (1997) *Cell*, **91**, 263–270.

Leutner, M., Gschwind, R.M., Liermann, J., Schwarz, C., Gemmecker, G. and Kessler, H. (1998) *J. Biomol. NMR*, **11**, 31–43.

Nitsch, M., Walz, J., Typke, D., Klumpp, M., Essen, L.-O. and Baumeister, W. (1998) *Nat. Struct. Biol.*, **5**, 855–857.

Sattler, M., Schleucher, J. and Griesinger, C. (1999) *Prog. NMR Spectrosc.*, **34**, 93–158.

Wishart, D.S., Bigam, C.G., Yao, J., Abildgaard, F., Dyson, H.J., Oldfield, E., Markley, J.L. and Sykes, B.D. (1995) *J. Biomol. NMR*, **6**, 135–140.