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Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N assignments and secondary structure of the high pH form of subunit c of the F_1F_o ATP synthase

Vinit K. Rastogi & Mark E. Girvin*
Biochemistry Department, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, U.S.A.

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Biological context

The F_1F_o ATP synthases use a transmembrane H^+-gradient to drive the synthesis of ATP from ADP and P_i during oxidative or photo-phosphorylation. Subunit c, and specifically its buried DCCD-reactive essential carboxyl residue Asp^{61}, are directly involved in H^+ transport. Changes in the ionization state of Asp^{61} of subunit c initiate the conformational changes leading to the release of ATP product at the catalytic sites of the enzyme. In order to understand the conformational changes leading to catalysis, we are studying the structure and dynamics of subunit c in the two protonation states of the Asp^{61} side chain. The high resolution structure of the Asp^{61}-protonated form (pH 5.0) of subunit c was solved recently (Girvin et al., 1998), and shows that the protein folds as a pair of interacting α-helices connected by a short structured loop. Here, we present the sequence-specific assignments and secondary structure of the high pH form (pH 8.0) of subunit c.

Methods and results

Uniformly isotopically labeled (^{15}N- or ^{13}C, ^{15}N-labeled) protein was prepared from an overproducing strain of *E. coli* as described earlier (Girvin and Fillingame, 1995). NMR samples were ∼2.0 mM ^{15}N- or $^{13}C^{15}N$-subunit c in 675 μl of 4:4:1 $CDCl_3$:CD_3OH:H_2O, 25 mM d_{11}-Tris, pH 8.0. The pH and sample stability were monitored by recording and comparing $^{1}H^{15}N$-HSQC spectra before and after flame sealing the NMR tube, and also intermittently between 3D NMR experiments.

All NMR experiments were performed at 300 K on a Bruker DRX-600 spectrometer. All experiments made use of pulsed field gradients for coherence selection and artifact suppression (Bax and Pochapsky, 1992), and utilized gradient sensitivity enhancement schemes wherever appropriate (Kay et al., 1992).

The following NMR experiments were recorded using the $^{13}C^{15}N$-labeled sample which aided in resonance assignments: $^{1}H^{15}N$-HSQC, CT-$^{1}H^{13}C$-HSQC, HNCO, $^{1}H^{15}N$ TOCSY-HSQC, H(CCO)NH, C(CO)NH, HNCACB, HCCH-COSY, HCCH-TOCSY, ^{1}H-TOCSY-ct-($^{13}C^{1}H$)-HMQC, $^{1}H^{15}N$ HMQC-NOESY-HSQC and $^{1}H^{15}N$ NOESY-HSQC (Bax and Grzesiek, 1993; Cavanagh et al., 1996). H^N-H^α coupling constants were determined from an HNHA experiment (Kuboniwa et al., 1994) on a uniformly ^{15}N-enriched sample.

Sequential resonance assignments were achieved through analysis of four 3D experiments: TOCSY-HSQC, H(CCO)NH, C(CO)NH and HNCACB. The first step was to group spin systems consisting of $^{1}HN(i)$, $^{15}N(i)$, $^{1}H_\alpha(i)$, $^{13}C_\alpha(i)$ and $^{13}C_\beta(i)$ making use of $^{1}H^{15}N$ TOCSY-HSQC and HNCACB experiments and $^{1}H_\alpha(i-1)$, $^{13}C_\alpha(i-1)$ and $^{13}C_\beta(i-1)$ making use of H(CCO)NH and C(CO)NH experiments, respectively. These spin systems were linked to each other by matching their respective $^{1}HN(i)$, $^{15}N(i)$ resonances. Degeneracies in $^{1}H^{13}C$ chemical shifts (e.g., for the 13 Ala, 12 Leu, 10 Gly and 8 Ile residues) and degeneracies in the sequence (e.g., Asp^7-Leu^8 and Asp^{44}-Leu^{45}, Ala^{14}-Val^{15} and Ala^{67}-Val^{68}, etc.) created some obstacles to this straightforward assignment procedure. The ambiguities arising due to these de-

*To whom correspondence should be addressed.

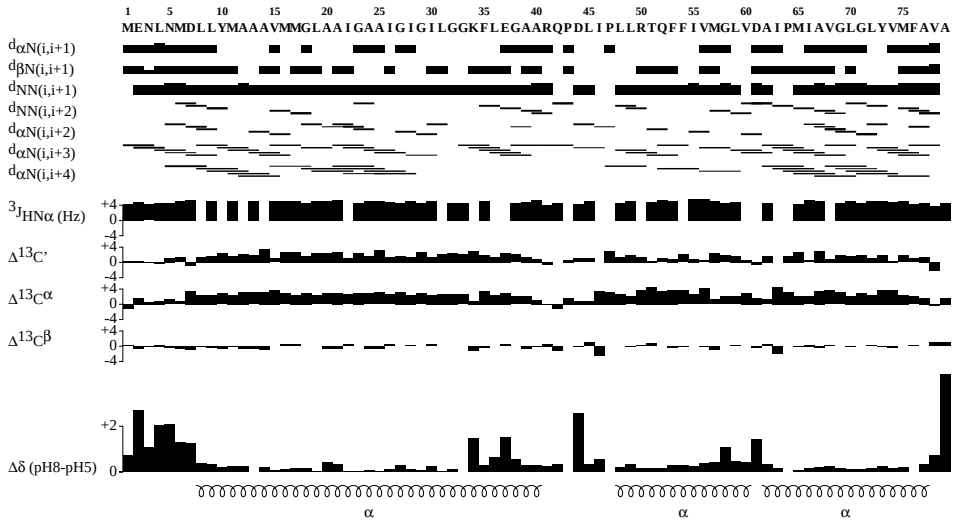

Figure 1. Backbone NOEs, $^3J_{HN\alpha}$ coupling constants, and secondary shifts of $^{13}C'$, $^{13}C_\alpha$, and $^{13}C_\beta$ for the high pH form of subunit c. A comparison of backbone chemical shifts at pH 8 with those at pH 5 is also shown. The changes in chemical shift for each backbone atom were normalized to the maximal change for that atom type (2.78 ppm for $^{13}C'$, 5.75 ppm for ^{15}N, 0.80 ppm for $^1H^N$, 2.37 ppm for $^{13}C_\alpha$, and 0.11 ppm for $^1H_\alpha$), and the absolute values of the changes were summed for each residue. Secondary structural elements for subunit c at pH 8 are depicted at the bottom.

generacies were easily resolved with the analysis of a $^1H^{15}N$ HMQC-NOESY-HSQC experiment and also by extending the assignment further along the protein sequence. The side-chain assignments were obtained from an HCCH-COSY experiment which also helped confirm the previous assignments. Aromatic side chain spin systems were assigned using a 3D ^{1}H-TOCSY-ct-($^{13}C^1$H)-HMQC (Zerbe et al., 1996).

Secondary structural elements were identified on the basis of chemical shifts, H^N-H^α coupling constants and medium range NOEs (Figure 1). From an examination of the deviations in the chemical shifts of $^{13}C'$ and $^{13}C_\alpha$ with respect to their random coil values, a predominance of α-helix all along the sequence was predicted. This was substantiated by the small H^N-H^α coupling constants (< 6 Hz) along the sequence and also by the NOE pattern. These helical segments correspond to those observed in the low pH form of the protein. Comparison of the backbone chemical shifts with those of the protein at pH 5, however, suggests that the protein undergoes some major conformational changes along the loop and around the Asp^{61} residue (Figure 1) as expected based on the models proposed earlier (Fillingame, 1990). The details of these conformational changes will emerge from determination of the complete structure of the protein at pH 8, and from studies of backbone dynamics at pH 5, 7, and 8.

Extent of assignments and data deposition

All ^{1}H, ^{15}N, and ^{13}C backbone resonances and most side chain resonances of subunit c at pH 8.0 have been assigned. Sequence-specific assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) database, accession number 4151.

References

Bax, A. and Grzesiek, S. (1993) *Acc. Chem. Res.*, **26**, 131–138.

Bax, A. and Pochapsky, S.S. (1992) *J. Magn. Reson.*, **99**, 638–643.

Cavanagh, J., Fairbrother, W.J., Palmer, A.G.I. and Skelton, N.J. (1996) *Protein NMR Spectroscopy: Principles and Practice*, Academic Press, San Diego, CA.

Fillingame, R.H. (1990) in *The Bacteria*. Vol. XII (Ed., Krulwich, T.A.), Academic Press, New York, NY, pp. 345–391.

Girvin, M.E. and Fillingame, R.H. (1995) *Biochemistry*, **34**, 1635–1645.

Girvin, M.E., Rastogi, V.K., Abildgaard, F., Markley, J.L. and Fillingame, R.H. (1998) *Biochemistry*, **37**, 8817–8824.

Kay, L.E., Keifer, P. and Saarinen, T. (1992) *J. Am. Chem. Soc.*, **114**, 10663–10665.

Kuboniwa, H., Grzesiek, S., Delaglio, F. and Bax, A. (1994) *J. Biomol. NMR*, **4**, 871–878.

Zerbe, O., Szyperski, T., Ottiger, M. and Wüthrich, K. (1996) *J. Biomol. NMR*, **7**, 99–106.