Biomol NMR Assign (2012) 6:27–29
DOI 10.1007/s12104-011-9318-1

ARTICLE

^{1}H, ^{13}C and ^{15}N resonance assignments of human muscle acylphosphatase

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Received: 5 March 2011 / Accepted: 23 May 2011 / Published online: 5 June 2011

Abstract Human muscle acylphosphatase (mAcP) is an enzyme with a ferrodoxin-like topology whose primary role is to hydrolyze the carboxyl-phosphate bonds of acylphosphates. The protein has been widely used as a model system for elucidating the molecular determinants of protein folding and misfolding. We present here the full NMR assignments of the backbone and side chains resonances of mAcP complexed with phosphate, thus providing an important resource for future solution-state NMR spectroscopic studies of the structure and dynamics of this protein in the contexts of protein folding and misfolding.

Keywords Human muscle acylphosphatase · Ferrodoxin-like fold · Hydrolase · Protein folding and misfolding

Biological context

Human muscle acylphosphatase (mAcP) is a small (~11 kDa) enzyme that specifically catalyzes the hydrolysis of the carboxyl-phosphate bond of acylphosphates (Stefani et al. 1997). mAcP has a ferredoxin-like topology, consisting of five β-strands (S1–S5) arranged in a single β-sheet and two α-helices (H1 and H2), and is produced in a wide variety of species and tissues. In the past two decades mAcP has attracted the interest of the biophysical community because of its functional relevance and also for the large number of studies on this protein that have contributed significantly to our understanding of the principles of protein folding (Chiti et al. 1999) and misfolding (Chiti et al. 2000, 2003). Indeed mAcP has proved to be an appropriate system to probe the generic mechanisms that result in normally soluble and stable proteins aggregating into insoluble thread-like amyloid fibrils (Chiti et al. 2000, 2003). These processes are of very considerable importance as they are linked to a group of fatal neurodegenerative disorders, including Alzheimer's and Parkinson's disease, as well as non-neuropathic diseases, including systemic amyloidoses and type II diabetes (Chiti and Dobson 2006). Despite this interest in folding and misfolding of mAcP, assignments of its NMR resonances have not previously been reported. The present work therefore opens to high-resolution NMR investigations designed to reveal the atomic details of the various steps in the protein folding and misfolding processes of mAcP that have been identified for this system by other biophysical techniques. The mAcP native structure analysed here is complexed with phosphate.

Methods and experiments

Sample preparation

Purification of mAcP was performed as described previously (Modesti et al. 1993). ^{1}H, ^{15}N, ^{13}C labelled protein

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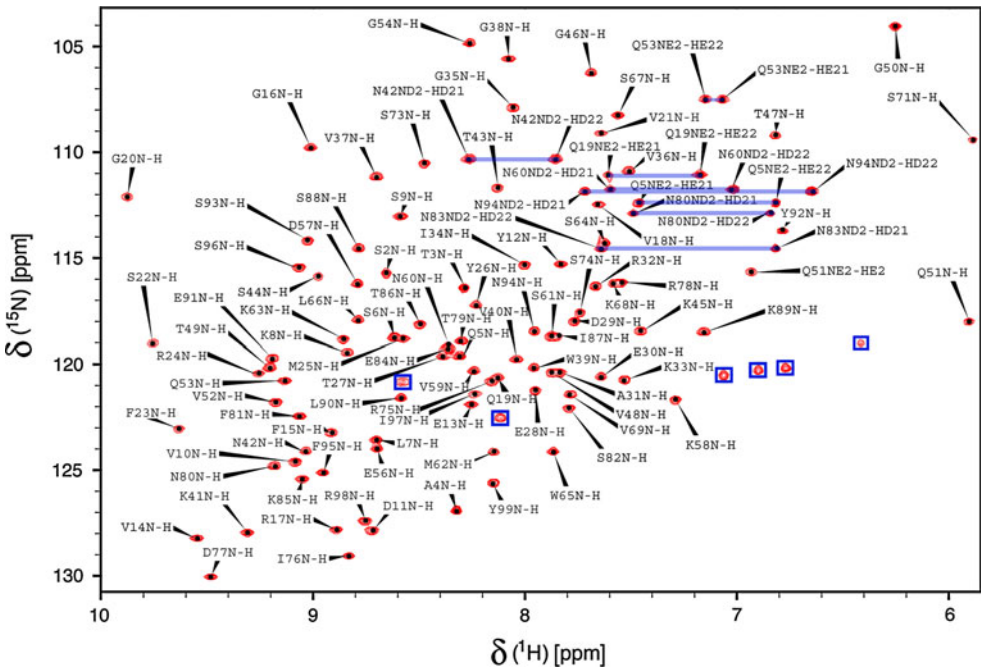

Fig. 1 Assigned 2D [^{15}N–^{1}H] HSQC spectrum of phosphate bound mAcP recorded at 25°C and at a ^{1}H frequency of 700 MHz. Aliased resonances from the six arginine side-chains are evidenced by blue squares. Paired amino side-chains NH correlations are linked by horizontal blue lines

was expressed as a GST fusion protein in the *E. coli* strain BL21-Gold(DE3) (Invitrogen), grown in minimal medium by using ^{15}N- enriched ammonium chloride and ^{13}C-enriched glucose, and purified using a glutathione column (Sigma–Aldrich). The GST/mAcP fusion protein was then cleaved with thrombin from human plasma (Sigma–Aldrich) in TRIS buffer. The eluted mAcP was then buffer exchanged into 30 mM ammonium carbonate buffer at pH 5.5 and then lyophilized. The percentage of ^{15}N and ^{13}C in the protein sample was checked by mass spectrometry, resulting in 96% for both the isotope types.

NMR spectroscopy

The resonance assignments have been defined by using a computer-aided procedure as described previously (Hsu and Dobson 2009). NMR data were recorded on ^{13}C/^{15}N labelled mAcP samples (250 μM protein buffered in 50 mM sodium phosphate at pH 5.5) at 25°C by using Bruker AVANCE spectrometers (500 and 700 MHz), all of which were equipped with cryogenic triple resonance probes.

[^{15}N–^{1}H] HSQC, HNCA, CBCA(CO)NH, HNCACB, HNCO, HN(CA)CO and HNHA spectra were recorded for $^{1}H^{\alpha}$,$^{13}C^{\alpha}$,$^{13}C^{\beta}$,^{1}HN,^{13}CO and ^{15}N assignments. In addition, a 3D ^{15}N-edited NOESY- HSQC spectrum was recorded at 25°C and at 700 MHz to assist resonance assignments in conjunction with visual inspection from a homology modelling structural model derived by using as template the X-ray structure of the human common type acylphosphatase (Yeung et al. 2006) (PDB code: 2VH7, 56% of sequence identity with mAcP). For side-chains: aliphatic side-chains assignments were determined by a combination of (H)C(CO)NH-TOCSY and H(CCO)NH-TOCSY spectra while aromatic side-chains assignments were obtained from 2D CBHD and CBHE experiments in conjunction with NOESY spectra. All NMR data were processed and analysed by TopSpin (Bruker BioSpin), NMRPipe and Sparky software packages (Fig. 1).

Extent of assignment, preliminary NMR analyses and data deposition

95 ^{1}H–^{15}N correlations were assigned, covering the entire protein (99 residues) except for those of proline residues (P55 and P72), the N-terminal residue and residue G70, which is solvent exposed in a loop region connecting helix H2 and strand S4 according to our homology model. $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and ^{13}CO resonances were assigned for 97, 97, 91 and 97 residues, respectively. We assigned resonances for all the expected side-chains, with an overall completeness of 93% of side-chains atoms.

From the analysis of the chemical shifts values of the $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$,^{1} HN, ^{13}CO and ^{15}N resonances we could estimate the backbone flexibility by means of the random coil index (RCI) (http://wishart.biology.ualberta.ca/rci/) (Berjanskii and Wishart 2007). The analysis indicates that the highest degree of flexibility in mAcP is exhibited in loop regions and in the N-terminal segment that precedes the strand S1. Notably, the small edge-strand S5 presents RCI values that are indicative of flexibility comparable

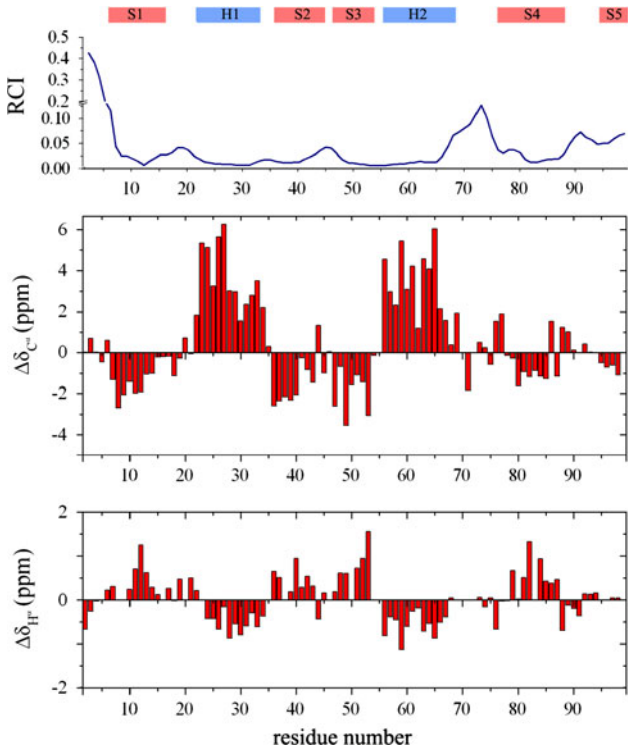

Fig. 2 Random Coil Index (RCI, http://wishart.biology.ualberta.ca/rci/) (Berjanskii and Wishart 2007) and Secondary chemical shifts of phosphate bound mAcP. The secondary chemical shifts are derived by subtracting the random coil shifts from the observed ones ($\Delta\delta = \delta_{\text{observed}} - \delta_{\text{random coil}}$). The sequence-dependent random coil shift values are calculated using CamCoil (De Simone et al. 2009) (http://www-vendruscolo.ch.cam.ac.uk/camcoil.php). The top of the diagram indicates the fragments of α-helices (*blue*) and β-strands (*red*), estimated from the homology model structure by using the program DSSP

with loop regions. α-helix and β-strands regions were estimated from secondary shifts by subtracting the random coil chemical shift values, estimated from the sequence by using the CamCoil method (De Simone et al. 2009) (http://www-vendruscolo.ch.cam.ac.uk/camcoil.php), from the measured chemical shifts of the native state mAcP. The secondary-shifts result in agreement with the predicted secondary structure elements from the homology modelling. The assignments have been deposited in the BMRB under the accession number 17505 (Fig. 2).

Acknowledgments This work was supported by grants from the MRC (to C.M.D.), EPSRC (to A.D.S. and C.M.D.), Marie Curie (to F.B.), HSFP (to S.T.H.) Italian Ministero dell'Istruzione dell'Università e della Ricerca (to F.C.), National Science Council of the Republic of China—Taiwan (to S.T.H.), BBSRC (to C.M.D. and M.V.) and the Royal Society (to M.V.).

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