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# Letter to the Editor: $^{1}H$, $^{13}C$, and $^{15}N$ resonance assignments of the phosphorylated enzyme IIB of the mannitol-specific phosphoenolpyruvate-dependent phosphotransferase system of *Escherichia coli*

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## Biological context

Enzyme $IIB^{mtl}$ is a catalytic domain of the mannitol-specific phosphoenolpyruvate dependent phosphotransferase system (PTS) of *E. coli*. The PTS consists of three catalytic proteins: enzyme I (EI) and the histidine-containing protein (HPr) are general PTS proteins, the enzyme II complex (EII) is carbohydrate-specific. After autophosphorylation of EI from PEP, the phosphoryl group is transferred to HPr, accepted by $IIA^{mtl}$ and finally transferred to C384 in the $IIB^{mtl}$ domain. The phosphorylation of $IIB^{mtl}$ triggers the rapid transport of the target carbohydrate by $IIC^{mtl}$ (Lolkema et al., 1991a, b) and its subsequent phosphorylation.

We present here the nearly complete assignment of the backbone and side-chain resonances for the phosphorylated C384S mutant protein.

## Methods and experiments

In order to circumvent stability problems due to oxidation of the active-site cysteine a mutant (C31S) was constructed. Note that residue 31 in our construct corresponds to residue 384 in the full-length $EII^{mtl}$. The construct was ligated into the pET-15b vector (Stratagene) and the *E. coli* strain BL21(DE3) with the plasmid was grown in M9 minimal medium containing 1 g $l^{-1}$ $(^{15}NH_4)_2SO_4$ (Campro Scientific) and 2 g $l^{-1}$ $^{13}C$-glucose (Cambridge Isotope Laboratories). Cell cultures were grown at 37 °C and induced with IPTG for 2 h. Cells were harvested by centrifugation, resuspended in 20 mM Tris–HCl, pH 8.0, 1 mM EDTA, 150 mM NaCl and 2 mM DTT and disrupted by French press. Cell debris were sedimented by ultracentrifugation and the supernatant solution was purified using a Ni-NTA agarose column (Qiagen). After washing out unbound proteins the $(His)_6$-tagged $IIB^{mtl}$ was eluted with 20 mM Tris–HCl, pH 8, 1 mM $NaN_3$, 1 mM DTT, 500 mM NaCl and 250 mM imidazole. To all fractions 1 mM EDTA was added and fractions containing the protein were dialysed against 20 mM Tris–HCl, pH 7, 1 mM EDTA, 1 mM DTT and 1 mM $NaN_3$.

The protein was phosphorylated by incubating the protein solution with HPr (6 μM), EI (0.5 μM), $IIA^{mtl}$ (3 μM), 5 mM $MgCl_2$ and 5 mM PEP for 3 h at room temperature. The reaction mixture was passed through a Q-sepharose fast flow column (Amersham Biosciences), equilibrated with 20 mM Tris–HCl, pH 7, 1 mM $NaN_3$ and 1 mM DTT. The column was washed with equilibration buffer containing 100 mM NaCl to fully recover the phosphorylated $IIB^{mtl}$.

Fractions containing the protein were pooled and the $(His)_6$-tag was cleaved by adding thrombin (bovine, 1821 U $mg^{-1}$, ICN biomedicals Inc.) (1:80 w/w of thrombin/protein). The $(His)_6$-tag was removed by passage over a Ni-NTA agarose

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column. After cleavage of the $(His)_6$-tag a few residues (G18-M21) remain attached to the protein of interest. Finally the protein was dialyzed against 20 mM Tris–acetate, pH 6.2, 1 mM EDTA, 1 mM $NaN_3$ and 3 mM DTT. A ~ 2 mM NMR sample was prepared using a 3 kDa limit concentrator (Millipore).

NMR measurements were performed at 20 °C on a Varian Unity INOVA 600 MHz spectrometer equipped with a pulsed field-gradient probe. All chemical shifts are relative to DSS (Wishart et al., 1995). 2D [$^{15}$N,$^{1}$H]-CPMG-HSQC (Mulder et al., 1996), $^{15}$N-edited NOESY-HSQC, 3D HNCA/HN(CO)CA, 3D HNCO/HN(CA)CO and 3D HNCACB/CBCA(CO)NH experiments (Sattler et al., 1999) were used to obtain the sequential backbone resonance assignment. Side-chain assignments were achieved with the 3D HBHA(CBCA)(CO)NH, 3D COCAH, 3D HC(C)H-TOCSY (mixing time 18 ms) and CβCγCδCεHε/CβCγCδHδ (Yamazaki et al., 1993) experiments.

All spectra were processed using an in-house developed NMR-package (Klaas Dijkstra, University of Groningen) for Python (Python Software Foundation, 2004) and analyzed using the program Sparky (Goddard and Kneller).

## Extent of assignments and data deposition

A nearly complete (> 97%) backbone assignment was obtained (Figure 1). Missing backbone assignments are $^{15}$N and $^{1}$H for all proline residues and some N-terminal residues (G18, S19, H20, M21 and H23). The carbon backbone resonances for residues G18, S19, H20 and P68 were tentatively assigned from the COCAH and HC(C)H-TOCSY experiments. Approximately 80% of the side-chain $^{1}$H and $^{13}$C resonances could be assigned. Unassigned side-chain resonances include the εCH3 groups of Met residues, Nε/Hε of Arg residues and side-chain amide groups of Arg and Lys residues. Due to overlap in the aliphatic regions the complete side-chain assignments for some Lys and Ile residues was not possible. $^{13}$C resonances of the aromatic rings were not assigned, $^{1}$H resonances were only partially assigned.

The determination of the secondary structure elements on the basis of $^{1}$Hα, $^{13}$Cα, $^{13}$C′ and $^{13}$Cβ chemical shifts was achieved using the Chemical Shift Index (Wishart and Sykes, 1994). The analysis of short range NOEs supported these findings: IIB$^{mtl}$ consists of three helices (residues 36–51, 81–87 and 102–115) and four β-strands (residues 25–31, 57–63, 72–76 and 93–98). The $^{1}$H, $^{13}$C and $^{15}$N chemical shifts have been deposited in the BioMagResBank (accession number 6267).

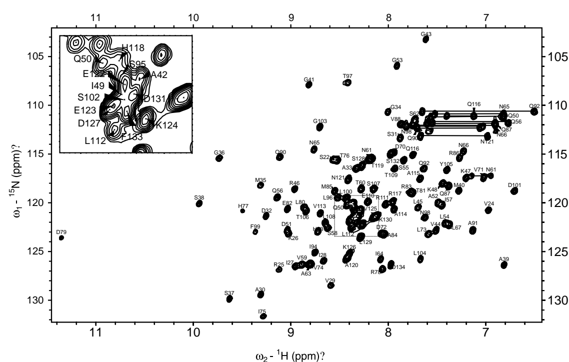


*Figure 1.* 2D [$^{15}$N,$^{1}$H]-cpmg-HSQC spectrum of uniformly $^{13}$C/$^{15}$N-labelled P-IIB$^{mtl}$. All peaks are annotated with the one-letter amino acid symbol and their position in the sequence. To convert to the residue number in the complete EII$^{mtl}$ sequence an offset of 353 must be added to the number of each residue in our construct. Cross peaks connected by a horizontal line correspond to side-chain $NH_2$ groups of Q and N residues.

## References

Goddard, T.D. and Kneller, D.G. *Sparky 3*, University of California, San Francisco.

Lolkema, J.S., Ten Hoeve-Duurkens, R.H., Swaving Dijkstra, D. and Robillard, G.T. (1991a) *Biochemistry*, **30**, 6716–6721.

Lolkema, J.S., Swaving Dijkstra, D., Ten Hoeve-Duurkens, R.H. and Robillard, G.T. (1991b) *Biochemistry*, **30**, 6721–6726.

Mulder, F.A.A., Spronk, C.A.E.M., Slijper, M., Kaptein, R. and Boelens, R. (1996) *J. Biomol. NMR*, **8**, 223–228.

Python Software Foundation (2004) *www.python.org*

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

Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR*, **4**, 171–180.

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.

Yamazaki, T. Forman-Kay, J.D. and Kay, L.E. (1993) *J. Am. Chem. Soc.*, **115**, 11054–11055.