# Letter to the Editor: Backbone resonance assignment of the 298 amino acid catalytic domain of protein tyrosine phosphatase 1B (PTP1B)

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## Biological context

Protein activation via tyrosine phosphorylation by tyrosine kinases and the concomitant deactivation/dephosphorylation by protein tyrosine phosphatases (PTPs) plays a central role in cellular signaling. Tyrosine phosphorylation regulates cell homeostasis by the control of gene-transcription, cell-cycle progression, cell growth, cell metabolism, immune response as well as programmed cell death (Walton et al., 1993; Zhang, 2002). Due to the low level of cellular phosphotyrosine these pathways and the respective enzymes were identified only rather recently: the first tyrosine kinase src by Hunter et al. (1980), the first protein tyrosine phosphatases by Nelson and Branton (1984). Receptor tyrosine kinases include the insulin receptor and a large variety of other growth factor receptors. Examples of PTPs include cdc25A responsible for cell cycle progression, CD45 activating B- and T-cells, the Vaccinia virus minimal variant VH1, YOP 2b required for the virulence of the pathogenic *Yersinia* genus of bacteria, and PTP1B, which is a potential target for diabetes and obesity treatment.

PTP1B can dephosphorylate a number of tyrosine kinases *in vitro* and in cell culture at the endoplasmic reticulum (ER) (Haj et al., 2002). *In vivo* specificity of this phosphatase for insulin receptor dephosphorylation was suggested by the finding that the active site mutant C215S copurifies with insulin receptors from eucaryotic cell lines (Seely et al., 1996; Bandyopadhyay et al., 1997). Association with the activated insulin receptor is mediated by three tyrosine residues in the catalytic domain that become phosphorylated upon insulin treatment (Bandyopadhyay et al., 1997). The importance of PTP1B in the regulation of glucose metabolism was underlined by the finding that homozygous PTP1B knockout in mice leads to strongly increased blood glucose clearance and hypersensitivity to insulin (Elchebly et al., 1999). Additionally, homo- and heterozygous knockouts proved to decrease weight gain by 50% on high fat diet. For these reasons, PTP1B appears as an attractive target for specific inhibitors that would counteract type II diabetes and obesity.

Full length human PTP1B consists of 435 residues. It encompasses the highly conserved 298 amino acid, N-terminal catalytic domain (PTP1B^cat), a proline rich domain and a C-terminal hydrophobic anchor that targets the protein to the membrane of the ER (Frangioni et al., 1992; Haj et al., 2002). The crystal structure of the PTP1B^cat construct 1-321 (Barford et al., 1994) shows that this domain consists of 12 β-strands and 8 α-helices. The catalytic residue C215 is located near the surface in a crevice between β12 and α4. Here we report the NMR resonance assignments of the conserved 298 amino acid PTP1B^cat domain in complex with the peptidic Novartis inhibitor CGS35385.

## Methods and experiments

Human PTP-1B (1-298) was cloned from a human hippocampal cDNA library (Clonetech) using PCR and placed into a pET 19-b vector (Novagen). Re-

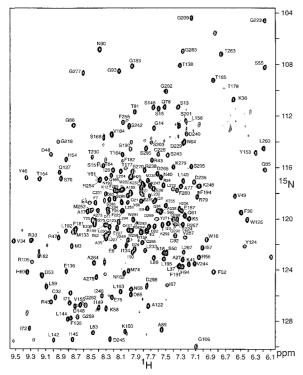*To whom correspondence should be addressed. E-mail: Stephan.Grzesiek@unibas.ch

*Figure 1.* $^{1}$H-$^{15}$N TROSY spectrum of PTP1Bcat at 293 K.

combinant, uniformly $^{2}$H ($\sim$ 60%), $^{15}$N ($>$ 95%), $^{13}$C ($>$ 95%) labeled PTP1B$^{cat}$ was expressed in *E. coli* strain BL21 (DE3), and the soluble protein was purified by cation followed by anion exchange chromatography. A 500 $\mu$l NMR sample was prepared containing 0.5 mM PTP1B$^{cat}$, 1 mM peptidic inhibitor CGS35385 (DADEXLIP-amide, X = phenylalanine-p-difluoromethyl phosphonate), 25 mM TRIS-d11, 10 mM DL-1,4-DTT-d10, 0.02% sodium azide, 50 mM NaCl, 95% $H_2O$/5% $D_2O$ at pH 7.5. The NMR tube was flame-sealed under nitrogen. PTP1Bcat tends to aggregate at concentrations higher than 0.5 mM and temperatures above 293 K. Peak dispersion was improved at pH 7.5 relative to pH 6.5. All spectra were recorded on Bruker DRX 600 and 800 spectrometers at 293 K. Assignments were derived from standard and TROSY versions of HN(CO)CA, HN(CA)CO, HN(CO)CACB, HNCACB, HNCA, HNCO, and $^{15}$N-edited NOESY experiments. $^{1}$H, $^{15}$N, and $^{13}$C chemical shifts were referenced relative to the frequency of the $^{2}$H lock resonance of water.

### Extent of assignment and data deposition

The assignments comprise 78% of all $^{1}$HN, $^{15}$N, $^{13}$C', $^{13}$C$^{\alpha}$ and $^{13}$C$^{\beta}$ resonances covering 244 of the 285

non-proline residues. Due to intermediate conformational exchange, almost all of the resonances for the remaining residues were broadened beyond detection in the three-dimensional spectra. A map of the missing assignments onto the crystal structure reveals that most of these residues surround the active site whereas a few others are located in turn and loop regions. Apparently, the active site undergoes conformational exchange despite the presence of the high affinity inhibitor CGS35385 (IC$_{50}$ = 100 nM). Very likely, this apparent plasticity of the active site is linked to the ability of PTP1B to accommodate a large variety of pTyr-containing substrates and the large structural rearrangements of the active site observed upon ligand binding (Zhang, 2002). Despite the difficulty in observing the active site, the assignments include at least 9 key residues (Y46, R47, D48, D181, F182, R254, R257, Q262, Q266) involved in substrate binding and turnover (Zhang, 2002). Chemical shifts have been deposited in the BMR data bank under accession number 5474.

### References

Bandyopadhyay, D., Kusari, A., Kenner, K.A., Liu, F., Chernoff, J., Gustafson, T.A. and Kusari, J. (1997) *J. Biol. Chem.*, **272**, 1639–1645.

Barford, D., Flint, A.J. and Tonks, N.K. (1994) *Science*, **263**, 1397–1404.

Elchebly, M., Payette, P., Michaliszyn, E., Cromlish, W., Collins, S., Loy, A.L., Normandin, D., Cheng, A., Himms-Hagen, J., Chan, C.C., Ramachandran, C., Gresser, M.J., Tremblay, M.L. and Kennedy, B.P. (1999) *Science*, **283**, 1544–1548.

Frangioni, J.V., Beahm, P.H., Shifrin, V., Jost, C.A. and Neel, B.G. (1992) *Cell*, **68**, 545–560.

Haj, F.G., Verveer, P.J., Squire, A., Neel, B.G. and Bastiaens, P.I. (2002) *Science*, **295**, 1708–1711.

Hunter, T. and Sefton, B.M. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 1311–1315.

Nelson, R.L. and Branton, P.E. (1984) *Mol. Cell Biol.*, **4**, 1003–1012.

Seely, B.L., Staubs, P.A., Reichart, D.R., Berhanu, P., Milarski, K.L., Saltiel, A.R., Kusari, J. and Olefsky, J.M. (1996) *Diabetes*, **45**, 1379–1385.

Walton, K.M. and Dixon, J.E. (1993) *Annu. Rev. Biochem.*, **62**, 101–120.

Zhang, Z.Y. (2002) *Annu. Rev. Pharmacol. Toxicol.*, **42**, 209–234.