Journal of Biomolecular NMR **30:** 229–230, 2004.
© 2004 Kluwer Academic Publishers. Printed in the Netherlands.

Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignment of the reduced form of thioredoxin h1 from Poplar, a CPPC active site variant

Nicolas Coudevylle[a], Aurélien Thureau[a], Christine Hemmerlin[a], Eric Gelhaye[b], Jean-Pierre Jacquot[b] & Manh-Thong Cung[a,*]

[a]*Laboratoire de Chimie-Physique Macromoléculaire, UMR 7568 CNRS-INPL, Groupe ENSIC, 1 rue Grandville, B.P. 451, 54001 Nancy Cedex, France;* [b]*Laboratoire Interaction Arbres Microorganismes, UMR 1136 INRA-UHP, Université Henri Poincaré Nancy-1 B.P. 239, 54506 Vandoeuvre Cedex, France*

Received 11 May 2004; Accepted 2 June 2004

Key words: disulfide oxidoreductase, resonance assignment, thioredoxin

Biological context

Thioredoxins belong to a superfamily of oxidoreductase, the function of which is either to create disulfide bonds (protein disulfide isomerases) or reduce disulfides bonds (glutaredoxin, thioredoxin) (Holmgren, 2000). These small molecular weight proteins (ca. 120 amino acids in the mature form for thioredoxin) are generally very stable and excellent catalyst for disulfide reduction. Although thioredoxins can be quite divergent in their primary structure, they seem to be related at the three-dimensional level, with all proteins characterized so far possessing a similar architecture named the thioredoxin fold (Eklund et al., 1984). Genome sequencing has revealed that there are multiple thioredoxin genes in plants (at least 20 in *Arabidopsis thaliana*) (Meyer et al., 2002). Although some of the sequences exhibit unusual mono cysteinic active sites, the vast majority of these sequences conforms to the WCGPC canonic active site. Unlike other organisms however, a significant fraction of the plant proteins exhibits a variant active site with two cysteines and the sequence WCPPC. These CPPC proteins have been studied biochemically in poplar and *Arabidopsis* and found to possess catalytic properties similar to those of the CGPC enzymes. The use of yeast mutants has suggested however that the CPPC enzymes may exhibits specificity in some oxidoreduction reactions that cannot be sustained by the CGPC isoforms (Brehelin et al., 2000). In order to understand the consequence of the G to P mutation on the structure and specificity of thioredoxin, we have overexpressed poplar thioredoxin h1 and purified several isotopically labelled samples. We report here the nearly complete assignments of ^{1}H, ^{13}C, ^{15}N nuclei as well as the particular secondary structures, a step before the full 3D structural resolution.

*To whom correspondence should be addressed. E-mail: Manh-Thong.Cung@ensic.inpl-nancy.fr

Methods and experiments

The coding sequence of thioredoxin h1 from *Populus trichocarpa* was cloned into the expression vector pET-Trxh1 and used to transform the expression strain BL21. ^{15}N and ^{15}N/^{13}C labelled samples were prepared by growing cells at 37 °C in minimal media M9, supplemented with ($^{15}NH_4$)Cl as the sole nitrogen source and with ^{13}C-labelled or not glucose, as the only carbon source. Purification and concentration were made as in Behm and Jacquot (2000). Sample purity and molecular mass (12 444 Da) were checked by SDS-PAGE and electrospray mass spectrometry, respectively.

The NMR sample contained 1.85 mM protein concentration (95% H_2O, 5% D_2O) in 50 mM phosphate buffer at pH 5.9. All spectra were acquired at 298 °K on Bruker DRX 600 MHz spectrometer equipped with a 3-axis TXI probe. Spectra were processed using the program XWINNMR (Bruker) and analysed with the program XEASY (Bartel et al., 1995). Backbone amide $^{1}H^{N}$, $^{1}H^{\alpha}$, ^{15}N, $^{13}C^{\alpha}$, $^{13}C'$ resonances were assigned using ^{1}H-^{15}N HSQC, HNCO, HN(CA)CO, HNCA, HN(CO)CA, CBCANH, CBCA(CO)NH and ^{1}H-^{15}N HSQC-TOCSY experiments, side-chain ^{1}H,

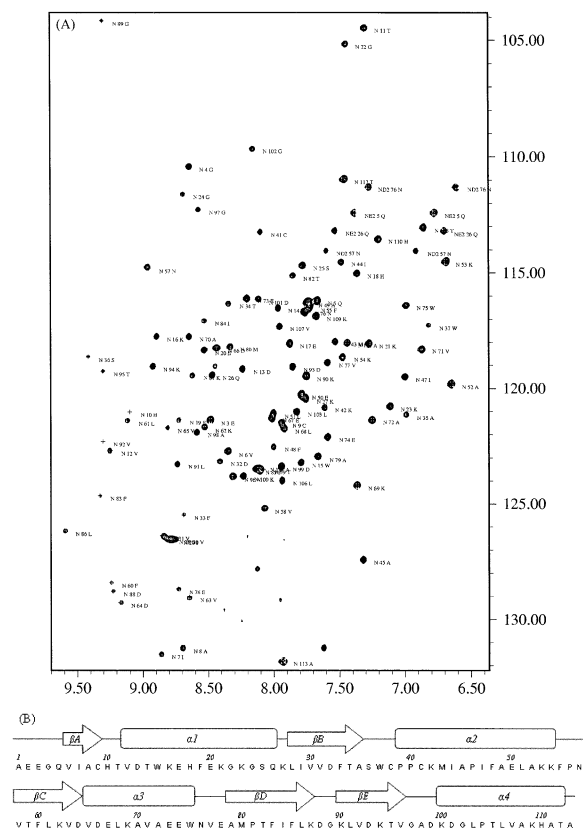

Figure 1. (A) ^{1}H-^{15}N HSQC spectrum of thioredoxin at 298°K. W side chain NH are outside of the region shown. (B) TALOS predicted secondary structures.

^{13}C resonances were assigned using ^{1}H-^{15}N HSQC-TOCSY and HCCH-TOCSY.

Torsions angles (ϕ, ψ) and secondary structure prediction are based on an analysis of the ^{1}H^N, ^{1}H$^\alpha$, ^{15}N, C$^\alpha$, C$'$ and C$^\beta$ chemical shifts using the TALOS program (Corneliscu et al., 1999).

Extent of assignments and data deposition

More than 96% of backbone, H$^\alpha$, H^N, N, C$^\alpha$, C$'$ and side-chain C, H nuclei have been assigned (i.e., 104/107 ^{15}N-H^N sites, 110/113 C$^\alpha$, 109/113 H$^\alpha$, 104/107 C$^\beta$, 109/113 C$'$, and 95% of the side-chain nuclei). Some peaks present in the ^{1}H-^{15}N HSQC spectrum (Figure 1A) were not found in the other heteronuclear 3D spectra. The TALOS predicted secondary structures (Figure 1B) exhibits a classical thioredoxin fold with 5 β-strands (31% of the primary structure) and 4 α-helices (49% of the primary structure). The CPPC variant active site does not seem to perturb significantly the overall conformation. It will be actually of great interest to complete this study by the assignment of the ^{1}H-^{13}C-, ^{1}H-^{15}N-HSQC NOESY spectra in order to get a high resolution structure of poplar thioredoxin.

Chemicals shifts were deposited in the BioMagResBank under access number BMRB-6079 (http://www.bmrb.wisc.edu).

Acknowledgements

This research was supported by the CNRS, the Universities of Nancy I and INPL. Access to the Bruker DRX 600 NMR facilities of the Service Commun de Biophysicochimie des Interactions, Nancy I was deeply appreciated.

References

Bartels, C., Xia, T., Billeter, M., Güntert, P. and Wüthrich, K. (1995) *J. Biomol. NMR*, **6**, 1–10.

Behm, M. and Jacquot, J.P. (2000) *Plant Physiol. Biochem.*, **38**, 363–369.

Brehelin, C., Mouaheb, N., Verdoucq, L., Lancelin, J.M. and Meyer, J.M. (2000) *J. Biol. Chem.*, **275**, 31641–31647.

Cornilescu, G., Delaglio, F. and Bax, A. (1999) *J. Biomol. NMR*, **13**, 289–302.

Elkund, H., Cambillau, N., Sjoberg, B.M., Holmgren, A., Jornvall, H., Hoog, J.O. and Branden, C.I. (1984) *EMBO J.*, **3**, 1443–1449.

Holmgren, A. (2000) *Antioxid Redox Signal*, **2**, 811–20.

Meyer, Y., Vignols, F. and Reichheld, J.P. (2002) *Meth. Enzymol.*, **347**, 394–402.