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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments and secondary structure of Tryparedoxin-I from *Crithidia fasciculata*

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Biological context

The thiol-disulfide oxidoreductase tryparedoxin (TXN; Nogoceke et al., 1997) is an essential enzyme of the hydroperoxide detoxification pathway of parasitic organisms such as *Trypanosoma*, *Crithidia* and *Leishmania* species, which comprise those responsible for a number of serious tropical diseases. As the special redox-cascade of this species differs from the glutathione-based systems of mammalians, the parasites' enzymes are potential targets for the development of specific inhibitors for use as trypanocidal drugs (Flohé et al., 1999). The main low molecular weight redox-mediator of the parasites is a bis-glutathionyl derivative of the trifunctional amine spermidine, called trypanothione (N1,N8-bis(glutathionyl)-spermidine). The compound is reduced by a NADPH-dependent trypanothione reductase and then acts as a reducing agent towards tryparedoxin. With its redox equivalents stored in the dithiol form of a WCPPCR motif, tryparedoxin is prepared for electron transfer to tryparedoxin peroxidase, the final enzyme of the parasites' hydroperoxide elimination system (Nogoceke et al., 1997). Compared to the mammalian system, TXN is only functionally analogous to thioredoxin, which usually contains a WCGPCK motif. Despite a similar catalytic mechanism and folding pattern, TXN is about 50% larger and shows limited sequence similarities to the thioredoxins (Hofmann et al., 2001). With regard to the evident structural differences and the characteristic substrate specificity, TXN should be inhibited with high selectivity by suitable compounds, which make the enzyme the most interesting drug target of this unique system.

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Methods and experiments

The TXN1 gene was heterologously expressed in *E. coli* BL21(DEB)pLys, strain 129 transformed with the plasmid pET22b(+)/TXNH6 (Guerrero et al., 1999) to yield a C-terminally His-tagged TXN derivative as described in detail elsewhere (A. Roß et al., submitted). The ^{15}N, ^{13}C double labeled Tryparedoxin-I (TXN1) from *Crithidia fasciculata* was studied by multidimensional, heteronuclear NMR spectroscopy. Most sequential information, although incomplete in parts, was achieved with the HNCA and HN(CO)CA experiments. Complementary assignment was carried out using CBCANH, CBCA(CO)NH, HAHB(CO)NH and ^{15}N-edited 3D-TOCSY data. The protein concentration was about 1 mM in 1 mM aqueous potassium phosphate, pH 6.5, containing 0.02% NaN_3 with additional 10% D_2O. Triple resonance NMR experiments were carried out on a Bruker Avance DMX 600 NMR spectrometer at 30 °C. Acquisition, processing and analysis of spectral data were performed with XWIN-NMR1.3 and Aurelia 2.7.9 (Bruker). A HCCH-TOCSY spectrum was recorded at 750 MHz on a Varian Unity INOVA 750 narrow-bore NMR spectrometer.

Secondary structure elements were determined by analysis of the chemical shifts of the backbone atoms and C^{β}, and by characteristic NOE resonances obtained from the ^{15}N-edited NOESY spectrum. Analy-

sis of the deviations from random coil values of $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and HNCO-derived $^{13}C'$ resonances according to the method of Wishart and Sykes (1994) gave results that allowed the prediction of helices from the $^{13}C^{\alpha}$ and $^{13}C'$ data and sheets from the $^{1}H^{\alpha}$ and $^{13}C^{\beta}$ data (Figure 1). Further information was afforded by the use of the TALOS software (Cornilescu et al., 1999), which allowed prediction of backbone angles in those regions with well defined secondary structure. Together with limited NOE data (about 440 restraints), structure calculations were performed with CNS (Brünger et al., 1998). As most NOEs were sequential short range, the calculation revealed distinct secondary structure elements, while the global folding has not converged completely at this stage.

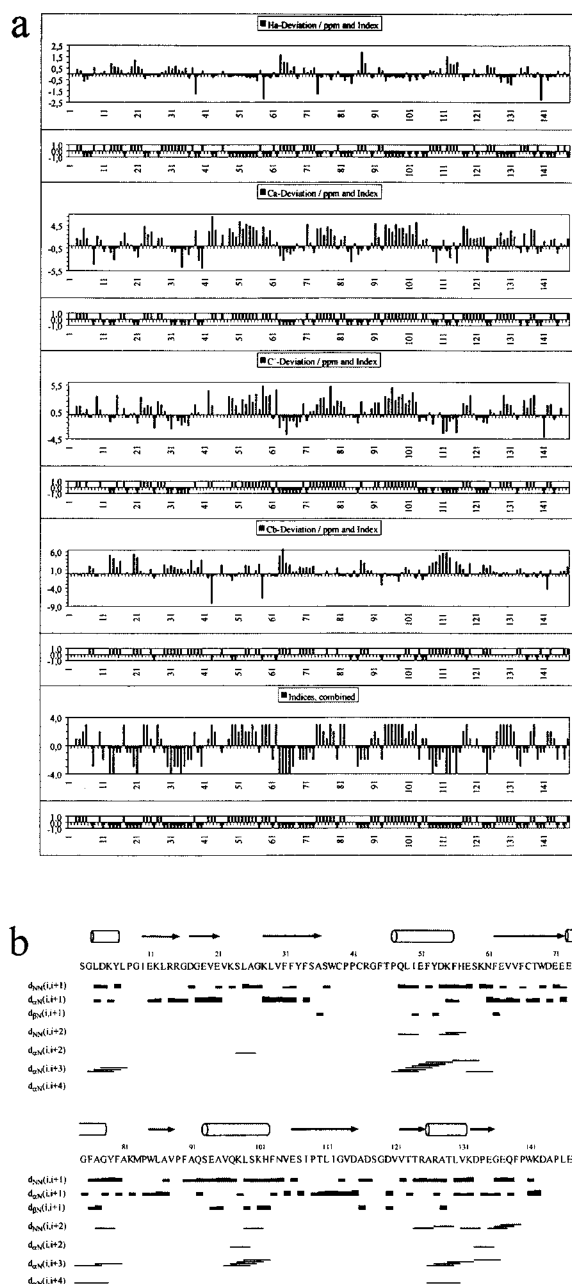

Figure 1. (a) Deviations of the measured chemical shifts from those of the random coil and indexed according to Wishart and Sykes (1994). The graphics show values of $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C'$ and $^{13}C^{\beta}$ from S1 to E147 of TXN1 and the combined indices with negative $^{13}C^{\beta}$-index values were filtered out. (b) Sequence of TXN1. Top: Secondary structure deduced from chemical shift deviations, NOE-contacts and NOE and TALOS-angle-based calculations with CNS. Bottom: Selected short range NOE-restraints used in structure calculations with CNS (scheme created with the program DYANA, Güntert et al., 1997).

Extent of assignments and data deposition

Out of the residues 1 to 147, backbone ^{15}N, $^{1}H^{\alpha}$, $^{13}C^{\alpha}$ and most $^{13}C'$ resonances were assigned for about 97% of non-Pro residues. For these, almost all of the $^{13}C^{\beta}$ and of the aliphatic proton resonances were identified, the latter derived from a HCCH-TOCSY spectrum. For nine of the ten Pro-residues, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and $^{13}C'$ resonances were obtained from HNCA, HN(CO)CA, CBCANH, CBCA(CO)NH and HNCO spectra. Residues S1, E11, G44 and T46 remain unassigned. Because of the structural flexibility near the active center, that leads to weak crosspeak intensities in the NOESY, assignments for the residues 41 to 45 are to be considered as uncertain. The assigned ^{1}H, ^{13}C and ^{15}N chemical shift values have been deposited in the BioMagResBank (http:/www.bmrb.wisc.edu).

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