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Letter to the Editor: Backbone H^N, N, C^α, C', and C^β assignment of the 6-phosphogluconolactonase, a 266-residue enzyme of the pentose-phosphate pathway from human parasite *Trypanosoma brucei*

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Biological context

In the cell, the pentose-phosphate pathway plays a crucial role by generating NADPH which serves as a hydrogen donor in biosynthetic processes. Furthermore, it also provides ribose 5-phosphate as a nucleic acid precursor and several metabolic intermediates, such as fructose 6-phosphate and glyceraldehyde 3-phosphate. The three first enzymes of the pathway are the glucose-6-phosphate dehydrogenase (G6PDH), the 6-phosphogluconolactonase (6PGL) and the 6-phosphogluconate dehydrogenase (6PGDH), respectively. Although sequences, catalytic activities, enzymatic mechanisms and tridimensional structures of some G6PDH and 6PGDH have been well documented (e.g., Barrett, 1997), very little information about 6PGL was available until the recent molecular and biochemical characterization of the human *pgl* gene (Collard et al., 1999). Moreover, the role of the 6PGL was not fully understood since its substrate (δ-6-phosphogluconolactone) was believed to undergo rapid spontaneous hydrolysis, suggesting that the presence of the 6PGL was useless in the pathway. Nevertheless, our functional study using ^{31}P and ^{13}C NMR spectroscopy revealed that 6PGL is required for efficient hydrolysis of the δ-6-phosphogluconolactone. In absence of 6PGL, slow spontaneous hydrolysis of δ-6-phosphogluconolactone, delays the flux of the patway. In addition, accumulation of this lactone, a high electrophilic compound, may be toxic by interfering with intracellular nucleophiles. Finally, the enzyme avoids rearrangement of δ-6-phosphogluconolactone into γ-6-phosphogluconolactone, considered as a 'dead end' of the pathway (Miclet et al., 2001). Since no structure is available today in the lactonase family, we have undertaken a 3D structure determination by NMR, in order to better understand the mechanism of action of this enzyme. Moreover, residues belonging to active site have not yet been identified in lactonase family. Assignment of the polypeptide backbone of the protein could provide a basis to identify the active site using interactions studies by NMR. Here we report the backbone H^N, N, C^α, C', C^β resonance assignment of the ^2H, ^{13}C and ^{15}N labelled 6PGL from the parasitic protozoa *Trypanosoma brucei*, a 266-residue protein (Duffieux et al., 2000). *T. brucei* is the causative agent of the human sleeping sickness in sub-Saharan Africa.

Methods and Experiments

All stable isotopes were purchased from Cambridge Isotope Laboratories or Euriso-Top. For simple (^{15}N) or double (^{15}N,^{13}C) isotopic labelling, *E. coli* growth was performed in minimal medium M9 supplemented with 0.1% $^{15}NH_4Cl$ or with 0.1% $^{15}NH_4Cl$ and 0.3% $^{13}C_6$ glucose. The expression of the uniformly deuterated protein was achieved by combining the adaptation of bacteria into a deuterated minimal medium M9 (0.1% $^{15}NH_4Cl$, 0.3% $^{13}C_6$ D-glucose, 99.8% $^2H_20$) (Gardner and Kay, 1998) with a recently described protocol allowing a high biomass production in unlabelled media before inoculation of labelled medium (Marley et al., 2001).

^{15}N-Leucine specific labelling was also performed, using M9 medium supplemented with 40 μg/ml of

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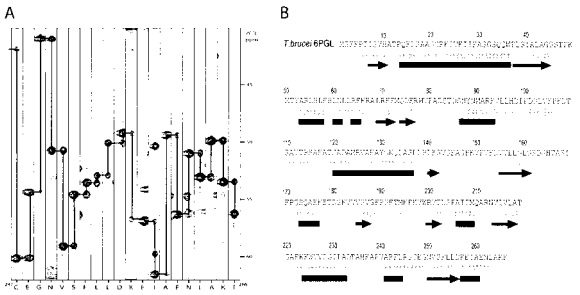

Figure 1. (A) Strips corresponding to the assignment of the last 20 residues of 6PGL, in the ^{1}H-^{15}N TROSY HNCA experiment recorded at 800 MHz, 25 °C and pH 6.0. *Tb*6PGL concentration was 1.4 mM. (B) Secondary structure elements predicted from ^{13}C and Hα chemical shifts with CSI program (in grey) and TALOS program (in black). Arrows represent predicted β sheets whereas lines are α helix.

the nineteen unlabelled amino acids (Sigma) and with 40 μg/ml of ^{15}N-Leucine. Recombinant *T. brucei* 6PGL has been expressed and purified as described previously (Duffieux et al., 2000). After extensive dialysis in 50 mM phosphate buffer pH 6.0, 200 mM NaCl and 1 mM β-mercaptoethanol, NMR samples were concentrated up to 1.4 mM of protein, using an 8003 Stirred Cell (Amicon). In the deuterated sample triple labelled protein, deuterium incorporation was 80.5%, as determined by mass spectroscopy.

NMR spectra were acquired at 25 °C and pH 6.0, on Bruker DRX600 and 800 MHz or Varian INOVA 600 MHz spectrometers equipped with triple-resonance (^{1}H, ^{13}C, ^{15}N) probes including z-gradients. The NMR experiments performed included 2D ^{15}N-TROSY, 3D d-HNCA, d-HN(CO)CA, d-HNCACB, d-HN(CO)CACB, d-HNCO, d-HN(CA)CO for the ^{2}H, ^{13}C, ^{15}N labelled sample, 3D HNHA, CBCACO(CA)HA and HBHA(CO)NH for the ^{13}C, ^{15}N labelled sample, and ^{15}N-TROSY for the ^{15}N-Leucine labelled sample. Spectra were processed on a Silicon Graphics workstation using the Gifa software (Pons et al., 1996). Processing included 90°-shifted sinebell window function, appropriate phase correction and zero filling, as well as linear prediction in the ^{15}N dimension. ^{1}H chemical shifts were referenced to external TSP at 0 ppm. ^{15}N and ^{13}C shifts were referenced indirectly using the $^{1}H/X$ frequency ratios of the zero point, according to Wishart et al. (1995).

Extent of assignments and data deposition

Ninety-seven % of the amino acids of the deuterated sample were assigned, residues F3-K4, M50, P190-S191, P196 and H236 being unassigned. Among assigned residues, 2% of the resonances of H^{N}, N, C′ or C^{β} were not unambiguously determined. Moreover, 80% of Hα resonances were assigned on the non-deuterated ^{13}C, ^{15}N sample. The strips corresponding to assignment of the last 20 residues are shown in Figure 1A, together with secondary structure predictions (Figure 1B) from CSI (Wishart and Sykes, 1994) and TALOS (Cornilescu et al., 1999). These predictions suggest the presence of 10 extended strands and 8 helices.

The ^{1}H, ^{13}C and ^{15}N chemical shifts for 6PGL have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 5468.

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