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Backbone assignment of double labelled 23.7 kDa phosphoglycerate mutase from *Schizosaccharomyces pombe*

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Biological context

Phosphoglycerate mutase (PGAM) is the glycolytic enzyme that catalyses the interconversion of 2-phosphoglycerate and 3-phosphoglycerate in the glycolytic pathway. In humans the functional enzyme exists as a homodimer of either 29-kDa or 30-kDa subunits (M-type or E-type respectively) whilst in yeasts it is found either as a tetramer (*Saccharomyces cerevisiae*), or as a monomer (*Schizosaccharomyces pombe*) (Nairn et al., 1994,1996). The PGAM from *S. pombe* (211 residues, 23.7 kDa), like the human PGAM, is dependent upon 2,3-bisphosphoglycerate (BPG) for activity. No crystal structure exists for the monomeric PGAM, nor for any PGAM in complex with its substrates or inhibitors. Therefore, high-resolution NMR studies of *S. pombe* PGAM will provide valuable insights into subunit interactions within oligomeric PGAMs, the binding of substrates and inhibitors, and the mode of catalysis.

Methods and Results

A ^{15}N,^{13}C-labelled sample of *S. pombe* PGAM was extracted from a transformed strain of *S. cerevisiae* (Nairn et al., 1996) grown on minimal medium containing 1 g/l ^{15}N ammonium sulphate, 4 g/l ^{13}C glucose, 32 mg/l uracil, 40 mg/l tryptophan and 40 mg/l histidine. The sample was prepared at pH 6.4 as a 0.6 mM solution of double labelled PGAM in 600 µl of H_2O/D_2O (90:10) containing 200 mM d_3-sodium acetate, 500 mM ammonium sulphate, and 2 mM sodium azide. Under these conditions more than 80% of enzyme activity was still present in a sample kept at 37 °C for three weeks. NMR spectra were recorded at 37 °C on a Varian INOVA-600 spectrometer equipped with a 5 mM z-gradient triple-resonance probe. The most useful experiments for the backbone assignment of PGAM proved to be CBCA(CO)NH and HNCACB (Muhandiram and Kay, 1994), together with their proton analogs HBHA(CBCACO)NH (Grzesiek and Bax, 1993) and HBHA(CBCA)NH (Wang et al., 1994). The additional experiments used for assignment were: HNCO, HNCA (Kay et al., 1994), HN(CO)CA (Grzesiek and Bax, 1992), HN(CA)CO (Engelke and Rüterjans, 1995), HCACO and CBCACOHA (Kay, 1993). In all experiments which involved detection of NH protons, the sensitivity-enhanced protocol was used. Several modifications were made, including combined use of nonselective and shaped ^{13}C pulses, return of the magnetisation of the water protons to the z-axis prior to acquisition without the aid of selective pulses, and elimination of the Bloch–Siegert shifts during the t_1-period. These modifications will be discussed elsewhere.

Due to overlap, sequential assignment based on the 3D HNCA and HN(CO)CA experiments was possible for only about 20% of the residues. Sequential connectivities were derived predominantly from CBCA(CO)NH and HNCACB experiments. HBHA(CBCACO)NH and HBHA(CBCA)NH experiments were essential for eliminating some of the sequential candidates obtained from the above experiments.

For illustrative purposes, the sequential assignment of residues 177 to 191 is shown in Figs. 1A and B. In Fig. 1A the NH strips from the CBCA(CO)NH and HNCACB experiments are overlaid. Vertical lines in each strip connect the pair of C^{α} and C^{β} signals from residue (i–1)

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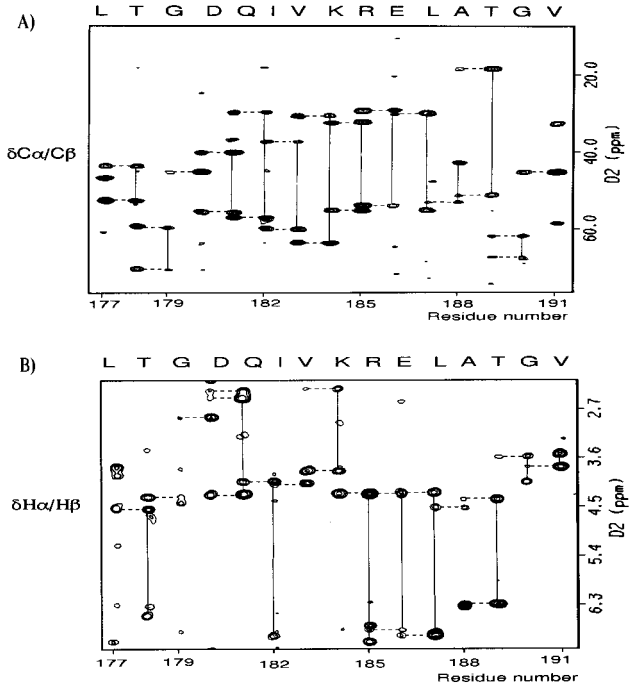

Fig. 1. Sequential assignment of residues 177–191. (A) Overlaid strips from CBCACONH and HNCACB experiments. Vertical lines in each strip connect a pair of C^{α} and C^{β} signals from residue (i–1) and horizontal lines show the sequential connectivity. (B) Overlaid strips from HBHA(CBCACO)NH and HBHA(CBCA)NH experiments with H^{α} and H^{β} chemical shifts in D2. Signals above 5.9 ppm are folded from the region below 2.3 ppm.

and the horizontal lines point to the equivalent pair of signals in residue i. Figure 1B shows the sequential connectivity of the same residues based on the pairs of α and β protons, obtained from HBHA(CBCACO)NH and HBHA(CBCA)NH experiments. In the set of CBCA(CO)NH and HNCACB spectra signals for the majority of residues were observed. In the less sensitive HNCACB experiment, 17 C^{β} and 12 C^{α} were not detected at all. Carbon chemical shifts of the missing residues were identified from the CBCA(CO)NH spectrum, where only two C^{β} resonances were not observed.

The sequential assignment thus obtained was verified by focusing on CO resonances in HNCO and HN(CA)CO spectra. Unfortunately, due to the low concentration of the sample and the inherently low sensitivity of the HN(CA)CO experiment many signals were very weak. It was therefore necessary to work simultaneously with the HCACO experiment.

Additional verification came from analysis of CBCA(CO)NH and CBCACOHA spectra. These spectra were searched for the same pair of C^{α},C^{β} carbons. For such a pair the corresponding NH chemical shifts extracted from the CBCA(CO)NH experiment were used to identify the CO frequency from the HNCO experiment, which was then tested against CO frequency in the CBCACOHA experiment.

Extent of assignments and data deposition

In total, assignments were obtained for 195 of the backbone residues, with 16 amino acids not detected or not showing sequential connectivity in the 3D experiments. Among the unassigned resonances were all four histidines. Out of the three tryptophans present in the amino acid sequence, only W^{111} was found in 3D experiments and this yielded only very weak signals. The culture medium was enriched with histidine, uracil and tryptophan, causing incorporation of unlabelled histidine and tryptophan amino acids into the protein. At least five NH signals from non-labelled residues were found by comparison of ^{15}N coupled and decoupled 1D spectra of the protein. Further proof for the failure to incorporate ^{13}C isotope into the histidines and tryptophans came from a 2D experiment correlating the C^{β} atom of aromatic amino acids with their δ protons (Yamazaki et al., 1993). Only one weak signal was observed in the histidine/tryptophan region (25–35 ppm). The six unlabelled amino acids complicated the sequential assignment, already interrupted by eight proline residues. We could not identify the NH resonances of the three residues following histidines in the amino acid sequence, but the C^{α}, C^{β}, and CO resonances of these residues appeared in those spectra in which they are correlated with i+1 residues. Other stretches of undetected residues were amino acids 71–73 and 93–95.

In conclusion, we have reported an almost complete NMR backbone assignment of *S. pombe* PGAM using a combination of triple resonance techniques. Table S1, containing chemical shift assignments, is available as supplementary material and will be deposited in BioMagResBank in Madison, WI, U.S.A.

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