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Letter to the Editor: Backbone and sequence-specific assignment of three forms of the lipoate-containing H-protein of the glycine decarboxylase complex

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Biological context

In plant and mammalian mitochondria the glycine decarboxylase complex (GDC) catalyses the oxidative decarboxylation of glycine in a multi-step reaction (Kikuchi and Hiraga, 1982; Douce and Neuburger, 1989). The complex consists of four proteins named P, H, T and L. The 14 kDa H-protein, which contains a lipoate molecule covalently bound to a lysine (K63), plays a central role in the reaction mechanism. Initially, the P-protein catalyzes the decarboxylation of glycine: the α-carboxyl is lost as CO_2 and the methylamine group is transferred onto an S atom of the oxidized lipoamide arm. The H-protein, loaded with methylamine, then interacts with the T-protein and the methylene group is transferred to a molecule of tetrahydrofolate. Formation of methylene–tetrahydrofolate takes place, and the H-protein is in the reduced form. Finally, the lipoate group is re-oxidized by the L-protein, thereby completing the catalytic cycle. In order to recycle methylene– tetrahydrofolate, the conversion, catalyzed by the serine hydroxymethyltransferase complex, of glycine and methylene–tetrahydrofolate to serine occurs.

All attempts to isolate the entire GDC complex have failed and available data seem to indicate that the H-protein behaves as a mobile substrate interacting successively with the different catalytic sites since its cofactor is oxidized, loaded with methylamine or reduced (Oliver et al., 1990; Douce et al., 1994). To gain insight into the glycine decarboxylase complex mechanism, we have initiated NMR structural studies of the H-protein from pea leaf.

In this note, we report the backbone assignments of the apoprotein H (Hapo), the oxidized H-protein (Hox) and the sequence-specific assignments of the form loaded with methylamine (Hmet).

Methods and results

^{15}N-labelled Hapo, Hox and Hmet samples were made by growing *Escherichia coli* on a minimal medium containing 1 g/l of ^{15}N-NH_4Cl. ^{15}N-^{13}C-labelled Hmet was obtained using a minimal medium containing 1 g/l of ^{15}N-NH_4Cl and 2 g/l [$^{13}C_6$]-glucose. The expression, lipoylation, methylamine loading and purification were achieved using techniques described previously (Guilhaudis et al., 1999). All samples were prepared in 50 mM potassium phosphate buffer, pH 5.5, 0.1 mM EDTA, 0.2% sodium azide and 10% D_2O. The final concentrations were respectively 2 mM (Hmet), 1.3 mM (Hox) and 1 mM (Hapo).

NMR spectra were recorded on a Bruker AMX-600 NMR spectrometer and on Varian Inova 400 and 600 MHz spectrometers equipped with a triple-resonance (^{1}H, ^{13}C, ^{15}N) probe including shielded z-gradients. The experiments were conducted initially

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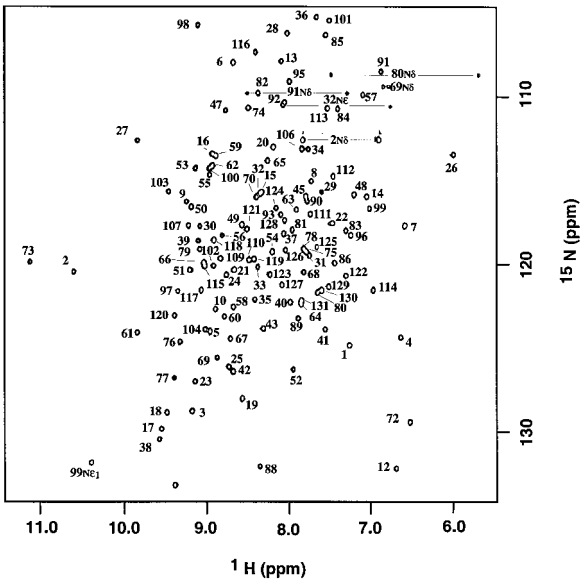

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum of Hmet. The number labels indicate the assignment at 291 K and pH 5.5. Side chain NH_2 resonances of asparagine and glutamine are connected by bars. The resonances 12, 72 and 88 are folded from the upfield region in ^{15}N.

at 35 °C for Hapo and at 18 °C for Hox and Hmet for reasons of sample stability (Guilhaudis et al., 1999). For assignments of the backbone resonances, 3D ^{15}N-edited NOESY-HSQC (150 ms) and TOCSY-HSQC (60 ms) spectra were acquired with 512×50×225 (^{1}H, ^{15}N, ^{1}H) complex points on the ^{15}N uniformly labelled samples. For Hapo, ^{1}H and ^{15}N assignments at 18 °C were obtained from ^{1}H-^{15}N HSQC recorded at 35, 30, 24 and 18 °C. For Hmet, confirmation of sequential connectivities was made from a 3D HNCA (Muhandiram and Kay, 1994) and assignments of non-aromatic side chains were obtained from a 3D HCCH-TOCSY (Kay et al., 1993). For the HNCA and HCCH-TOCSY experiments 512×50×100 (^{1}H, ^{15}N, ^{13}C) and 512×100×128 (^{1}H, ^{13}C, ^{1}H) complex points were acquired, respectively. Aromatic spin systems were assigned using ^{1}H-^{1}H NOESY spectra and a ^{1}H-^{13}C HSQC experiment optimised for the aromatic resonances.

All data were processed using Felix (MSI technologies). Proton chemical shifts were reported with respect to the H_2O signal relative to DSS. The ^{15}N and ^{13}C chemical shifts were referenced indirectly using the ^{1}H/X frequency ratios of the zero-point: 0.101329118 (^{15}N) and 0.251449536 (^{13}C) (Wishart et al., 1995).

Extent of assignments and data deposition

The ^{1}H-^{15}N HSQC spectrum of the ^{15}N-labelled Hmet is shown in Figure 1. For Hmet, we have assigned all the protonated ^{15}N resonances of the backbone and from asparagine, glutamine and tryptophan side chains except for Trp 15. With the exception of the residues 1, 46 and 52, 95.3% of the non-labile ^{1}H resonances and 95.7% of the protonated ^{13}C (the aromatic carbons are not included) have been assigned. The ^{13}Cα resonances for residues 1, 46 and 52 have been assigned. Assignment of the ^{13}C and ^{1}H resonances of the CH_2 unit of the methylamine group has been previously reported (Guilhaudis et al., 1999).

Assignments were obtained unambiguously for 95.1% and 97.6% of non-prolyl backbone residues of Hapo and Hox respectively. Sequence-specific assignments for Hmet and backbone assignments for Hox and Hapo have been deposited in the BioMagResBank database (accession numbers: Hmet: 4336, Hox: 4337, Hapo: 4338).

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