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# Sequence-specific assignments of the inner lipoyl domain of human pyruvate dehydrogenase

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## Biological context

The pyruvate dehydrogenase (PDH) complex plays a central role in glucose metabolism by catalyzing the first irreversible reaction in the mitochondrial oxidation of pyruvate to acetyl-CoA. PDH activity is reduced in experimental models of diabetes and non-insulin dependent diabetes mellitus (NIDDM). The activity of the PDH complex is regulated by the reversible phosphorylation of serine residues of its E1 subunits. This phosphorylation is achieved by binding of the complex to PDH kinases (PDHK) or PDH phosphatases. In order for PDHK to optimally phosphorylate the E1 subunit, thereby inactivating the PDH complex, it must be bound to the E2 subunit. Thus activation of the complex can be brought about by inhibition of the kinases, making PDH kinase an attractive target in NIDDM.

The PDH complex consists of multiple copies of several component proteins, including a decarboxylase (E1 subunit), a transacetylase (E2), and a dehydrogenase (E3). The mammalian E2 has three small domains – two lipoyl domains, $E2_{L1}$ and $E2_{L2}$, and an E1-binding domain, which are connected to each other by alanine- and proline-rich hinge regions. The lipoyl domains have a lipoyl cofactor bound to a specific lysine residue. The binding of the inner lipoyl domain ($E2_{L2}$) to the kinase is a precursor for the phosphorylation of the E1 subunit of PDH (Hucho et al., 1972). Therefore, interrupting the binding between the lipoyl domain ($E2_{L2}$) and PDHK is a potential pharmacological strategy towards activation of the PDH complex. We have undertaken the study of the solution structure of human $E2_{L2}$ both free and bound to PDHK. The ultimate goal would be to design an antagonist to the $E2_{L2}$/PDHK binding. In this note, we report the sequence-specific assignments of $E2_{L2}$ in its free form.

The $E2_{L2}$ domain that has been studied contains 104 residues of which the first 12 residues comprise a flag tag which is used as a marker in the biological assays. The sequence is as follows: DY KDDDDKSYPP HMQVLLPALS PTMTMGTVQR WEKKVGEKLS EGDLLAEIET DKATIGFEVQ EEGYLAKILV PEGTRDVPLG TPLCIIVEKE ADISAFADYR PT.

## Methods and Results

The construct was cloned by RT-PCR from human skeletal muscle RNA. Overexpression was achieved using recombinant *E. coli* BL21 DE3/pLysS/pET17b/$E2_{L2}$-il-flag. $^{15}$N-labeled $E2_{L2}$ and $^{15}$N,$^{13}$C-labeled $E2_{L2}$ and $^{15}$N-Leu-labeled $E2_{L2}$ were made by growing the *E. coli* using standard labeling techniques.

NMR spectra were recorded on a Bruker AMX-500 NMR spectrometer using 1–2 mM samples in 50 mM phosphate buffer, 5 mM deuterated dithiothreitol and 50 mM sodium chloride at a pH of 5.5 at temperatures of 288, 298 and 308 K. 3D $^{15}$N-edited NOESY-HSQC (100 ms) and TOCSY-HSQC (70 ms) spectra were recorded with gradients (Muhandiram and Kay, 1994) and contained most of the expected correlations, yielding a majority of the assignments. However, constant time HNCA and HNCOCA experiments (Grzesiek and Bax, 1992) were required on the doubly labeled sample in order to sort out some residual ambiguities. $^{1}$H, $^{15}$N and $^{13}$C dimensions were sampled over 6666.66, 1760 and 3268 Hz, respectively. For the NOESY-HSQC and TOCSY-HSQC experiments, $1024\times128\times16$ complex points were acquired.

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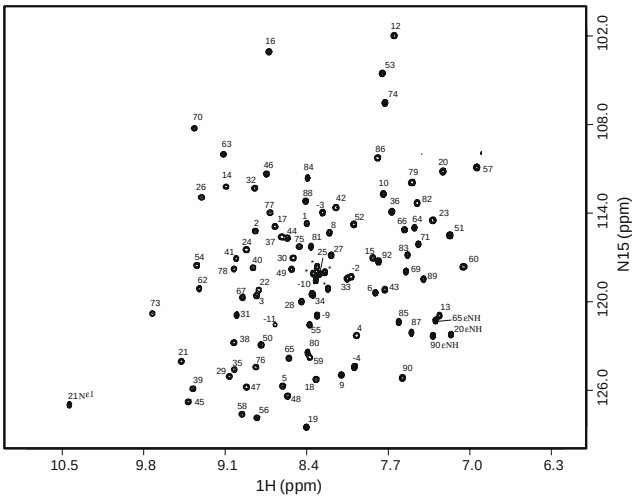


Fig. 1. $^{1}H$-$^{15}N$ HSQC of human $E2_{L2}$ at 298 K, pH 5.5. Peaks are labelled according to the residue number in the sequence. The numbering starts from the 13th residue, as explained in the text. Peaks labelled with negative numbers belong to the flag tag and peaks that could not be assigned have been marked with an asterisk. Side chains of arginines (which are folded over) and the tryptophan residue are also indicated.

For the HNCA and HNCOCA experiments, $1024 \times 16 \times 32$ ($^{1}H$, $^{15}N$, $^{13}C$) complex points were acquired.

All data were processed using FELIX (software from MSI). The FIDs were typically treated with low-frequency deconvolution to remove the residual water signal and with a 70° shifted squared sine-bell apodizing function. Final dimensions of the processed NOESY-HSQC and TOCSY-HSQC matrices were $512 \times 512 \times 64$ ($^{1}H$, $^{1}H$, $^{15}N$) points, while the HNCA and HNCOCA matrices were $512 \times 128 \times 64$ ($^{1}H$, $^{13}C$, $^{15}N$) points. The proton spectra were referenced to DSS at 0.00 ppm. $^{15}N$ and $^{13}C$ resonances were referenced indirectly to DSS using frequency ratios (Wishart et al., 1995).

The $^{1}H$,$^{15}N$ HSQC spectrum of $E2_{L2}$ is shown in Fig. 1. The numbering starts from the 13th residue in the sequence given above, since it is the first residue of the lipoyl domain. Using standard methods for sequence-specific assignments (Wüthrich, 1986), all non-prolyl residues –4 to 92 have been assigned. The α-protons of prolines 19, 61 and 68 have been identified by the cross peaks from the sequential amide protons in the NOESY spectrum. The α-carbon assignments of the prolines (except for $Pro^{91}$) were obtained from the HNCA/HNCOCA spectra.

The first 12 residues that comprise the flag tag are floppy in solution and therefore only some of those residues could be assigned based on limited TOCSY data. For purposes of structure determination, only residues 1 to 92 are relevant and will be discussed here. All 86 non-prolyl residues gave correlations to the $H^{\alpha}$ and $H^{\beta}$ protons. About 75% of the residues gave correlations to $H^{\gamma}$ and $H^{\delta}$ protons. Based on the analysis of the TOCSY data, all valine, threonine, alanine and glycine spin systems were identified and were used as a starting point for sequential assignments. In addition, a $^{15}N$[Leu] $E2_{L2}$ sample aided in the assignment of the 10 leucine amide protons in the protein.

The amide protons fall within the range 6.89–9.74 ppm. The α-protons fall within the range 3.25–5.20 ppm with the exception of $Glu^{80}$, whose α-proton resonates at 2.85 ppm. Thus, more than 95% of the backbone protons have been assigned and >85% of side-chain assignments have been made. A table of chemical shifts will be deposited with the BioMagResBank Database. A plot of the deviation of the proton and carbon chemical shifts from the random coil values (Wishart and Sykes, 1994) shows that the protein is mainly β-sheet in character with the 12 C-terminal residues involved in a nascent helix (not shown). This indicates that this lipoyl domain is structurally quite similar to other lipoyl domains reported in the literature (Ricaud et al., 1996; Berg et al., 1997).

## Extent of assignments and data deposition

Secondary and three-dimensional structure determination is underway and will be reported elsewhere. In addition, the structure determination of the $E2_{L2}$ subunit when it is bound to the kinase is planned upon availability of PDH kinase.

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