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Solution NMR Structure of Yeast Succinate Dehydrogenase Flavinylation Factor Sdh5 Reveals a Putative Sdh1 Binding Site

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Abstract

The yeast mitochondrial protein Sdh5 is required for the covalent attachment of flavin adenine dinucleotide (FAD) to protein Sdh1, a subunit of the hetero-tetrameric enzyme succinate dehydrogenase (SDH). The NMR structure of Sdh5 represents the first eukaryotic structure of the Pfam family PF03937 and reveals a conserved surface region, which likely represents a putative Sdh1-Sdh5 interaction interface. Point mutations in this region result in the loss of covalent flavinylation of Sdh1. Moreover, backbone chemical shift perturbation measurements showed that Sdh5 does not bind FAD *in vitro*, indicating that it does not function as simple cofactor transporter *in vivo*.

Succinate dehydrogenase (SDH; E.C. 1.3.5.1), also known as succinate-coenzyme Q reductase (SQR) or respiratory complex II, represents a central enzyme complex of the tricarboxylic acid (Krebs) cycle. SDH occurs in mitochondria and bacteria, and couples the oxidation of succinate to fumarate with the reduction of ubiquinone to ubiquinol. Subsequently, ubiquinol is re-oxidized to generate ATP by oxidative phosphorylation. ^{1,2} Mitochondrial SDHs are hetero-tetrameric proteins: the catalytic subunit SdhA (Sdh1 in yeast), SdhB (Sdh2) containing iron-sulfur clusters, and SdhC (Sdh3) and SdhD (Sdh4), which anchor SDH in the inner mitochondrial membrane. In humans, SDH deficiencies have been associated with a number of hereditary diseases, including certain types of cancer.^{3,4}

Accession codes. Sdh5 structure is deposited in the Protein Data Bank as entry 2LM4. Chemical shifts and NOE peak lists are deposited in BioMagResBank as entry 18098.

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Yeast mitochondria represent a valuable model system for studying human mitochondrial diseases and developing the molecular and structural biology of SDH in particular.⁵⁻⁷

Functional SDH features a covalent attachment of FAD to the Sdh1 subunit, ⁸ for which yeast mitochondrial matrix protein Sdh5 (UniProtKB ID Q08230), aka succinate dehydrogenase assembly factor 2 (SDHAF2) or early meiotic induction factor 5 (EMI5), is required. ⁹ It has been hypothesized that Sdh5 either directly catalyzes the attachment, or serves as a chaperone enabling autocatalytic formation of the covalent link.

Sdh5 belongs to the large Pfam¹⁰ protein family PF03937, currently comprising 1304 members. Based on the findings of Hao et al.,⁹ PF03937 was assigned functionally as 'Flavinylation factors of SDH'. Sdh5 was selected as a target by the Mitochondrial Protein Partnership (MPP; http://www.mitoproteins.org) and assigned for structure determination to the Northeast Structural Genomics Consortium (NESG; http://www.nesg.org; Sdh5 ID: YT682A). MPP and NESG are centers of the US Protein Structure Initiative (PSI:Biology) of the United States National Institutes of Health.

Here we report the high-quality NMR structure (Figure 1A; Table S1) of Sdh5(55-152) representing the mature form of Sdh5 after cleavage of the mitochondrial signal peptide and terminal segments predicted to be disordered being partially removed. It is the first structure of a eukaryotic member of PF03937 and represents a five-helix bundle (Figure 1B): α -helix I (residues 62-73), II (79-95), III (98-108), IV (112-119), and V(136-147). Residue conservation analysis with the program ConSurf¹¹ of Sdh5 homologs identified with a single PSI-BLAST¹² search reveals a well-defined region of conserved surface residues (Figures 1C, S1A) located in α -helices I and II, including the bend connecting them, as well as tips of α -helices III and IV. The conserved region is comprised of a mostly positive surface (Arg 68, 74, Lys 75), a negatively charged periphery (Glu 60, 80, Asp 82, 105, 112, 114), and a hydrophobic patch (Ile78, Leu 79, 83) (Figure 1D).

A search of the PDB database for structurally similar proteins using the DALI server¹³ identified as top hits the structures of three bacterial proteins likewise belonging to PF03937, two which have been solved previously by NESG: NMA1147¹⁴ from *N. meningitides* (1PUZ, deposited by NESG on June 25 2003: Z-score 8.1, RMSD of C^{α} atoms = 2.9 Å for 80 aligned residues and 23% sequence identity), Ygfy¹⁵ from *E. coli* (1X6I, deposited by the structural genomics 'Structure 2 Function' project on August 8 2004: Z-score 9.8, RMSD of C^{α} atoms = 2.6 Å for 84 aligned residues and 19% sequence identity), and VC2471 from *V. cholera* (2JR5, deposited by NESG on June 20 2007: Z-score 6.6, RMSD of C^{α} atoms = 2.9 Å for 83 aligned residues and 18% sequence identity). The remaining DALI hits were of low significance (Z-scores < 4.2), a finding which confirms the original assessment¹⁴ that the five-helix bundle first described for NMA1147¹⁴ is of 'distinct' nature.

The novel modeling leverage, ^{16,17} *i.e.*, the number of new protein structures that can be reliably modeled based on experimental templates, calculated with a sequence alignment PSI-BLAST¹² E-value cut-off of smaller that $10^{-20} / 10^{-10}$ (corresponding, respectively, to about 50% / 30% sequence identity) for the four structures of PF03937 are: NMA1147 (267/703 models, 33.9% 89.2% coverage), YgfY (55/2 additional models, 7.0%/0.2% novel coverage, 40.9%/89.4% total coverage), VC2471 (26/1; 3.3%/0.1%; 44.2%/89.5%), and Sdh5 (16/73; 9.3%/2.0%, 53.4%/91.6%). Hence, while the structure of NMA1147 alone provided nearly complete structural coverage at about 30% sequence identity, the additional structures provide valuable leverage for obtaining high-quality homology models at about 50% sequence identity.

Structure-based sequence alignment (Figure 2A) of proteins Sdh5, Ygfy, NMA1147 and VC2471 shows that all residues conserved among the four proteins are located in the conserved surface area identified based on sequence alignments alone (Figure 1C), and essentially coincides with the PF03937 domain boundary. Operon and regulon prediction¹⁹ using the MicrobesOnline server (http://www.microbesonline.org) suggests that the transcription of NMA1147 and VC2471 is co-regulated with the genes encoding the SDH subunits. This supports the current functional assignment that all members of PF03937 mediate SDH flavinylation, which is further supported by the finding that the spatial arrangement of the conserved residues is likewise highly conserved (Figure 2B): the mean RMSD values calculated between the C^a atoms of polypeptide segments comprising the conserved residues in Sdh5 (71-74 and 76-121) and the bacterial homologues (NMA1147: 1.8 Å, YgfY: 1.3 Å, VC2471: 2.4 Å) are significantly lower than the corresponding values calculated for all aligned residues as reported by DALI (NMA1147: 2.9 Å for 80 residues; Ygfy: 2.6 Å for 84 residues; VC2471: 2.9 Å for 83 residues).

We evaluated the functional importance of the conserved surface region of Sdh5 (Figure 1C) by mutagenesis of Arg 68→Glu (R68E), a charge reversal of a strongly conserved Arg, Tyr 71 - Asp (Y71D), substitution of a loosely conserved surface-exposed Tyr to a negatively charged Asp, and Trp 113-Ala (W113A), a change of a non-conserved Trp within the conserved area. Yeast cells harboring any of the mutant alleles lacked growth on nonfermentable carbon source indicating a respiratory defect (Figure 3A). Furthermore, steadystate Sdh1 flavinyation analysis of purified mitochondria from these cells indicated a complete, or near complete, disruption of covalent flavinylation (Figure 3B). This defect in flavinylation is not due to unstable Sdh1 levels: steady-state levels of SDH subunit (Sdh1, Sdh2 and Sdh3) in R68E and Y71D mutants are near normal as indicated by immunoblots of purified mitochondria (Figure 3B). Steady-state levels of the mutant Sdh5 reveal an enhanced level of the R68E mutant and attenuation of the Y71D and W113A mutant proteins. To ensure the covalent flavinylation defect was not due to decreased Sdh5 levels in the Y71D and W113A mutants (perhaps due to structural perturbation and subsequent degradation), we overexpressed Sdh1 and observed enhanced stability of the W113A Sdh5 mutant, suggesting that the Sdh5 mutant is destabilized by the attenuation in its interaction with Sdh1. Sdh1 overexpression, however, did not alter the covalent flavinylation defect in cells with W113A Sdh5. Lastly, a double substitution of Glu 98,99→Lys that lies along a negative patch, but on the other face from the conserved region, led to neither growth nor flavinylation defects. These results greatly support the hypothesis that the conserved area represents a binding site for Sdh1.

Furthermore, we recorded 2D [¹⁵N,¹H]-HSQC spectra of Sdh5 in the presence of FAD or FADH₂. Even at a two-fold excess of either cofactor no significant perturbation of backbone ¹H^N and ¹⁵N chemical shifts was registered (Figure S2). Thus, Sdh5 apparently does not bind FAD or FADH₂ *in vitro*, indicating that Sdh5 does not function as a simple cofactor transporter *in vivo*.

Finally, the structure of Sdh5 yields new hypotheses to rationalize the fact that the Gly 78—Arg mutation in human Sdh5 is a genetic marker of type 2 hereditary paraganglioma (PGL2). While the wild-type hSdh5 is fully functional in yeast models, the mutant is incapable to promote the covalent attachment of FAD to Sdh1. Since the corresponding Gly 77 in Sdh5 belongs to the highly conserved surface region (Figure 1C, 2A), the mutation might directly disrupt the Sdh1-Sdh5 interaction. Alternatively, the mutation may prevent proper folding of Sdh5: Gly 77 is located in the tight bend connecting α-helices I and II (Figure 1).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Supporting Information. Detailed description of sample production, structure determination, and FAD titration, as well as Table S1, and Figures S1 and S2 are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS

FAD flavin adenine dinucleotide

NESG Northeast Structural Genomics Consortium

NOE nuclear Overhauser effect

PDB Protein Data Bank

RMSD root mean square deviation
SDH succinate dehydrogenase

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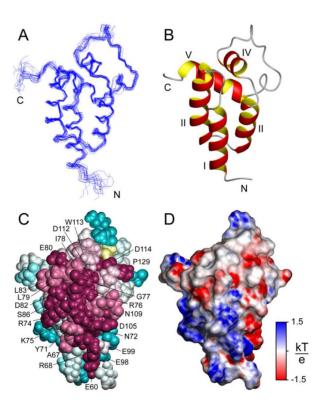


Figure 1. A) Conformers representing the structure of Sdh5 after superposition of the C^{α} atoms of the helices for minimal RMSD. Residues 43-58 and 151-152 of the disordered N- and C-terminal polypeptide segments were omitted, and the termini are labeled as "N" and "C". (B) Ribbon diagram of residues 59-150 of the lowest-energy conformer of Sdh5: α -helices are shown in red and yellow, other polypeptide segments are in gray. (C) Space-filling representation of residues 59-150 for the same conformer showing the degree of residue conservation from cyan (most variable) to burgundy (most conserved) and yellow indicating lack of statistical significance. (D) Surface electrostatic potential representation of residues 59-150 (see also Figure S1).

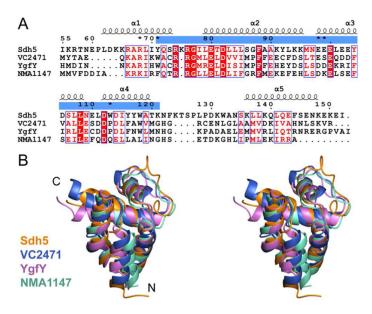


Figure 2.A) Structure-based sequence alignment of Sdh5(55-152), VC2471, YgfY and NMA1147 generated using the 'Superimpose_CE' algorithm of the program Strap (http://3d-alignment.eu) and rendered with ESPript. ¹⁸ Similar residues are shown in red and strictly conserved residues are shown in white of red background. Residue numbers and regular secondary structure elements of Sdh5 are displayed above; asterisks indicate point mutations of Figure 3; the blue area indicates the PF03937 domain boundary. (B) Stereo view of ribbon diagrams of YgfY and the lowest-energy conformers of Sdh5, VC2471, NMA11447 superimposed for minimal pairwise RMSD between C^a atoms of Sdh5 residues 71-74 and 76-121 and the corresponding C^a atoms of the other three proteins according to the structure based sequence alignment in (A).

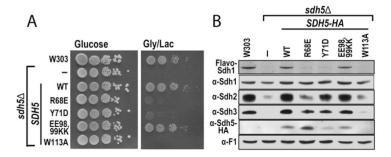


Figure 3. A) Growth test of yeast *S. cerevisiae* strains harboring the indicated point mutations on fermentative (glucose) and respiratory (glycerol/lactate) carbon sources. (B) Steady-state Sdh1 covalent flavinylation levels (Flavo-Sdh1) and SDH subunit levels in mitochondria prepared from strains in panel (A); α –F1 is loading control.