

Backbone ^1H , ^{13}C , ^{15}N NMR assignments of the unliganded and substrate ternary complex forms of mevalonate diphosphate decarboxylase from *Streptococcus pneumoniae*

Guido Reuther · Richard Harris · Mark Girvin ·
Thomas S. Leyh

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Abstract Mevalonate diphosphate decarboxylase (MDD) catalyzes the ATP-dependent decarboxylation of diphosphomevalonate (DPM) to produce isopentenyl diphosphate (IPP), the molecular “building block” for more than 25,000 distinct isoprenoids, including cholesterol, steroid hormones and terpenoids. Here, we present the first backbone assignment of *Streptococcus pneumoniae* MDD in the unliganded state and in a ternary complex with DPM and AMPPCP—a nucleotide analogue unable to transfer the γ -phosphoryl group. The secondary chemical shifts for the unliganded form are in good agreement with the crystal structure of *Streptococcus pyogenes* (~70% sequence identity). The addition of substrate and nucleotide to the enzyme results in chemical shift changes of cross peaks that correspond to residues in the binding pocket.

Keywords Mevalonate diphosphate decarboxylase (MDD) · NMR resonance assignments

Biological context

The mevalonate pathway is composed of a series of three enzymes that provide the cell with molecular building blocks that are polymerized enzymatically into isoprenoids—a vast family of compounds, estimated to contain ~25,000 distinct natural products that provide numerous metabolic functions (Eisenreich et al. 1998). Isoprenoids tether proteins to membranes, transport electrons among redox centers, and are used to decorate heme (Eisenreich et al. 1998); they are the precursors of steroids, carotenoids, bile acids (Wang and Ohnuma 2000; Fujihashi et al. 2001) and therapeutics, such as taxol and artemisinin.

S. pneumoniae MDD (35.5 kDa) is the third enzyme in the mevalonate pathway—which is essential for the survival of the organism in lung and serum (Wilding et al. 2000). MDD catalyzes the ATP-dependent decarboxylation of mevalonate 5-diphosphate (DPM)—an allosteric inhibitor that potently inactivates mevalonate kinase from *S. pneumoniae*, but does not affect the human enzyme at physiologically relevant concentrations (Andreassi et al. 2004). With the goal of developing a new class of antibiotics against *S. pneumoniae*, efforts are underway to develop DPM analogues that inhibit both the streptococcal MDD and mevalonate kinase (Kudoh et al. 2010; Lefurgy et al. 2010). Toward this end, we are engaged in determining the structure, and assessing the dynamics, of liganded and unliganded forms of MDD by NMR. Currently, six X-ray structures of MDD are available in the PDB—none of them contains ligands (PDB ID: 2GS8, 2HK2, 2HKE, 3F0 N, 3D4 J, 1FI4). Among these structures, *S. pneumoniae* MDD is most similar to the *S. pyogenes* enzyme (70% sequence identity). The structure of the *S. pneumoniae* enzyme was modeled from the *S. pyogenes* structure (2GS8) using *Phyre* (Kelley and Sternberg 2009).

G. Reuther · T. S. Leyh (✉)
Department of Microbiology and Immunology,
Albert-Einstein-College of Medicine of Yeshiva University,
1300 Morris Park Avenue, Bronx, NY 10461-1926, USA
e-mail: tsleyh@gmail.com

R. Harris · M. Girvin
Department of Biochemistry, Albert-Einstein-College
of Medicine of Yeshiva University, 1300 Morris Park Avenue,
Bronx, NY 10461-1926, USA

Materials and experiments

Sample preparation

Uniformly [$^2\text{H}/^{13}\text{C}/^{15}\text{N}$] labeled MDD was using an *E. coli* expression system, and purified using a slightly modified, published protocol (Pilloff et al. 2003; Andreassi et al. 2004). Protein was overexpressed with a polyhistidine-GST tag in BL21(DE3) competent cells grown in M63 minimal media (100% D_2O) with $^{15}\text{NH}_4\text{Cl}$ (2 g/l) and $^{13}\text{C}_6\text{D}_7$ -glucose (2 g/l) as sole nitrogen and carbon source, respectively. Protein purification was achieved by nickel ion affinity chromatography followed by a glutathione—Sephacrose column. The polyhistidine-GST tag was removed using PreScission protease during overnight dialysis against 25 mM HEPES, 50 mM KCl, 2.0 mM DTT, pH 7.5. The tag and protease were then separated from MDD by passing the dialysate over a glutathione—

Sephacrose resin. The purity of the protein samples ($\geq 95\%$) was assessed by SDS-PAGE.

NMR spectroscopy

NMR samples contained 1 mM MDD, 50 mM HEPES (pH 7.5), 50 mM KCl, 1.0 mM DTT and 10% D_2O (v/v). The ternary complex was prepared by the addition of 10.0 mM AMPPCP, 1.2 mM DPM and 11.0 mM Mg^{2+} . A standard series of 3D TROSY triple resonance experiments (HNCA, HN(CA)CB, HN(CO)CA, HN(COCA)CB, HNCO and HN(CA)CO) were used to obtain nearly complete ^1H , ^{13}C and ^{15}N backbone assignments of MDD in the unliganded and ternary-complex states. NMR spectra were recorded at 298 K on either a Varian Inova 600 MHz or Bruker Avance 800 MHz spectrometers, processed using NMRPipe (Delaglio et al. 1995), and analyzed using CcpNmr

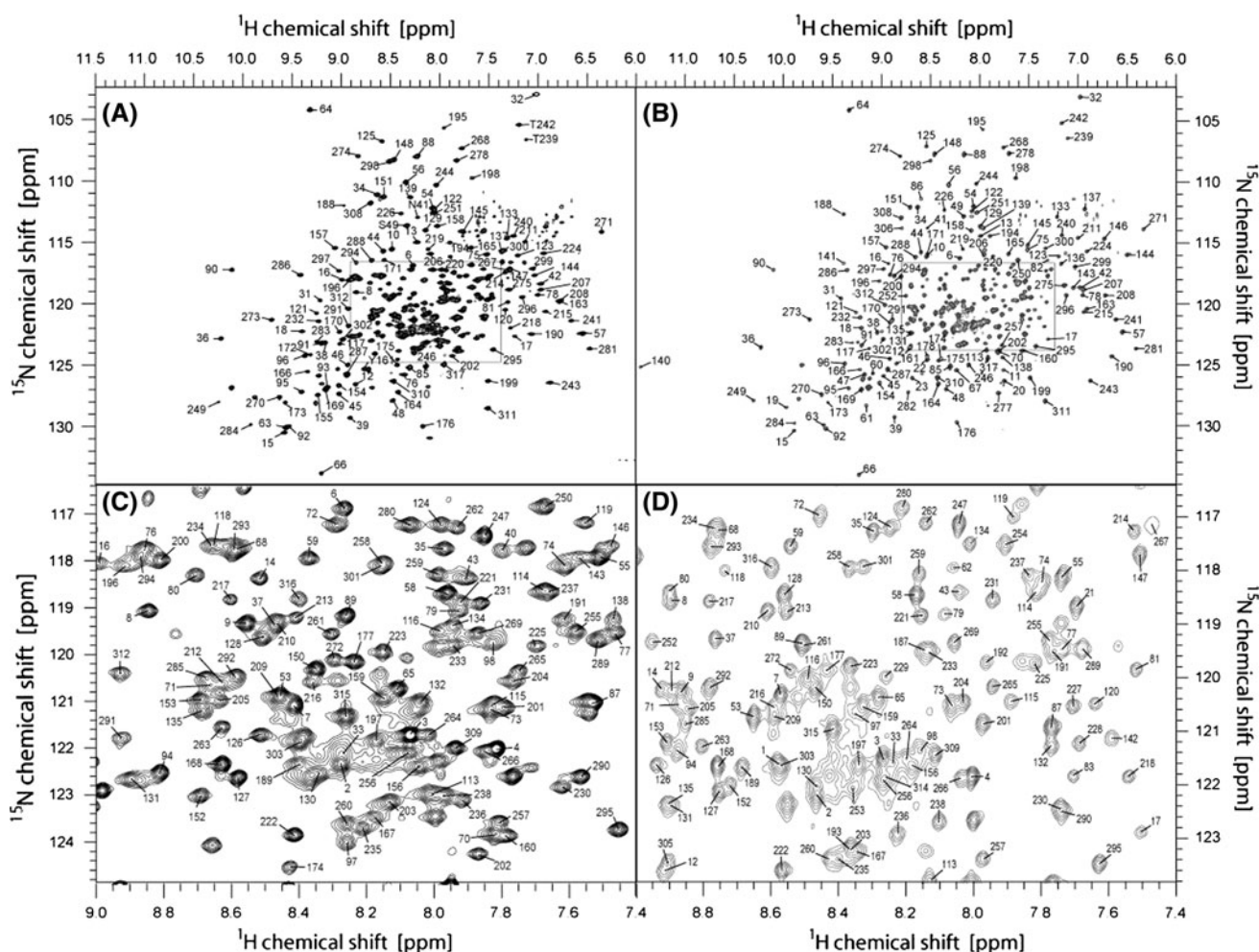


Fig. 1 Assigned 2D ^1H , ^{15}N TROSY spectra of [^2H , ^{13}C , ^{15}N] MDD. **a,c** Unliganded MDD. **b,d** MDD in complex with DPM and AMPPCP. Sample conditions: 90% H_2O , 10% D_2O , Hepes (25 mM, pH 7.5), 298 K

Analysis (Vranken et al. 2005). Chemical shifts were referenced to DSS (Wishart et al. 1995).

Extent of assignments and data deposition

The analysis of the triple resonance spectra obtained from the unliganded form of MDD led to the identification and assignment of 265 of the 306 non-proline MDD backbone ^{15}N and amide proton resonances (86%). An assigned 2D ^{13}C -decoupled ^1H - ^{15}N TROSY spectrum of [^2H , ^{13}C , ^{15}N] MDD in 90% H_2O at pH 7.5, recorded at proton frequency of 800 MHz is shown in Fig. 1a. In addition to the amide resonance assignments, we were able to obtain 92, 86, and 91% of the $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and ^{13}CO chemical shifts, respectively. The secondary structure of MDD was predicted from the secondary chemical shift values of $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and ^{13}CO . The $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$ shifts were corrected for the deuterium effect (Venters et al. 1996). The difference of $(\Delta\text{C}\alpha - \Delta\text{C}\beta)$ is shown in Fig. 2b for MDD in the apo-state. The predicted secondary structure elements (Fig. 2c) are in good agreement with those for a homology model of MDD (Fig. 2a) derived from the crystal structure of MDD from *S. pyogenes* (Kelley and Sternberg 2009). Slight differences may result from missing data for the unassigned residues. The missing ^{13}C resonances in the heteronuclear triple resonance spectra suggest that most of the unassigned residues are missing due to intermediate conformational exchange. The majority of the unassigned residues localized to the loop regions surrounding the expected binding pocket of the enzyme.

The assigned ^1H , ^{15}N TROSY spectrum for the enzyme in the ternary complex with substrate DPM and nucleotide

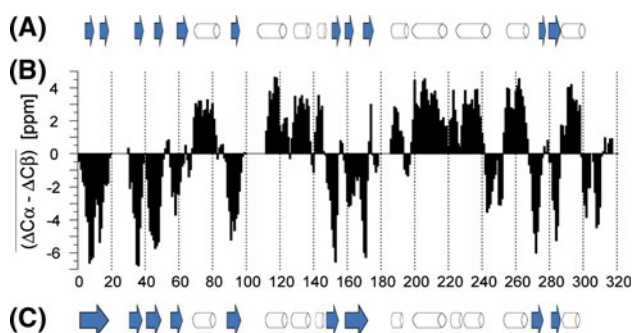


Fig. 2 MDD Secondary Structure. **a** Secondary structure predicted from a model of the pneumoniae enzyme derived, by homology modeling, from the crystal structure of the *S. pyogenes* MDD (Kelley and Sternberg 2009), which is 70% identical to the pneumoniae MDD. α -helices are represented by barrels; β -sheets by arrows. **b** Difference of chemical-shift deviation from random coil $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$. Values are averaged over three consecutive residues. Unassigned residues were given a value of zero. **c** The consensus result from CSI (Wishart and Sykes 1994)

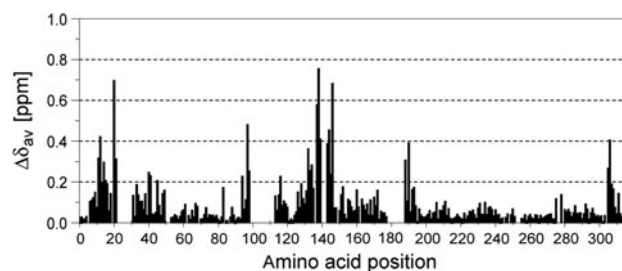


Fig. 3 Average chemical shift change $\delta_{av} = \sqrt{(\Delta H^2 + 1/5\Delta N^2)}/2$ of amide cross peaks between unliganded and ternary-complex forms of MDD (pH 7.5 and 298 K). Zero values correspond to unassigned residues

analogue AMPPCP is shown in Fig. 1 (b, d). The triple resonance 3D-spectra show more cross peaks and higher signal-to-noise ratio compared to that of the unliganded state. This increased number of correlations suggests that MDD becomes more rigid upon binding of the ligand; consequently, we were able to assign 278 out of 306 ^{15}N backbone and amide proton resonances of MDD in the ternary complex (91%), and we obtained 92, 91, and 92% of $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and ^{13}CO assignments, respectively. Chemical shift changes of the amide cross peaks were observed with the binding of the ligands (Fig. 3). Residues with the largest chemical shift changes, $\Delta\delta > 0.3$ (A12, W20, G21, N97, A132, F137, A138, S139, S143, R144, F146, G188, K190 and S306) are located in the predicted binding pocket of the enzyme. Further NMR investigation will enable the development of a structural model of the enzyme in the unliganded and complexed states. In addition, full resonance assignments will enable the investigation of dynamic changes of the enzyme upon ligand binding. Structural and dynamic information will be integrated to provide a better understanding of the mechanism of this interesting decarboxylation reaction. The backbone ^1H , $^{13}\text{C}\alpha$, ^{15}N , and ^{13}CO chemical shifts including $^{13}\text{C}\beta$ resonances of MDD for the unliganded and ternary-complex forms of MDD have been deposited in the BMRB (accession numbers 16,919 and 16,916, respectively).

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References

Andreassi JL, Dabovic K et al (2004) Streptococcus pneumoniae isoprenoid biosynthesis is downregulated by diphosphomevalonate: an antimicrobial target. *Biochemistry* 43(51):16461–16466

- Delaglio F, Grzesiek S et al (1995) Nmrpipe—a multidimensional spectral processing system based on unix pipes. *J Biomol NMR* 6(3):277–293
- Eisenreich W, Schwarz M et al (1998) The deoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms. *Chem Biol* 5(9):R221–R233
- Fujihashi M, Zhang YW et al (2001) Crystal structure of cis-prenyl chain elongating enzyme, undecaprenyl diphosphate synthase. *Proc Natl Acad Sci U S A* 98(8):4337–4342
- Kelley LA, Sternberg MJE (2009) Protein structure prediction on the web: a case study using the Phyre server. *Nat Protoc* 4(3):363–371
- Kudoh T, Park CS et al (2010) Mevalonate analogues as substrates of enzymes in the isoprenoid biosynthetic pathway of *Streptococcus pneumoniae*. *Bioorg Med Chem* 18(3):1124–1134
- Lefurgy ST, Rodriguez SB et al (2010) Probing ligand-binding pockets of the mevalonate pathway enzymes from *Streptococcus pneumoniae*. *J Biol Chem* 285:20654–20663
- Pilloff D, Dabovic K et al (2003) The kinetic mechanism of phosphomevalonate kinase. *J Biol Chem* 278(7):4510–4515
- Venters RA, Farmer BT 2nd et al (1996) Characterizing the use of perdeuteration in NMR studies of large proteins: ^{13}C , ^{15}N and ^1H assignments of human carbonic anhydrase II. *J Mol Biol* 264(5):1101–1116
- Vranken WF, Boucher W et al (2005) The CCPN data model for NMR spectroscopy: development of a software pipeline. *Proteins-Struct Funct Bioinform* 59(4):687–696
- Wang KC, Ohnuma S (2000) Isoprenyl diphosphate synthases. *Biochim Biophys Acta* 1529(1–3):33–48
- Wilding EI, Brown JR et al (2000) Identification, evolution, and essentiality of the mevalonate pathway for isopentenyl diphosphate biosynthesis in gram-positive cocci. *J Bacteriol* 182(15):4319–4327
- Wishart DS, Sykes BD (1994) Chemical-shifts as a tool for structure determination. *Methods Enzymol* 239:363–392
- Wishart DS, Bigam CG et al (1995) H-1, C-13 and N-15 chemical-shift referencing in biomolecular NMR. *J Biomol NMR* 6(2):135–140