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¹H, ¹⁵N and ¹³C resonance assignment of imidazole glycerol phosphate (IGP) synthase protein HisF from *Thermotoga maritima*

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Abstract

HisF comprises one half of the heterodimeric protein complex IGP synthase responsible for the fifth step of histidine biosynthesis. Here we report backbone and sidechain assignments necessary for characterization of protein dynamics involved in the allosteric mechanism of IGP synthase.

Keywords

NMR Assignments; HisF; IGP synthase; thermophile

Biological Context

Imidazole glycerol phosphate (IGP) synthase is a heterodimeric complex comprised of two folded proteins, HisF and HisH, which catalyzes the fifth step in histidine biosynthesis (Smith and Ames 1964; Beismann-Driemeyer and Sterner 2001). HisH (201 residues) is a type I glutamine amidotransferase responsible for the hydrolysis of glutamine to yield ammonia and glutamate (Beismann-Driemeyer and Sterner 2001). The ammonia product likely travels through the HisH protein interior to the HisH/HisF interface where it passes through a proposed gate and down the center of the HisF (253 residues) β -barrel to where it reacts with N'-[(5'phosphoribulosyl)formimino]-5-amino-imidazole-4-carboxamide-ribonucleotide (PRFAR) to yield the histidine precursor IGP (Lang, Thoma et al. 2000; Douangamath, Walker et al. 2002). Despite being separated by 30 Å, ligand binding in the active site of HisF has been shown to increase the k_{cat}/K_m for glutamine hydrolysis in HisH by 4900-fold (Myers, Amaro et al. 2005). Analysis of residue conservation reveals an extensive network connecting the two active sites and point mutations of non-catalytic residues in this region have been shown to dramatically affect both the rate of catalysis and coupling of the two reactions (Myers, Jensen et al. 2003). Together these data suggest that conformational changes may play a critical role in the coupling of the active sites of the two proteins. HisF and HisH are independently folded proteins, which facilitates NMR resonances assignments of each in isolation. In addition, the thermostability of the *T. maritima* enzyme used in this study aids in the NMR experiments, which can be performed at higher temperatures. Here we present the NMR assignments for H^N , N^H , C^α , and C^β resonances from the *T. maritima* HisF enzyme. These data will provide a basis for the study of protein dynamics involved in the function of IGP synthase and provide a model system for understanding long-range allosteric mechanisms in proteins.

Methods and Experiments

The full length gene for HisF was cloned from *Thermotoga maritima* genomic DNA into the pET14b(+) vector and expressed in Rosetta(DE3) cells (Novagen). Labeled samples were grown in fully deuterated M9 minimal media supplemented with ^{15}N ammonia chloride and ^{13}C glucose. Cells were grown at 37°C until the OD_{600} reached 0.9 at which point the temperature was reduced to 30°C and the cells were allowed to grow for an additional 14 hours. Protein purification followed a modified published protocol (Thoma, Obmolova et al. 1999). Harvested cells were lysed by sonication, heat shocked at 60°C and the HisF remaining in the supernatant was purified by column chromatography with HiTrapQ HP and S-200 gel filtration columns (GE Biosciences). Protein fractions greater than 98% purity were dialyzed into 10 mM MES pH 6.8, 50 mM KCl, 1 mM EDTA, 5% $^2\text{H}_2\text{O}$ (NMR buffer) and concentrated to 0.3-0.5 mM for NMR spectroscopic study.

All NMR data were collected on a Varian Inova 600 MHz spectrometer at 30°C equipped with pulsed field gradients and a triple resonance probe. Backbone assignments were completed using the following TROSY (Pervushin, Riek et al. 1997) triple resonance experiments: HN(CA)CB, HN(COCA)CB, HNCA, HN(CO)CA, HNCO, HN(CA)CO. All spectra were processed in NMRPipe (Delaglio, Grzesiek et al. 1995) and analyzed with Sparky (Kneller and Kuntz 1993).

Assignment and Data Deposition

The assigned ^1H - ^{15}N TROSY-HSQC spectrum for HisF is shown in Figure 1. 96.8% of the backbone ^1H - ^{15}N resonances have been assigned. Resonances for 100% of all C^α and C^β atoms and 94.4% of all backbone carbonyl atoms were assigned. Plotting the chemical shift difference for $^{13}\text{C}^\alpha$ resonances referenced to the average random coil values as a function of residue position reveals close secondary structural agreement with the published crystal structure as shown in Figure 2 (Lang, Thoma et al. 2000). A list of the ^1H , ^{13}C and ^{15}N chemical shifts has been deposited into the BioMagResBank (<http://www.bmrb.wisc.edu/>) under accession number BMRB - 15741.

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References

- Beismann-Driemeyer S, Sterner R. Imidazole glycerol phosphate synthase from *Thermotoga maritima* - Quaternary structure, steady-state kinetics, and reaction mechanism of the bienzyme complex. *Journal of Biological Chemistry* 2001;276(23):20387–20396. [PubMed: 11264293]
- Delaglio F, Grzesiek S, et al. Nmrpipe - a Multidimensional Spectral Processing System Based on Unix Pipes. *Journal of Biomolecular Nmr* 1995;6(3):277–293. [PubMed: 8520220]
- Douangamath A, Walker M, et al. Structural evidence for ammonia tunneling across the (beta alpha) (8) barrel of the imidazole glycerol phosphate synthase bienzyme complex. *Structure* 2002;10(2): 185–193. [PubMed: 11839304]
- Kneller DG, Kuntz ID. Ucsf Sparky - an Nmr Display, Annotation and Assignment Tool. *Journal of Cellular Biochemistry* 1993:254–254.
- Lang D, Thoma R, et al. Structural evidence for evolution of the beta/alpha barrel scaffold by gene duplication and fusion. *Science* 2000;289(5484):1546–1550. [PubMed: 10968789]
- Myers RS, Amaro RE, et al. Reaction coupling through interdomain contacts in imidazole glycerol phosphate synthase. *Biochemistry* 2005;44(36):11974–11985. [PubMed: 16142895]

- Myers RS, Jensen JR, et al. Substrate-induced changes in the ammonia channel for imidazole glycerol phosphate synthase. *Biochemistry* 2003;42(23):7013–7022. [PubMed: 12795596]
- Pervushin K, Riek R, et al. Attenuated T-2 relaxation by mutual cancellation of dipole-dipole coupling and chemical shift anisotropy indicates an avenue to NMR structures of very large biological macromolecules in solution. *Proceedings of the National Academy of Sciences of the United States of America* 1997;94(23):12366–12371. [PubMed: 9356455]
- Smith DWE, Ames BN. Intermediates in Early Steps of Histidine Biosynthesis. *Journal of Biological Chemistry* 1964;239(6):1848. [PubMed: 14213364]
- Thoma R, Obmolova G, et al. Efficient expression, purification and crystallisation of two hyperthermostable enzymes of histidine biosynthesis. *Febs Letters* 1999;454(1-2):1–6. [PubMed: 10413084]
- Wishart DS, Sykes BD. The ¹³C chemical-shift index: a simple method for the identification of protein secondary structure using ¹³C chemical-shift data. *Journal of Biomolecular NMR* 1994;4(2):171–180. [PubMed: 8019132]

Fig. 1. shows the assigned ^1H - ^{15}N TROSY HSQC spectrum of *Thermotoga maritima* HisF collected at 303 K and 600 MHz. Residue specific backbone assignments are shown at indicated ^1H and ^{15}N frequencies.

Fig. 2. shows the difference between measured $^{13}\text{C}^\alpha$ chemical shifts for HisF residues and average random coil chemical shifts. Positive chemical shift differences map closely with alpha helical regions in the crystal structure, while negative values match closely with beta sheets.