

Crystal Structure of the ATP-binding Subunit of an ABC Transporter

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INTRODUCTION

ATP-binding cassette (ABC) transporters (also known as traffic ATPases) form a large family of proteins responsible for movement of biochemical compounds through cell membranes. Members of the ABC transporter superfamily are widely found in prokaryotes and eukaryotes. Among the eukaryotic members, several are medically important proteins, such as the cystic fibrosis transmembrane conductance regulator (CFTR), the P-glycoprotein (MDR: multi-drug resistance protein), and the transporter associated with antigen processing (Tap1/Tap2). ABC transporters have two highly conserved ATP-binding domains and two transmembrane domains [1]. We report the crystal structure at 1.5 Å resolution of HisP [2], the ATP-binding subunit of the histidine permease, an ABC transporter from *Salmonella typhimurium*, and correlate the crystal structure of HisP with the biochemical, genetic, and biophysical properties of the wild type and of numerous mutant HisP proteins as well as with the properties of mutant CFTR and MDR proteins.

RESULTS AND DISCUSSION

HisP crystallizes in space group $P4_32_12$ with cell parameters $67 \times 67 \times 149$ Å. The crystal structure of HisP was determined by four-wavelength Multiple Anomalous Diffraction experiment [3] conducted at ALS beamline 5.0.2. The current model contains residues 5 to 262 of HisP_(his6), one ATP, three possible anions modeled as Cl⁻, and 322 solvent molecules. The overall shape of the crystal structure of the HisP monomer is that of an "L" with two thick arms (arm I and arm II, Fig. 1); the ATP-binding pocket is near the end of arm I (Fig. 2). A six-stranded β -sheet spans both arms of the "L" with a domain of $\alpha + \beta$ type on one side (within arm I) and a domain of mostly α -helices on the other side (within arm II) of the sheet. The overall fold of the structure is different from that of any known protein. However, the topology of the ATP binding pocket is close to that of RecA [4] and of the α - and β -subunits of bovine F1 ATPase [5]. In agreement with the notion that HisP forms a dimer *in vivo*, a dimer consisting of two monomers related by a two-fold axis is also found in the crystal structure of HisP (Fig.1). All available biochemical data about HisP in the HisQMP₂ complex are compatible with the characteristics of HisP in the crystal structure. The structure of HisP provides a basis for understanding properties those of defective CFTR proteins. This kind of information may eventually lead to a treatment for cystic fibrosis. Moreover, the ability to correlate the

properties of CFTR mutants with the crystal structure of HisP indicates that HisP is a good model for the nucleotide-binding domains of ABC transporters in general.

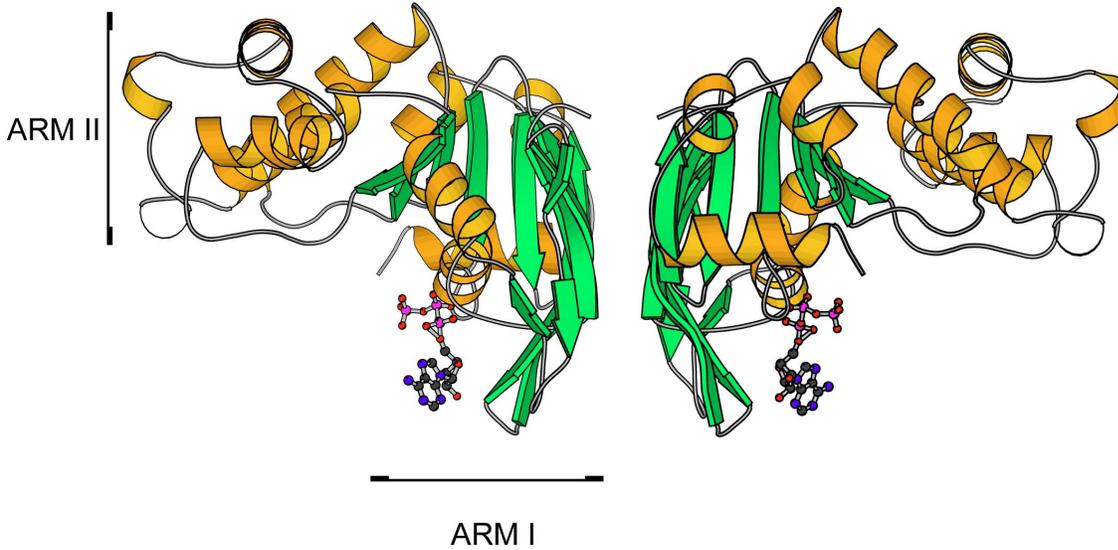


Figure 1. View of the HisP dimer along an axis perpendicular to its two-fold axis. The thickness of arm II is about 25\AA , comparable to that of membrane.

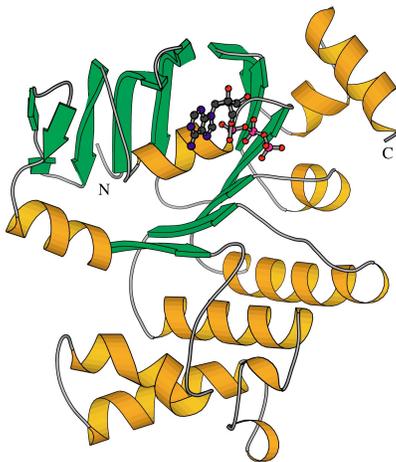


Figure 2. The crystal structure of HisP. This is a view of one monomer from the bottom of arm I, as seen in Figure 1, towards arm II, showing the ATP-binding pocket. The protein and the bound ATP are in "ribbon" and "ball-and-stick" representations, respectively. The N- and C- termini of HisP are indicated.

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