MAD Analysis of FHIT at the Structural Biology Center*

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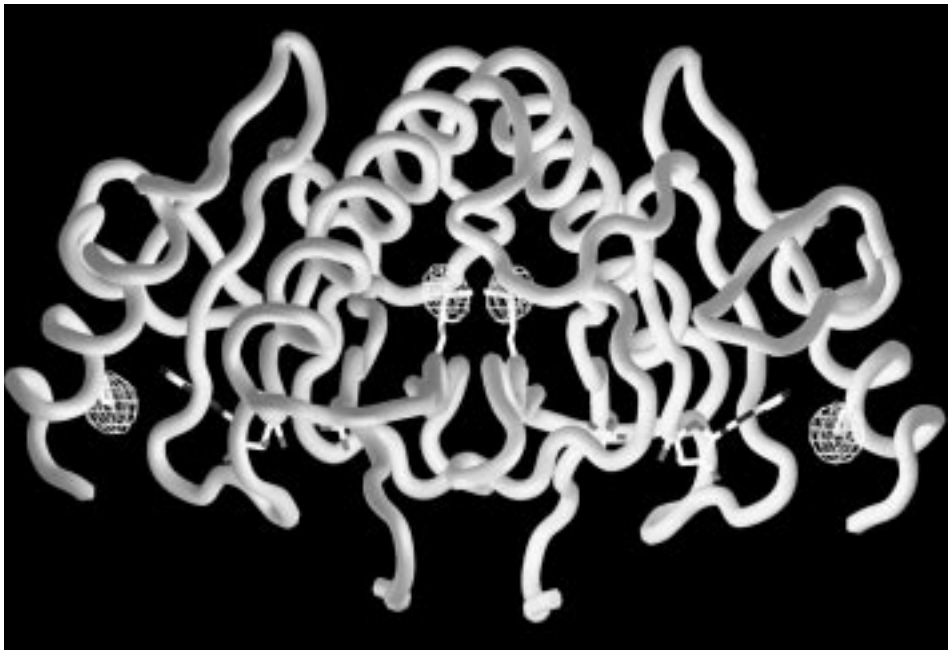

The three-dimensional crystal structure of the putative tumor suppressive fragile histidine triad protein, determined to 1.9-Å resolution by a group using multiwavelength anomalous diffraction data of the seleno-methionyl protein (white cages mark four Se sites).

The three-dimensional multiwavelength anomalous diffraction (MAD) structure of the fragile histidine triad (FHIT) protein, a member of a large and highly conserved family of proteins known as the histidine triad (HIT) family of proteins,[1,2] has been determined by a group using the 19-ID beamline at the Advanced Photon Source (APS). Although their in vivo function is still unknown, HIT proteins have been implicated in the binding and hydrolysis of nucleotide polyphosphates. It is likely that such a highly conserved family of enzymes, shown by the high sequence similarity among organisms ranging from mycoplasma, archae, bacteria, plants, and humans, are involved in some critical and ubiquitous biochemical pathway that remains undiscovered, several features of which were later identified by members of the same group (C.D. Lima, M. G. Klein, W.A. Hendrickson, "Structure-based Analysis of Catalysis and Substrate Definition in the HIT Protein Family," Science 278, 286-290, [1997]).

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FHIT, a gene that resides within a fragile site of human chromosome 3, has been postulated to be a human tumor suppressor gene. Unlike the p53 gene, few point mutations have been discovered in the coding regions of the FHIT gene. Instead, disruptions and translocations seem to be the major factors that alter the FHIT translation product. These alterations usually affect only one copy of the FHIT gene in the genome, leaving one full-length copy available in the cell. On the basis of the structure of the FHIT protein, it is unlikely that truncated forms of the protein resulting from altered FHIT transcripts would be capable of folding and interacting with the full-length gene product, thus eliminating the possibility of a dominant negative phenotype when one altered FHIT transcript exists in the cell with one full-length copy of the gene.

This study provides the first structure of FHIT, a diverse member of the HIT family of proteins, and suggests that distant members within the HIT superfamily share a similar overall fold and catalytic mechanism, some features of which can be extended to and inferred from the GalT family of proteins. As the catalytic activity observed *in vitro* demonstrates the ability of these enzymes to hydrolyze their substrates, it is likely that these enzymes could behave as nucleotidyl phosphotransferases through a pathway mediated via a covalent enzyme intermediate — several features of which can be inferred from the FHIT-nucleotide-analog complex. These studies support a mechanism in which the nucleophilic attack by respective histidines in various HIT family members at the α-phosphorus atom would result in a transient covalently attached nucleotidyl-protein intermediate before hydrolysis of the phosphoramidate bond.

Experimental Techniques

The Structural Biology Center (SBC) beamline 19-ID at the APS utilizes an undulator insertion device to generate very bright x-rays in narrow tunable harmonic peaks. The APS operates at suitably high particle energy to provide the first undulator harmonic in the x-ray range for MAD experiments at the K edge of selenium. The structure of FHIT has been determined by MAD phasing[3] using the selenomethionyl protein[4] in both its free and adenosine/sulfate-bound forms. The data for free and ligand-bound forms of FHIT were measured with the SBC APS1 3x3 charge-coupled device (CCD) array detector[5] and a FUJI image plate (IP) system, respectively. Both four-wavelength experiments took advantage of the ability to tune the peak of the harmonic by altering the gap width of the undulator during data collection, as has been done previously.[6] Three gap widths were chosen to center the peak of the first harmonic at the wavelength of choice. A single setting sufficed for peak and inflection points of the selenium K edge, which are very close together. Both experiments utilized crystals of the selenomethionyl-FHIT protein, although one of the crystals was also soaked in an adenosine/vanadate mixture in order to obtain a complex between protein and nucleoside. The nucleoside soak severely limited the diffraction quality of this sample in comparison with the free form of the protein. Although this enabled two independent structure determinations, it hampered the ability to compare results from the IP system and the CCD detector directly.

An almost complete set of phases was obtained by MADSYS[3] analysis to 2.8 Å spacings for the FHIT-adenosine/sulfate complex IP data set. This gave a fully interpretable Fourier map, whereupon the chain was traced and all ordered amino acid residues were positioned. A partially complete phase set was calculated to 2.0 Å spacings for the free FHIT CCD-data set, which resulted in an incomplete and disconnected electron density map. The limited extent of this data set was due to a time constraint, which prevented the completion of the experiment, and to an abundance of saturated low-angle reflections ($d > 5.0$ Å). The addition of phased reflections out to 5.0 Å spacings from the IP data set and density modification[7] at 2.0 Å yielded readily interpretable maps at 2.0 Å resolution, which allowed the peptide chain to be retraced and sidechains to be positioned.[8] Although these differences make it difficult to compare the strategies and detectors used in the two experiments, the merging and phasing statistics from both experiments demonstrate that undulator beamline 19-ID and the CCD detector are capable of producing data of sufficient quality for accurate MAD phasing. Ultimately, the native structure was refined against a more complete and higher resolution data set that was measured at beamline X4A at the National Synchrotron Light Source.

Description of the FHIT Structure

The electron density for native FHIT was well-enough defined to complete the model for residues 2-106 and 127-147, but not for the intervening segment or the first residue. Elution of FHIT upon size exclusion chromatography suggests that it is a dimer in solution and, although there is only one chain per asymmetric unit in the crystal, a dimer interaction similar to that previously reported for the PKCI dimer[9] is observed across a crystallographic twofold axis. The overall structure of the FHIT protomer can be described as a general α + β type and further subclassed as an α + β meander fold.[10] The FHIT protomer contains two helices, A and B, and seven β strands. Strands 3 to 7 form a five-stranded antiparallel sheet and antiparallel strands 1 and 2 form a β hairpin across from and at an angle to the other sheet. In the protomer, helix A packs on one side of the five-stranded antiparallel sheet. Helix

B packs on the same side of the molecule as helix A and primarily interacts with strands 3 and 4 and the loop connecting strands 2 and 3. Two protomers are brought together in the dimer by interactions between helices A and A′ and by the formation of a ten-stranded antiparallel sheet comprising the respective five-stranded antiparallel sheet of each protomer. A common hydrophobic core is formed from the protomer interactions within the context of the dimer. The extensive dimer interface occupies 2374 Å^2 per dimer compared to the total dimer surface area of 11239 Å^2 as computed with a probe radius of 1.4 Å.[11]

With the structure of FHIT at hand, the deletion of specific exons from the FHIT locus, which in turn codes for the aberrant FHIT transcripts observed in many cancer cell lines and primary tumors, can be analyzed in terms of protein structure.[12-16] Several of these aberrant transcripts have been sequenced, and details of the specific deleterious effects on FHIT transcripts have been described. Most of the observed deletions in the FHIT gene either truncate or eliminate the coding regions. Specific exons are deleted in some cases, leading to the possibility of a fused protein. Analysis of any of these deletions mapped onto the three-dimensional structure lead us to believe that none of these mutant proteins would fold and dimerize with the full-length FHIT protein; thus, it is unlikely if not impossible to have a dominant negative phenotype when one copy of the gene is found to be aberrant in length or sequence. This does not exclude the possibility of dose-dependent effects of FHIT expression when one copy of the gene has been disrupted. ❍

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