CRYSTAL STRUCTURE OF THE HEXAMERIC TRAFFIC ATPase OF THE HELICOBACTER PYLORI TYPE IV SECRETION SYSTEM: INSIGHTS INTO FUNCTION AND MODE OF ACTION

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The type IV secretion system of Helicobacter pylori consists of 10-15 proteins responsible for transport of the transforming protein CagA into target epithelial cells. Secretion of CagA crucially depends on the hexameric ATPase, HP0525, a member of VirB11-PulE family. The first crystal structure of the protein, as a binary complex of HP0525 bound to ADP, unveiled the hexameric HP0525 with the shape of a closed grapple where the claws of the grapple, formed by the C-terminal domains, are coming together to form the base of the chamber (Yeo et al., 2000). Each monomer consists of two domains formed by the N- and C-terminal halves of the sequence. ADP is bound at the interface between the two domains. In the hexamer, the N- and C-terminal domains form two rings, which together form a chamber open on one side and closed on the other. Current studies to gain insights into the function and mode of action of the protein are in progress. We further determined the structures of non-liganded protein and a complex with ATPγS. While the ATPγS binary complex is virtually indistinguishable from the ADP-bound form, the structure of apo-HP0525 exhibits significant conformational differences. Apo-HP0525 is an asymmetric hexameric assembly with each subunit displaying various degrees of displacement of the N-terminal domain about the ATP binding site. These three structures suggest that nucleotide binding is likely responsible for the observed conformational changes, and provide a structural basis for the function and mode of action of this essential bacterial ATPase.

Keywords: TYPE IV SECRETION SYSTEM HP0525 CRYSTAL STRUCTURE

ATOMIC RESOLUTION STRUCTURE OF RESTING AND INTERMEDIATE STATES OF NITRIC OXIDE REDUCTASE (CYTOCHROME P450nor)

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Crystal structures of nitric oxide reductase, cytochrome P450nor (P450nor), in the ferric resting and the ferrous carbonmonoxy (CO) states have been determined at 1.00 and 1.05 Å resolution, respectively. P450nor consists of 403 amino acid residues (46 kDa), and is one of the largest proteins refined to this resolution so far. The final models have conventional R-factors of 10.2 % (ferric resting) and 11.7 % (ferrous CO), with mean coordinate errors of 0.028 (ferric resting) and 0.030 Å (ferrous CO) as calculated from inversion of the full positional least-squares matrix. The crystal in the ferric nitric oxide (NO) state is readily reduced by X-ray irradiation at 0.7 Å within 120 minutes at 100K. The spectral change was monitored by using on-line microspectrophotometer. X-ray structural analysis of the ferric NO and its reduced intermediate states at 1.4 Å resolution revealed changes of coordination structure at heme-NO moiety, and of network of water molecules which may provide protons for the reduction of NO as well.

Keywords: ATOMIC RESOLUTION NITRIC OXIDE REDUCTASE INTERMEDIATE STATE

ATOMIC RESOLUTION STRUCTURE OF PHOTOPROTEIN OBELIN BOUND WITH DIFFERENT SUBSTRATES: NEW ASPECTS ABOUT FUNCTION

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Recombinant obelin from Obelia longissima in complex with coelenterazine h, displays some altered biochemical properties compared to the natural obelin-coelenterazine complex. A complete data set to 1.17Å has been collected at 100 K, using 1.0Å X-rays at a synchrotron source. The space group is *C*2 with unit-cell dimensions a = 83.021Å, b = 54.341Å, c = 52.442Å, β = 112.15°. The crystal structure has been determined by molecular replacement using the native obelin-coelenterazine complex (PDB code 1EL4) as the search model and refined to 1.17Å resolution. The electron density maps show clear electron density corresponding to two oxygen atoms at the *C*2-position of the coelenterazine h ligand, in contrast with the electron density observed at this position in the native obelin -coelenterazine complex. Details of the structure analysis and a comparison between the native and coelenterazine h structures will be presented.

This work is supported by funding from the University of Georgia Research Foundation, the Office of Naval Research and the Russian Academy of Sciences.

Keywords: PHOTOPROTEIN, OBELIN, ATOMIC RESOLUTION STRUCTURE

CRYSTAL STRUCTURE OF POPLAR PLASTOCYANIN b

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Poplar plastocyanin exists in two isoforms, PCa and PCb, in ratio 1:1[1]. They exibit significant differences in their UV-vis absorption[2] and Raman spectra[3], and in the pH-dependence of the redox-potential [4]. The PCa structure was solved previously [5]. We determined the crystal structure of PCb both in the reduced (pH 4,6,8) and oxidized states (pH 4,6) at high resolution between 1.35Å-1.65Å. In addition, we refined the PCa structure at ultra-high resolution,1.05Å, in the reduced and oxidized states (pH 4,6,8). The sequences of PCa and PCb differ in 12 a.a. residues disposed mainly in the southern end of the protein. Even outside this range,the structural models of PCa and PCb show substantial conformational changes with r.m.s. deviations that exceed by an order of magnitude those between the oxidized and reduced structures of PCa. The most pronounced differences between the PCa and PCb structures are observed in the segment from 34 to 65. A further difference occurs in the Cu-ligand geometry in the reduced state of low-pH structure. In PCb,sulfate anion is hydrogen bonded to $N\varepsilon_2$-His87 and prevents the imidazole ring from swinging away, as it is the case in PCa. This structural difference may explain the weaker pH-dependence of the redox potential of PCb[4].

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Keywords: X-RAY STRUCTURE PLASTOCYANIN