

rossmann-like domain

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### Crystallographic studies of ferredoxin-NAD(P)<sup>+</sup> reductase from *Chlorobium tepidum*

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Ferredoxin-NAD(P)<sup>+</sup> reductase (FNR) is a key enzyme that catalyzes the photoreduction of NAD(P)<sup>+</sup> to generate NAD(P)H during the final step of the photosynthetic electron-transport chain. FNR from the green sulfur bacterium *Chlorobium tepidum* is a homodimeric enzyme with a molecular weight of 90 kDa; it shares a high level of amino-acid sequence identity to thioredoxin reductase rather than to conventional plant-type FNRs. In order to understand the structural basis of the ferredoxin-dependency of this unique photosynthetic FNR, *C. tepidum* FNR has been heterologously expressed, purified and crystallized. The *C. tepidum* FNR was crystallized in two forms, I and II, from screening conditions consisting of 20% PEG 3350 containing 200 mM ammonium sulfate or diammonium tartrate as precipitant. Form I crystals belong to the orthorhombic space group C222<sub>1</sub>, with unit cell parameters  $a = 100.5$ ,  $b = 128.0$ ,  $c = 128.4$  Å. Assuming the presence of one dimer in the asymmetric unit, the Matthews coefficient ( $V_M$ ) is  $2.6$  Å<sup>3</sup>/Da corresponding to a solvent content of 53.2%. Form II crystals belong to the tetragonal space group  $P4_122$  or  $P4_322$ , with unit-cell parameters  $a = b = 82.0$ ,  $c = 162.7$  Å. The  $V_M$  value of  $3.5$  Å<sup>3</sup>/Da indicates that the form II crystal contains one protein molecule per asymmetric unit. Diffraction data were collected from a form I crystal to 2.4 Å resolution on the synchrotron-radiation beamline NW12 at the Photon Factory.

Keywords: FNR, ferredoxin, thioredoxin reductase

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### The crystal structure of the staphylococcal amidase AmiE reveals the active site of a metalloenzyme

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Staphylococci are gram-positive bacteria that mainly colonise human skin and the upper respiratory tract. While most staphylococcal infections are averted by a working immune system, they can be life threatening in immunocompromised hosts. Among the most common diseases are endocarditis, pneumonia and the toxic shock syndrome. The ability of staphylococci to form biofilms upon attachment to polystyrene surfaces is another pathogenic factor and especially relevant in transplantation of medical prostheses. Peptidoglycan (PGN) hydrolases such as the major autolysin AtlE from *Staphylococcus epidermidis* play an important role in cell wall turnover, which renders them appealing targets for drug design.

During cell division AtlE splits the equatorial septum, thus allowing the formation of daughter cells. Deletion mutants exhibit a severely disordered division pattern and are biofilm-negative. We have determined the crystal structure of the catalytic domain AmiE, a N-acetylmuramyl-L-alanine amidase at 1.7Å resolution. The protein adopts a globular fold, with several  $\alpha$ -helices surrounding a central  $\beta$ -sheet. Alignments with homologous proteins revealed a conserved surface cleft, which is capable of incorporating a PGN-like ligand. A divalent cation is bound in the active site and likely participates in catalysis. Analysis of the architecture of the binding site and the location of key residues allow us to postulate a mechanism of function, which is likely to be that of a metalloenzyme. Mutations of amino acids directly involved in catalysis resulted in severe changes of adjacent loops and a loss of activity. The high-resolution structure of AmiE advances our understanding in terms of ligand binding and enzymatic function, thus providing an excellent base for future drug design.

Keywords: AmiE, amidase, peptidoglycan

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### Structure of the inactive mutant of arabinanase complexed with oligosaccharides

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The three-dimensional structure of the thermostable endo- $\alpha$ -1,5-L-arabinanase, ABN-TS, from a strain of *Bacillus thermodynamitricans* TS-3, has been determined at 1.9 Å resolution. We have started the structure analysis of the inactive mutant of ABN-TS complexed with oligosaccharides to elucidate the substrate recognition and reaction mechanism of the enzyme. ABN-TS and the inactive mutant D27A were expressed in *E. coli* as His-tag fusion proteins at their C-termini. They were purified by Ni-affinity, anion-exchange, and size-exclusion chromatographic techniques. The activity of the purified WT (ABN-TS with His-tag) was assayed using debranched arabinan as a substrate by the Somogyi-Nelson method. The WT showed the same catalytic activity as the native ABN-TS, while the mutant D27A showed only very weak activity. The crystals of the inactive mutant complexed with oligosaccharides were prepared by co-crystallization and soaking methods using PEG8000 as a precipitant. Data collection and structure analysis are now in progress.

Keywords: arabinanase, glycoside hydrolase, crystal structure analysis

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### Structure of endo-1,5- $\alpha$ -L-arabinanase from *Penicillium chrysogenum*

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