

agents, anti-Alzheimer drugs, and insecticides. A better understanding of its molecular mode of action thus goes beyond academic interest, and may be beneficial to the design of anti-Alzheimer drugs, antidotes against OP poisoning and novel insecticides. We have solved crystal structures of conjugates with *Torpedo californica* AChE (*TcAChE*) of the potent nerve agent, soman. Soman reacts covalently with the active-site serine to form an initial conjugate (the 'non-aged' conjugate), that can be reactivated by suitable oximes. This conjugate subsequently undergoes a dealkylation reaction known as 'aging', that produce a conjugate that can no longer be reactivated (the 'aged conjugate') [1]. X-ray crystallography data collection performed at the ESRF (Grenoble, France), permitted solution of the structure of both conjugates. Since 'aging' reaction is fast at room temperature, the crystal-soaking time prior to cryo-cooling had to be reduced to minutes in order to trap the 'non-aged' conjugate. The structures obtained supply a structural basis for rational designing of more oxime reactivators that may serve as antidotes of OP intoxication.

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MS03 P13

Crystallographic study of *E. coli* WrbA protein in complex with its flavin cofactor
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The currently studied protein WrbA from *Escherichia coli* belongs to a new family of multimeric flavoproteins [1,2], that contain flavin mononucleotide (FMN) as a physiological cofactor. The latest observations revealed that *E. coli* WrbA and its homologues exhibit the NAD(P)H:quinone oxidoreductase activity, which is proposed to be implicated in protection mechanisms against oxidative stress [3]. Crystallographic study of the *E. coli* WrbA is aimed at proper characterization of the new protein family with respect to its recently revealed oxidoreductase activity. Recombinant WrbA protein was expressed in *E. coli* CY15071(IDE3) cells and purified as described previously [2]. Pure WrbA protein was obtained without bound cofactor, because of the loss of FMN during purification. The complex of WrbA protein with the FMN cofactor for crystallization was prepared by incubation of pure WrbA with equimolar concentration of FMN. Crystallization was carried out at 285 K by the sitting-drop vapor-diffusion technique, with droplets containing equal parts of protein and precipitant solution. Yellow, well-formed tetragonal crystals of WrbA protein in complex with its flavin cofactor were obtained from several crystallization conditions. Yielding of diffraction-

quality crystals without additional optimization steps shows the positive influence of FMN cofactor on crystallization of WrbA protein. This effect reflects FMN as a potential stabilizer of the protein structure. Diffraction data for the crystals reported here were collected at 100 K at synchrotron DESY, beamline X13 (EMBL) with an X-ray wavelength of 0.805 Å. The different crystallization conditions yielded two forms of primitive tetragonal crystals having different space groups (P43212 ; P41212) and different unit-cell dimensions (a = b = 94.351 Å, c = 175.371 Å; a = b = 61.125 Å, c = 168.375 Å; respectively). The resolutions of 2.60 Å and 1.99 Å, respectively, were reached. The diffraction limit of the better-diffracting crystal represents the best resolution reported to date for WrbA protein in complex with FMN. Solving of the structures from both data sets is in progress.

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MS03 P14

Carbohydrate-binding of the starch binding domain of *Rhizopus oryzae* glucoamylase in complex with β-cyclodextrin and maltoheptaose
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Glucoamylase hydrolyses starch and polysaccharides to β-D-glucose. *Rhizopus oryzae* glucoamylase consists of two functional domains, an N-terminal starch binding domain (SBD) and a C-terminal catalytic domain. The two domains are connected by an O-glycosylated linker. The crystal structures of the SBD in complex with a cyclic carbohydrate, β-cyclodextrine, and a linear carbohydrate, maltoheptaose, were determined. The overall structures of the SBD complexes belong to a β-sandwich fold with an immunoglobulin-like structure and two carbohydrate-binding sites were observed. Site I is created by several conserved aromatic residues, Trp47, Tyr83, and Tyr94, to form a broad, flat, and firm hydrophobic surface. In addition to the hydrophobic interaction, numerous asparagines residues are involved in the hydrophilic interactions to carbohydrate. Site II is built up by Tyr32 and Phe58 to produce a protruded and narrow binding environment. Site I undergoes a bigger conformational change than site II upon the carbohydrate binding.