s1.m8.p4 **Crystal Structure of the Type III Secretion Chaperone SycT from *Yersinia enterocolitica*.** Carina Buettner, Hartmut Niemann and Dirk Heinz, *Division of Structural Biology, German Research Centre for Biotechnology (GBF), Mascheroder Weg 1, D-38124 Braunschweig, Germany. E-mail: cbu@gbf.de*

Keywords: Type III secretion; Chaperone; Bacterial pathogenesis

Numerous invasive pathogenic bacteria, among them the human pathogenic *Yersinia* spp. (*Y. pestis, Y. enterocolitica, Y. pseudotuberculosis*), use a so-called contact-dependent or Type III secretion (TTS) system to deliver multiple virulence effector proteins directly into the eucaryotic host cell cytosol. *Yersinia*-injected virulence effector proteins are termed Yops (*Yersinia* outer proteins). After translocation into the host cell, they interfere with signalling pathways involved in the regulation of inflammation and cytoskeletal dynamics. Several of these TTS effector proteins require for their effectual translocation specific cytosolic chaperones that bind to distinct domains of the cytotoxin. These customized chaperones are small (14-19 kDa), acidic (pI~4-5) proteins but differ in sequence. Although their definite role is still mysterious, diverse functions have been proposed. They are suggested to serve as anti-aggregation and stabilizing reagents. Furthermore they are proposed to maintain their cognate substrates in a secretion-competent state, to establish an hierarchy on translocation and to act as secretion pilots. We are interested in the molecular characteristics of the components of Type III secretion systems to understand their functionalities with regard to infection biology. Recently we successfully crystallized the chaperone SycT from *Yersinia enterocolitica*, a widely distributed causative agent of gastrointestinal infection. SycT is the specific chaperone of YopT, a cysteine protease inactivating membrane-associated Rho-GTPases in macrophages and thereby forestalling phagocytosis of the bacterial pathogen. The chaperone was heterologously produced in E. coli. The determination of the three-dimensional protein structure by X-Ray crystallography is in progress.

s1.m8.p5 **Substrate and dioxygen binding to an endospore coat laccase from *Bacillus subtilis.*** Francisco J. Enguita, David Marçal, Lígia O. Martins, Rosa Grenha, Isabel Bento, Gonçalo Gato, Peter F. Lindley and Maria Arménia Carrondo, *Instituto de Tecnologia Química e Biológica, Oeiras, Portugal. E-mail: carrondo@itqb.unl.pt*

Keywords: Laccase; Bacillus; Endospore

Endospores produced by the Gram positive soil bacterium *Bacillus subtilis* are shielded by a proteinaceous coat formed by over thirty structural components which self-assemble into a lamellar inner coat and a thicker striated electrodense outer coat. The 65 kDa CotA protein is an abundant component of the outer coat layer. CotA is a highly thermostable multicopper oxidase with laccase activity, whose assembly into the coat is required for spore resistance against hydrogen peroxide and UV light. We have reported previously the structure of the native CotA laccase solved by the MAD method using the copper anomalous signal [1,2]. The CotA laccase has been also crystallised in the presence of the non-catalytic co-oxidant, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) [ABTS] and the structure determined using synchrotron radiation [3]. The binding site for this adduct is well defined and indicates how ABTS in conjunction with laccases could act as an oxidative mediator towards non-phenolic moieties. In addition, a dioxygen moiety is clearly defined within the solvent channel oriented towards one of the T3 copper atoms in the trinuclear centre. The present study shows that cryo-freezing of crystals of the CotA laccase from *B. subtilis* soaked with ABTS for one hour induce the ABTS to form a stable complex with the laccase. Soaking times longer than this appear to cause dissociation of the ABTS and substantial loss of copper from the trinuclear centre. The enzyme has probably been trapped in a dormant form with an dioxygen molecule in the solvent channel awaiting binding to one of the T3 copper atoms. When the enzyme receives further electrons, the dioxygen will move closer to the copper and will be reduced to two molecules of water. Clearly more detailed studies are required to define the precise mechanisms of both dioxygen reduction and the role of oxidative mediators. We are currently investigating the reaction of CotA laccase crystals with other laccase activity mediators and substrates.

[1] Enguita, F.J., Matias, P.M., Martins, L.O., Placido, D., Henriques, A.O., Carrondo, M.A. (2002) *Acta Cryst*. D**58**, 1490-1493.
[2] Enguita, F.J., Martins, L.O., Henriques, A.O., Carrondo, M.A. (2003) *J. Biol. Chem*.. **278**, 19416-19425.
[3] Enguita, F.J., Marçal, D, Martins, L.O., Grenha, R., Henriques, A.O., Lindley, P.F., Carrondo, M.A. (2003) *J. Biol. Chem*. (In press).