CRYSTALLIZATION OF KILLER PROTEIN SPOIISA AND ITS ANTIDOTE SPOIISB FROM *BACILLUS SUBTILIS*

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The *B. subtilis spoIISA* gene encodes a 248-residue protein containing three predicted transmembrane domains [2] with the last two-thirds of protein being located in the cytoplasm. The *spoIISB* gene codes for a hydrophilic 56-residue protein. None of these proteins shares any sequentional similarity to a protein of known function, providing no clue to their function and evolutionary origin. The *spoIISB* translation start codon overlaps the *spoIISA* translation stop codon what is a strong indication that the two genes constitute an operon [1].

A null mutation in *spoIISB* leads to the strong sporulation defect, whereas disruption of either *spoIISA* or whole *spoIIS* locus has no effect on sporulation. Altogether, this facts indicates that a) *SpoIISA* prevents normal progression of the sporulation process; b) *SpoIISB* neutralizes the action of *SpoIISA*; and c) *spoIIS* locus does not play essential role in sporulation process. The strain carrying the *spoIISB* null allele does not exhibit any obvious defect during exponential growth. This immunity of exponentially growing cells to the absence of *SpoIISB* most likely reflects the existence of a threshold concentration below which *SpoIISA* does not significantly impair cell viability, since the induced expression of additional *spoIISA* gene copy led to rapid drop in optipcal density of exponential phase cell [1].

Since it has structural features of an integral membrane protein, *SpoIISA* could act as a holin and allow some endolysin to gain access to the peptidoglycan [3]. Local solubilization of the cell wall would lead to membrane disruption and consequently to the large plasmolysis zones which were observed by electron microscopy [1]. However, *SpoIISA* does not show any similarity to known holins and is significantly larger than holins identified so far [3]. It is therefore quite possible that the cytoplasmic membrane itself is the target of the toxic action of *SpoIISA*.

In our work we over-expressed cytosolic part of *SpoIISA* His-tag fusion protein together with intact *SpoIISB* protein in *Escherichia coli*. The both proteins were purified using single step metal chelate affinity chromatography, and therefore isolated proteins formed stable complex, which indicates their specific interactions. The gel filtration and electrophoresis experiments showed that the most abundant form of the complex is oligomer consisting of two SpoIISA and two SpoIISB molecules. This observation confirms the results gain using genetic complementation experiments, which predicted that SpoIIA acts as an oligomer [1]. The purified SpoIISA-SpoIISB protein complex was used for crystallization trials.

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SHORTCUTS TO MUSHROOMS: NMR AND MS ANALYSES OF FUNGAL PYRANOSE OXIDASE

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Pyranose 2-oxidase (P2O, EC 1.1.3.10), a fungal periplasmic homotetrameric flavoprotein (~300 kDa), has received increased attention due to its potential analytical and biotechnological applications [1]. This enzyme catalyzes C-2/C-3 oxidation of numerous sugars to their corresponding dicarbonyl derivatives (aldos-2-uloses or glycosid-3-uloses), accompanied with the reduction of flavin adenine dinucleotide (FAD), an obligatory cofactor. P2O has a great biotechnological potential as a catalyst in the key step of C-2 oxidation of D-glucose and D-galactose in the production of modern low-caloric sweeteners D-fructose and D-tagatose.

Our research on the P2O from the fungus *Trametes multicolor* followed three subjects. The first one was the study of the enzyme substrate specificity and characterization of its reaction products by spectral analyses (NMR, FAB mass spectrometry) [2,3]. Further, we applied MALDI mass spectrometry with post-source decay (PSD) analysis to determine sequence segments suitable for designing PCR primers for cloning cDNA corresponding to the P2O gene [4]. Finally, we elucidated the structure of the P2O flavin-binding domain, which is of importance for understanding the enzyme reaction mechanisms and possible optimized application. The combination of PSD-MALDI MS and electrospray ion trap mass spectrometry (ESI IT-MS) on the isolated flavopeptide identified flavopeptide sequence, flavin type and flavin linkage site. The type of the aminoacyl flavin covalent link was determined by NMR spectroscopy resulting in the structure STXW with X = 8 -(N^3-histidyl)-FAD [5].