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STRUCTURAL AND BINDING PROPERTIES OF A QUADRUPLE EPITOPE REGULATING TAU PROTEIN OLIGOMERIZATION

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Polymerization of tau protein and the deposition of insoluble tau lesions highly correlate with the cognitive decline in Alzheimer's disease and related tauopathies [1]. The most probable nuclei of tau protein aggregation are the hexapeptides VQIINK, VQIVYK and other two segments, each localized in one of the microtubule-binding repeats of tau [2, 3]. Recently we have described an anti-tau monoclonal antibody, DC8E8, which effectively blocked tau-tau interaction by binding four highly homologous epitopes in the immediate vicinity of aggregation-promoting hexapeptides [4]. The sequence of DC8E8 epitope served as a base for the construction of an active vaccine, which is currently under clinical development [5].

In the present study we aimed to answer the questions about the sequence and structure requirements of DC8E8-based immunomodulation. We have determined the kinetics of DC8E8 binding to each of its four binding sites on the tau protein molecule. Further, we have crystallized DC8E8 Fab fragment and its complexes with several tau peptides and solved the structure by X-ray crystallography. Finally, we validated our structural findings by alanine-scanning mutagenesis of DC8E8 paratope residues to confirm antibody contact sites. Obtained results allowed mechanistic insights into DC8E8 inhibitory activity.

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L5

ASYMMETRIC CELL DIVISION DURING SPORULATION IN *BACILLUS SUBTILIS*

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Bacillus subtilis is a Gram-positive microorganism which is able to differentiate during process called sporulation. A hallmark of sporulation in *B. subtilis* is the polar cell division. As occurs during vegetative cell division, the tubulin-like GTPase FtsZ forms a ring-like structure at mid-cell. At the onset of sporulation, however, the division apparatus is not assembled at this site, instead the Z-ring migrates from mid-cell on a spiral trajectory to the two cell poles in a process that depends on sporulation-specific overexpression of *ftsAZ* and the presence of SpoIIE. SpoIIE colocalizes with the polar Z-rings. One of the Z-rings matures into the sporulation septum while the other dissolves. Asymmetric cell division otherwise appears to involve the same set of proteins as constitute the divisome during vegetative cell division. However, the resulting sporulation septum is much thinner. Interestingly, SpoIIE is the only sporulation-specific protein whose deletion or mutation causes changes in the ultrastructure of the asymmetric septum. *spoIIE* null mutants are defective in sporulation and at low frequency give rise to aberrantly thick asymmetric septa [1]. Accompanying these morphological changes is a coordinated programme of differential gene expression, involving intercellular signalling processes, that leads to the activation of the RNA polymerase sigma factors, ^{F} and ^{G} in the forespore and ^{E} and ^{K} in the mother cell [2].

SpoIIE from *B. subtilis* is an 827 residue protein that consists of three regions. It has 10 putative membrane-spanning segments (region I) at its amino terminus and a PP2C-type phosphatase domain (region III) at its C-terminus. The central region II is required for localisation of SpoIIE to the divisome and its reported interaction with FtsZ. The structure of the PP2C phosphatase domain of SpoIIE was already solved [3]. In contrast, the structure of N-terminal two-thirds of SpoIIE and the character of its interactions with partner proteins are unknown. We re-

cently identified a new partner of SpoIIE, the cytoskeletal protein, RodZ, which is essential for cell shape determination. This interaction is additionally required for asymmetric septum formation and sporulation. Presently we try to employ a new method of "slimfield" microscopy to study the oligomeric state of SpoIIE in live cells and during its different roles in asymmetric septum site recognition and its formation, activation of ^{F} and forespore engulfment.

Although, SpoIIE has a critical function in determining the site of formation of the sporulation septum, it is not understood (i) how it localises to the polar septum (ii) how it causes FtsZ to relocalise from mid-cell to the polar site (iii) what role SpoIIE plays in septal thinning, (iv) how its SpoIIAA~P phosphatase activity is controlled so that ^{F} acitivation is delayed until the septum is completed (v) what role SpoIIE playes in SpoIIQ-SpoIIIAH channel formation. How, SpoIIE brings about activation of ^{F} in the forespore but not in the mother cell has been the subject of great interest. Plausible mechanisms have been invoked based on (i) preferential SpoIIE localisation on the forespore face of the septum, (ii) transient gene asymmetry leading to accumulation of a SpoIIE inhibitor in the mother cell and (iii) the volume difference between the compartments leading to higher specific activity of equipartitioned SpoIIE, but this question is not fully resolved [4].

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L6

INTERACTION OF THE MASON-PFIZER MONKEY VIRUS MATRIX PROTEIN AND ITS BUDDING DEFICIENT MUTANTS WITH THE PLASMA MEMBRANE

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Mason-Pfizer monkey virus (M-PMV) is a simple Betaretrovirus and is often used as a model organism for studying the late phase of the retroviral life cycle because the formation of the immature viral particle and budding are spatially separated. Retroviral matrix proteins play a key role in the transport of viral precursor proteins inside the cell and in their interactions with cellular membrane which is an important prerequisite for the final step in the viral budding and maturation.

We have studied the interactions of the M-PMV wild-type MA and its two budding deficient mutants (T41I/T78I and Y28F/Y67F) with water soluble phospholipids with shorter fatty acid residues and liposomes consisted of phospholipids with naturally long fatty acid chains. The interactions were monitored by several experimental techniques, such as liposomes pelleting assays, NMR spectroscopy and MicroScale Thermophoresis. We found that the affinity of M-PMV MA proteins to both water soluble phospholipids and liposomes is much lower than in HIV-1 MA and therefore, the conclusion is that M-PMV might adopt a different membrane binding mechanism than HIV-1.

L7

CALCIUM-DRIVEN FOLDING OF RTX DOMAIN -ROLLS RATCHETS TRANSLOCATION OF RTX PROTEINS THROUGH TYPE I SECRETION DUCTS

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Type I secretion system (T1SS) conduits span the envelope of Gram-negative bacteria and enable single step translocation of proteins, directly from bacterial cytosol into extracellular space and without a periplasmic secretion intermediate. Most of the T1SS substrates belong to 'Repeats-in-Toxin' (RTX) proteins characteristic of the presence of the C-terminal secretion signal preceded by single or several blocks of calcium-binding tandem RTX motifs with a prototypical consensus sequence GGxGxDxxx. The RTX repeats remain unfolded inside the Ca^{2+}-depleted bacterial cytosol until their release outside of the bacterial cell into Ca^{2+}-rich environment (>1 mM) where bind Ca^{2+} ions and fold into a -roll structure. While the proton motive force and the hydrolysis of ATP by the ABC transporter subunit of T1SS are essential for promoting initial movement of the substrate through the conduit, the mechanism underlying translocation of often very large RTX proteins (>1,000 residues) is unknown. Here we show that Ca^{2+}-dependent folding of the emerging C-terminal RTX repeats outside the cells acts as an efficient intramolecular ratchet during translocation of the *Bordetella pertussis* adenylate cyclase toxin (CyaA) through the T1SS conduit. Folding of the RTX repeats is initiated by formation of a structurally conserved C-terminal folding nucleus that governs the vectorial folding of the RTX repeats from the carboxy- towards the amino-terminus of the polypeptide. Sequential assembly of RTX repeats into -roll continuously prevents backsliding of the substrate through the T1SS duct indicating that a passive diffusion (Brownian ratchet) is sufficient to achieve translocation. These results provide new insights into secretion and activity of major virulence factors of important pathogens, such as the whooping cough agent *Bordetella pertussis* that is re-emerging in the most developed countries.

L8

STRUCTURE-FUNCTION RELATIONSHIP OF PARADOXICALLY THERMOSTABLE HALOALKANE DEHALOGENASE OF PSYCHROPHILIC ORIGIN

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Extremophilic organisms represent unexplored resource of biotechnologically exploitable enzymes that can work in harsh conditions, e.g., low or high temperatures, presence of organic solvents and inorganic salts [1]. Novel haloalkane dehalogenase DmxA originates from *Marinobacter* sp. ELB17 isolated form Antarctic lake [2]. Despite its psychrophilic origin, the enzyme exhibits unusually high melting temperature (T_m = 65.9 ± 0.1 °C) among so far characterized wild-type haloalkane dehalogenases. DmxA also possesses broad substrate specificity and high enantioselectivity towards -substituted bromoalkanes (E > 100) and brominated esters (E > 200). Gel permeation chromatography, native gel electrophoresis and X-ray crystallography revealed that the enzyme exists in the solution in monomer-dimer equilibrium due to cysteine bridge formation between individual subunits of DmxA dimer. Since cysteine bridges are often responsible for protein stability, we examined its role in DmxA stability by testing enzyme activity and stability under reducing conditions and also by site directed mutagenesis. Although we suc-

cessfully disrupted the bridge, thermal stability and catalytic properties of the enzyme remained unaffected. DmxA also differs from other family members by one of two halide-stabilizing residues (Gln instead of Asn). According to the orientation of Gln in the enzyme structure, it was found that the residue can contribute to enzyme stability by extra hydrogen interactions with surrounding amino acids. In order to investigate the impact of unusual halide stabilizing residue on DmxA stability, unique Gln residue was replaced by commonly present Asn. While introduced mutation significantly changed substrate specificity and catalytic activity, it had a negligible effect on DmxA stability. We further examined accessibility of the access tunnels and hydrophobic pocket of the enzyme. Molecular dynamics followed by CAVER [3] analysis revealed that DmxA has very narrow tunnels (with average bottleneck radius 1.0 to 1.2 Å) that are mostly closed during the simulations. In order to investigate the hypothesis that narrow tunnels are responsible for high thermostability of the enzyme, two bulky residues located at the tunnel mouth were replaced by smaller residues. The resulting mutant exhibited 9 °C lower thermostability than the wild-type enzyme. Simultaneously, introduced mutations in the tunnel affect enzyme activity, substrate specificity and enantioselectivity. These results suggest that narrow tunnels in DmxA contribute to its paradoxical stability.

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L9

DEVELOPMENT OF A NEW PROTEIN SCAFFOLD FOR DIRECTED EVOLUTION

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Directed *in vitro* evolution, e.g. phage display or ribosome display are powerful techniques to develop protein molecules binding with high affinity and specificity to medically important target biomolecules. These protein binders may serve as an alternative to antibodies, now usually preferred for use as protein therapeutics and diagnostics. Currently, a few tens of proteins with stable folds are used as so called scaffolds for directed evolution. We decided to enrich this repertoire by independent engineering a new stable, non-immunogenic scaffold capable of extensive mutations on its surface. We evaluated several protein structures deposited in PDB by computational mutation analysis and experimental characterization of their stability to discover a novel effective scaffold. A protein structure was selected to be used as scaffold for establishing a highly diverse library consisting of 10 mutable sites theoretically generating 10^{13} protein variants, the actual complexity of the purchased DNA library is estimated to be still reasonable 10^{10} variants. After 3 rounds of ribosome display selection, we obtained binders targeting an immune-related protein with 5uM affinity as determined by MST. We assume that after forth and fifth rounds of ribosome display, respectively, the affinity of new binders will increase to sub-micromolar range suitable for evolution of a binder with potential to be developed into a medically applicable product.

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