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Insights into the structural background and mechanism of inhibition of dUTPases by a proteinaceous inhibitor

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The dUTPase enzyme family plays a key role in maintaining genomic integrity throughout preventing uracil incorporation into DNA (1). Their importance is underlined by their presence in almost all type of organisms from bacteria to primates. Their dysfunction poses severe consequences to most cells or organisms, since increased uracil incorporation leads to DNA double strand breaks and eventually cell death via hypersensitization of the uracil base excision repair process. Therefore dUTPases are regarded as chemotherapeutic targets in certain diseases like tuberculosis and malaria, and also in some aspects of cancer treatment (2,3). To design effective, species-specific dUTPase inhibitors with minimal side-effects, it is essential to explore carefully any structural differences among dUTPase homologues encoded by different species.

A recently discovered staphylococcal protein termed Stl is able to inhibit a couple of dUTPase homologues (4,5). However, its inhibitory potential may be different according to species-specific structural differences among dUTPases. We have pointed out that protein Stl which was originally identified as a bacterial repressor is also able to form a stable protein complex with the eukaryotic *Drosophila melanogaster* dUTPase and inhibit remarkably its enzymatic activity (6).

Our results indicated that the amino acid side-chains being involved in this interaction are partially evolutionarily conserved among bacterial and eukaryotic dUTPase homologues. Interestingly, we have also found that while Stl forms a strong complex with *Escherichia coli* dUTPase, still, no inhibition of this dUTPase was observed – this is exceptional among the numerous investigated complexes (4,5,6,7). Consequently we became committed to uncover the structural bases for the lack of enzymatic inhibition upon complex formation in the *E. coli* dUTPase.

Relying on our recent results regarding the interaction surface of the human dUTPase and protein Stl (7), we designed a rational mutation screen. We identified specific amino acid side-chains in the *E. coli* dUTPase sequence as potentially relevant factors for the lack of inhibition, and found that among the generated mutants, two became sensitized to Stl-induced inhibition. We successfully grew crystals from these mutants and obtained a complete X-ray diffraction dataset at 2.5 Å resolution for one of these. The structure is being solved and will be presented at the conference - it is expected to provide key novel insights into the mechanism of Stl induced dUTPase inhibition.

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