[MS5-P46] Structural analysis of Phi11 Staphylococcal dUTPase

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Genome integrity requires well-controlled cellular pools of nucleotides. deoxyuridin-triphosphatases (dUTPases) are responsible for regulating cellular deoxyuridine-triphosphate (dUTP) levels and providing deoxyuridin- monophosphate dUMP for the deoxythymidine-triphosphate dTTP biosynthesis. In Staphylococcus, phage dUTPases are also suggested to be involved in a moonlighting function also regulating expression of pathogenicity island genes. Transfer of virulence genes in Staphylococci is under control by repressor proteins (e.g. Stl) and was recently reported to be activated by helper phage dUTPases. Investigation of the molecular mechanism proves the removal of the Stl protein from its cognate DNA by direct binding to the helper phage dUTPase. Phage dUTPase sequences include a specific insertion not found in other organisms. The 2.1 Å resolution X-ray crystal structure of Φ11phage dUTPase trimer reveals the complete localization of the three phage-specific inserts, each folding into a small β-pleated mini-domain reaching out from the dUTPase core surface. The insert mini-domains jointly coordinate one unique Mg2+ ion at the entrance to the threefold inner channel. Structural results provide an explanation for the role of Asp95, suggested to have functional significance in moonlighting activity, through the metal ion coordinating moiety, potentially involved in correct positioning of the insert. Enzyme kinetics on wild type and mutant constructs show that the insert has no major role in dUTP binding or cleavage and provide description of the elementary steps (fast binding of substrate and release of product). In conclusion, the structural and kinetic data allow insights into both phage-specific characteristics and generally conserved traits of Φ11 phage dUTPase.

References:

[1] Leveles, I., Rona, G., Zagyva, I., Bendes, A., Harmat, V. & Vertessy, B. G. (2011). Acta Crystallogr Sect F Struct Biol Cryst Commun 67, 1411-1413.
[2] Vertessy, B. G. & Toth, J. (2009). Acc Chem Res 42, 97-106.
[3]Pecsi, I., Leveles, I., Harmat, V., Vertessy, B. G. & Toth, J. (2010). Nucleic Acids Res 38, 7179-7186.
[4] Tormo-Mas, M. A., Mir, I., Shrestha, A., Tallent, S. M., Campoy, S., Lasa, I., Barbe, J., Novick, R. P., Christie, G. E. & Penades, J. R. (2010). Nature 465, 779-782.
[5]Tormo-Mas, M. A., Donderis, J., Garcia-Caballer, M., Alt, A., Mir-Sanchis, I., Marina, A. & Penades, J. R. (2013). Mol Cell.

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