Acanthamoeba polyphaga Mimivirus proteome

Proposal 30-01-761
Proposal 30-01-742

The complete sequence of the largest known double-stranded DNA virus, Acanthamoeba polyphaga mimivirus, was determined (Raoult et al., 2005) and revealed numerous genes not expected to be found in a virus. A comprehensive structural and functional study of these gene products was initiated (Abergel et al., 2005; Jeudy et al., 2005) both to better understand their role for the virus physiology and to get some clues on the origin of DNA viruses.

The *Acanthamoeba polyphaga* mimivirus Nucleoside diphosphate kinase (NDKapm):

Among the proteins never identified before in a viral genome, Acanthamoeba polyphaga mimivirus includes a Nucleoside Diphosphate Kinase (NDK). The NDKs are required for the synthesis of nucleoside triphosphates (NTP) other than ATP (EC: 2.7.4.6). They are non specific enzymes active on both purine and pyrimidine, ribo- or desoxyribonucleotides and they can provide NTPs or dNTPs for nucleic acid synthesis, CTP for lipid synthesis, UTP for polysaccharide synthesis and GTP for protein elongation, signal transduction and microtubule polymerization.
We cloned and expressed the NDK using Escherichia coli expression system (Rosetta(DE3)pLysS) and 2 crystal forms of the purified recombinant protein have been produced. Crystals belongs to the cubic space group, P213 with cell dimensions of 99.425 (Jeudy et al., 2005). The crystal structure of the Mimivirus NDK native protein has been solved using a data set collected on a MAR CCD camera at the European Synchrotron Radiation facility (ID29 beamline) at a wavelength of 0.97563 Å (figure 1).

Figure 1: Structure of NDKapm. Monomers are represented with different colors.

Enzymatic activity measurements on the recombinant NDKapm revealed its preferential affinity for deoxypyrimidine nucleotides. This property might represent an adaptation of NDKapm to the production of the limiting TTP deoxynucleotide required for the replication of the large A+T rich (72%) viral genome. The NDKapm might also assume a role in dUTP detoxification to compensate for the surprising absence of Mimivirus dUTPase (deoxyuridine triphosphate pyrophosphatase) an important enzyme conserved in most viruses. Although the phylogenetic analysis of NDK sequences sampled through organisms from the three domains of life is only partially informative, it favors an ancestral origin for NDKapm over a recent acquisition from a eukaryotic organism by horizontal gene transfer (Jeudy et al., 2006).
In order to elucidate the molecular basis of this peculiar affinity of the NDKapm for dYTP we produced crystals of the complexes with dTTP and dGTP. Data have been collected on the

BM30A and ID23 beamlines. The R107 residue could explain the lower affinity of the NDKapm enzyme for purine versus pyrimidine residues due to the difference in size of these nucleotides. Along the same line, the N62 residue replacing a serine residue in other NDK sequences could explain the decrease in affinity of the Mimivirus enzyme for the ribo- versus desoxyribo-nucleotides (figure 2, manuscript in preparation).

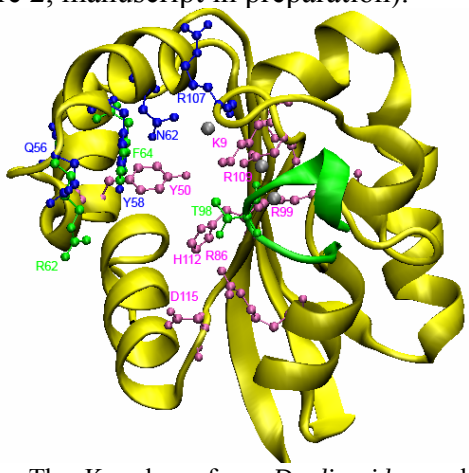

Figure 2: Structure of NDKapm. The Kpn loop from *D. discoideum* absent from the viral sequence is represented in green. Conserved residues between all NDK active sites are colored in pink and residues specific of the NDKapm are colored in blue

The *Acanthamoeba polyphaga* mimivirus Tyrosyl tRNA synthetase (TyrRSapm):

The analysis of the mimivirus genome also revealed the unique presence of genes encoding proteins involved in translation including 4 amino-acyl tRNA synthetases. To understand their role for the viral cycle and the origin of these genes never encountered before in a viral genome we cloned and expressed them.

We first tried to solve the TyrRSapm structure using molecular replacement based on homology modelling. These crystals belong to the monoclinic space group P21 (Abergel et al., 2005). However refinement was difficult to perform due to the quality of the electronic density map and we thus decided to combine this preliminary result with phases obtained from a MAD dataset collected on the BM30A beamline.

The crystals of the selenomethionyl substituted protein produced low resolution data and were fast decaying under X-ray exposure. They belong to the orthorhombic space group (a=63.25, b=107.19, c=148.67 Å) with one biological dimer per asymmetric unit. Even though, a full 4 Å resolution MAD dataset was collected. AutoSHARP was used to obtain initial phases and twenty three selenium atoms were located, refined and solvent-flattening was performed to improved phases. The resulting electronic density map was used to superimpose in TURBO-FRODO the model of the TyrRS structure previously obtained from the native dataset onto the refined selenium atoms positions. A round of manual building was performed to better fit the initial model in the electronic density map and to remove the portion of the model that were not in density.

The unrefined structure was then used to identify a molecular replacement solution (AMoRe) with a 2.2 Å dataset of native TyrRS in complex with tyrosinol and ATP. These crystal also belong to the orthorhombic space group. The model was further refined by using rigid body refinement followed by several rounds of positional refinement in CNS with manual rebuilding using TURBO-FRODO. Mimivirus TyrRSapm, as expected from its sequence similarity with other TyrRSs, exhibits the typical fold of the TyrRS core domain and is more similar to the archeal type with a N-terminus Rossmann-fold catalytic domain, an α-helical domain and no extra C-terminal domain. The protein was co-crystallized with both ATP and the non reactive tyrosine analogue tyrosinol and its structure solved at 2.2 Å resolution. While

tyrosinol was clearly located in the electronic density map, only residual density appears at the ATP binding site probably due to its instability under the rather acidic crystallization conditions (pH 5.5). The position of the residues contacting tyrosinol in the TyrRSapm structure were found to be superimposable to their counterparts in the archeal structures.

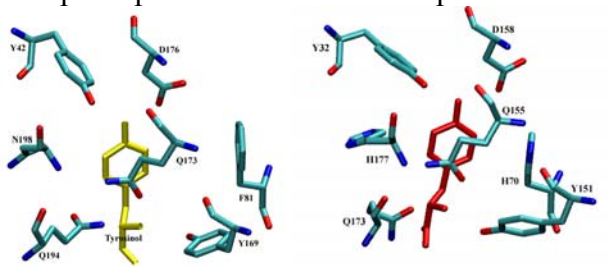

Figure 3: comparison of the TyrRSapm ligand binding site (left) with the archeal Methanococcus jannaschii TyrRS binding site (right).

Like all other TyrRSs, TyrRSapm also crystallizes as an homodimer. The superposition of the TyrRSapm structure with all other available TyrRS dimeric structures highlights a major difference of the viral dimer. While the first monomer superimposes very well with the first monomer of other TyrRSs (RMSD < 1.6 Å based on Cα superimposition), the second one is rotated by around 90 degrees relative to the second monomer in the other TyrRSs (Figures 4). Despite this significant conformation change, the TyrRSapm is fully active and specific of eukaryotic/archeal tRNAs (Manuscript in preparation). Some hypotheses have been proposed to explain the tRNA recognition despite the dimer differences. To elucidate the mechanism of the viral enzyme recognition of the tRNA we are currently trying to produce crystals of the TyrRS/tRNA complex.

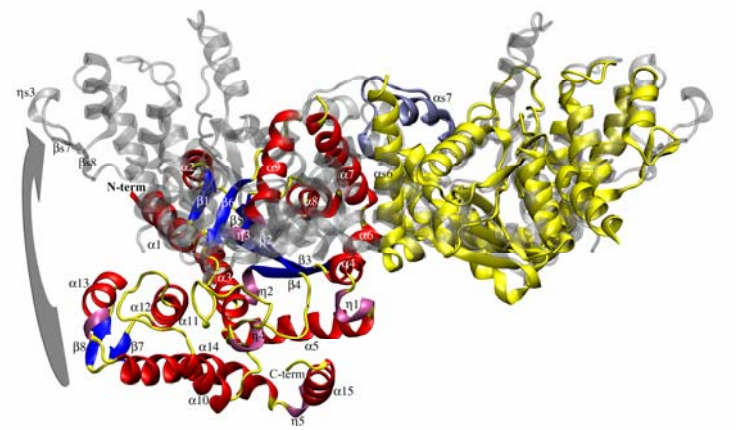

Figure 4: Dimer of $TyrRS_{apm}$ (first monomer yellow; second monomer red, blue and purple) superimposed on the archeal dimer (grey, transparent)

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