

CRYSTALLIZATION AND PRELIMINARY X-RAY CRYSTALLOGRAPHIC STUDIES OF dUTPase FROM EQUINE INFECTIOUS ANEMIA VIRUS

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Abstract: Deoxyuridine 5'-triphosphate nucleotidohydrolase (dUTPase), an enzyme in the nucleotide metabolism, from the retrovirus Equine Infectious Anemia Virus (EIAV) has been crystallized from PEG mixtures at neutral pH using a vapour diffusion method. Crystals of native enzyme belong to the space group R32, while crystals formed in the presence of Sr²⁺ and dUDP, a substrate analogue, belong to the space group P4₁32 or P4₃32. Both forms are suitable for high resolution X-ray structure analyses.

Introduction: By catalyzing the hydrolysis of dUTP to dUMP and pyrophosphate [1], the Mg²⁺-dependent enzyme dUTPase plays dual roles in nucleotide metabolism and DNA replication. The main functions of the enzyme are the provision of a pool of dUMP, a precursor in the biosynthesis of thymidine nucleotides, and the prevention of misincorporation of uracil into DNA, a task achieved by the maintenance of the cellular concentrations of dUTP at minute levels [2]. The importance of dUTPase is suggested by the presence of the enzyme in a wide variety of organisms, prokaryotes as well as eukaryotes. In addition, many viruses encode their own dUTPases despite the presence of a host enzyme. EIAV does, for example, encode its own dUTPase even though it has the smallest and simplest genome of the lentiviruses characterized so far. The existence of viral dUTPases indicates that the enzyme is important in the viral life cycle, an assumption supported by the observation that the viability of certain viruses, including EIAV, decreases in nonproliferating cells if the viral dUTPase is inactivated [3].

Equine Infectious Anemia, EIA, the disease caused by EIAV, is considered a worldwide disease of great economic importance, affecting exclusively members of the family *Equidae*. Clinical features of the disease include fever, hemorrhage and recurring cycles of anemia. Given the lack of an effective vaccine against EIA, the presence of a viral dUTPase and the economic impact of the disease when affecting domestic horses, the EIAV dUTPase seems as a suitable target for antiviral drugs. The design of such drugs would be facilitated by knowledge about the structural features of EIAV dUTPase and we have therefore crystallized the enzyme and initiated a structural determination.

Materials and methods: EIAV dUTPase used in crystallization experiments was overproduced as a recombinant enzyme and purified according to [4]. The pooled peak fractions, containing active dUTPase, were dialyzed extensively against 10 mM 3-[N-Morpholino]propanesulfonic acid, pH 7.2, containing 1 mM β -mercaptoethanol, and then concentrated to 20-40 mg/ml. Favourable conditions for crystal formation were established using the screening method described in [5]. Crystals were obtained under several different conditions. The use of PEGs as precipitants was chosen for further investigation because the presence of PEGs seems to promote growth of crystals to sufficient size and quality for X-ray analysis. PEGs do, in addition, have a possible use as cryo-protectants. The crystallization experiments were performed using the vapour diffusion technique [6] with sitting drops. Each droplet, with a total volume of 10 μ l, was placed in a Micro-Bridge® (Crystal microsystems, Oxford), encased in a well of a Linbro plate and equilibrated against 1 ml of reservoir solution. Native crystals were grown using 15-20% PEG 400 or PEG 3400 in 0.1 M imidazole malate buffer, the pH range being 6.5-7.5. The protein concentration in the drop varied between 1.5-6 μ g/ μ l. The droplets were mixed and stored at 4°C, crystals appeared after 3-5 months.

Crystals of enzyme grown in the presence of Sr^{2+} and dUDP were obtained using 20-25% PEG 400 as precipitant in 0.1 M imidazole malate buffer in the pH range 6.5-7.0. The protein concentration in the drop was 3 μ g/ μ l, the concentrations of metal salt (SrCl_2) and substrate analogue were 20 mM and 5 mM, respectively. Mixing of the droplets were done with the Linbro plates placed on ice at room temperature, the drops were then transferred to 4°C and left standing for a fortnight for crystal formation.

Crystals were mounted in quartz capillaries in the conventional way. Data were collected at 4°C at the EMBL Outstation at DESY, Hamburg, using the experimental station X11 with a wavelength of 0.93Å for native crystals and the experimental station BW7B using a wavelength of 0.885Å for crystals presumed to contain Sr^{2+} -dUDP. The DENZO program [7] was used to determine the space groups and cell parameters.

Results: Native crystals, shaped like irregular plates or rhombohedrons (Fig. 1), require long growth periods and are difficult to reproduce. The crystals usually reach a maximum size of 0.25x0.25x0.15 mm, and diffract X-rays to beyond 1.7Å resolution. A 97.5% complete data set to 1.9Å resolution has been collected. Native crystals belong to the trigonal space group R32, the cell parameters being $a=b=86\text{Å}$ and $c=95\text{Å}$, and the asymmetric unit contains one monomer. The specific volume is estimated to be $2.30\text{Å}^3/\text{Da}$, using a molecular mass of 14678 Da/monomer.

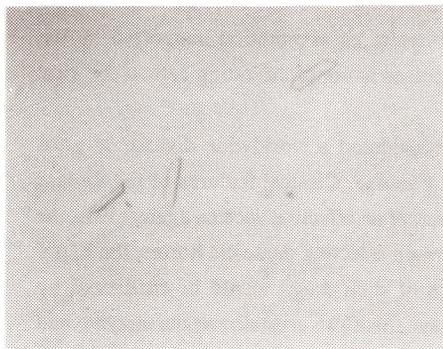


Fig. 1: Crystals of native EIAV dUTPase

Crystals of enzyme grown in the presence of Sr^{2+} and dUDP are, in contrast to the native ones, easy to obtain reproducibly and in a shorter period of time, they usually appear after a fortnight. These crystals appear to be cubes (Fig. 2) but are in fact dodecahedrons, each side being 0.25 mm long. They diffract X-rays to about 2\AA resolution and a 100% complete data set has been collected. The crystals belong to the cubic space group $P4_132$ or $P4_332$ with $a=b=c=106.7\text{\AA}$, there is one monomer per asymmetric unit, and the crystals have a specific volume of $3.45\text{\AA}^3/\text{Da}$. Recent experiments have confirmed that Sr^{2+} is indeed present in the crystal. Conditions for cryo measurements have been established and these measurements will commence soon.



Fig. 2: Crystal of EIAV dUTPase grown in the presence of Sr^{2+} and dUDP

Discussion: Data have been collected on crystals of native and presumably complexed enzyme to high resolution, thus enabling us to initiate a structure determination of EIAV dUTPase. We now have several options available of how to solve the structure. One of these would be to utilize, as a search model, the only 3-D structure of a dUTPase known so far, that of the *E.coli* enzyme [8]. The EIAV and the *E.coli* enzymes show 24.8 % sequence identity and they are both trimers. Another approach would be the use of heavy-atom derivatives and a search for such derivatives is in progress. Yet another interesting possibility is the utilization of the Sr^{2+} for anomalous scattering phase determination, the confirmation of the presence of the metal ion in the crystal being a first step in that direction.

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