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# Crystallization and preliminary X-ray analysis of human dUTPase

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## Abstract

Human dUTPase, expressed in *Escherichia coli*, has been crystallized. Single crystals were obtained by the vapour-diffusion technique using 2-propanol and PEG 4000 as precipitants. The enzyme was co-crystallized with the substrate dUTP and a metal chelator EDTA to prevent hydrolysis of the substrate. The crystals belong to the orthorombic space group $P2_12_12_1$ with cell dimensions $a = 67.51$, $b = 68.26$ and $c = 91.00$ Å. The crystallographic asymmetric unit contains one trimer of identical subunits.

## 1. Abbreviations

dUTPase, deoxyuridine triphosphate nucleotidohydrolase (E.C. 3.6.1.23); dUTP, 2′-deoxyuridine 5′-triphosphate; dUMP, 2′-deoxyuridine 5′-monophosphate; *E. coli*, *Escherichia coli*; EDTA, ethylenediaminetetraacetic acid; PEG, polyethylene glycol; MOPS, 3-[*N*-morpholino]-propanesulfonic acid; DTT, dithiothreitol.

## 2. Introduction

The enzyme dUTPase specifically hydrolyzes dUTP to dUMP and pyrophosphate and has a crucial role in the pyrimidine metabolism and DNA replication (Tye, Nyman, Lehman, Hochhauser & Weiss, 1977; Kornberg & Baker, 1991). The presence of dUTPase has been shown to be important for the cell life cycle and essential for the viability of *E. coli* and *Saccharomyces cerevisiae* (El-Hajj, Zhang & Weiss, 1988; Gadsden, McIntosh, Game, Wilson & Haynes, 1993). dUTPases are found in a variety of eukaryotic and prokaryotic organisms as well as in many viruses like herpes viruses, poxviruses, and certain retroviruses (McIntosh, Ager, Gadsden & Haynes, 1992; Climie *et al.*, 1994; Björnberg *et al.*, 1993; Bergman, Björnberg, Nord, Nyman & Rosengren, 1994). Comparisons of the primary structures of dUTPase from different sources have revealed five conserved regions, scattered along the sequence (McGeoch, 1990). X-ray crystal structures of *E. coli* dUTPase, native and in complex with a substrate analogue, show that at least four of these motifs come together in the quaternary structure forming the active site (Cedergren-Zeppezauer, Larsson, Nyman, Dauter & Wilson, 1992; Larsson, Svensson & Nyman, 1996).

The human dUTPase, similar to the *E. coli* enzyme, is a trimer (Climie *et al.*, 1994). The subunit molecular masses for the enzymes are 16.6 and 16.0 kDa, respectively (McIntosh *et al.*, 1992; Climie et al., 1994; Lundberg, Thoresson, Karlström & Nyman, 1983). The amino-acid sequence identity is 35%, largely concentrated into the five motifs (McIntosh *et al.*, 1992). The human enzyme, like the viral dUTPases, are subjects of medical interest as potential targets for drugs. Structural information about these enzymes should then be of pertinent interest. Here, we describe the preparation and some properties of crystals of the human dUTPase suitable for X-ray diffraction studies. We intend to solve the structure of the human dUTPase by molecular-replacement calculations using the coordinates from our previous structural studies of the *E. coli* enzyme as a search model.

Fig. 1. Crystals of human dUTPase grown in the presence of dUTP and EDTA. Precipitation agent was a mixture of 2-propanol and PEG. The crystal has the dimensions $0.3 \times 0.1 \times 0.1$ mm.

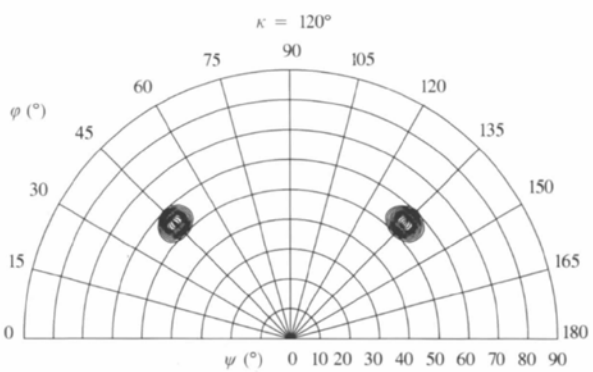


Fig. 2. The self-rotation function calculated at $\kappa = 120°$ using the *MERLOT* program system (Fitzgerald, 1988). The peaks correspond to the direction of the non-crystallographic threefold axis of the trimers in the unit cell. Only one of the peaks is unique, the second is generated by symmetry. The crystallographic $c$ axis points upwards along $\psi = 0°$, the $b$ axis along $\varphi = 90$ and $\psi = 90°$ and the $a$ axis along $\varphi = 180$ and $\psi = 90°$.

## 3. Materials and methods

### 3.1. *Crystallization*

The human enzyme was purified according to Climie *et al.* (1994). Samples of enzyme (5 mg ml$^{-1}$), stored in 25 m*M* $KH_2PO_3/K_2HPO_3$, pH 6.1, 25 m*M* NaCl and 5 m*M* DTT at 253 K, were dialyzed against 10 m*M* MOPS, pH 7.0, 0.006% Na azide and 0.1% 2-mercaptoethanol. Crystallization conditions were screened in various buffer systems at different pH and temperatures (Crystal Screen, Hampton Research, USA). Among precipitation agents tried, 2-propanol and 2-propanol mixed with PEG were found to be the most favourable.

Crystals were obtained from vapour-diffusion experiments in sitting drops. EDTA and dUTP were added to the enzyme solution (final concentrations of 250 μ*M* and 1 m*M*, respectively). Enzyme solution (4 μl) was mixed with an equal volume of the reservoir solution, containing 0.1 *M* Na Hepes, pH 7.5, 10% 2-propanol, 20% PEG 4000, 0.006% Na azide, 0.1% 2-mercaptoethanol and 250 μ*M* EDTA. The drops were equilibrated at 293 K against 1 ml of reservoir solution. Crystals up to 0.5 mm in length and 0.2 mm in the other dimensions (Fig. 1) appeared after one month of incubation.

### 3.2. *X-ray analysis*

Data were collected from a single crystal on a Rigaku RU200BEH rotating Cu anode. Diffraction patterns were collected with a Siemens X1000 area detector system. Cell parameters and space group were determined with the use of *XDS* (Kabsch, 1993).

## 4. Results and discussion

The crystals belong to the space group $P2_12_12_1$ with cell dimensions $a = 67.51$, $b = 68.26$, $c = 91.00$ Å. A data set with a maximum resolution of 3.3 Å and 92% completeness for $I > \sigma(I)$ was collected from a single crystal. The asymmetric unit of the crystals contains a trimer of identical subunits with a molecular mass of $3 \times 16\,600$ Da. Calculation of $V_m$ gives 2.14 Å$^3$ Da$^{-1}$, a normal value for globular proteins (Matthews, 1968). The solvent content is approximately 43%. The existence of a trimer in the asymmetric unit is further validated by self-rotation functions using the program *MERLOT* (Fitzgerald, 1988). Large peaks are seen at $\kappa = 120$ (Fig. 2). No other peaks originating from non-crystallographic symmetry are found.

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