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Expression, Purification, Crystallization and Preliminary X-ray Studies of a Deoxycytidylate Deaminase From *Streptococcus mutans**

HOU Hai-Feng[1,2], GAO Zeng-Qiang[1,2], XU Jian-Hua[1], XU Rui[1,2], LI Li-Qin[1,2], LI Lan-Fen[3], LIANG Yu-He[3], SU Xiao-Dong[3], LIU Peng[1], XIAN Ding-Chang[1], DONG Yu-Hui[1]**

([1]*Beijing Synchrotron Radiation Facility, Institute of High Energy Physics, The Chinese Academy of Sciences, Beijing* 100049, China;
[2]*Graduate School of The Chinese Academy of Sciences, Beijing* 100049, China;
[3]*College of Life Science, Peking University, Beijing* 100871, China)

Abstract Deoxycytidylate (dCMP) deaminase is an enzyme belonged to dCMP cyt deam family. The dCMP deaminase from *Streptococcus mutans* UA159 was cloned and expressed in *E. coli*, and purified to homogeneity. The FPLC size exclusion chromatography analysis reveals that the *S. mutans* dCMP deaminase forms hexamer in solution. The protein was crystallized using hanging drop vapour-diffusion method and diffracted to a resolution of 3.1 Å. The diffraction data were collected at BSRF beamline 3W1A. The crystals belong to $P2_13$ space group, with unit cell parameters $a = b = c = 113.2$ Å, $\alpha = \beta = \gamma = 90°$. Assuming there are two subunits per asymmetric unit, the Matthews coefficient is 3.6 $Å^3 \cdot Da^{-1}$. This is the first crystallization report of the wild-type deoxycytidylate deaminase.

Key words deoxycytidylate deaminase, *Streptococcus mutans*, allosteric regulation, crystallization

Wild-type deoxycytidylate deaminase, a hexameric allosteric enzyme binding zinc ion, which is required for catalytic activity, belongs to dCMP cyt deam family according to Pfam search[1]. The activity of the enzyme is allosterically regulated by the ratio of dCTP to dTTP[2,3], not only in eukaryotic cells but also in T-even phage-infected *Escherichia coli*, with dCTP acting as an activator and dTTP as an inhibitor. Deoxycytidylate deaminase catalyzes the deamination of dCMP to dUMP, providing the nucleotide substrate for thymidylate synthase, which is important in DNA synthesis and cancer chemotherapy[4,5]. dCMP deaminase exists in most eukaryotic and bacterial organisms. There are two classes in these dCMP deaminases (EC 3.5.4.12). Mammalian dCMP deaminase contains a single zinc ion per subunit for catalytic activity, while bacteria dCMP deaminase contains two zinc ions per monomer, one is related to the catalytic action indispensably and the other seems to be important for the structural integrity of the enzyme and for binding to the phosphate group of the substrate. Both classes function in hexmeric form[6,7]. In the past decade, dCMP deaminase from T4-bacteriophage was studied extensively[8~10] in enzyme activities and mutation properties. The dCMP deaminases from T4-bacteriophage and *S. mutans* show significant similarities in two sequence segments, which share 31.6% identity. One of the segments locates at the catalytic site of the T4-bacteriophage dCMP deaminase according to the structure [11]. We purified and crystallized dCMP deaminase from *S. mutant* successfully, and report in this paper the preliminary crystallographic studies of the first crystallized wild-type dCMP deaminase. The determination of dCMP deaminase structure may provide clue of detailed studies of catalytic mechanism and substrate binding properties, also a possible model for the mechanism for the allosteric regulation of the dCMP deaminase.

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**Corresponding author . Tel: 86-10-88233090, Fax: 86-10-88233201, E-mail: dongyh@ihep.ac.cn

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1 Materials and methods

1.1 Protein expression and purification

The dCMP deaminase gene was amplified from *Streptococcus mutans* UA159 genomic DNA by polymerase chain reaction (PCR), using primers comEB-F (5′ cgcggatccatgacgaatagactttcttggc 3′) and comEB-R (5′ cggctcgagttacttttcacctaatttca 3′). The PCR-amplified fragment was then ligated to expression vector pET28a (Novagen), which contains N-terminal His6 tag. The recombinant dCMP deaminase was expressed in *E. coli* strain BL21 (DE3). The transformed cells were first cultured overnight in 40 ml Luria Broth (LB) media with 50 g/ml kanamycin, then transferred to 1 L LB media with 50 g/ml kanamycin and grown at 37℃. When optical density (A_{600}) reached to 0.6～0.8, the cells were induced with 1 mmol/L isopropyl β-D-thiogalactopyranoside (IPTG) for 4 h. The cells were harvested by centrifugation at 6 000 r/min for 10 min. Bacteria were suspended with buffer of 20 mmol/L Tris-HCl pH 8.0, 500 mmol/L NaCl (buffer A), 1 mmol/L phenymethanesulfonyl fluoride (PMSF). After disruption of the cells by sonication, the cell debris was removed by centrifugation at 16 000 r/min for 45 min. The soluble lysis fraction was loaded onto a nickel-chelation column (5 ml, Amersham) equilibrated in buffer A and the targeted protein was then eluted by a liner gradient of 10 to 500 mmol/L imidazole in buffer A. The dCMP deaminase fractions were collected and concentrated by ultrafiltration (30 000MWCO) in an Amicon cell (Millipore, CA, USA). The protein is sensitive to ion strength and insoluble until the concetration of NaCl reachs 500 mmol/L in the buffer. So we load the protein solution onto a Superdex 200 (120 ml, Amersham) gel-filtration column equilibrated in 20 mmol/L Tris-HCl buffer pH 8.0, 500 mmol/L NaCl. All the purification was carried out in room temperature on an ÄKTA Purifier System (Amersham).

1.2 Oligomeric and homogeneous condition

The protein purity was monitored by SDS-PAGE and showed a molecular mass of 20 ku. The molecular mass estimated from gel-filtration chromatography is about ～118 ku, which indicates that the His-dCMP deaminase is a hexameric protein. The result is in consistant with previous work [12]. The dCMP deaminase was concentrated to 16 g/L, determined by Bio-Rad protein assay kit, and stored as frozen aliquots at 193K.

1.3 Crystallization

The concentration of the dCMP deaminase used for crystallization screening was 16 g/L and 8 g/L, respectively, in the buffer containing 20 mmol/L Tris-HCl pH 7.5, 500 mmol/L NaCl. The initial screening were carried out by hanging drop vapour-diffusion mehods using Hampton Research Crystal Screen 1 and 2 (Hampton Research, Riverside, CA, USA) at room temperature, 1 μl of protein solution was mixed with 1 μl of reservoir solution and equilibrated against 500 μl of reservoir solution in a 24-well culture plate. Good shaped crystals were obtained in many conditions (about 30) after about 2 h, but the diffraction resolution is very poor, only about 7 Å. After optimizing the concentration of the precipitants and ion strength, the resolution of the diffraction is about 5 Å under the condition of 1.5 mol/L $(NH_4)_2SO_4$, 0.1 mol/L Tris pH 8.5, 15%(*v*/*v*) glycerol. In order to improve the diffraction ability, dehydration, a post-crystallization method was used. We add glycerol in the reservoir solution to a final concentration of 25%, and the diffraction ability of the crystal was improved to 3.1Å after 24 h. The photo of single crystal is shown below (Figure 1).

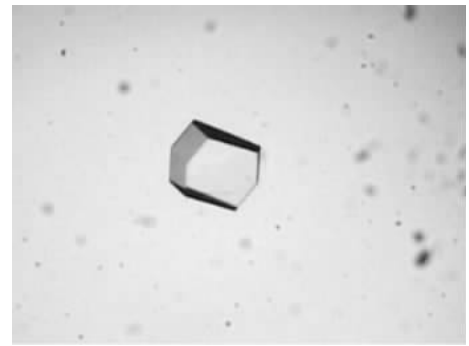

Fig. 1 His-dCMP deaminase crystal grown by the hanging-drop method in 1.2 mol/L $(NH_4)_2SO_4$, 0.1 mol/L Tris pH 8.5, 15% (*v*/*v*) glycerol after dehydration using glycerol at 289 K

1.4 X-ray data collection and analysis

Diffraction data of dCMP deaminase crystal were collected at BSRF (Beijing Synchrotron Radiation Facility), beamline 3W1A at 100 K. To optimize the Zn anomalous signal for Zn MAD, the source wavelength was set to 1.282 9 Å (for peak), 1.283 5 Å (for edge) and 1.200 Å (for remote), respectively, according to the XAFS (data was not shown). The

diffraction image was shown in Figure 2. The data were collected using a MAR 165 CCD detector in 1.0 oscillation steps over a range of 110°. The data were processed, scaled and merged using DENZO and SCALEPACK from HKL2000 program package[13].

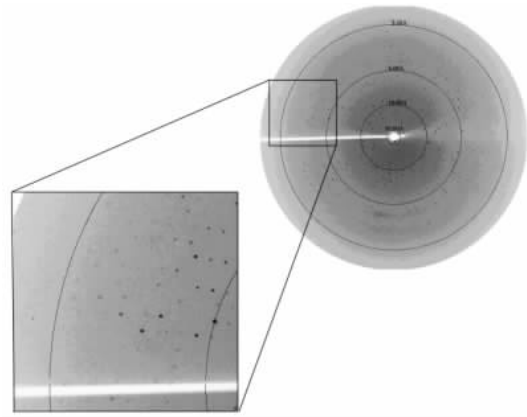

Fig. 2 Diffraction pattern of the *S. mutans* dCMP deaminase with 3.1 Å diffraction resolution

2 Results and discussion

We have succeeded in the expression, purification and crystallization of the *S. mutans* dCMP deaminase. The enzyme eluted on a Superdex 200 size-exclution column as a homohexamer which was satisfied by initial result. The purified protein migrated as a single 20 ku (150 residuals) on 15% SDS-PAGE gel.

Initially, crystals were grown by hanging-drop vapour-diffusion methods using Hampton Research Crystal Screen 1 and 2. About 30 solutions of Hampton Research Crystal Screen 1 and 2 produced well looking crystals. After optimizing the certain condition, well diffracted single crystals are obtained from 1.2mol/L $(NH_4)_2SO_4$, 0.1 mol/L Tris pH 8.5, 15% (*v*/*v*) glycerol. The crystal of dCMP deaminase diffracted to 3.1 Å and belonged to space group $P2_13$ with unit-cell parameters $a = b = c = 113.2$ Å, $\alpha = \beta = \gamma = 90°$. Assuming the presence of two polypeptides per asymmetric unit, the calculated Matthews coefficient V_M value was 3.6 $Å^3\ Da^{-1}$, with a solvent content of 66.2%(Table 1). Structure determination by Zn-MAD is underway.

Table 1 Crystallographic parameters and data-collection statistics

	Peak	Edge	Remote
Wavelength/Å	1.282 9	1.2835	1.2000
Space group	$P2_13$	$P2_13$	$P2_13$
Resolution/Å	50.0～3.1 (3.2～3.1)	50.0～3.1 (3.2～3.1)	50.0～3.2 (3.3～3.2)
Completeness/%	100 (99.9)	100(100)	100(100)
R_{merge}/%[1)]	11.0(54.9)	10.6(52.2)	11.6(44.1)
$I/\sigma(I)$	26.7(4.9)	27.3(5.0)	23.9(6.0)
Mosaicity	0.6°	0.6°	0.6°
Unit-cell parameters/Å	$a = b = c = 113.2$	$a = b = c = 113.2$	$a = b = c = 113.2$
No. of reflections	122 071	122 084	101 969
Redundancy	13.5(13.5)	13.5(13.5)	12.4(12.4)
No. of unique reflections	9 042	9 043	8 223
Mol/Asym		2	
V_M / ($Å^3 \cdot Da^{-1}$)[2)]		3.6	
Solvent content / %		66.2	

Values in parentheses are for the highest resolution shell. [1)] $R_{merge} = \sum |I_{obs} - I_{avg}| / \sum I_{obs}$ where the summation is over all reflections. [2)] Matthews coefficient[14].

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来自于变性链球菌的脱氧胞嘧啶核苷酸脱氨酶的表达，纯化，结晶以及初步晶体学研究 *

侯海峰[1,2] 高增强[1,2] 徐建华[1] 许 锐[1,2] 李丽琴[1,2]
李兰芬[3] 梁宇和[3] 苏晓东[3] 刘 鹏[1] 冼鼎昌[1] 董宇辉[1]**
([1]中国科学院高能物理研究所，北京同步辐射实验室，北京 100049；
[2]中国科学院研究生院，北京 100049；[3]北京大学生命科学学院，北京 100871)

摘要 脱氧胞嘧啶核苷酸脱氨酶属于脱氧胞苷酸脱氨家族. 对来自于变性链球菌 UA159 的脱氧胞嘧啶核苷酸脱氨酶进行了克隆，在大肠杆菌中进行了表达，最后纯化. 快速液相分子排阻色谱分析表明这种酶在溶液中形成六聚体. 利用悬滴气相扩散技术获得了这个蛋白的晶体. 在北京同步辐射的 3W1A 线站，收集了衍射分辨率到达 3.1 Å 的数据. 这个晶体属于 $P2_13$ 空间群，其晶胞参数为 $a=b=c=113.2$ Å，$\alpha=\beta=\gamma=90°$. 计算可得马修斯系数为 3.6 Å^3·Da^{-1}，据此可估计在一个不对称单位中含有两个单亚基. 据目前所知，这是第一个关于野生型的脱氧胞嘧啶核苷酸脱氨酶的结晶学报道.

关键词 脱氧胞嘧啶核苷酸脱氨酶，变性链球菌，变构调控，结晶
学科分类号 Q51，O72

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** 通讯联系人. Tel: 010-88233090, Fax: 010-88233201, E-mail: dongyh@ihep.ac.cn
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