

Crystal structure analysis of monomeric isocitrate dehydrogenase

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The Krebs cycle is a common metabolic pathway to completely oxidize fuel molecules. Isocitrate dehydrogenase (IDH) catalyzes the oxidative decarboxylation step of isocitrate to yield α -ketoglutarate and CO_2 in the Krebs cycle. The α -ketoglutarate is known to be a key substance in biosyntheses of cell constituents via reductive amination to glutamate. Although most bacterial IDHs are NADP⁺-dependent enzymes and form a dimeric structure with molecular weights of 40-50 kDa per subunit, monomeric IDHs with molecular weights of 80-100 kDa have been found in a few species of bacteria. Recent success in cloning and sequencing the gene encoding monomeric IDH has shown that the amino acid sequences of monomeric and dimeric IDHs are completely different from one another.

We obtained very thin crystals of monomeric IDH from *Azotobacter vinelandii* by hanging-drop vapor-diffusion method at 293 K (Figure 1). IDH solution was prepared with a concentration of 9.0 mg/ml in 0.1 M HEPES pH 7.0 and 10.0 % glycerol. The reservoir solution consisted of 0.1 M HEPES

pH 7.0, 26.0 % PEG 6000, 20.0 % glycerol, 4.0 mM isocitrate and 4.0 mM MnCl_2 . Hanging drops were formed from 2.5 μl IDH solution and 2.5 μl reservoir solution.

Preliminary X-ray diffraction experiment shows that these thin crystals belong to a monoclinic system; the space group was determined to be $P2_1$ with unit cell dimensions of $a=120$, $b=109$, $c=122$ Å and $\beta=90^\circ$. The asymmetric unit contains four molecules of monomeric IDH corresponding to a V_M value of 2.5 Å³Da⁻¹ and a solvent content of 50.6 %.

We also collected MAD data set up to 3.1 Å based on the absorption spectrum for Mn K -edge. The diffraction data were processed with MOSFLM and SCALA (CCP4 suite). The search of the Mn sites is now in progress.

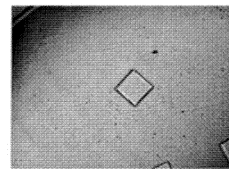


Figure 1
Very thin crystals ($0.2 \times 0.2 \times 0.01$ mm³) of monomeric IDH.

Table 1 Processing statistics

	Crystal I		Crystal II	
	Peak	Edge	Remote	
Wavelength (Å)	1.00000	1.89197	1.89342	1.88564
Resolution range (Å)	40.0-2.60 (2.74-2.60)	40.0-3.10 (3.27-3.10)	40.0-3.10 (3.27-3.10)	40.0-3.10 (3.27-3.10)
Observed reflections	355,527	226,131	223,525	213,325
Unique reflections	94,844	51,471	50,918	48,635
Completeness (%)	99.1 (98.7)	89.7 (59.2)	88.7 (54.9)	84.8 (39.0)
Redundancy	3.7 (3.6)	4.4 (2.1)	4.4 (1.9)	4.4 (1.6)
$I/\sigma(I)$	8.2 (2.6)	8.9 (3.9)	8.5 (3.8)	8.5 (3.8)
R_{meas} (%)	9.4	9.0 (26.5)	9.2 (27.1)	9.4 (27.6)
R_{int} (%)		6.1 (13.9)	6.4 (13.5)	-

*The numbers in parentheses refer to data in highest resolution shell.

Crystal Structure Analysis of Ribonuclease MC1 Complexed with Substrate Analog at Ultra-high Resolution

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Ribonuclease (RNase) MC1 isolated from seeds of bitter melon (*Momordica charantia*) consists of 190 amino acids with a relative molecular weight of 21,222 Da, and belongs to the RNase T2 family. MC1 has absolute specificity to the 5'-side of uridine that differs from RNases grouped in T2 family, and cleaves a phosphodiester bond of NpU (where N is either A, C or U). We have already determined the structure of MC1 complexed with 2'-uridine monophosphate (UMP) or 3'UMP from the X-ray crystallographic analysis. To get insight into the interaction between MC1 and substrate *in vivo*, we study the crystal structure of MC1-5'UMP complex at high resolution.

RNase MC1 complexed with 5'UMP was crystallized using the hanging-drop vapor diffusion method. The crystals of MC1-5'UMP complex were grown within four days to a size of $0.5 \times 0.2 \times 0.2$ mm³ against reservoir containing 0.1M sodium cacodylate (pH6.1), 0.2M sodium acetate with 24% PEG8000 and 10mM 5'UMP.

Data set of the single crystal complexed with 5'UMP was collected up to 1.0Å resolution using MARCCD detector at BL41XU under 100K.

The diffraction data were processed with MOSFLM and SCALA (CCP4 suite). The summary of data collection and processing is shown in Table 1. The crystals of MC1-

5'UMP complex belongs to the same space group $P2_12_12_1$ with the native crystal, but the cell dimensions ($a=39.16$ Å, $b=63.49$ Å and $c=75.54$ Å) are significantly different (cell dimensions of native crystals are $a=38.76$ Å, $b=67.69$ Å and $c=75.42$ Å).

The complex structure of MC1-5'UMP was determined by the molecular replacement method using program AMoRe (CCP4 suite), and a search model was constructed from the native structure of MC1. The structure was refined to an R-factor of 20.2% and R-free-factor of 20.7% for 10-1.30Å resolution region by program CNS.

Table 1. Crystallographic data of RNase MC1 - 5'UMP complex

Wavelength (Å)	0.70
Resolution (Å)	19.8-1.30
Observed reflections	534879
Unique reflections	45935
Completeness (%)	99.9(99.4)
Multiplicity	7.1 (7.0)
Averaged $I/\sigma(I)$	8.7 (2.4)
R_{mean} (%)	5.2(24.0)

Values in parentheses are for the highest resolution cell(1.37-1.30Å).