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Crystallization and Preliminary X-Ray Diffraction Studies of *N*-Acyl-D-glucosamine 2-Epimerase from Porcine Kidney

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***N*-Acyl-D-glucosamine 2-epimerase from porcine kidney, which was cloned and expressed in *Escherichia coli*, was crystallized by the vapor-diffusion method, using polyethylene glycol and ammonium acetate as precipitants. The crystals were resistant to X-ray radiation damage and diffracted to more than 2.0 Å resolution. The diffraction pattern indicated that the crystals belong to the orthorhombic system, space group $P2_12_12_1$, with unit-cell dimensions of $a=78.1$, $b=97.2$, and $c=100.7$ Å. It is supposed that the asymmetric unit consists of two *N*-acyl-D-glucosamine 2-epimerase molecules. Collection of data on the native crystals indicated that they are suitable for X-ray structural analysis.**

Key words: *N*-acyl-D-glucosamine 2-epimerase, crystallization, porcine kidney, renin-binding protein, X-ray crystallography.

An *N*-acyl-D-glucosamine 2-epimerase (GlcNAc 2-epimerase) [EC. 5.1.3.8] that catalyzes the conversion of *N*-acetyl-D-glucosamine (GlcNAc) to *N*-acetyl-D-mannosamine (ManNAc) has been found in porcine kidney, and rat kidney, liver, spleen, brain, intestinal mucosa, thymus, pancreas, and salivary gland (*1, 2*). Ghosh and Roseman (*2*), and Datta (*3*) partially purified the enzyme from porcine kidney and found that its activity is modulated by a catalytic amount of ATP. ATP was not essential for the enzyme reaction, but the activity of the enzyme was enhanced about 20-fold in the presence of ATP or deoxy-ATP (*2, 4*). Datta (*3*) also reported that GlcNAc 2-epimerase possesses two distinct interaction sites, a catalytic site for the substrate and an allosteric site for ATP. We have purified GlcNAc 2-epimerase to homogeneity from porcine kidneys and cloned the gene for the enzyme in *Escherichia coli* (*4*). The enzyme is a dimer of identical subunits, each with a molecular weight of about 46 kDa. The nucleotide sequence of the cDNA was determined, and the predicted 402 amino acid residues were characterized by the presence of a leucine zipper motif and by the absence of a hydrophobic amino-terminal sequence indicative of a signal sequence. There is a potential asparagine-linked glycosylation site conforming to the consensus sequence of Asn-X-Ser, although no glycosyl residues were detected in the purified GlcNAc 2-epimerase from porcine kidney. Comparison of the nucleotide and amino acid sequences of the GlcNAc 2-epimerase with those of other known genes revealed striking similarity to the renin-binding protein (RnBP) (*5, 6*). The identities of the nucleotide and amino acid sequences of the enzyme to those of RnBP from porcine kidney were 99.6 and 99.0%, respectively (*4*). Furthermore, the amino acid sequence of the enzyme was highly homologous to those of other RnBPs from human and rat kidneys, the identities being 87.8 and 83.1%, respectively (*7*). That GlcNAc 2-epimerase is a RnBP was further confirmed by its ability to bind porcine kidney renin and to inhibit renin activity (*4*). GlcNAc 2-epimerase, as a RnBP, possesses unique hydrodynamic features such as a leucine zipper motif, which was identified as a key structure for the formation of a heterodimer of renin and one of the subunits of RnBP (*8*). Therefore, the crystal structure of GlcNAc 2-epimerase was required to clarify the underlying mechanisms of not only the GlcNAc 2-epimerase reaction, but also the renin-binding ability. In this paper, we report the crystallization of GlcNAc 2-epimerase from porcine kidney and some results of preliminary crystallographic characterization.

GlcNAc 2-epimerase from porcine kidney was expressed in *E. coli* and purified by the method previously described (*4*). The hanging drop vapor-diffusion method was used for crystallization. Protein solutions were prepared in 10 mM Tris-HCl buffer (pH 7.2). Polyethylene glycol (PEG) and ammonium acetate were used as precipitant agents. Droplets (10 μl) of a protein solution (5 mg/ml) comprising 5.5% (w/v) PEG #6000, 100 mM ammonium acetate, and 50 mM sodium citrate buffer (pH 5.0) were pipetted onto plastic coverslips, and then vapor-equilibrated at 20°C with a reservoir solution (1.0 ml) comprising 11% (w/v) PEG #6000, 200 mM ammonium acetate, and 100 mM sodium citrate buffer (pH 5.0). Prismatic, colorless crystals appeared after 20 days incubation and grew to a maximum size of 0.8 mm (Fig. 1).

A crystal was mounted in a quartz capillary with a trace amount of mother liquor. Still and precession photographs were taken at room temperature with a Nonius precession camera using Ni-filtered CuKα radiation, which was generated with a Rigaku X-ray generator operating at 40 kV and

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Abbreviations: GlcNAc, *N*-acetyl-D-glucosamine; ManNAc, *N*-acetyl-D-mannosamine; PEG, polyethylene glycol.

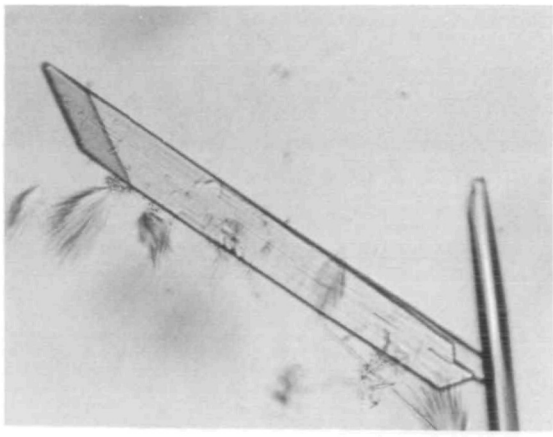

Fig. 1. A single crystal of GlcNAc 2-epimerase.

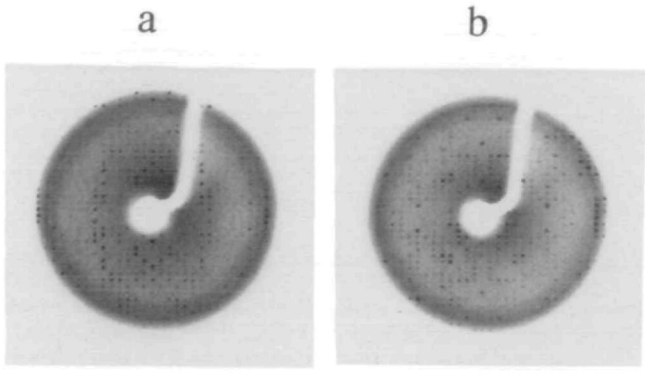

Fig. 2. **Precession photographs of a crystal of GlcNAc 2-epimerase.** The crystal-to-film distance was 60 mm, the precession angle 8.0 degrees and the exposure time 8 h. a, *hk*0 zone; b, *h*0*l* zone.

20 mA. The crystal-to-film distance was set at 60 mm. On still photographs, which were obtained by exposure for 20 min, reflections extended to at least 2.0 Å resolution and did not decay for more than 72 h. Precession photographs (Fig. 2) showed that the crystal belonged to the orthorhombic system.

The systematic absence of reflections ($h00$, $0k0$, $00l$) indicated that the space group is $P2_12_12_1$. The unit-cell dimensions are $a=78.1$, $b=97.2$, and $c=100.7$ Å, with a unit-cell volume of 764,446 Å^3. Assuming that there is one dimer per asymmetric unit of the cell, the crystal volume per unit molecular mass (V_m) and the solvent volume fraction (V_{sol}) were estimated to be 2.1 Å^3/Da and 0.58, respectively. These values are within the ranges of those of typical protein crystals (*9*).

Intensity data for the native crystals were collected with a Siemens HI-STAR detector on a MAC Science M18XHF rotating anode generator. Table I shows the strategy and statistics for the data collected. The diffractions up to 1.92 Å were collected with 5.6% R-sym and 82% completeness. These data indicate the good quality of the crystals for X-ray structural analysis. A search for heavy atom derivatives is now being carried out to elucidate the high-resolution structure of GlcNAc 2-epimerase.

TABLE I. **Strategy and statistics of data collected.**

Detector	Siemens HI-STAR
Crystals used	1
Cell dimensions	$a=78.1$, $b=97.2$, $c=100.7$ Å
Detector distance	15 cm
Step size	*0.25 degree/scan*
Scan period	60 s/scan
Scans	ϕ-scan 360°($2\theta=28°$, $\chi=0°$)
	ϕ-scan 360°($2\theta=10°$, $\chi=0°$)
	ω-scan 50° ($2\theta=28°$, $\chi=45°$)
	ω-scan 50° ($2\theta=28°$, $\chi=90°$)
Collected reflections	124,509
Unique reflections	49,059
Resolution	1.92 Å
R-sym	5.6%
Completeness	82.1%

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