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Crystallization and preliminary crystallographic investigation of porcine quinolinate phosphoribosyltransferase

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Abstract

Quinolinate phosphoribosyltransferase (QPRT), purified from hog liver, has been crystallized using PEG 8000 as the precipitant. The crystals form long hexagonal rods in the space group $P6_322$ with cell dimensions $a = b = 121.7$, $c = 94.5$ Å. Based on the unit-cell dimensions and the calculated molecular mass of 33 500 Da, the Matthews coefficient suggests one molecule per asymmetric unit (V_m = 3.45 Å^3 Da^{-1}; 64% solvent). Three native data sets were collected to a resolution of 2.5 Å and merged to provide a set that is 94.7% complete, with an R_{sym} value of 9.6%.

1. Introduction

The *de novo* biosynthesis of nicotinamide adenine dinucleotide (NAD) proceeds from tryptophan, aspartate and glycerol or related compounds depending on the specific system (Iwai & Taguchi, 1973). Quinolinate phosphoribosyltransferase (QPRT) catalyzes the stoichiometric conversion of quinolinate (QUIN) to nicotinic acid mononucleotide and carbon dioxide in the presence of phosphoribosyl pyrophosphate (PRPP) and the divalent metal magnesium (Musick, 1981). In this reaction, the cleavage of the pyrophosphate moiety of PRPP is accompanied by the anomeric inversion of the ribofuranose ring resulting in *β-N* nicotinic acid mononucleotide. QUIN and Mg-PRPP are the sole reactants in all organisms, although the synthesis varies between plant, mammalian and bacterial systems (Mann & Byerrum, 1974; Taguchi & Iwai, 1976). QPRT has been homogeneously isolated from a variety of hosts including bacteria, rat, hog, castor bean, 'Shiitake' mushroom, beef and human (Foster, Zinkind & Schwarcz, 1985; Taguchi & Iwai, 1974, 1975; Shibata & Iwai, 1980; Okuno, White & Schwarcz, 1988). The substrate QUIN is a rigid structural analogue of glutamate and aspartate that excites the mammalian central nervous system neurons by activating the *N*-methyl-D-aspartate (NMDA) receptor. (Schwarcz & Kohler, 1983). Intracerebral injections of QUIN in nanomolar quantities cause a selective pattern of nerve cell death in certain regions of the brain (Schwarcz, Whetsell & Mangano, 1983) and causes a characteristic pattern of seizure activity related to epilepsy. Since this QUIN-induced neuropathology is similar to the damage observed in certain neurodegenerative disorders such as Huntington's disease and epilepsy, the identification of QUIN as a natural constituent of human and animal brain has lent support to the hypothesis that an increase of endogenous QUIN levels may be an etiological factor in these diseases (Foster & Schwarcz, 1985; Foster, Whetsell, Bird, & Schwarcz, 1985; Schwarcz, Speciale, Okuno, French & Kohler, 1986). QPRT provides the only route for the metabolism of QUIN. Knowledge of the three-dimensional structure will help define the catalytic mechanism and provide needed structural information on the phosphoribosyltransferase (PRTase) family of enzymes. To date, only two of the ten phosphoribosyltransferases, orotate PRTase (OPRT) and hypoxanthine-guanine PRTase (HGPRT), have been structurally resolved (Scapin, Grubmeyer & Sacchettini, 1994; Eads, Scapin, Xu, Grubmeyer & Sacchettini, 1994). Crystals that have been produced previously (Musick, 1977) using ammonium sulfate as precipitant were not of diffraction quality. Here we describe conditions for the crystallization of QPRT using PEG 8000 as the precipitant and their preliminary characterization using X-ray diffraction techniques.

2. Crystallization and diffraction data collection

Methods used to purify hog liver QPRT have been described (Taguchi & Iwai, 1975) and we followed the same with some modifications to increase purity and yield. A potassium phosphate buffer (*A*) was used throughout the purification. The hog liver (10 kg) was sliced and homogenized in a blender and the connective tissue removed using a wire screen. The crude homogenate was then adjusted to pH 5.5 using acetic acid. After standing overnight, the solution was centrifuged at 10 000*g* for 15 min. The supernatant pII was adjusted to 7.0 using 10% ammonia. Ammonium sulfate was added until 40% saturation, with the pH maintained at 7.0 with 10% ammonia. The precipitant was collected by centrifugation, dissolved in 300 ml of 5 m*M* buffer *A*, and dialyzed overnight. The solution was then applied to a DEAE–cellulose column. Elution was performed by applying a linear gradient from 0.005 to 0.3 *M* of buffer *A*. Active fractions were pooled and ammonium sulfate was added to 60% saturation. The precipitant was collected by centrifugation and dissolved in 30 ml of 5 m*M* buffer *A*. The solution was then dialyzed overnight and the supernatant was applied to a DEAE–cellulose column. Ammonium sulfate with 40% saturation was added to the elutant and the precipitant was collected by centrifugation. This procedure was repeated once more and the precipitant was collected dissolved in 5 m*M* buffer *A*. The total amount of protein was 550 mg with a yield of 65%.

Crystals of QPRT were obtained at room temperature using the vapor-diffusion technique with hanging drops. Crystals were seen growing within 3 d at room temperature as long hexagonal rods. The protein concentration was 22 mg ml^{-1}. 3 µl of protein were mixed with 3 µl of solution taken from reservoir and equilibrated with 1.0 ml reservoir containing 50 m*M* Tris (pH 7.9), 0.1792 *M* ammonium citrate, and the precipitating agent PEG 8000 at 14–20%(*w*/*v*) concentrations. Rod-shaped crystals continued to grow over a period of two weeks until their dimensions were 0.25 × 0.25 × 1.0 mm, and were suitable for

Table 1. *Native QPRT diffraction statistics*

Crystal	No. of frames	Total No. of observations	No. of unique reflections	Completeness (%)†	R_{sym}‡
QPRT0130	40	127062	12782	81.9	0.099
QPRT0203	30	100676	11208	71.7	0.106
QPRT0204	30	95315	10669	71.6	0.098
Merged	100	323053	14845	94.7	0.096

† $I/\sigma = 1.0$ at 2.5 Å. ‡ $R_{sym} = |I_{obs} - I_{avg}|/I_{avg}$.

X-ray diffraction studies. Crystals of QPRT were characterized on a R-AXIS IIc image-plate detector, mounted on Rigaku RU-200 rotating-anode source operating at 40 kV, 100 mA with Cu $K\alpha$ radiation. The crystals diffracted to 2.5 Å. Results are summarized in Table 1. The angle of oscillation was 1°, the crystal-to-detector distance was 150 mm, and exposure time was 1800 s per frame. Three crystals were mounted in different orientations in order to get a complete native data set. The oscillation frames were indexed and scaled using the R-AXIS IIc software. In the highest resolution shell, 38.6% of the reflections had $I/\sigma > 3$. The space group was determined to be $P6_322$ with unit-cell dimensions $a = b = 121.7$, $c = 94.5$ Å. The calculated Matthews coefficient of 3.45 Å^3 Da^{-1} suggests one molecule per asymmetric unit (M_r = 33 500 Da; Iwai & Taguchi, 1980), with a solvent content of 64% in the unit cell (Matthews, 1968). In the search for useful heavy-atom derivatives, the crystals are being soaked with a variety of heavy atoms, including mercury, platinum and lead compounds. A complex containing Mg^{2+}-PRPP and the competitive inhibitor phthalic acid is also being soaked into the crystals to provide information about the active site.

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