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Crystallization and preliminary X-ray crystallographic analysis of human nucleoside diphosphate kinase A

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Human nucleoside diphosphate kinase A catalyzes phosphoryl transfer and acts as a suppressor of metastasis. It has been crystallized using 2-methyl-2,4-pentanediol as a precipitant at 288 K. The crystal is monoclinic, belonging to the space group $P2_1$, with unit-cell parameters a = 74.21, b = 78.11, c = 82.29 Å, β = 101.33°. The asymmetric unit contains a homohexamer, with a corresponding crystal volume per protein mass (V_m) of 2.27 Å^3 Da^{-1} and a solvent content of 46%. Native X-ray data to 2.15 Å resolution have been collected using synchrotron X-rays.

1. Introduction

Nucleoside diphosphate (NDP) kinase catalyzes the transfer of the γ-phosphoryl group from a nucleoside triphosphate to a nucleoside diphosphate using ATP as a major phosphate donor (Parks & Agarwal, 1973). The reaction has a ping-pong mechanism *via* a high-energy phosphohistidine intermediate. As the enzyme accepts all common nucleotides and deoxynucleotides as substrates, it plays a key role in maintaining cellular pools of all nucleoside triphosphates. NDP kinase also plays a crucial role in the regulation of several fundamental cellular processes such as development, inhibition of cell differentiation, oncogene transformation and signal transduction (Biggs *et al.*, 1990; Okabe-Kado *et al.*, 1992). The crystal structures of NDP kinases from *Dictyostelium discoideum* (Dumas *et al.*, 1992), *Drosophila melanogaster* (Chiadmi *et al.*, 1993), *Myxococcus xanthus* (Williams *et al.*, 1993) and bovine retina (Abdulaev *et al.*, 1998) as well as that of human NDP kinase B encoded by the *nm23-H2* gene (Moréra *et al.*, 1995; Webb *et al.*, 1995) have been reported. Although these structures share the similar $\beta\alpha\beta\beta\alpha\beta$ topology for the subunit fold, prokaryotic and eukaryotic NDP kinases are tetramers and hexamers, respectively, in quaternary structure.

Human NDP kinase A, encoded by the *nm23-H1* gene, is closely related in amino-acid sequence (88% identity) to NDP kinase B, but displays significant differences in cellular functions, isoelectric point and mobility on SDS–PAGE. Transfection of *nm23-H1* cDNA into highly metastatic cell lines resulted in a significant reduction of metastatic potential *in vivo*, indicating a possible role of human NDP kinase A as a metastasis suppressor (Leone *et al.*, 1991; Kantor *et al.*, 1993). Despite a strong similarity to human NDP kinase B in primary sequence, human NDP kinase A, a hexamer of identical 152-residue subunits (subunit M_r = 17 149), is an interesting target for structure elucidation owing to its distinct functional roles. In order to provide a structural basis for understanding the functional differences between human NDP kinase A and kinase B, missing information on the three-dimensional structure of NDP kinase A needs to be obtained. As the first step toward its structure determination, crystals of recombinant human NDP kinase A diffracting to at least 2.15 Å resolution have been produced. Here, we report the crystallization conditions and preliminary X-ray data.

2. Protein preparation and crystallization

The recombinant human NDP kinase A was overexpressed and purified as described previously (Kim *et al.*, 1997). The purified enzyme was dialyzed against 10 m*M* ADA–NaOH at pH 6.5 and concentrated by ultrafiltration (Amicon, YM30). Crystallization was performed by the hanging-drop vapour-diffusion method at 288 K using 24-well tissue culture plates (Hampton Research). Each hanging drop on a siliconized cover slip was prepared by mixing 3 µl each of the protein solution (at 17 mg ml^{-1}) and the reservoir solution. It was placed over 1 ml of the reservoir solution. The protein concentration was estimated by measuring the absorbance at 280 nm, employing a correspondence of 1 mg ml^{-1} concentration to an A_{280} of 0.237 for 1 cm path length. Initial crystallization trials were set up using Crystal Screens I and II and MembFac conditions (Hampton Research). Microcrystals obtained from 2-methyl-2,4-pentanediol (MPD) were further optimized. Under the optimized reservoir conditions

Table 1
Synchrotron data-collection statistics.

Values in parentheses refer to the highest resolution shell.

Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 74.21, b = 78.11, c = 82.29, β = 101.33
Resolution range (Å)	50–2.15 (2.25–2.15)
No. of unique reflections	48679 (5479)
$I/\sigma(I)$	19.1 (3.0)
Redundancy	2.7 (2.3)
Data completeness (%)	90.9 (73.8)
$R_{\rm merge}$† (%)	6.0 (29.2)

† $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of reflection h, $\sum_h$ is the sum over all reflections, and $\sum_i$ is the sum over i measurements of reflection h.

100 m*M* MES–KOH, 30–34%(*v*/*v*) MPD at pH 6.35, crystals of approximate dimensions 0.6 × 0.3 × 0.1 mm grew within 3 d (Fig. 1).

3. X-ray crystallographic studies

Native X-ray diffraction data were collected to 2.15 Å resolution at 100 K using a Weissenberg camera for macromolecular crystallography at the BL-6A experimental station of the Photon Factory, Tsukuba, Japan (Sakabe, 1991). The crystals could be directly frozen owing to the high MPD concentration during crystallization. The wavelength of the synchrotron X-rays was 1.000 Å and a 0.2 mm collimator was used. Two image plates (20 × 40 cm, Fuji BAIII) were placed at a distance of 429.7 mm from the crystal. The oscillation range per frame was 3.5°, with a speed of 2.0° s^{-1} and a coupling constant of 1.5° mm^{-1}. An overlap of 0.5° was allowed between contiguous frames. A total of 60 frames were collected, with either 28 or 31.5 s exposure per frame, covering 180° rotation of the crystal around an arbitrary axis. The diffraction patterns recorded on the image plates were digitized with an off-line Fuji BA100 scanner. The raw data were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

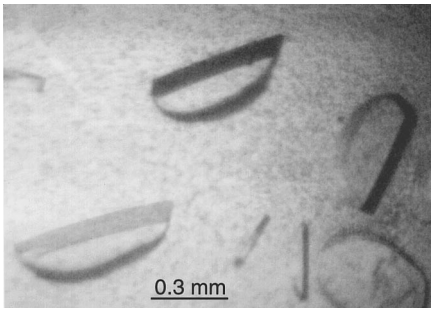

Figure 1
Monoclinic crystals of human NDP kinase A. Approximate dimensions are 0.6 × 0.3 × 0.1 mm.

Table 1 summarizes the statistics for the synchrotron data collection. The synchrotron data consist of 153 045 measurements of 48 679 unique reflections, with an $R_{\rm merge}$ (on intensity) of 6.0% (rejecting 2.7% outliers). The systematic absences indicated that the crystals belong to the monoclinic space group $P2_1$, with unit-cell parameters $a = 74.21\ (24), b = 78.11\ (20), c = 82.29\ (29)$ Å, $\beta = 101.33\ (22)°$. The asymmetric unit contains a homohexameric molecule of human NDP kinase A, giving a crystal volume per protein mass (V_m) of 2.27 $Å^3\ Da^{-1}$ and a solvent content of 46%. These values are within the frequently observed ranges for protein crystals (Matthews, 1968). The structure will be solved by molecular replacement.

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