crystallization papers

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

Kyeongsik Min,[a]† Sun Young Kim,[b]† Hyun Kyu Song,[a] Changsoo Chang,[a] Seung-Je Cho,[a] Jinho Moon,[a] Jin Kuk Yang,[a] Jae Young Lee,[a] Kong-Joo Lee[b] and Se Won Suh[a]*

[a]Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742, South Korea, and [b]College of Pharmacy, Ewha Women's University, Seoul 120-750, South Korea

† KM and SYK contributed equally to this work.

Correspondence e-mail: sewonsuh@snu.ac.kr

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, $\beta = 101.33°$. 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, °)	$a = 74.21$, $b = 78.11$, $c = 82.29$, $\beta = 101.33$
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_{merge}† (%)	6.0 (29.2)

† $R_{merge} = \sum_h \sum_i |I(h)_i - \langle I(h) \rangle| / \sum_h \sum_i I(h)_i$, 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 mM MES–KOH, 30–34%(v/v) MPD at pH 6.35, crystals of approximate dimensions $0.6 \times 0.3 \times 0.1$ 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
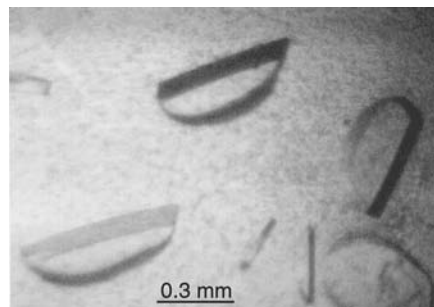

Figure 1
Monoclinic crystals of human NDP kinase A. Approximate dimensions are $0.6 \times 0.3 \times 0.1$ mm.

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).

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_{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.

References

Abdulaev, N. G., Karaschuk, G. N., Ladner, J. E., Kakuev, D. L., Yakhyaev, A. V., Tordova, M., Gaidarov, I. O., Popov, V. I., Fujiwara, J. H., Chinchilla, D., Eisenstein, E., Gilliland, G. L. & Ridge, K. D. (1998). *Biochemistry*, **37**, 13958–13967.

Biggs, J., Hersperger, E., Steeg, P. S., Liotta, L. A. & Shearn, A. (1990). *Cell*, **63**, 933–940.

Chiadmi, M., Moréra, S., Lascu, I., Dumas, C., Le Bras, G., Véron, M. & Janin, J. (1993). *Structure*, **1**, 283–293.

Dumas, C., Lascu, I., Moréra, S., Glaser, P., Fourme, R., Wallet, V., Lacombe, M.-L., Véron, M. & Janin, J. (1992). *EMBO J.* **11**, 3203–3208.

Kantor, J. D., McCormick, B., Steeg, P. S. & Zetter, B. R. (1993). *Cancer Res.* **53**, 1971–1973.

Kim, S. Y., Chang, K. H., Doh, H.-J., Jung, J. A., Kim, E., Sim, C. J. & Lee, K.-J. (1997). *Mol. Cells*, **7**, 630–634.

Leone, A., Flatow, U., King, C. R., Sandeen, M. A., Margulies, I. M., Liotta, L. A. & Steeg, P. S. (1991). *Cell*, **65**, 25–35.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Moréra, S., Lacombe, M.-L., Yingwu, X., Lebras, G. & Janin, J. (1995). *Structure*, **3**, 1307–1314.

Okabe-Kado, J., Kosukabe, T., Honma, Y., Hayashi, M., Henzel, W. J. & Hozumi, M. (1992). *Biochem. Biophys. Res. Commun.* **182**, 987–994.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Parks, R. E. & Agarwal, R. P. (1973). *The Enzymes*, Vol. 8, edited by P. D. Boyer, pp. 307–334. New York: Academic Press.

Sakabe, N. (1991). *Nucl. Instrum. Methods A*, **303**, 448–463.

Webb, P. A., Perisic, O., Mendola, C. E., Backer, J. M. & Williams, R. L. (1995). *J. Mol. Biol.* **251**, 574–587.

Williams, R. L., Oren, D. A., Muñoz-Dorado, J., Inouye, S., Inouye, M. & Arnold, E. (1993). *J. Mol. Biol.* **234**, 1230–1247.