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New crystal forms of *Escherichia coli* and *Saccharomyces cerevisiae* soluble inorganic pyrophosphatases.

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Abstract

We have obtained new crystal forms of *Escherichia coli* and *Saccharomyces cerevisiae* soluble inorganic pyrophosphatase with and without substrate, competitive inhibitor and divalent cation. They diffract to higher resolution than any forms previously reported. The best *E. coli* crystals are in space group *R*32 with cell dimensions of 111.4 × 111.4 × 76.6 Å and diffract to 2.0 Å. The best *S. cerevisiae* crystals were grown from a mixture of PEG 1000 and 4000 in the presence of metal ions. They are in space group $P2_12_12_1$, have cell dimensions of 54.2 × 68.5 × 161.7 Å and diffract to 1.8 Å.

Introduction

Soluble inorganic pyrophosphatase (E.C. 3.6.1.1, PPase) is a ubiquitous enzyme that catalyses the interconversion of inorganic pyrophosphate (PP_i) and phosphate (P_i). As a result, it shifts the overall equilibrium for many key biosynthetic processes in the cell, including nucleic acid and protein synthesis (Kornberg, 1962), and may thus have an important role in evolution by affecting how accurately DNA molecules are replicated (Kukko-Kalske & Heinonen, 1985). It is also an essential enzyme in bacteria and yeast (Chen *et al.*, 1990; Lundin, Baltscheffsky & Ronne, 1991).

All known PPases require divalent cations and Mg^{2+} conveys the highest activity. Free Mg^{2+} activates the enzyme and Mg_2PP_i is the substrate (Cooperman, 1982). PPase is thus a good model enzyme for studying the mechanism of P—O—P hydrolysis and for studying the role of metal ions in catalysis. The best studied PPases are the *S. cerevisiae* (Y-PPase) and *E. coli* (E-PPase) enzymes (Cooperman, Baykov & Lahti, 1992). E-PPase and other bacterial PPases are homohexamers with a monomeric molecular weight of about 20 kDa; Y-PPase and other eukaryotic PPases are homodimers with a monomeric molecular weight of about 30 kDa. Even though bacterial and eukaryotic PPases have very similar mechanisms, their sequences are not very similar – typically around 25% identity. The genes for E-PPase (Lahti *et al.*, 1988) and Y-PPase (Kolakowski, Schlösser & Cooperman, 1988) have been cloned and site-directed mutagenesis and enzymological studies on them are now underway (Lahti *et al.*, 1990; Lahti *et al.*, 1991, Käpylä *et al.*, 1995; Salminen *et al.*, 1995; Heikinheimo *et al.*, 1995). The structure of *apo*-Y-PPase is known at 3 Å resolution (Terzyan *et al.*, 1984) and a preliminary report of the 2.35 Å structure of Y-PPase complexed with manganese phosphate has appeared (Chirgadze *et al.*, 1991); *apo*-E-PPase has been crystallized (Josse & Wong, 1971) and its structure solved at medium (2.7 Å) resolution (Kankare *et al.*, 1994). The only other PPase structure known is that of *Thermus thermophilus* PPase, recently determined at 2.0 Å resolution by Teplyakov *et al.* (1994).

We are using site-directed mutagenesis, enzymology and crystallography to understand the enzyme mechanism of PPases in as much detail as possible, and so have obtained crystals of E-PPase and Y-PPase that diffract to very high resolution with and without divalent cation, and with and without substrate or competitive inhibitor. We report here the first crystal forms of E-PPase that diffract beyond 2.5 Å resolution and the first crystal forms of Y-PPase that diffract beyond 2 Å resolution.

Materials and methods

Y-PPase crystallization

Y-PPase protein was produced in *E. coli* and purified by ion exchange and gel filtration as described elsewhere (Heikinheimo *et al.*, 1995). Before crystallization, the protein buffer was changed to 50 m*M* MES, pH 6.0, with 2 m*M* $MnCl_2$ by twice diluting and reconcentrating the protein in Centricon 30 tubes (Amicon Inc., USA). The final protein concentration was 15–20 mg ml^{-1}, determined by UV absorption at 280 nm (Kunitz, 1952). Crystallization conditions were first screened with the sparse-matrix method of Jancarik & Kim (1991). We found PEG to be an effective crystallizing agent for Y-PPase and eventually obtained two new crystal forms, YP1 and YP2 (Table 1). We grew them at 277 K in 8 µl hanging drops (McPherson, 1985) containing a 1:1 mixture of protein and reservoir solution.

The crystal form YP1 grew best above a reservoir solution containing 100 m*M* Tris–HCl, pH 7.2, when both drop and well contained a mixture of PEG 1000 and 4000. The protein concentration was 20 mg ml^{-1}. The same crystals could be grown using only PEG 4000 but the addition of PEG 1000 decreased the mosaicity of the crystals and increased the resolution. The ratio of PEG 1000 to PEG 4000 was 4:5, and the final concentration of PEG 4000 in the reservoir was between 6 and 12%. The morphology of the crystals was irregular but they diffracted to at least 1.8 Å resolution. They appeared within two weeks and grew rapidly up to 0.4 × 0.4 × 0.9 mm. Unfortunately, the YP1 crystals did not grow in the presence of phosphate and became disordered when soaked in solution containing phosphate. To study the binding of PP_i and metals to the active site, we had to find another crystal form that would also diffract to high resolution. YP2 was grown with the same buffers, but with MEP 550 (polyethylene glycol methyl ether, $M_r = 550$; Aldrich, Germany) as a precipitating agent.

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Table 1. *Reported crystal forms of inorganic pyrophosphatases*

	Space group	Unit-cell dimensions (Å)*	Resolution (Å)	Reference
E-PPase	*R*32	110.4 × 110.4 × 153.0	2.7	Kankare *et al.*, 1994
E-PPase (EP1)	*R*32	110.6 × 110.6 × 154.7	2.0	This work
E-PPase (EP2)	*R*32	111.4 × 111.4 (0.029) × 76.6 (0.018)	2.0	This work
Thermus thermophilus PPase	*R*32	110.3 × 110.3 × 82.0	2.0	Obmolova, Kuranova & Teplyakov, 1993
Y-PPase	$P2_1$	70.4 × 95.4 × 52.2, $\beta = 99.8°$	2.5	Bunick, McKenna, Colton & Voet, 1974
Y-PPase	$P2_12_12_1$	116.0 × 106.8 × 56.2	2.35	Chirgadze *et al.*, 1990
Y-PPase (YP1)	$P2_12_12_1$	54.2 (0.038) × 68.5 (0.080) × 161.7 (0.194)	1.8	This work
Y-PPase (YP2)	$P2_12_12_1$	115.9 × 106.1 × 50.3	2.0	This work

* Estimated standard deviation values are included in parentheses for those new crystal forms where data was collected from more than two crystals.

The protein concentration was 15 mg ml^{-1}, and the precipitant concentration in the well was between 21 and 25%. We could also grow these crystals with 5 or 10 m*M* phosphate in the drop or with the inhibitors $O_3PCH(OH)PO_3$ (hydroxymethane bisphosphonate) (Cooperman & Chiu, 1973) or $CaPP_i$ (Moe & Butler, 1972) at drop concentrations of 0.1 and 0.05 m*M*, respectively. The metal ion in all experiments was Mn^{2+} at a concentration of 1 m*M*.

E-PPase crystallization

E-PPase has previously been crystallized from a protein solution containing 100 m*M* potassium phosphate buffer, pH 6.4, mixed together 1:1 with reservoir solution containing about 50% saturated ammonium sulfate. Crystals grew in a week at 310 K but not at all at lower temperatures. The crystals were not ideal, however. They diffracted only to 2.7 Å (Kankare *et al.*, 1994); Mg^{2+} and Mn^{2+} could not be included in either the crystallization solutions or in soaking solutions because ternary salts precipitated out (Adrian Goldman, unpublished results).

E-PPase was purified from an overexpressing clone as described elsewhere (Salminen *et al.*, 1995). Before crystallization, the protein buffer was changed to 25 m*M* Tris–HCl, pH 8.0, by twice diluting and concentrating in Centricon 30 tubes (Amicon Inc., USA), the protein was concentrated to 12–30 mg ml^{-1} and 2–4 m*M* $MnCl_2$ was added. We used the sparse-matrix sampling method for initial screening (Jancarik & Kim, 1991). Crystals were grown at room temperature in hanging drops (McPherson, 1985). Protein solution was mixed 1:1 with the reservoir solution. Two of the screening conditions were further modified to obtain the crystal forms EP1 and EP2.

The EP1 crystals are a much improved version of the original E-PPase crystal form (Josse & Wong, 1971; Kankare *et al.*, 1994). They grew best with the reservoir solution containing 100 m*M* HEPES buffer pH 7.0, from 0.8 to 1.0 *M* sodium citrate and 4 m*M* KH_2PO_4, but also grew from 100 m*M* sodium citrate buffer pH 5.6, 1 *M* ammonium phosphate and 4 m*M* KH_2PO_4. Crystals also grew if 0.1 m*M* $O_3PCH(OH)PO_3$ or 0.1 m*M* $CaPP_i$ was added to the drop. The crystals were either cubic or trigonal pyramidal in shape, appeared within a week and grew to their final size of 0.5 × 0.4 × 0.25 mm in three weeks. The EP2 crystals grew with the reservoir solution containing 2–15% PEG 6000, 0.8–1.0 *M* lithium sulfate and 4–50 m*M* KH_2PO_4, with or without 0.05 m*M* $O_3PCH(OH)PO_3$ or 0.1 m*M* $CaPP_i$. The crystals grew and looked like the EP1 crystals but have different cell dimensions (Table 1). The biggest crystals were 0.5 × 0.45 × 0.3 mm.

Data collection

Single crystals were mounted in glass capillary tubes and data collected using our R-AXIS IIC image-plate area detector (Sato, Yamamoto, Imada & Katsube, 1992) mounted on a Rigaku RU-200B rotating-anode X-ray source using Cu $K\alpha$ radiation (50 kV, 180 mA). We used the R-AXIS 3.40V software to determine the unit-cell dimensions. The space groups suggested by the R-AXIS software were checked by analysing collected diffraction data. For the orthorhombic Y-PPase crystals, we listed all the axial reflections and looked for possible systematic absences. For the rhombohedral E-PPase crystals, we checked that the pattern of systematic absences in the predicted oscillation matched the observed diffraction pattern. The data were processed with the R-AXIS software and different data sets scaled together in *PROTEIN* (Steigemann, 1974, 1991).

Results and discussion

Y-PPase

The crystal form YP1 diffracts to 1.8 Å, belongs to the orthorhombic space group $P2_12_12_1$ and has cell dimensions 54.2 × 68.5 × 161.7 Å. Data were collected in 2° frames to 2.0 Å resolution. The data set was 80% complete to 2.3 Å resolution and the overall R_{merge} was 8.2%. The crystal form YP2 has unit-cell dimensions of 115.9 × 106.1 × 50.3 Å and belongs to the space group $P2_12_12_1$. Chirgadze *et al.* (1990) have earlier reported a co-crystal of the Y-PPase–$Mn_3(P_i)_2$ complex which had the same space group but unit-cell dimensions 116.0 × 106.8 × 56.2 Å (Table 1). We believe that YP2 is a more tightly packed version of the published crystal form. YP2 crystals diffract to at least 2.0 Å and are suitable for three-dimensional structure determination.

E-PPase

Both of the E-PPase crystal forms diffract to 2.0 Å resolution. EP1 has unit-cell dimensions 110.6 × 110.6 × 154.7 Å and EP2 has unit-cell dimensions 111.4 × 111.4 × 76.6 Å. Both belong to space group *R*32 and are suitable for three-dimensional structure determination. EP1 is a better diffracting version of the crystal form reported by Kankare *et al.* (1994). They have shown that it has two 20 kDa subunits in the asymmetric unit, related by a non-crystallographic twofold axis passing through the *c* axis parallel to the crystallographic *a* axis at a height of 0.219 in fractional coordinates. Because the *a* and *b* axes are the same in both crystal forms and the *c* axis of EP2 is half of that of EP1, EP2 can only have one subunit in the asymmetric unit and the centre of the E-PPase hexamer must therefore be at the crystallographic origin. The completeness of the EP1 data set was 80% to 2.5 Å resolution and the R_{merge} was 6.0%. We have also collected data from EP2 crystals grown in the presence of 2 m*M* Mn^{2+} and 25 m*M* P_i. The data set was 93% complete to 2.0 Å resolution with an R_{merge} of 9.5%.

The structure determination from these crystals is under way and should, in combination with our ongoing mutagenesis and enzymological studies (Käpylä *et al.*, 1995; Salminen *et al.*, 1995; Heikinheimo *et al.*, 1995), yield a detailed picture of the enzyme mechanism in and evolutionary relationships among inorganic pyrophosphatases.

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References

BUNICK, G., MCKENNA, G. P., COLTON, R. & VOET, D. (1974). *J. Biol. Chem.* **249**, 4647–4649.

CHEN, J., BREVET, A., FROMAN, M., LEVEQUE, F., SCHMITTER, J.-M., BLANQUET, S. & PLATEAU, P. (1990). *J. Bacteriol.* **172**, 5686–5689.

CHIRGADZE, N. Y., KURANOVA, I. P., NEVSKAYA, N. A., TEPLYAKOV, A. V., WILSON, K., STROKOPYTOV, B. V., ARUTYUNYAN, É. G. & KHENE, V. (1991). *Sov. Phys. Crystallogr.* **36**, 128–132.

CHIRGADZE, N. Y., STROKOPYTOV, B. V., KURANOVA, I. P., ARUTYUNYAN, É. G., HÖHNE, W. & HAUSDORFF, G. (1990). *Sov. Phys. Crystallogr.* **35**, 451–453.

COOPERMAN, B. S. (1982). *Methods Enzymol.* **87**, 526–548.

COOPERMAN, B. S., BAYKOV, A. A. & LAHTI, R. (1992). *Trends Biol. Sci.* **17**, 262–266.

COOPERMAN, B. S. & CHIU, Y. C. (1973). *Biochemistry*, **12**, 1670–1682.

HEIKINHEIMO, P. *et al.* (1995). In preparation.

JANCARIK, J. & KIM, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.

JOSSE, J. & WONG, S. C. K. (1971). *The Enzymes*, Vol. 4, edited by P. D. BOYER, pp. 499–527. New York: Academic Press.

KANKARE, J., NEAL, G. S., SALMINEN, T., GLUMOFF, T., COOPERMAN, B., LAHTI, R. & GOLDMAN, A. (1994). *Protein Eng.* **7**, 823–830.

KÄPYLÄ, J., HYYTIÄ, T., LAHTI, R., GOLDMAN, A., BAYKOV, A. & COOPERMAN, B. (1995). *Biochemistry*. In the press.

KOLAKOWSKI, L. F., SCHÖLSSER, M. & COOPERMAN, B. S. (1988). *Nucleic Acids Res.* **16**, 10441–10452.

KORNBERG, A. (1962). *Horizons in Biochemistry*, edited by M. KASHA & B. PULLMAN, pp. 251. New York: Academic Press.

KUKKO-KALSKE, E. & HEINONEN, J. (1985). *Int. J. Biochem.* **17**, 575–580.

KUNITZ, M. (1952). *J. Gen. Physiol.* **35**, 423–450.

LAHTI, R., KOLAKOWSKI, L. F. JR, HEINONEN, J., VIHINEN, M., POHJANOKSA, K. & COOPERMAN, B. S. (1990). *Biochim. Biophys. Acta*, **1038**, 338–345.

LAHTI, R., PERÄLÄ, M., HEIKINHEIMO, P., PITKÄRANTA, T., KUKKO-KALSKE, E. & HEINONEN, J. (1991). *J. Gen. Microbiol.* **137**, 2517–2523.

LAHTI, R., PITKÄRANTA, T., VALVE, E., ILTA, I., KUKKO-KALSKE, E. & HEINONEN, J. (1988). *J. Bacteriol.* **170**, 5901–5907.

LUNDIN, M., BALTSCHEFFSKY, H. & RONNE, H. (1991). *J. Biol. Chem.* **266**, 12168–12172.

MCPHERSON, A. (1985). *Methods Enzymol.* **114**, 112–120.

MOE, O. A. & BUTLER, L. G. (1972). *J. Biol. Chem.* **247**, 7315–7319.

OBMOLOVA, G., KURANOVA, I. & TEPLYAKOV, A. (1993). *J. Mol. Biol.* **232**, 312–313.

SALMINEN, T., KÄPYLÄ, J., HEIKINHEIMO, P., KANKARE, J. GOLDMAN, A., HEINONEN, J., BAYKOV, A., COOPERMAN, B. & LAHTI, R. (1995). *Biochemistry*. In the press.

SATO, M., YAMAMOTO, M., IMADA, K. & KATSUBE, Y. (1992). *J. Appl. Cryst.* **25**, 348–357.

STEIGEMANN, W. (1974). PhD thesis, Technical Univ. of Munich, Germany.

STEIGEMANN, W. (1991). *Crystallographic Computing*, edited by D. MORAS, A. D. PODJARNY & J.-C. THIERRY, pp. 115–125. Oxford Univ. Press.

TEPLYAKOV, A., OBLOMOVA, G., WILSON, K. S., ISHII, K., KAJI, H., SAMEJIMA, T. & KURANOVA, I. (1994). *Protein Sci.* **3**, 1098–1107

TERZYAN, S. S., VORONOVA, A. A., SMIRNOVA, E. A., KURANOVA, I. P., NEKRASOV, Y. V., ARUTYUNYAN, É. G., VAINSHTEIN, B. K., HÖHNE, W. & HANSEN, G. (1984). *Bioorg. Khim.* **10**, 1469-1482.