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Crystallization and preliminary X-ray diffraction analysis of the regulatory subunit of human protein kinase CK2

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Protein kinase CK2 is a tetramer composed of two α catalytic subunits and two β regulatory subunits. A C-terminal truncated form of the β subunit has been overproduced in *Escherichia coli* and purified to homogeneity. Two crystal forms of the truncated protein which diffract to at least 2 Å resolution have been obtained. Form I belongs to the monoclinic space group $P2_1$, with unit-cell parameters a = 49.9, b = 92.9, c = 53.7 Å, β = 96.3°, and yields plate-like crystals. Form II belongs to the tetragonal space group $P4_22_12$, with unit-cell parameters a = 132.19, b = 132.19, c = 63.79 Å, and produces rod-shaped crystals. Both crystal forms have a functional dimer in the crystal asymmetric unit.

1. Introduction

Protein kinase CK2 is a heterotetrameric serine/threonine protein kinase made by the association of three dissimilar subunits, the 35–44 kDa catalytic subunits α and α' and the 24–29 kDa β subunit, to generate native structures exhibiting the stoichiometries $\alpha_2\beta_2$, $\alpha'_2\beta_2$ and $\alpha\alpha'\beta_2$. Despite the large number of substrates of CK2, the exact physiological role of this protein kinase is not clear (Allende & Allende, 1995; Pinna & Meggio, 1997). However, it was unambiguously shown that CK2 is essential for the viability of *Saccharomyces cerevisiae* (Padmanabha *et al.*, 1990; Glover, 1998), *S. pombe* (Snell & Nurse, 1994) and *Dictyostelium discoidium* (Kikkawa *et al.*, 1992). In addition, a large body of observations support the implication of CK2 in cell division (Pinna & Meggio, 1997) and cell transformation (Pena *et al.*, 1983; Brunati *et al.*, 1986; ole-MoiYoi *et al.*, 1993).

The β subunit of CK2 does not share sequence similarity with any other known protein, with the notable exception of the product of the Stellate gene of *Drosophila*. Typically, β subunits from vertebrates and insects are composed of 215 amino acids exhibiting high sequence conservation. The β subunit usually stimulates the enzymatic activity of the α catalytic subunit by a factor of five to ten (Cochet & Chambaz, 1983; Filhol *et al.*, 1991), although with a few substrates it down-regulates the kinase activity (Meggio *et al.*, 1992), suggesting that it can change the specificity of the interaction of CK2 with substrates and inhibitors.

A number of structural elements responsible for the biochemical properties and putative functions of the β subunit have been mapped (Leroy *et al.*, 1999). The N-terminal region contains two autophosphorylation sites on serine in positions 2 and 3 and a conserved acidic stretch encompassing residues 51–80, which is mainly responsible for the down-regulation of CK2 activity and has the potential to bind polycationic effectors (Leroy *et al.*, 1997). The β subunit contains four conserved cysteines (109, 114, 137, 140) arranged in a sequence fulfilling the requirement of a potential metal-binding motif, most probably a zinc-finger motif (Allende & Allende, 1995). Application of the two-hybrid system has allowed the delineation of subdomains which are involved in the dimerization of the β subunit (residues 155–165) and in the β–α interaction (residues 170–190; Gietz *et al.*, 1995; Kusk *et al.*, 1995).

The β subunit is also responsible for the reversible polymerization of the holoenzyme observed *in vitro* (Valero *et al.*, 1995), and preliminary observations indicate that a C-terminal region of the protein (residues 183–215) is at least partially responsible for this oligomerization (Leroy *et al.*, 1999). The increasing number of newly identified proteins which interact with the β subunit opens up new perspectives on the regulation of this kinase. Among the interacting partners identified so far are some protein kinases such as Mos (Chen *et al.*, 1997) and A-Raf (Boldyreff & Issinger, 1997), implying that in addition to its role in controlling the activity of CK2, the β subunit may also participate in the regulation of other serine/threonine kinases.

The crystal structure of the recombinant catalytic subunit (α) of CK2 from maize has been recently solved, highlighting peculiar features of this enzyme that are unusual among protein kinases (Niefind *et al.*, 1998). The

generation of the tetrameric CK2 is achieved exclusively by the ability of the β subunit to form homo- and heterodimers with catalytic subunits, while the two catalytic subunits make no contact with each other (Gietz *et al.*, 1995).

To gain an insight into the β-subunit structure at the atomic level and to understand its multiple functions, an X-ray crystallographic study of the protein was undertaken. Owing to the strong propensity of recombinant β subunit (β1–215) to irreversibly aggregate, a C-terminally truncated form of the protein (β1–182) was chosen because of its high stability. To date there are no structural data for the β subunit of CK2, and this C-terminally truncated form of the protein seems a reasonable model to use to obtain useful information on its molecular organization into functional subdomains (Leroy *et al.*, 1999).

Firstly, we describe here the production and purification of the human β1–182 as a highly purified protein. The purified protein is a dimer in solution and interacts with the catalytic α subunit, but with a reduced affinity. Secondly, we report the crystallization and preliminary results towards the determination of the structure of this protein.

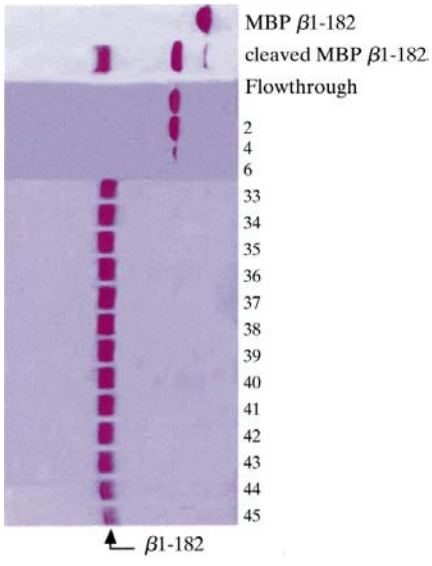

Figure 1
Purification of recombinant β1–182 on a heparin–Sepharose column. The MBP–(β1–182) fusion protein was cleaved with factor Xa protease and loaded on a heparin–Sepharose column. The β1–182 protein was then eluted from the column using a linear gradient of 0.1–1.0 *M* NaCl. Aliquots of the collected fractions (lanes 1–45) were analyzed by electrophoresis on a 15% SDS polyacrylamide gel. The position of the β1–182 protein is indicated on the bottom line.

2. Materials and methods

2.1. Protein expression and purification

The β1–182 protein was fused to the maltose-binding protein by cloning into the polylinker of the pMal C2 vector (Protein Fusion and Purification System, New England Biolabs; Guan *et al.*, 1988). The resulting recombinant vector was used to transform *E. coli* strain BL 21. After induction with 0.3 m*M* IPTG and growth for 2 h, the bacterial pellet was resuspended in cold lysis buffer (10 m*M* phosphate, 30 m*M* NaCl, 0.25% Tween 20, 10 m*M* β-mercaptoethanol, 10 m*M* EDTA, 10 m*M* EGTA, pH 7, containing a cocktail of protease inhibitors). After sonication, the lysate was adjusted to 0.5 *M* NaCl and subjected to centrifugation at 9000*g* for 20 min. The supernatant was loaded onto an amylose resin column and the fusion protein was eluted with 10 m*M* maltose added to the column buffer (10 m*M* phosphate, 0.5 *M* NaCl, 1 m*M* sodium azide, 2 m*M* EDTA, 1 m*M* EGTA, 10 m*M* DTT, pH 7). The purified fusion protein was cleaved with the protease factor Xa at a protease:protein ratio of 1:100(*w*/*w*). After 4 h at 298 K, the solution was diluted twice with TDG buffer (20 m*M* Tris–HCl, 10 m*M* DTT, 2% glycerol) containing 100 mg ml^{-1} leupeptine and loaded onto a heparin–Sepharose column. The β1–182 protein was eluted from the column using a linear gradient from 0.1 to 1 *M* NaCl (Fig. 1). The purified protein was concentrated to 20–28 mg ml^{-1} using a Centriprep concentrator (Amicon Co.) with a 30 kDa cutoff and stored in aliquots at 193 K. The sample was checked by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and by mass spectrometry. Gel-filtration experiments carried out on a Superose 12 column (Pharmacia) indicated that the protein is a dimer in solution.

2.2. Crystallization

The hanging-drop vapour-diffusion method (McPherson, 1982) was used to screen crystallization conditions. The first crystals were obtained using a sparse-matrix kit (Hampton Research), condition 6 of screen I (30% PEG 4000, 0.1 *M* Tris–HCl pH 8.5, 0.2 *M* $MgCl_2$). Optimum conditions were found using a protein concentration of 18 mg ml^{-1}. Plate-like crystals (Fig. 2*a*) formed after 3 d equilibration at 281 K against a well solution of polyethylene glycol monomethylether 5000 (14–20%) in Bicine buffer pH 9.2, 0.46 *M* $MgCl_2$, 10 m*M* DTT and 3% dioxane. After one month of equilibration, needle-like crystals also appeared in the drop. This crystal form was improved to produce rod-shaped crystals by adding 0.4 *M* NaCl to the above conditions (Fig. 2*b*).

Interestingly, while the first crystal form was very sensitive to any manipulations, the second one could easily withstand irradiation, soaking in heavy-atom derivative solutions and cryo-cooling.

2.3. Crystallographic diffraction data collection

Diffraction data on the truncated β subunit of CK2 were collected at 277 K using a MAR Research image-plate detector (180 mm) and Cu $K\alpha$ radiation from a Rigaku rotating-anode generator operating at 40 kV and 100 mA. Reflections could be measured to 3.2 and 2.65 Å for form I and form II, respectively (Table 1). Whereas the resolution did not seem to decay appreciably for form II, it was only possible to obtain a partially complete data set for form I.

3. Results and discussion

Crystallization of the full-length β subunit of CK2 was unsuccessful; the protein is highly unstable and forms irreversible aggregates

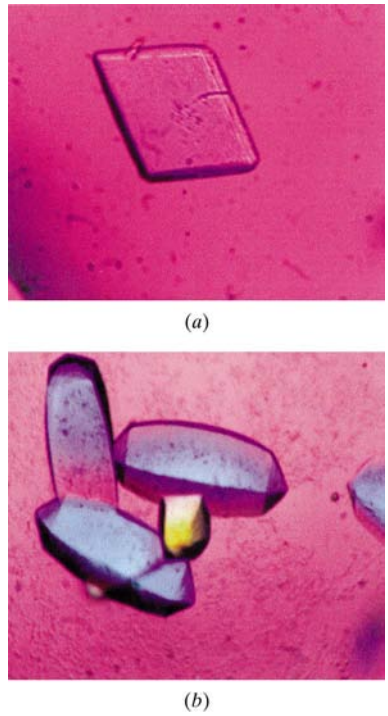

Figure 2
Crystals of the β1–182 regulatory subunit of human protein kinase CK2. (*a*) Monoclinic crystal, space group $P2_1$, grown at 281 K in Bicine buffer pH 9.2. Crystal size is 0.3 × 0.3 × 0.15 mm. (*b*) Tetragonal crystals, space group $P4_22_12$, grown by adding 0.4 *M* NaCl to the conditions used for (*a*). The crystal size is 0.1 × 0.1 × 0.2 mm.

Table 1
Data-collection statistics for the regulatory subunit of CKII.

Statistics for the highest resolution shell are given in parentheses.

	Form I	Form II
Lattice	Monoclinic	Tetragonal
Space group	$P2_1$	$P4_22_12$
Resolution limit (Å)	3.2	2.65
$R_{\rm merge}$† (%)	10.2 (23.0)	8.6 (17.2)
Completeness (%)	74 (78.2)	99.1 (99.0)
Redundancy	2 (2.1)	8.7 (8.5)
$I/\sigma(I)$	8.4 (4.7)	37.0 (17.5)
Molecules in cell	4	16

† $R_{\rm merge} = \sum |I(k) - \langle I\rangle| / \sum I(k)$, where $I(k)$ is the value of the kth measurement of the intensity of a reflection, $\langle I\rangle$ is the mean value of the intensity of that reflection and the summation is over all measurements.

at all ionic strengths. However, during the purification of the protein it was observed that its spontaneous degradation yielded an end product corresponding to amino-acid residues 1–182 as determined by mass-spectrometric analysis. The cDNA coding for this stable fragment was cloned and expressed in *E. coli* as a fusion protein with the maltose-binding protein (MBP). The affinity-purified protein was cleaved with the protease factor Xa and the β1–182 fragment was purified to homogeneity using heparin–Sepharose chromatography (Fig. 1). The purified protein was stable and highly soluble; concentrations as high as 28 mg ml^{-1} could be achieved. Two crystal forms of the protein suitable for X-ray diffraction were grown (Fig. 2). The typical sizes of form I and form II crystals were 0.3 × 0.3 × 0.15 and 0.1 × 0.1 × 0.2 mm, respectively. Form I belongs to the monoclinic space group $P2_1$ with unit-cell parameters a = 49.9, b = 92.9, c = 53.7 Å, β = 96.3°. Form II belongs to the tetragonal space group $P4_22_12$ with unit-cell parameters a = 132.19, b = 132.19, c = 63.79 Å. A native data set was collected for both crystal forms (Table 1). The data were reduced and scaled with the *DENZO* package (Otwinowski, 1993) and gave overall $R_{\rm sym}$ values of 10.2 and 8% for form I and form II, respectively. The self-rotation function suggests that there is a dimer in the asymmetric unit (a.u.) in each form. Assuming a partial specific volume of 0.73 cm^3 g^{-1} and a dimer in the a.u., the solvent contents would be 58 and 63% for form I and form II, respectively. These values are in the range observed for protein crystals (Matthews, 1968). Both crystal forms were tested on the ID14 beamline at ESRF and diffracted beyond 2 Å resolution.

An attempt will be made to solve the structure by multiple anomalous dispersion methods, making use of selenomethionyl proteins produced by recombinant DNA techniques (Hendrickson *et al.*, 1990). The results of this analysis may well help in the understanding of the structure–function relationship of this protein and allow refinement of our knowledge of the molecular basis of its multiple functions.

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References

Allende, J. E. & Allende, C. C. (1995). *FASEB J.* **9**, 313–323.
Boldyreff, B. & Issinger, O. G. (1997). *FEBS Lett.* **403**, 197–199.
Brunati, A. M., Saggioro, D., Chieco-Bianchi, L. & Pinna, L. A. (1986). *FEBS Lett.* **206**, 59–63.
Chen, M., Li, D., Krebs, E. G. & Cooper, J. A. (1997). *Mol. Cell Biol.* **17**, 1904–1912.
Cochet, C. & Chambaz, E. M. (1983). *J. Biol. Chem.* **258**, 1403–1406.
Filhol, O., Cochet, C., Wedegaertner, P., Gill, G. N. & Chambaz, E. M. (1991). *Biochemistry*, **30**, 11133–11140.
Gietz, R. D., Graham, K. C. & Litchfield, D. W. (1995). *J. Biol. Chem.* **270**, 13017–13021.
Glover, C. V. (1998). *Prog. Nucleic Acid. Res. Mol. Biol.* **59**, 95–133.
Guan, C. di, Li, P., Riggs, P. D. & Inouye, H. (1988). *Gene*, **67**, 21–30.
Hendrickson, W. A., Horton, J. R. & LeMaster, D. M. (1990). *EMBO J.* **9**, 1665–1672.
Kikkawa, U., Mann, S. K., Firtel, R. A. & Hunter, T. (1992). *Mol. Cell Biol.* **12**, 5711–5723.
Kusk, M., Bendixen, C., Duno, M., Westergaard, O. & Thomsen, B. (1995). *J. Mol. Biol.* **253**, 703–711.
Leroy, D., Filhol, O., Quintaine, N., Sarrouilhe, D., Loue-MacKenbach, P., Chambaz, E. M. & Cochet, C. (1999). In the press.
Leroy, D., Heriche, J. K., Filhol, O., Chambaz, E. M. & Cochet, C. (1997). *J. Biol. Chem.* **272**, 20820–20827.
McPherson, A. (1982). *Preparation and Analysis of Protein Crystals*, pp. 1–371. New York: John Wiley & Sons.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Meggio, F., Boldyreff, B., Marin, O., Pinna, L. A. & Issinger, O. G. (1992). *Eur. J. Biochem.* **204**, 293–297.
Niefind, K., Guerra, B., Pinna, L. A., Issinger, O. G. & Schomburg, D. (1998). *EMBO J.* **17**, 2451–2462.
ole-MoiYoi, O. K., Brown, W. C., Iams, K. P., Nayar, A., Tsukamoto, T. & Macklin, M. D. (1993). *EMBO J.* **12**, 1621–1631.
Otwinowski, Z. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 56–62. Warrington: Daresbury Laboratory.
Padmanabha, R., Chen-Wu, J. L., Hanna, D. E. & Glover, C. V. (1990). *Mol. Cell Biol.* **10**, 4089–4099.
Pena, J. M., Itarte, E., Domingo, A. & Cusso, R. (1983). *Cancer Res.* **43**, 1172–1175.
Pinna, L. A. & Meggio, F. (1997). *Prog. Cell Cycle Res.* **3**, 77–97.
Snell, V. & Nurse, P. (1994). *EMBO J.* **13**, 2066–2074.
Valero, E., De Bonis, S., Filhol, O., Wade, R. H., Langowski, J., Chambaz, E. M. & Cochet, C. (1995). *J. Biol. Chem.* **270**, 8345–8352.