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Expression, purification and crystallization of the catalytic subunit of protein kinase CK2 from *Zea mays*

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Abstract

The catalytic (α) subunit of protein kinase CK2 (CK2α) was originally cloned and overexpressed in the *Escherichia coli* strain pT7-7/BL21(DE3). The protein has been purified to homogeneity and crystallized. The crystals belong to the monoclinic space group $C2$, they have unit-cell parameters a = 142.6, b = 61.3, c = 45.6 Å, β = 103.3° and diffract X-rays to at least 2.0 Å resolution. The calculated crystal packing parameter is V_m = 2.47 Å^3 Da^{-1} suggesting that one CK2α molecule is contained in the asymmetric unit and that the solvent content of the unit cell is 50%.

1. Introduction

Protein kinase CK2 (former name casein kinase II) is a heterotetrameric ($\alpha_2\beta_2$ or $\alpha\alpha'\beta_2$) Ser/Thr protein kinase which specifically phosphorylates the sequence motif S/T*XX*D/E (Pinna, 1990). *In vitro* numerous different protein substrates of CK2 are known (Allende & Allende, 1995). The exact physiological role of CK2 is not clear although it is known that the enzyme is involved in cell proliferation (Pinna, 1990; Münstermann *et al.*, 1990).

Although CK2 belongs to the large superfamily of eukaryotic protein kinases, it possesses some features which are unusual among those enzymes: (1) while most Ser/Thr kinases are basophilic CK2 prefers acidic phosphorylation sites; (2) in contrast to most other kinases CK2 accepts both ATP and GTP as phosphoryl donors; (3) the regulatory properties of CK2 are poorly understood. No second messengers like cAMP, cGMP or Ca^{2+} are known, and the quaternary structure of the enzyme is so stable that it is probably constitutively active and finely tuned for its activity.

To gain an insight into CK2 at the atomic level and to understand its unusual properties, an X-ray crystallographic study of the enzyme was performed. Recombinant CK2α from maize (rmCK2α) was chosen for its high stability in contrast to the recombinant human CK2α (rhCK2α).

As a first step we describe here the production of the catalytic (α) subunit of CK2 (CK2α) from *Zea mays* to get a highly purified protein and its subsequent crystallization. Maize CK2α was cloned and sequenced by Dobrowolska *et al.* (1991). The protein consists of 332 amino acids corresponding to a calculated molecular mass of 39 228 Da and its sequence is highly conserved from yeast to man. Furthermore, though no CK2β subunit is known in maize so far, recombinant maize CK2α can be combined with human CK2β to form a stable and active tetrameric complex (Boldyreff *et al.*, 1993). For these reasons maize CK2α seems to be a reasonable model to obtain useful information about the human enzyme apart from its specific value in context of maize metabolism.

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2. Experimental procedure

2.1. *Expression and purification of rmCK2 α subunit*

The rmCK2α was expressed in a bacterial expression system pT7-7/BL21(DE3) (Boldyreff *et al.*, 1993) and purified to homogeneity according to Grankowski *et al.*, 1991) *i.e.* 20 g bacterial pellet of *Escherichia coli* culture, were suspended in 200 ml of 25 m*M* Tris–HCl pH 8.5, 7 m*M* β-mercaptoethanol, 0.2 m*M* phenylmethylsulfonylfluoride (PMSF) (buffer *A*). After sonication and centrifugation at 30 000*g* the supernatant (~1.2 g) was filtered and loaded onto a P11 phosphocellulose (Whatman) column (200 ml). A 1.5 l linear gradient in the range 300–1000 m*M* NaCl in buffer *A* was applied.

rmCKα-containing fractions were collected, concentrated and equilibrated in 25 m*M* Tris–HCl pH 8.5, 1 *M* NaCl, 50%(*v*/*v*) glycerol solution and loaded onto Superose 6 (Pharmacia). In contrast to Grankowski *et al.* (1991) a totally newly designed purification scheme was introduced after the above mentioned gel-filtration step.

After gel filtration the purest fractions were collected and equilibrated in buffer *A* plus 100 m*M* NaCl and loaded onto Resource S (Pharmacia). A 160 ml linear gradient from 100 to 500 m*M* NaCl was applied. Again the purest fractions were collected, concentrated and dialysed in buffer *A* with the addition of 500 m*M* NaCl, 20%(*w*/*v*) PEG 20 000.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) analysis showed an apparently homogeneous enzyme preparation, the total yield of the preparation was 27 mg (20 g bacteria)$^{-1}$.

2.2. *Protein kinase assay*

20 µl column fractions were assayed at 310 K in a mixture containing 25 m*M* Tris–HCl pH 8.5, 20 m*M* NaCl, 5 m*M* $MgCl_2$, 1 m*M* DTT, 50 µ*M* (γ-^{32}P) ATP (specific activity 1000–2000 counts min^{-1} pmol^{-1}), 190 µ*M* synthetic peptide substrate (RRRDDDSDDD) in a total volume of 50 µl. The reaction was stopped on ice. 30 µl of the reaction mixture was spotted onto P81 phosphocellulose paper as already described (Kuenzel & Krebs, 1985; Kuenzel *et al.*, 1987).

1 U of rmCKα is defined as the amount of activity necessary to transfer 1 µmol phosphate min^{-1} into the synthetic peptide substrate at 310 K.

Table 1. *Native data set statistics of maize CK2α crystals*

Total number of observations: 67 224; number of outlier rejections: 1081

High-resolution limit (Å)	No. of unique reflections	$I/\sigma(I)$	Completeness (%)	Multiplicity	R_{sym} (%)
6.07	989	13.1	100.0	5.0	9.3
4.35	1659	12.8	99.8	5.0	10.4
3.57	2085	11.7	99.2	4.6	12.2
3.10	2484	9.9	100.0	4.1	13.1
2.78	2787	7.7	100.0	3.6	14.9
2.54	3028	5.6	98.9	3.0	16.0
2.35	3032	3.7	91.2	1.9	16.5
2.20	2832	2.3	79.9	1.5	17.7
2.08	2476	1.7	65.0	1.3	21.5
1.97	966	1.1	23.5	1.1	30.8
Total	22338	6.5	80.3	3.0	11.9

2.3. *Gel electrophoresis and western blot analysis*

Aliquots of either partially or totally purified rmCK2 were subjected to 12.5% SDS–PAGE. The gels were either stained with Coomassie Blue or transferred onto nitrocellulose as described earlier (Issinger *et al.*, 1988).

2.4. *Protein determination*

Protein concentration was evaluated on the basis of the method of Bradford (1976) using bovine serum albumin as standard and Bio Rad reagent.

2.5. *Crystallization*

Purified rmCK2α was gently concentrated by dialysis against a solution of 20% PEG 20 000. For crystallization setups rmCK2α was stored in a stock solution containing 8 mg ml^{-1} protein in 500 m*M* NaCl, 25 m*M* Tris–HCl, 7 m*M* 2-mercaptoethanol, pH 8.5.

All crystallization experiments were performed according to the sitting-drop variant of the vapour-diffusion method (Ducruix & Giegé, 1992) using Cryschem crystallization plates. For each setup temperatures of 292, 285 and 277 K were tested in parallel. Generally a 3 µl droplet of rmCK2α stock solution was mixed with equal volumes of firstly 6 m*M* ATP in water, secondly 1.5 m*M* $MgCl_2$ in water and thirdly reservoir solution composed as described below. Each well contained 1 ml of reservoir solution. Initial crystallization conditions were established by a factorial experimental approach described by Jancarik & Kim (1991) using a Biomek-1000 automated laboratory station (Beckmann, Mannheim, Germany). Subsequently the initial conditions were refined by systematic variation of the pH value and the concentration of the precipitating agent.

2.6. *X-ray diffraction experiment*

RmCK2α crystals were characterized by X-ray diffraction. Diffraction data were collected with a Siemens electronic area detector and Cu *Kα* radiation. The X-rays were generated by a Rigaku rotating anode, operating at 50 kV and 100 mA. They were monochromated by a graphite lattice and parallelized by a 0.3 mm collimator. RmCK2α crystals were mounted in glass capillaries on a Rigaku three-axis goniometer. The crystal-to-detector distance was 120 mm. The width of each oscillation frame was 10 min arc in ω with an exposure time of 10 min. All measurements were carried out at 283 K. The data were processed using the *XENGEN* 2.0 software (Howard *et al.*, 1987) and the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

The purification of rmCK2α was one of the critical steps in order to obtain a pure and stable protein present in solution at a high concentration. As it is shown in Fig. 1, the Resource S chromatography was an important new element in the purification process leading to a narrow elution profile which allows the collection of pure rmCK2α fractions. Stability of the enzyme in solution was enhanced by the subsequent concentration step involving dialysis against polyethylene glycol (PEG) 20 000.

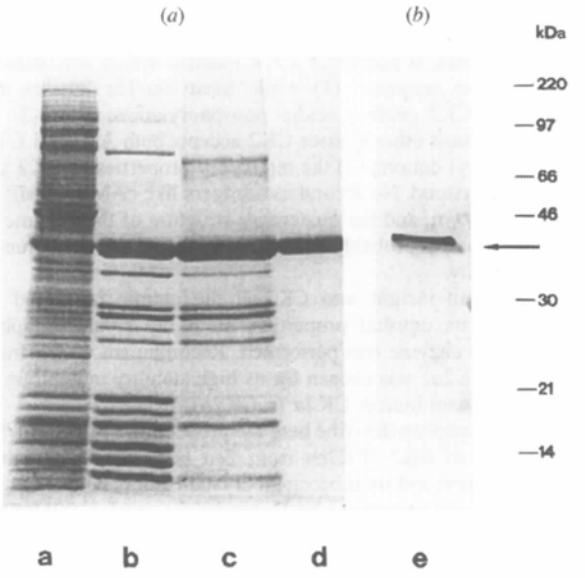

Fig. 1. Purification of the recombinant maize CK2α subunit (rmCK2α). (*a*) 12.5% SDS–PAGE analysis. Lane *a*, crude extract (20 µg); lane *b*, eluate from phosphocellulose column (20 µg); lane *c*, eluate from Superose 6 column (20 µg); lane *d*, eluate from Resource S column (5 µg). (*b*) Western blot analysis. Lane *e*, 100 ng of pure rmCK2α.

Compact rmCK2α crystals with good diffraction qualities could be found (Fig. 2). They grew at 292 K within 2 weeks with the following optimal reservoir composition: 25% PEG 4000, 200 m*M* sodium acetate, 100 m*M* Tris–HCl, pH 8.0. The typical size of these crystals was 0.3 × 0.3 × 0.5 mm. RmCK2α crystals tend to be twinned and aggregated, a tendency that is somewhat but not completely suppressed by the presence of sodium acetate. An alternative additive to achieve this effect is 2-propanol.

The crystals were stable in the X-ray beam for about 3 d during the diffraction experiments. They belong to the monoclinic space group C2 with lattice constants of a = 142.6, b = 61.3, c = 45.6 Å, β = 103.3° (Table 1) and diffract to at least 2.0 Å resolution. Assuming one rmCK2α molecule per asymmetric unit this lattice leads to a plausible V_m value of 2.47 Å^3 Da^{-1} corresponding to a solvent content of 50% of the crystallographic unit cell (Matthews, 1968).

A native data set of monoclinic rmCK2α crystals was collected (Table 1). The quality of the data set probably suffered from radiation, resulting in a signal-to-noise ratio [$I/\sigma(I)$] of less than 3 in the resolution shells higher than 2.3 Å. Therefore, the resolution limit was set at 2.3 Å. A lower resolution, *e.g.* 2.0 Å, possibly could have been achieved by using shorter exposure times, cryogenic freezing and one crystal.

Fig. 2. Monoclinic crystals of recombinant maize CK2α-subunit.

As with the structure of human cyclin-dependent kinase 2 (De Bondt *et al.*, 1993) a search model is available that shares 33.7% sequence identity with rmCK2α. We are confident in being able to solve the rmCK2α structure by molecular replacement. Details about the structure determination together with a description and discussion of the structure will be published in a further paper.

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