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Communication

Different properties of four molecular forms of protein kinase CK2 from *Saccharomyces cerevisiae*

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CK2 is a pleiotropic constitutively active serine/threonine protein kinase composed of two catalytic α- and two regulatory β-subunits, whose regulation is still not well understood. It seems to play an essential role in regulation of many cellular processes. Four active forms of CK2, composed of αα′ββ′, α_2ββ′, α'_2ββ′, and a free α′-subunit were isolated from wild-type yeast and strains containing a single deletion of the catalytic subunit. Each species exhibits properties typical for CK2, but they differ in substrate specificity and sensitivity to inhibitors. This suggests that each CK2 isomer may regulate different process or may differ in the way of its regulation.

Keywords: protein kinase CK2, isoenzyme specificity, protein phosphorylation, yeast

Protein phosphorylation represents a mechanism of regulation controlling practically all aspects of cell life. Phosphorylation regulates a number of important cell processes, including cell metabolism, response to external stimuli, cell cycle, transcription and translation, cell proliferation, differentiation and transformation (Edelman *et al*., 1987; Krebs, 1994). This covalent modification is carried out by protein kinases and induces conformational changes in the target polypeptide causing changes in its activity (an inhibitory or stimulatory effect).

Protein kinase CK2 is the most pleiotropic protein kinase known and is able to phosphorylate *in vitro* over 300 proteins (Meggio & Pinna, 2003), whose phospho-acceptor sites are specified by multiple acidic residues downstream from the phosphorylable amino acid. The acidic side chain at position n + 3 plays the most prominent role in susceptibility to CK2 phosphorylation (Pinna, 1990).

It has become clear that protein kinase CK2 plays an important role in the cell, regulating physiological as well as pathological processes, such as cancer development, signal transduction, transcriptional control, proliferation, and cell cycle control (reviewed in Litchfield, 2003). CK2 is essential to cell viability and appears to be implicated in global processes such as tRNA and rRNA synthesis (Ghavidel & Schultz, 2001), apoptosis (Guo *et al*., 2001), cell survival (Ahmed *et al*., 2002), and transformation (Tawfic *et al*., 2001).

Such pleiotropicity may account for the unique property of CK2 of being constitutively active, either in its heterotetrameric form, composed of two catalytic (α and/or α′) subunits and two regulatory β-subunits, or in its monomeric form. In many organisms, distinct isoenzymatic forms of the catalytic subunit of CK2 have been identified (Lozeman *et al*., 1990; Glover, 1998; Litchfield, 2003). In humans, two isoforms of the catalytic subunit, designated CK2α and CK2α′, have been well characterized, and a third one, the CK2α″ isoenzyme, highly expressed in liver, has been identified recently (Hilgard *et al*., 2002). Only a single regulatory CK2β subunit has been identified in mammals, but multiple forms of CK2β in simple eukaryotes (Pinna, 1990; Glover, 1998; Litchfield, 2003).

Yeast CK2 is composed of two catalytic (α and α′) and two regulatory (β and β′) subunits (Meggio *et al*., 1986; Glover, 1998). In our latest work we showed that in *Saccharomyces cerevisiae*

Abbreviations: CK2, protein kinase CK2, casein kinase type 2; CK2α and CK2α′, α and α′ subunits of CK2; DMSO, dimethylsulfoxide; DRB, 5,6-dichloro-1-(β-D-ribofuranosyl)-benzimidazole; IPTG, isopropyl β-D-thiogalactoside; PMSF, phenylmethylsylfonyl fluoride; rP2A, rP2B, recombinant yeast acidic ribosomal proteins P2A, P2B; SOD1, Cu-Zn superoxide dismutase; TBBt, 4,5,6,7-tetrabromo-1*H*-benzotriazole; TBBz, 4,5,6,7-tetrabromo-1*H*-benzimidazole.

two naturally occurring forms of CK2 exist simultaneously, the active free catalytic subunit CK2α′ and the holoenzyme hCK2 (Abramczyk *et al*., 2003) which may form tetrameric structures $\alpha\alpha'\beta\beta'$, $\alpha_2\beta\beta'$ or $\alpha'_2\beta\beta'$.

The present paper is a continuation of our studies on the structure and function of yeast protein kinase CK2. We report here the purification of different CK2 isoenzymes from *S. cerevisiae* mutants and compare their biochemical properties. A possible correlation between CK2 molecular composition and catalytic activity as well as cell function is discussed.

MATERIALS AND METHODS

Strains and growth conditions. *S. cerevisiae* W303 (**a**/α; *leu2, trp1, ura3, his3, ade2, kan1*) was grown under aerobic conditions in YPD medium (yeast extract, peptone, and glucose) at 28°C to exponential growth phase. *S cerevisiae cka1Δ* (gene YIL035c), *cka2Δ* (gene YOR061w), *ckb1Δ* (gene YGL019w), and *ckb2*Δ (gene YOR039w) single deletion strains (Y01428, Y01837, Y04387 and Y01815, respectively) were from Euroscarf Collection Centre.

Protein kinases. Different molecular forms of protein kinase CK2 with the subunit composition of $\alpha\alpha'\beta\beta'$, $\alpha_2\beta\beta'$ and $\alpha'_2\beta\beta'$ were obtained from yeast strains W303, Y01428 and Y01837, respectively. Native CK2 holoenzymes were isolated and purified from yeast postribosomal fraction as previously described (Szyszka *et al*., 1986) using two-step ion exchange chromatography on DEAE- and P-cellulose followed by Heparin-Sepharose and α-Casein-Sepharose affinity column chromatography. The free CK2α′ subunit was isolated according to the previously described procedure (Abramczyk *et al*., 2003). Protein kinase activities were tested under standard conditions with exogenous casein or bovine calmodulin, or with endogenous protein substrates (80S ribosomes, recombinant P2A and P2B) as previously described (Zieliński *et al*., 2002) using either [γ-^{32}P]ATP or [γ-^{32}P]GTP as a phosphate donor.

Kinetic parameters. Michaelis constants K_m were determined for CK2 nucleotide substrates (ATP or GTP) and rP2B protein with the use of the Lineweaver-Burk plot method. The same method was used for determination of V_{max} values for CK2 isoforms, ATP and protein substrates (casein, rP2A, rP2B, 80S ribosomes, bovine calmodulin).

Inhibition constants, K_i were determined for inhibitors (DRB, TBBt, TBBz, heparin and SOD1) *vs* CK2 isoforms with the use of Dixon plots.

Glycerol gradients. Ultracentrifugation of purified yeast CK2 holoenzymes was conducted in a preformed 15–40% glycerol linear density gradient in 20 mM Tris/HCl, pH 7.5, 150 mM NaCl, 6 mM β-mercaptoethanol, 1 mM EDTA and 0.5 mM PMSF, at 30000 r.p.m. for 40 h in a Sorval TH641 rotor. Fractions (0.4 ml) were collected by upward displacement and assayed for protein kinase activity.

Recombinant proteins. SOD1 — The PCR amplified *Nde*I–*Sal*I restriction fragment of yeast SOD1 was cloned into respective restriction sites of the pT7-7 vector. The plasmid was overexpressed in *Escherichia coli* BLD21*trx*B (DE3) after addition of IPTG to a final concentration of 0.1 mM. For purification under native conditions after 5 h of growth, the cells were centrifuged and subsequently sonificated in a solution containing 50 mM Tris/HCl, pH 7.5, 300 mM NaCl, 6 mM β-mercaptoethanol and 5 mM PMSF. Purification of recombinant SOD1 from *E. coli* protein extract was performed as described before (Zieliński *et al*., 2002).

rP2A and rP2B — Recombinant acidic ribosomal proteins P2A and P2B were purified according to a previously described method (Tchorzewski *et al*., 1999).

CK2 inhibitors. TBBt — 4,5,6,7-Tetrabromo-1*H*-benzotriazole and TBBz — 4,5,6,7-tetrabromo-1*H*-benzimidazole were synthesized as previously described (Borowski *et al*., 2003; Zień *et al*., 2003a). TBBt and TBBz were dissolved in DMSO to obtain stock solutions, which were diluted with water or buffer solution to the desired concentrations. The possible effects of low concentrations of DMSO on the enzymatic assays were eliminated by suitable controls.

Other procedures. Protein concentration was determined as previously described (Bradford, 1970) with bovine serum albumin as a standard.

RESULTS

M_r of yeast holoenzymes

In order to evaluate the M_r of the three molecular forms of native yeast CK2, the holoenzymes were subjected to glycerol gradient ultracentrifugation as described in Materials and Methods. All CK2 isoenzymes exhibited similar mobility (not shown), and their M_r values were estimated at 150000 for $\alpha\alpha'\beta\beta'$, 155000–160000 for $\alpha_2\beta\beta'$ and 140000–145000 for $\alpha'_2\beta\beta'$, values in agreement with those expected from the M_r of single CK2 subunits: 45000 for α, 39000 for α′, 32000 for β and 30000 for β′.

ATP binding site

Protein kinase CK2 has been shown to be a second messenger independent phosphotransferase with an exceptional ability to utilize both ATP and GTP as phosphate donors (Pinna, 1990; Litch-

Table 1. K_i values for inhibition of different molecular form of yeast CK2 with selected inhibitory compounds[a]

Inhibitor	K_i [a] Form of CK2			
	$\alpha\alpha'\beta\beta'$	$\alpha_2\beta\beta'$	$\alpha'_2\beta\beta'$	α'
DRB	32 ± 3	53 ± 4	38 ± 3	>200
TBBt	0.6 ± 0.05	3.5 ± 0.3	1.4 ± 0.1	0.1 ± 0.02
TBBz	0.5 ± 0.05	3.1 ± 0.3	5.4 ± 0.5	36 ± 3
Heparin	0.10 ± 0.005	0.09 ± 0.005	0.09 ± 0.005	>20
SOD1	>50	7.2 ± 0.3	3.0 ± 0.2	2.6 ± 0.2

[a]K_i values are in μM except for heparin where it is in μg/ml. Values are expressed as means ± S.D. of at least three separate determinations performed in duplicate or triplicate.

Table 2. Substrate specificity of the four forms of yeast CK2[a]

Protein substrate	V_{max} [pmol/min per mg of protein substrate] Form of CK2			
	$\alpha\alpha'\beta\beta'$	$\alpha_2\beta\beta'$	$\alpha'_2\beta\beta'$	α'
rP2B	540 ± 25	188 ± 10	453 ± 20	320 ± 15
80S ribosomes	225 ± 12	95 ± 5	184 ± 10	110 ± 8
Casein	392 ± 18	23 ± 2	530 ± 25	4.5 ± 0.3
Bovine calmodulin	50 ± 5	34 ± 3	78 ± 8	857 ± 30

[a]V_{max} values are expressed as means ± S.D. of at least three separate determinations performed in duplicate or triplicate.

field, 2003). All three molecular forms of yeast CK2 holoenzyme and free CK2α' were examined for their capacity to bind both phosphodonors. Three isoforms of CK2: $\alpha\alpha'\beta\beta'$, $\alpha'_2\beta\beta'$ and free α' show similar ATP/GTP binding ratio, while $\alpha'_2\beta\beta'$ shows quite a low affinity for GTP. They bind ATP with a K_m of 7.5 μM, 4.5 μM, 8.5 μM and 11.0 μM, and GTP with a K_m of 52 μM, 50 μM, 70 μM and 51 μM, respectively, for CK2 isoforms $\alpha\alpha'\beta\beta'$, $\alpha_2\beta\beta'$, $\alpha'_2\beta\beta'$ and α'.

As previously shown, halogenated benzimidazoles and benzotriazoles, compounds competitive with respect to the ATP/GTP binding site, are specific and effective inhibitors of protein kinase CK2 (Zień *et al*., 2003a; 2003b). As can be seen in Fig. 1 and Table 1, the efficiency of DRB, TBBt and TBBz in inhibiting the activity of the four examined forms of CK2 is different. TBBt shows a high affinity for the ATP binding site of free CK2α' and the $\alpha\alpha'\beta\beta'$ form, whereas TBBz binds with a high affinity only to the $\alpha\alpha'\beta\beta'$ form of CK2.

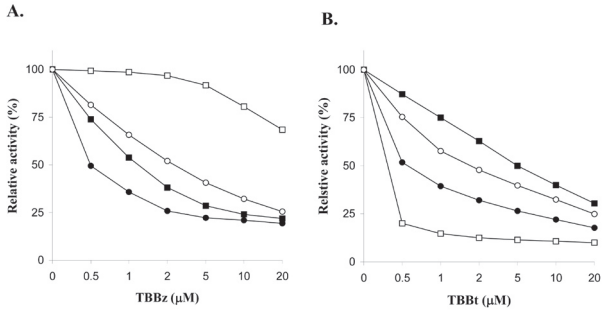

Figure 1. Influence of TBBz (A) and TBBt (B) on the activities of different molecular forms of protein kinase CK2 from yeast.
Protein kinase activity measurements were performed for 15 min at 30ºC in a medium (50 μl final volume) containing 50 mM Tris/HCl buffer, pH 7.5, 10 mM Mg^{2+}, [γ-^{32}P]ATP (specific radioactivity 500–1000 c.p.m./pmol), 4 U of the appropriate form of CK2: $\alpha\alpha'\beta\beta'$ (●), $\alpha_2\beta\beta'$ (■), $\alpha'_2\beta\beta'$ (○) or α' (□), and 0.2 mg/ml of rP2B.

Heparin and SOD1

A number of compounds have been proposed to regulate CK2 (Pinna, 1990; Tuazon & Traugh, 1991; Litchfield, 2003). It has long been known from *in vitro* studies that CK2 is inhibited by negatively charged compounds such as heparin, and is activated by positively charged compounds such as polyamines or basic polypeptides. At concentrations below 1 μg/ml heparin has little effect on protein kinases other than CK2 (Hathaway *et al*, 1980; Tuazon & Traugh, 1991). Heparin along with GTP has been utilized as a diagnostic tool to define an enzyme as protein kinase CK2 (Tuazon & Traugh, 1991). As shown in all oligomeric forms of CK2 are effectively inhibited by heparin, unlike the monomeric CK2α' which is inhibited by this polyanionic compound only at much higher concentrations.

In our previous study we have shown that yeast Cu-Zn superoxide dismutase can interact with free CK2α' and form a complex as an inactive phosphotransferase (Zieliński *et al*., 2002; Abramczyk *et al*., 2003). As shown, SOD1 at a concentration similar to that observed for the CK2α' shows inhibitory activity also for the $\alpha'_2\beta\beta'$ form. The K_i value of $\alpha'_2\beta\beta'$ kinase for SOD1 equals 7 μM, whereas the $\alpha\alpha'\beta\beta'$

holoenzyme is inhibited only at higher concentrations of dismutase.

The substrate specificity of protein kinase CK2 isoforms

Protein kinase CK2 appears to reside in a variety of cellular compartments where it participates in the phosphorylation and regulation of a broad array of cellular protein targets (Glover, 1998; Pinna, 1999; Ahmed *et al*., 2002; Litchfield, 2003; Meggio & Pinna, 2003).

In our report we analyse four different forms of yeast protein kinase CK2. The differences in substrate specificity of these isoenzymes with regard to selected protein substrates are shown in Table 2. As can be seen, each molecular form of CK2 has its own preferences for protein substrates. It appears that the activity of the protein kinases towards a particular substrate depends on the composition of the holoenzyme as well as on the presence of the regulatory β/β′ subunits.

DISCUSSION

Protein kinase CK2 is ubiquitously distributed in eukaryotic organisms where it appears to exist as a tetrameric complex consisting of two catalytic α and two regulatory β subunits (Pinna, 1990; Ahmed *et al*., 2002; Litchfield, 2003; Filhol, 2004).

Yeast CK2 consists of two catalytic (α and α′) and two regulatory (β and β′) subunits (Glover, 1998) which, as shown in the present work, can form at least four different enzymatic forms. At this point it should be noted that besides the CK2 forms αα′ββ′, α_2ββ′and α'_2ββ′ isolated from the wild-type and *cka1Δ* and *cka2Δ* single deletion strains we have also tested strains having single deletions in the regulatory β or β′ genes. In the last two cases we did not observe CK2 holoenzyme activities (not shown), which probably means that CK2 isoenzymes composed of catalytic α and/or α′ subunits with homodimers of regulatory β_2 or β'_2 subunits are not formed or are not present in cell cytoplasm (not shown). Similar results were observed by Dr. C.V. Glover (personal communication).

The four CK2 forms present in yeast cells differ in their protein substrate specificity (Table 2) as well as in the sensitivity to specific modulators (Fig. 1, Table 1). Bearing in mind that in humans there are three forms of CK2 catalytic subunits, α, α′ and α″ (Hilgard *et al*., 2002), simple mathematical calculation indicates that they may form at least six different forms of holoenzyme. In addition, as shown in yeast (Abramczyk *et al*., 2003), the catalytic subunits of CK2 may be active independently of β subunits.

CK2 has been shown to reside in a variety of cellular compartments where it participates in phosphorylation of over 300 currently known protein substrates (Meggio & Pinna, 2003). Therefore, it is not surprising that CK2 is involved in regulation of many cellular functions such as DNA replication, transcription, translation, cell metabolism, cell growth and proliferation, and many others (Pinna, 1990; 2003; Ahmed *et al*. 2002; Litchfield, 2003; Meggio & Pinna, 2003; Unger *et al*., 2004). As it has been shown in our report such high pleiotropicity of CK2 may be due to its ability to exist in many different isoenzymatic forms. In addition, each subunit of CK2 might interact with additional regulatory proteins changing its substrate specificity and in many cases subcellular localization (Litchfield, 2003; Bibby & Litchfield, 2005). According to numerous authors (reviewed in Lichfield, 2003) such interactions between regulatory proteins and the catalytic α/α′ or the regulatory β subunits of CK2 can change the structure of the enzyme (binding or catalytic centre) and as a result change CK2 specificity. Previously (Zień *et al*., 2003a; 2003b) as well as in the results shown in this paper we have observed differences in protein substrate specificity as well as sensitivity to different inhibitors between the different isoenzymes.

The observed differences in the properties of individual molecular forms of CK2 can be taken advantage in designing specific inhibitors for their selective inhibition, especially for the forms engaged in the regulation of processes potentially pathogenic for the cell. As can be seen in Fig. 1A, 0.5 μM TBBz can inhibit selectively the αα′ββ′ form of CK2, while 0.2 μM TBBt (Fig. 1B) can effectively inhibit only the free CK2α′ subunit.

From this perspective, it will be interesting to study in detail the differences in the molecular structure of all the forms of CK2 and the mechanisms of their regulation in the cell as well as their cellular targets. Recent reports give us information regarding phosphorylation and the regulatory role of CK2 but without description of the holoenzyme composition (Pinna, 1990; 2003; Ahmed *et al*., 2002; Litchfield, 2003; Meggio & Pinna, 2003; Unger *et al*., 2004). Now is the time to ask which form of CK2 regulates what? This question has special importance in the cases of CK2-regulated processes affecting cell survival and apoptosis and in pathological processes, such as cancer cases or viral infections where the anti-apoptotic CK2 activity may act as an element enforcing cell proliferation.

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REFERENCES

Abramczyk O, Zień P, Zieliński R, Pilecki M, Helman U, Szyszka R (2003) *Biochem Biophys Res Commun* **307**: 31–40.
Ahmed K, Gerber DA, Cochet C (2002) *Trends Cell Biol* **12**: 226–230.
Bibby A, Litchfield DW (2005) *Int J Biol Sci* **1**: 67–79.
Borowski P, Deinert J, Schalinski S, Bretner M, Ginalski K, Kulikowski T, Shugar D (2003) *Eur J Biochem* **270**: 1645–1653.
Bradford MM (1970) *Anal Biochem* **72**: 248–254.
Edelman AM, Blumenthal DK, Krebs EG (1987) *Annu Rev Biochem* **56**: 567–613.
Filhol O, Martiel J-L, Cochet C (2004) *EMBO Rep* **5**: 351–355.
Ghavidel A, Schultz MC (2001) *Cell* **106**: 575–584.
Glover CVC (1998) *Prog Nucleic Acid Res Mol Biol* **59**: 95–133.
Guo C, Yu S, Davis AT, Wang H, Green JE, Ahmed KA (2001) *J Biol Chem* **276**: 5992–5999.
Hathaway GM, Lubben TH, Traugh JA (1980) *J Biol Chem* **255**: 8038–8041.
Hilgard P, Huang T, Wolkoff AW, Stockert RJ (2002) *Am J Physiol Cell Physiol* **282**: C472–C483.
Krebs EG (1994) *Trends Biochem Sci* **19**: 439–444.
Litchfield DW (2003) *Biochem J* **369**: 1–15.
Lozeman FJ, Litchfield DW, Piening C, Takio K, Walsh KA, Krebs EG (1990) *Biochemistry* **29**: 8436–8447.
Meggio F, Pinna LA (2003) *FASEB J* **17**: 349–368.
Meggio F, Grankowski N, Kudlicki W, Szyszka R, Gąsior E, Pinna LA (1986) *Eur J Biochem* **159**: 31–38.
Pinna LA (1990) *Biochim Biophys Acta* **1054**: 267–284.
Pinna LA (2003) *Acc Chem Res* **36**: 378–384.
Szyszka R, Łopaczynski W, Gałasiński W, Grankowski N, Gąsior E (1986) *Acta Biochim Polon* **23**: 39–46.
Szyszka R, Grankowski N, Felczak K, Shugar D (1995) *Biochem Biophys Res Commun* **208**: 418–424.
Tawfic S, Yu S, Wang H, Faust R, Davis A, Ahmed K (2001) *Histol Histopathol* **16**: 573–582.
Tchórzewski M, Boguszewska A, Abramczyk D, Grankowski N (1999) *Protein Expr Purif* **15**: 40–47.
Tuazon PT, Traugh JA (1991) *Adv Second Messenger Phosphoprotein Res* **23**: 123–164.
Unger GM, Davis AT, Slaton JW, Ahmed K (2004) *Curr Cancer Drug Targets* **4**: 77–84.
Zieliński R, Pilecki M, Kubiński K, Zień P, Hellman U, Szyszka R (2002) *Biochem Biophys Res Commun* **296**: 1310–1316.
Zień P, Bretner M, Szyszka R, Shugar D (2003a) *Biochem Biophys Res Commun* **306**: 129–133.
Zień P, Abramczyk O, Domańska K, Bretner M, Szyszka R (2003b) *Biochem Biophys Res Commun* **312**: 623–628.