Crystal structure of the human protein kinase CK2 regulatory subunit reveals its zinc finger-mediated dimerization

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Protein kinase CK2 is a tetramer composed of two α catalytic subunits and two β regulatory subunits. The structure of a C-terminal truncated form of the human β subunit has been determined by X-ray crystallography to 1.7 Å resolution. One dimer is observed in the asymmetric unit of the crystal. The most striking feature of the structure is the presence of a zinc finger mediating the dimerization. The monomer structure consists of two domains, one entirely α -helical and one including the zinc finger. The dimer has a crescent shape holding a highly acidic region at both ends. We propose that this acidic region is involved in the interactions with the polyamines and/or catalytic subunits. Interestingly, conserved amino acid residues among β subunit sequences are clustered along one linear ridge that wraps around the entire dimer. This feature suggests that protein partners may interact with the dimer through a stretch of residues in an extended conformation.

Keywords: MAD method/protein kinase CK2/regulatory subunit/X-ray structure/zinc finger

Introduction

Among the hundreds of protein kinases known to date, Ser/Thr protein kinase CK2 is an essential component in living cells, as clearly demonstrated by the lethal effect of its gene disruption in yeast (Padmanabha *et al.*, 1990). However, understanding of its mechanism of action at the molecular level remains elusive (for reviews, see Pinna, 1990; Issinger, 1993; Allende and Allende, 1995). When purified from many sources, CK2 is composed of two catalytic (α and/or α') and two regulatory β subunits forming extremely stable heterotetramers ($\alpha_2\beta_2$, $\alpha'\beta_2$ or $\alpha\alpha'\beta_2$). The β subunits form homodimers which are building blocks to generate heterotetramers with the catalytic subunits, while the two catalytic subunits in the holoenzyme make no contacts with each other (Gietz *et al.*, 1995). Several functional studies have raised particular interest in CK2 and its subunits: it is essential for cell

viability and is believed to play a central role in cellular regulation (Issinger, 1993); however, its physiological regulation remains unknown (Allende and Allende, 1995). The recently solved crystal structure of a CK2 α -ATP complex provides structure-based clues as to the regulation and the apparent constitutive active nature of the enzyme (Niefind *et al.*, 1998).

The β regulatory subunit of CK2 (CK2 β) is ubiquitous in the eukaryotic kingdom; it ranges in size from 26 to 42 kDa. Yeast expresses two CK2 β proteins (Bidwai *et al.*, 1994), and three functional homologues have been identified in *Arabidopsis thaliana* (Sugano *et al.*, 1998), whereas the genome of higher eukaryotes encodes a single protein. A striking feature is that the protein has no sequence homologies with other known proteins except the stellate protein from *Drosophila melanogaster* (Bozzetti *et al.*, 1995). The β subunit was discovered initially as a regulatory subunit of protein kinase CK2. Indeed there is some biochemical evidence to support a role for the β subunit in regulating the substrate specificity (Meggio *et al.*, 1992) and possibly the subcellular localization of the holoenzyme (Sarrouilhe *et al.*, 1998). In this view, the molecular organization of CK2 is reminiscent of the catalytic and targeting subunit association seen in cAMP-dependent protein kinase, cyclin-dependent kinases and phosphatases (Hubbard and Cohen, 1993). More recently, it was observed that CK2 β also has the potential to regulate several protein kinases, such as c-Mos (Chen *et al.*, 1997), A-Raf (Boldyreff and Issinger, 1997) and p90 Rsk (M.Frodin, unpublished data), that play important roles in cell proliferation.

Sustained efforts at dissecting the β subunit into several domains with distinct functions have implicated specific regions of the protein in specific functions (Marin *et al.*, 1995; Leroy *et al.*, 1999a). The N-terminal region appears to represent a pseudo-substrate segment containing two autophosphorylation sites (Ser2 and Ser3) (Boldyreff *et al.*, 1993b). This segment is in tandem with a polyamine-binding domain responsible for both the down-regulation of CK2 activity and its activation by polybasic compounds (Filhol *et al.*, 1991; Leroy *et al.*, 1994, 1995, 1997a,b; Marin *et al.*, 1995).

Mutational analysis disclosed that important glutamic residues lying in the polyacidic region of the β subunit are involved in the binding of polyamine molecules and allowed the delineation of an autonomous binding domain (Leroy *et al.*, 1997b). Furthermore, this regulatory domain was shown to mediate the association of the CK2 holoenzyme with plasma membrane (Sarrouilhe *et al.*, 1998). Recent biochemical and genetic data clearly indicate that the regulatory β subunit is required for the assembly of a fully active and functional enzyme both *in vitro* and *in vivo* (Filhol *et al.*, 1991; Meggio *et al.*, 1992; Roussou and Draetta, 1994). In addition, it was observed that, under

optimal catalytic conditions, the CK2 holoenzyme forms a ring-like polymeric structure in a process that requires the β subunit. This indicates that this subunit also plays an important role in the molecular organization of the enzyme (Valero *et al.*, 1995). Two-hybrid studies as well as peptide-based immunomapping methods have provided conflicting results on the delineation of regions of the β subunit required for β - β homo- and β - α heterodimerization (Kusk *et al.*, 1995; Boldyreff *et al.*, 1996; Krehan *et al.*, 1996, 1998). The C-terminal 33 residues of the β subunit appear to play a role in the oligomerization of the kinase (Leroy *et al.*, 1999a). In addition, several important cellular proteins may bind to CK2 β , suggesting the presence of specific regions involved in the binding activity of this protein (Grein *et al.*, 1999). Altogether, these observations support the notion that the β subunit of CK2 may be a modular protein made by the association of interdependent domains involved in its multiple functions. However, sequence analysis of CK2 β and comparisons with sequences of other proteins have not revealed recognizable motifs or domains. Furthermore, a deeper understanding of the architecture and mechanism of action of CK2 β has been impeded greatly by the lack of three-dimensional structural information. We now present the crystal structure of CK2 β lacking only its C-terminal region. The structure lays bare the molecular architecture of a β subunit dimer and reveals the presence of an acidic ligand-binding groove that is fused into a contiguous structural element that includes a zinc finger-binding motif which controls the dimer formation.

Results

Structure determination

For crystallization purposes, 33 residues at the C-terminus that are responsible for the oligomerization of the tetramer have been deleted (Chantalat *et al.*, 1999). The resulting sequence corresponds to five out of six coding exons in the nucleotide sequence of the human CK2 β gene (Voss *et al.*, 1991). The resulting protein retains the wild-type protein's ability to interact with the catalytic α subunit, albeit with a lower affinity (Boldyreff *et al.*, 1993b).

Residues 1–182 of the human CK2 β subunit were expressed in *Escherichia coli* in fusion with the maltose-binding protein (MBP) for purification. The affinity-purified protein was cleaved and the 1–182 fragment (referred to as CK2 β^{δ}) was purified to homogeneity using heparin–Sepharose chromatography. Crystallization trials yielded two types of crystals with different space groups (Chantalat *et al.*, 1999). Both crystal forms contain two monomers in the asymmetric unit. The structure was determined by multiple anomalous dispersion (MAD) phasing techniques using X-ray diffraction data. These data were collected on beam line BM14 at the European Synchrotron Radiation Facility (ESRF-Grenoble) from tetragonal crystals of a selenomethionyl form of the protein (cell constants are $a = b = 132.23$ Å, $c = 63.78$ Å, space group $P4_22_1$). The resulting electron density map was of high quality, allowing the polypeptide chain to be traced without difficulty (Figure 1). The final structure was refined to a crystallographic R -factor of 19.3% at a resolution of 1.74 Å. Crystallographic statistics are reported in Tables I and II. The current model for the

Fig. 1. View of the experimental electron density at 2 Å resolution showing the region near the two tryptophan residues (W9 and W12) of the protein. The final model is represented. Protein bonds and water molecules are shown by thick lines and stars, respectively. The picture was drawn with O (Jones *et al.*, 1991).

Table I. Data reduction and phasing statistics

	Edge	Peak	Remote
Data collection			
Wavelength (Å)	0.9786	0.97842	0.88
f' , f''	–12, 3	–7, 5.5	–2, 2
Resolution (Å)	2.0	2.0	1.7
Total data	145 882	144 741	634 684
Unique data	37 728	37 386	62 119
Redundancy	3.9	3.9	10.2
Completeness (%) ^a	97.6 (92.5)	97.5 (92.2)	99.5 (99.7)
$I > 3\sigma(I)$	89.5 (73.2)	90.1 (74.1)	84.8 (57.7)
$R_{\text{sym}}(\%)^b$	4.2 (10.6)	4.2 (10.5)	4.3 (32.6)
Phasing			
Phasing power		1.49 (1.00)	3.32 (2.33)
R_{Cullis}^c		0.76	0.49
R_{Cullis}^d (ano)	0.72	0.50	0.55
FOM ^e before solvent flattening (to 2 Å)	0.61		
FOM after solvent flattening (to 2 Å)	0.75		

^aValues in parentheses correspond to the highest resolution shell.

^b $R_{\text{sym}} = \sum |I - I_j| / \sum I_j$

^c R_{Cullis} (dispersive) = $\sum |F_{\text{ph}} - F_p + F_{\text{hc}}| / \sum |F_{\text{ph}} - F_p|$ for centric reflections.

^d R_{Cullis} (anomalous) = $\sum |D_{\text{ph}} - D_{\text{phc}}| / \sum |D_{\text{ph}}|$ for acentric reflections.

^eFOM, figure of merit.

Table II. Refinement statistics

No. of reflections	57 967
R_{work} (R_{free}) ^a	19.33% (21.77%)
R.m.s.d. ^b bonds (Å)	0.011
R.m.s.d. angles (°)	1.3
R.m.s.d. NCS ^c (Å) C α atoms	0.301
Quality of Ramachandran plot	
Percentage of residues in most favoured regions	96%
Percentage of residues in additional allowed regions	4%
No. of atoms [average B -value (Å ²)]	
Protein	2721 [23.1]
Water	321 [31.5]
Ion	3 [22.8]

^a R calculated on 10% of data excluded from refinement.

^bR.m.s.d., root-mean-square deviation.

^cNon-crystallographic symmetry.

Fig. 2. Ribbon representations of the CK2 β^{δ} dimer. The β strands and one 3_{10} helix (η_1) are shown as pink arrows and a pink coil, respectively. The α and other 3_{10} helices are represented as green coils, loops as brown lines and the acidic loop as dashed brown lines. The zinc atoms and cysteine ligands are shown in ball-and-stick, blue and yellow, respectively. Figures 2, 4 and 5 were drawn with MOLSCRIPT (Kraulis, 1991) and RASTER 3D (Bacon and Anderson, 1988). (A) Stereo view along the 2-fold axis. (B) The view in (A) rotated by 90°, the 2-fold axis is vertical.

asymmetric unit contains two CK2 β^{δ} subunits, two zinc ions, one magnesium ion and 321 water molecules. It includes all but residues 1–6, 60–65 and 180–182 for monomer A and 1–5, 59–66 and 177–182 for monomer B. Residues at both ends of the monomer are not visible in the electron density map and are presumed to be disordered. The two loops including residues 60–65 have an ill-defined density.

Monomer structure

The compact monomer is an ovoid-shaped molecule with approximate dimensions of $57 \times 34 \times 35$ Å and contains a total of six α helices of more than one turn and three β strands. Figure 2 shows the relative position of a monomer in the dimer. Secondary structural elements were assigned on the basis of characteristic hydrogen bonding patterns (DSSP; Kabsch and Sander, 1983). The overall topology of the CK2 β^{δ} monomer is unlike that of any known protein. An analysis performed using DALI (Holm and Sander, 1993), involving searches with the entire molecule, showed no structural homology with other known proteins. Domains I and II described below were also submitted to

the same algorithm. This search revealed that domain II was structurally related to TFIIS (PDB code: 1tfi; Qian *et al.*, 1993) with a Z-score of 2.8 and an r.m.s.d. of 2.8 Å. The sequence identity between TFIIS and the domain II of CK2 β^{δ} is only 10%. The CK2 β^{δ} monomer consists of two domains that are packed closely together. Domain I (residues 5–104) is entirely α -helical (α_1 , α_2 , α_3 , α_4 and α_5). A prominent feature of this region is a structure that resembles the letter 'L' with helix α_4 (residues 66–87) forming the 40 Å stem and helix α_5 (residues 91–102) forming the base (Figure 2B). The arrangement of these two helices is rather unusual since α_4 and α_5 form a 95° angle and interact very little. In contrast, helices α_1 , α_2 and α_3 wrap around helix α_4 holding a protruding acidic loop (residues 55–66). The N-terminal region is packed against helix α_4 , making close contacts with the acidic loop and extending at the surface of the molecule.

Domain II (residues 105–161) exhibits a three-stranded antiparallel β -sheet in which a single Zn^{2+} is co-ordinated with tetrahedral geometry. As anticipated, four conserved cysteines (Cys109, Cys114, Cys137 and Cys140) are involved in a Zn^{2+} -binding motif. Helix α_6 (residues

163–170) makes contacts with both β 1 of the zinc finger motif and helix α 5. Residues 170–190 have been implicated in the binding of the catalytic subunit CK2 α . Indeed, the crystallized form (1–182) of the protein partially binds the catalytic subunit (25% of the binding capacity of full-length CK2 β). However, in the structure, residues 170–177 which are part of the α – β subunit interface extend away from the bulk of the protein. The C-terminus of CK2 β is variable in length, extending beyond Lys177 for 38 residues in the human and *Drosophila* protein and for >58 residues in *Caenorhabditis elegans* CK2 β .

The acidic binding groove

A representation of the molecular surface of CK2 β^{δ} is displayed in Figure 3. The view clearly shows that the two ends of the dimer fold as compact and very acidic regions. Although residues Asp60–Asn65 are not well defined in the electron density map, they were modelled using the O loop database (Jones *et al.*, 1991). Twenty of the total 28 glutamic and aspartic residues cluster in domain I. All acidic residues contribute to a surface area of >3600 Å², which represents >40% of the total surface area of the CK2 β^{δ} monomer. The most remarkable feature of this region is an extended acidic groove formed by helices α 1 and α 3, an acidic loop (residues 55–64) and the N-terminus of helix α 4. The groove is ~35 Å long, 7 Å wide and 4.5 Å deep. Hydrophobic residues (Trp9, Trp12, Phe13 and Leu16) delineate one side of the groove. Six glutamic and five aspartic residues surround the rest of the groove surface. The negatively charged residues forming the groove are Asp51 in helix α 3, Asp55, Glu57, Asp59, Glu60, Glu61, Glu63 and Asp64 in the acidic loop and Asp70, Glu73 and Glu77 in helix α 4. Since the acidic loop is not well defined in the electron density, this part of the groove must be very mobile and flexible. Binding of cations, aliphatic cationic components or positively charged proteins can alter the size and the shape of the groove. Interestingly, the segment (Arg47–Asp55) located in helix α 3 is described as a potential ‘destruction box’: a sequence present in mitotic cyclins that is required for their ubiquitin-mediated proteolysis in a cell cycle-specific manner (Allende and Allende, 1995). It is noteworthy that in the structure, Arg47, Gln48 and Asp55 are located at the surface of the protein (Figure 2B).

The acidic stretch (residues 51–70) was previously proposed to represent a region involved in the down-regulation of CK2 activity and in its stimulation by polybasic ligands (Filhol *et al.*, 1991; Boldyreff *et al.*, 1993a, 1994; Leroy *et al.*, 1997a,b). Site-directed mutagenesis of CK2 β has suggested that specific acidic residues in this region, with special reference to Asp55, Glu57, Glu60, Glu61 and Glu63, are clearly responsible for an intrinsic down-regulation of CK2 activity. This effect may occur through interactions with the highly conserved basic cluster (helix α c) located in the active site cleft of the CK2 catalytic subunit (Krehan *et al.*, 1996; Leroy *et al.*, 1997b; Niefind *et al.*, 1998). Indeed, in the CK2 β^{δ} structure, these five acidic residues form a loop at the surface of the protein suggesting a localized contact between CK2 β and basic peptide segments. Similarly, it has been suggested that the N-terminus of CK2 β , which includes two autophosphorylation sites (Ser2 and Ser3), should be exposed at the surface of the molecule to interact with

the catalytic cleft of the α subunit (Boldyreff *et al.*, 1993a). Therefore, it has been postulated that the autophosphorylation sites should be functionally related to and possibly physically associated with the acidic cluster responsible for the down-regulatory properties of CK2 β (Boldyreff *et al.*, 1994). Indeed, the structure suggests that the acidic stretch forms a protruding binding surface, clustering along the N-terminal sequence and extending together at the surface of one side of the protomer.

Modelling of polyamine–CK2 β contacts

Polyamines are aliphatic cationic components of all living cells; putrescine and spermidine are ubiquitous while spermine appears confined to nucleated eukaryotic cells (Cochet and Chambaz, 1983). Using a series of spermine analogues, we have previously investigated the structural requirements of the CK2 polyamine-binding site.

A photoaffinity labelling method was used to identify a spermine-binding site on the CK2 holoenzyme. From this study, a structural model was proposed in which two of the four positive charges of the spermine molecule are able to interact with both glutamic acid residues 73 and 77. The remaining free positive charges of spermine probably interact with one or two acidic residues located in the loop 51–64 (Leroy *et al.*, 1995). Since the loop is flexible and contains seven acidic residues, polyamines of different length can bind to the groove. Furthermore, a direct correlation between the binding of spermine and the activation of CK2 was strengthened by the observation that the binding of a spermine molecule to the β subunit induces a conformational change in the holoenzyme (Leroy *et al.*, 1997b). Therefore, we decided to model the interaction of a spermine molecule with the CK2 β molecule by manual docking. Among a series of potential fits and assuming that spermine binds to the acidic region of CK2 β , one fit had the best complementary and atomic interactions compatible with the affinity labelling data, involving Asp55, Glu73 and Glu77.

Zn²⁺ co-ordination and overall folding properties

The primary structure of the human CK2 β subunit contains six cysteines, but only Cys109, Cys114, Cys137 and Cys140 are invariant whereas Cys14 and Cys23 are not. The experimental electron density maps allowed the tracing of a polypeptide chain that positioned the invariant cysteines within co-ordination distance of a zinc ion. The structure illustrated in Figure 2 shows that the four conserved cysteine residues are indeed involved in a Zn²⁺-binding motif. The protein segment (Phe105–Pro146) on its own forms a zinc finger motif located in domain II. The zinc ion is tetrahedrally ligated to Cys109, Cys114, Cys137 and Cys140 at distances of ~2.3 Å. To date, several structures that contain one or more zinc finger motifs have been determined by X-ray crystallography or NMR. Thus the geometry of the interaction between Zn²⁺ and different co-ordinating residues is well characterized (Berg, 1990). There are several types of fingers, categorized by the nature and spacing of their Zn²⁺-chelating residues (MacKay *et al.*, 1998). In the CK2 β^{δ} structure, the invariant cysteines co-ordinate Zn²⁺ and the motif is made by a three-stranded β sheet (β 1, β 2 and β 3). However, unlike the classical zinc finger, no α helix is observed. As mentioned above, the motif (105–146) is

Fig. 3. Molecular surface of the CK2 β^{δ} dimer coloured according to electrostatic potential ranging from deep blue (+) to red (−). The view is along the 2-fold axis going into the concave surface of the crescent.

Fig. 4. Ribbon representations of the zinc finger domains of CK2 β^{δ} and of transcriptional elongation factor TFIIIS. The β strands are shown as pink arrows, loops as brown lines and zinc as a blue sphere.

remarkably and unexpectedly similar in topology to the previously characterized zinc finger (named the Zn $^{2+}$ ribbon) present in the transcriptional elongation factor TFIIIS (Qian *et al.*, 1993) and in the RNA polymerase II subunit 9 RPB9 (Wang *et al.*, 1998). The three β strands and the P loop that form the core of this Zn $^{2+}$ -binding motif of CK2 β show a strikingly similar topology to the structure depicted for TFIIIS (Figure 4). In CK2 β , the twisted loop (Gly123–Met132) extends on the protein surface and stacks with residues 155–161 leading to helix $\alpha 6$. Important interactions involve two conserved residues Asp126 and Asp155. Asp126 OD1 interacts with Thr161 O γ , and Asp126 OD2 with Thr161 NH. Asp155 is almost

Fig. 5. Ribbon representation of the dimer interface. The β strands are shown as pink arrows, loops as brown lines and zinc as a blue sphere. Amino acid side chains and the water molecule involved in the protein–protein interaction, and zinc ligands are represented in ball-and-stick in black, red and yellow, respectively.

buried and makes strong hydrogen bonds with three peptide nitrogens (OD2 with NH129; OD1 with NH156 and NH157). The Zn $^{2+}$ -binding site is well ordered and contains two non-canonical zinc finger loops (Cys109–Pro110–Arg111–Val112–Tyr113–Cys114 and Cys137–Pro138–Lys139–Cys140).

The knuckles of the Zn $^{2+}$ -binding site of CK2 β are similar to those described for TFIIIS. The β sheet surface of the CK2 β zinc finger motif is remarkable for its hydrophobicity, with 13 hydrophobic side chains defining the central surface. This feature clearly rules out the possibility that the CK2 β monomer plays a functional role on its own. The zinc finger motif makes many hydrophobic interactions with domain II and the zinc finger motif of the other monomer (see below). On the contrary, the zinc finger motif of TFIIIS is a highly soluble nucleic acid-binding domain (235–280) of the transcriptional elongation factor. In CK2 β , the charges of the side chains of Glu115, Asp105, Lys147, Arg111, Asp142 and Lys134 are scattered at the edge of the zinc finger motif. The concentration of conserved residues in the zinc finger motif (residues 105–146) is high: 14 out of 42 positions are absolutely conserved and the remaining 28 positions are highly conserved. Differences primarily cluster at the C-terminus of the loop (124–132). A similar overall comparison can be made with the zinc finger motif of RBP9.

The dimer structure

Unlike the full-length protein, the purified core fragment of CK2 β lacking its C-terminal region does not aggregate. However, a size exclusion chromatography analysis showed that the protein is a dimer in solution (results not shown). The two different crystal forms obtained contain two monomers in the asymmetric unit. In each crystal form, criteria such as number of contacts and extent of

interaction between monomers allowed selection of the same dimer, which can be assumed to reflect the native association. Therefore, we assume that the self-association of the β subunit in a stable dimeric structure should reflect a physiologically important interaction and the crystal structure is in accord with the native dimeric state of CK2 β^{δ} in solution. Figure 5 illustrates the characteristics of the dimer interface. Two CK2 β^{δ} monomers pack against each other generating a crescent-shaped dimer (Figure 2B).

In the dimer, the Zn^{2+} -binding motifs interact significantly with one another forming the protein–protein interface. The molecular dimer interface is mostly hydrophobic since 65% of the residues in this region are non-polar. Several conserved hydrophobic residues make van der Waals interactions that form several contacts between the two monomers. In this core region of the interface, the most significant contacts are Pro110, Tyr144 and Lys147 of one monomer, which make hydrophobic contacts with their counterpart residues on the other monomer. Other hydrophobic interactions can be found away from the 2-fold axis, such as ValA112 with ValB143 and LeuB124. There are also hydrogen bonds between main chain atoms: Pro110 O...Thr145 N and Val143 O...Val112 N. Two important water molecules are involved in the protein–protein interface. One is on the 2-fold axis and makes hydrogen bonds with the OH of Tyr144 of both monomers. The other one interacts with ValB143 O, TyrA113 N and AspB142 OD1 (Table III). About 16 water molecules ($B \leq 40 \text{ \AA}^2$) are located in the groove of the crescent and contribute to the stabilization of the dimer.

Most of the interactions between two CK2 β^{δ} monomers are contacts between residues that are localized at the edge of the Cys109–X4–Cys114 element and on the β 3 strand. These two regions of the zinc finger motif form a continuous interface that buries a total area of $540 \text{ \AA}^2$ for each monomer and defines the interface between two CK2 β^{δ} molecules. Approximately 5.6% of the overall surface of a monomer contributes to the dimer interface. In a systematic comparison of the structure of oligomeric proteins, Janin *et al.* (1988) reported that interface areas in small dimers range from $670 \text{ \AA}^2$ in superoxide dismutase (M_r 15 710 Da) to $3470 \text{ \AA}^2$ in phosphorylase a (M_r 95 410 Da). This analysis shows no simple correlation between the molecular weight of the monomer and the interface area. Nevertheless, the value of $540 \text{ \AA}^2$ observed for CK2 β^{δ} is clearly small, even less than values found in several protease–protease inhibitor complexes (Hirono *et al.*, 1984) or lysozyme–antibody complex (Amit *et al.*, 1986). In these complexes, interface areas are of the order of $700 \text{ \AA}^2$. However, on average, one hydrogen bond per $200 \text{ \AA}^2$ is found at a dimer interface (Janin *et al.*, 1988). Since eight hydrogen bonds are observed in the present structure, the loss in energy of interaction between monomers may be compensated for by five extra hydrogen bonds. The implication of the zinc finger motif in the dimerization of CK2 β was confirmed further by the observation that a mutant form of CK2 β in which Cys109 and Cys114 were replaced by serine residues behaved as a monomeric protein (results not shown), indicating that the Zn^{2+} -binding motif is critical for dimerization. Although there is no evidence for a direct involvement of SH groups in the regulation of CK2 activity, the structure and the monomeric form of the Cys/Ser mutants clearly

Table III. Hydrogen bonds found in the interface^a

Type of interactions	Residues	Residues
Between main chain atoms	Pro110 O Val143 O	Thr145 N Val112 N
Water-mediated	Wat 69	Val143 O Tyr113 N Asp142 OD1
Salt bridges	Wat 145 Arg111 NE Arg111 NH2	Tyr144 OH Asp142 OD2 Asp142 OD2

^aAll distances are between 2.8 and 3.1 Å.

reveal that the self-association of the β subunit relies on the integrity of the zinc finger motif.

It is estimated that perhaps 1% of all mammalian genes encode zinc finger proteins. They were characterized initially as DNA-binding motifs in transcription factors, but recent reports suggest that they may mediate homodimerization or interaction with other proteins (MacKay *et al.*, 1998). Thus CK2 β joins the expanding subset of self-associating zinc finger proteins and its structure provides the first picture of a zinc finger-mediated protein dimerization.

Sequence conservation

Eleven sequences of CK2 β are available in databases (<http://wit.mcs.anl.gov/wit2>). For clarity, only six of them are shown in Figure 6. They are the most divergent compared with the human sequence (44–77% identity). Alignments of CK2 β protein sequences reveal a high degree of homology among these proteins and clearly suggest that their three-dimensional structures will be similar to the human protein (Figure 6). A related sequence from *D.melanogaster* has 39% identity with the human CK2 β and corresponds to the stellate protein, which has an unknown function and interestingly also lacks the C-terminal domain. In the CK2 β sequences, 58 residues are identical in all species. These residues (coloured in red in Figures 6 and 7) form patches that wrap around the entire molecule, including the dimerization interface.

Some of these conserved residues are buried in the structure, indicating that they most probably play a role in determining the structural framework of CK2 β : this is particularly clear for residues forming the C-terminus of helix α 4. For example, His84 is completely buried in the monomer and makes two strong hydrogen bonds with the carbonyl oxygen of Tyr158 and the peptide bond nitrogen of Cys23. However, some other conserved residues are exposed and form notable surface clusters: the first one, defined by Asp32, Phe34, Asn35 and Arg86 (Figure 7, patch 1), sits on the back of the acidic groove projecting away from the rest of the protein. This cluster is connected to a second group of conserved residues, which forms a helical groove (or ridge) rolling around the surface of the CK2 β^{δ} dimer. The main residues contributing to this ridge (Figure 7, ridge 1) are: Glu24, Ile10, Cys23, Lys100, Phe22, Phe21, Glu20, Asp105, Gly107, Ser148, Pro146, Lys147, Pro110 and Tyr144 of each monomer. The side chains of nine of these residues are accessible to solvent and therefore can be envisaged to accommodate multiple protein ligands previously identified as CK2 β binding partners.

Fig. 6. Sequence alignment of proteins related to CK2 β . The first line represents secondary structure assignment according to the program DSSP. The following lines are sequences for: *Homo sapiens*, *D.melanogaster*, *C.elegans*, *A.thaliana*, *Schizosaccharomyces pombe*, *S.cerevisiae* and the stellate protein from *D.melanogaster*. Residues which are conserved in the first six sequences are marked in red and in all sequences are boxed in red. The acidic loop (55–66) and the zinc finger motif (105–146) are underlined with horizontal stripes in red and yellow, respectively. Cysteine ligands of the zinc are in green. The figure was generated by using ESPript available at the Web site: www.ipbs.fr/ESPrpt/

Another invariant region located between the $\beta 1$ and $\beta 2$ sheets of the zinc finger motif (Gly123–Ile127) is not involved in the formation of the interface between two CK2 β^{δ} monomers. The highly conserved and solvent-accessible residues in this region extend onto the molecular surface of the CK2 β^{δ} dimer generating potential sites of interaction with other proteins. This is corroborated by the observation that native CK2 β is recognized specifically in this region by a monoclonal antibody (Nastainczyk *et al.*, 1995).

Regions of considerable divergence can also be seen on the sequence alignment (Figure 6); this includes the C-terminal segment that starts after helix $\alpha 6$ with Val170 and ends with Lys176. This segment, together with the C-terminal domain which is not present in the structure, shows very little sequence conservation between CK2 β molecules from different species. In the CK2 β sequences, two large insertions are observed. First, two sequences from *A.thaliana* (SP/P40228 and SP/P40229, 84% identity) have an 88 amino acid extension at the N-terminus. Secondly, in *Saccharomyces cerevisiae*, an insertion of 30 amino acids is found in domain I between the acidic loop (55–66) and helix $\alpha 4$ (Figure 6). Interestingly, the insertion has 11 acidic residues and four basic residues. The latter are clustered at the C-terminal part of the insertion. These features clearly support a model for the yeast CK2 β in which the crescent shape of the dimer and the acidic loop are conserved (Figure 2A). The main difference from the human CK2 β resides in the length of the crescent arm. Thus, these divergent segments tend to be found in areas that are distant from the core fragment described in the present structure.

Implications for CK2 β functions

Biochemical characterization of binding affinities for deletion constructs of CK2 β has identified the minimal parts necessary for stable complex formation with the catalytic

Fig. 7. The molecular surface of CK2 β^{δ} showing the solvent-exposed patches of conserved residues. The colour scheme for conserved residues matches that in Figure 6. The orientation is the same as in Figure 3.

α subunit. It has been suggested that residues from position 175 to 193 serve as an important interaction site for CK2 α (Boldyreff *et al.*, 1993b). This segment of CK2 β lies just at the C-terminus of our fragment, and is indeed exposed in the structure. Biochemical data and yeast two-hybrid studies have provided evidence that unlike the α subunits,

CK2 β subunits can interact directly (Gietz *et al.*, 1995). Assembly of tetrameric CK2 is probably an ordered process where the formation of a CK2 β dimer could represent the first step preceding the incorporation of the catalytic subunits. In addition, multiple interacting sites are predicted at the α - β interface (unpublished results). Since α - β heterodimers have never been observed, the CK2 β dimer is probably the only competent form for efficient α subunit binding. One possibility would be that upon dimerization, the CK2 β monomer undergoes conformational changes that are required for a correct α subunit binding. However, the structure of the CK2 β monomer shows a compact protein with no obvious linker region providing conformational flexibility. Alternatively, we propose that the CK2 β dimer could provide a rigid framework in which one catalytic subunit could span both CK2 β subunits in the dimer, generating intimate interactions and conformational changes in the catalytic subunit. Changes in the conformation of the CK2 catalytic subunit would be reminiscent of those seen in the activation of cyclin-dependent protein kinases (Jeffrey *et al.*, 1995).

When expressed alone, recombinant CK2 β^{δ} is a dimer in solution both in low and high salt buffer, suggesting that hydrophobic interactions may play a central role in the dimerization. Indeed the structure highlights the implication of specific hydrophobic segments of the zinc finger motif in the tight interaction between two CK2 β^{δ} molecules. It is noteworthy that only the Cys-X4-Cys element and the β 3 sheet are involved in the formation of the dimeric interface and, therefore, some of the remaining segments of the zinc finger motif are accessible for interactions with other molecules (Figure 5). The structure of the CK2 β^{δ} dimer also reveals, around its outer surface, a set of grooves that may provide multiple interaction sites for the variety of observed protein partners. The highly acidic nature of domain I creates a continuous negatively charged surface on one side of the CK2 β^{δ} dimer (Figure 3). This view is highly suggestive of a long-range electrostatic interaction between this protein and a positively charged host protein. In domain I, the size of the acidic groove suggests that it may serve as a docking site for stretches of basic polypeptides from a partner protein. Interestingly, it has been observed that the isolated region of CK2 β^{δ} extending from residue Asp51 to Pro110 exhibited a full binding activity for polyamines, reflecting a functional folding of this region of the protein (Leroy *et al.*, 1997a). Indeed, it was proposed previously that this domain might interact with and down-regulate the CK2 catalytic subunit. In addition to the tight interaction between the C-terminal segment (175–193) of CK2 β and the α catalytic subunit, the complex may be stabilized by electrostatic interactions between the positively charged region located on the α c helix of the catalytic subunit (residues 69–81) (Niefind *et al.*, 1998) and the highly negative stretch of the β subunit (Leroy *et al.*, 1997b). The resulting closed conformation would be responsible for the steric occlusion of the catalytic site and the down-regulation effect of the β subunit. The binding of polybasic ligands to this domain would release this intra-steric inhibition making the catalytic cleft accessible to large protein substrates and resulting in an open fully active conformation of the kinase. This makes it conceivable that proteins with suitable high-affinity basic motifs might,

upon binding to the acidic groove of CK2 β , overcome the down-regulation of the CK2 activity by this subunit. Interestingly, several proteins known to interact with CK2 β do so through a segment of CK2 β containing the acidic groove. For instance, the growth suppressor protein p53 was shown to interact with an internal region of CK2 β between residues 72 and 149 (Appel *et al.*, 1995) and the interaction was competed away by polyamines, reflecting a possible involvement of the acidic groove in the interaction (Filhol *et al.*, 1992). Similarly, it was observed that the cell cycle inhibitor p21^{WAF1} competes with p53 for the binding to CK2 β , suggesting that the two proteins may bind to a common or overlapping domain of CK2 β (Gotz *et al.*, 1996). Interestingly, p21^{WAF1} harbours a polybasic stretch in its C-terminus that may bind to the acidic groove of CK2 β . Fibroblast growth factor-2 (bFGF) was shown to bind to the regulatory subunit of CK2 and to activate its catalytic activity (Bonnet *et al.*, 1996). Similarly, polyamines compete with bFGF for binding to CK2 β , suggesting that the binding site for bFGF lies in the acidic groove (unpublished observations). In addition, the acidic domain of CK2 β may also bind to specific membrane proteins since it was shown to mediate the association of the CK2 holoenzyme with plasma membrane (Sarrouilhe *et al.*, 1998). Thus the acidic domain may represent a common docking site for several proteins.

In contrast, the binding site of topoisomerase II on CK2 β (Bojanowski *et al.*, 1993; Leroy *et al.*, 1999b) does not involve the acidic domain. The invariant region between the β 1 and β 2 strands that is not involved in the dimerization interface may represent a potential domain to interact with topoisomerase II.

Conclusions

CK2 β appears to be a unique protein with a new fold and containing a large amount of highly conserved residues that most likely represent the results of a functionally restricted evolution. The CK2 β^{δ} structure reported here provides the first information about this protein which is endowed with a common function of regulating different serine/threonine protein kinases. The structure gives a first glimpse of the molecular architecture of the dimeric core of CK2 β , which contains major determinants suspected of serving regulatory functions. As anticipated, the CK2 β monomer contains a zinc finger motif and the structure reveals, for the first time, how this motif can mediate the highly stable homodimerization of a protein. Another attractive structural feature of the CK2 β dimer is the presence of two acidic grooves at both ends of the molecule generating a highly anisotropic electrostatic potential around the dimeric protein. The structure of the acidic groove suggests a potential role as a docking domain for polyamines or CK2 β partners. Increasing evidence indicates that CK2 holoenzyme formation should involve extensive interactions between several regions of the CK2 catalytic and regulatory subunits. Validation of this point urgently awaits the structural analysis of an appropriate α 2 β 2 tetramer.

Materials and methods

Native and selenomethionyl protein production

Full details of the expression and purification of CK2 β^{δ} have been described previously (Chantalat *et al.*, 1999). Briefly, human CK2 β^{δ}

(residues 1–182) was expressed as a folded fusion protein in *E. coli* by the pMalc2 expression vector (New England Biolabs). Purification of the MBP–CK2 β^S protein fusion was achieved by chromatography on an amylose column. The fusion protein was cut with the protease factor Xa and the CK2 β^S separated from the MBP by chromatography on heparin–Sephacrose. The purified protein was concentrated to 20–28 mg/ml using a centrprep concentrator (Amicon Co.) and stored in aliquots at -80°C .

Seleno-L-methionine-labelled protein was purified similarly from *E. coli* B834 (DE3) cells, grown in defined medium prepared as reported before (LeMaster and Richards, 1985). A 300 ml pre-culture was used to inoculate 2 l of medium additionally supplemented with 25 mg/l DL-seleno-methionine. The cells were grown at 37°C to an $\text{OD}_{600} = 0.5$, and protein expression was induced for 2 h by addition of 0.1 mM isopropyl- β -D-thiogalactopyranoside (IPTG). All purification steps for this material were done in the presence of 10 mM β -mercaptoethanol and 1 mM EGTA to prevent oxidation of the selenomethionine. High-resolution electrospray ionization mass spectrometry was consistent with a polypeptide of the expected sequence (calculated mass for eight methionine residues = 21 354 Da, observed mass 21 356 Da) and confirmed a very high selenium incorporation in the selenomethionine-substituted protein (data not shown).

Crystallization

Crystallization of the Se-met CK2 β^S subunit was first tried under conditions similar to those already reported (Chantalat *et al.*, 1999). Under these conditions, small and twinned crystals were obtained. To grow larger crystals, it was necessary to increase the MgCl_2 concentration from 0.4 to 1 M. The main effect of the MgCl_2 was to increase the solubility of the protein. Under those conditions, it was possible to produce high quality crystals that diffracted to higher resolution than the native ones.

Data collection and processing

The crystals were soaked for a few minutes in a cryo-protecting solution containing polyethylene glycol monomethyl ether 5 kDa, 1 M MgCl_2 , 100 mM Bicine pH 9 and 20% glycerol. A single crystal was then mounted on a loop transferred on the goniometer head and kept at 100 K in a nitrogen stream. The MAD data were collected on the selenomethionyl-substituted crystals at three wavelengths around the K absorption edge of selenium (beamline BM14, ESRF-Grenoble, France). A 345 mm MAR image plate detector was used. The bipyramidal crystal could be oriented readily with the 4-fold axis aligned along the spindle axis. This allowed collection of Friedel mates very close in time. For each data set, 45° were collected starting with the *ac* plane perpendicular to the X-ray beam. For the high resolution data set at the remote wavelength, the crystal was misaligned by $\sim 15^\circ$ and another 45° was collected. All data (Table I) were integrated and reduced with the DENZO/SCALEPACK programs (Otwinowski and Minor, 1997). Data to 2.5 Å resolution at the three wavelengths were input into SOLVE (Terwilliger and Berendzen, 1997) to locate the selenium atoms. The program provided the positions for 15 out of the 16 atoms expected in the asymmetric unit. The remaining selenium atom, corresponding to the last methionine at position 169, was assumed to be disordered. The final phases from SOLVE resulted in a figure of merit of 0.77 up to 2.5 Å.

Structure determination and refinement

To improve the experimental phases further, the scaled and reduced intensity data were converted to amplitudes using TRUNCATE (CCP No. 4, 1994), and cross-wavelength scaling was performed using SCALEIT (CCP N°4, 1994), treating λ_{edge} as native data (Table I). The anomalous data were treated as a special case of multiple isomorphous replacement (Ramakrishnan and Biou, 1997). Phase calculation and heavy atom refinement with MLPHARE (CCP No. 4, 1994) gave a final figure of merit of 0.61 up to 2.0 Å (Table I). Owing to the high quality of the MAD map to 2 Å, it was decided to try the newly released version of wARP (Perrakis *et al.*, 1997) to build the model automatically. With this procedure, it was possible to assign 70 and 50% of the main and side chain atoms, respectively, into the density without any manual intervention. The program could have built most of the final model if it had not been misled by the zinc finger domain, which makes a closed piece of electron density caused by the links between the zinc ion and the four cysteines. The rest of the model was built in the MAD map using O (Jones *et al.*, 1991). The refinement was done with CNS (Brünger *et al.*, 1998) using the λ_{remote} data set to 1.74 Å, with the maximum likelihood target function. The program was set up to compute automatically a cross-validated σ_a estimate and the weighting scheme between the X-ray refinement target and the geometric energy function.

Corrections for a flat bulk solvent and for anisotropy in the data were also applied. The σ_a weighted maps obtained from the subsequent refined models were used for further model building. The MAD phases were modified and extended to 1.74 Å with SOLOMON (CCP No. 4, 1994) and the resulting map was used to check the refinement process. The first waters were added conservatively to peaks of $2F_o - F_c$ density $>2\sigma$ and that were making at least one hydrogen bond with a protein atom or another water molecule. In the final stages, the sigma cut-off was decreased to 1σ and water molecules with a B -factor $>60 \text{ Å}^2$ were removed. The final refined model at 1.74 Å resolution has a crystallographic R_{work} of 19.3% and an R_{free} of 21.8% and consists of 169 and 166 residues for molecules A and B, respectively, 321 water molecules, two zinc ions and one magnesium ion (Table II). As the electron density for residues 1–6, 60–65 and 180–182 of monomer A and 1–5, 59–66 and 177–182 of molecule B was very weak or not visible, they were not included in the model. All non-glycine residues are in the 'most favoured' or in 'additionally allowed' ϕ/ψ regions of the Ramachandran plot according to PROCHECK (Laskowski *et al.*, 1993) classification (Table II). Also, the overall stereochemistry yields higher scores in all categories when compared with structures refined at similar resolution.

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