Cooperative Modulation of Protein Kinase CK2 by Separate Domains of Its Regulatory β-Subunit†

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ABSTRACT: Protein kinase CK2 ("casein kinase 2") holoenzyme is composed of two catalytic (α and/or α′) and two regulatory β-subunits. A truncated form of the β-subunit lacking its C-terminal region (βΔ171−215) has lost the ability to stably associate with the catalytic subunits and to display a number of properties which are mediated by structural elements still present in its sequence, notably down-regulation of catalytic activity, autophosphorylation, and responsiveness to polycationic effectors. All these functions are restored by simultaneous addition of a synthetic peptide reproducing the deleted fragment, β170−215, which is able to associate with the catalytic subunits and to stimulate catalytic activity. This peptide includes a segment displaying significant sequence similarity with a region of cyclin A which interacts with the PSTAIRE motif of CDK2 eliciting its catalytic activity. A peptide reproducing this sequence (β181−203), but not its derivative in which three nonpolar side chains have been replaced by polar ones, interacts with the α-subunit and stimulates its catalytic activity; it also partially restores the ability of truncated βΔ171−215 to autophosphorylate. These data disclose the essential role of a structural module located between residues 181 and 203 in conferring regulatory properties to the β-subunit of CK2.

The acronym CK2[1] has been adopted to indicate a ubiquitous and essential Ser/Thr protein kinase previously termed "casein kinase 2" after its ability to phosphorylate casein in vitro, although it has no functional link with casein at all. CK2 is one of the most pleiotropic protein kinases, with possibly more than 200 protein substrates known to date *(1, 2)*, many of which have been shown to undergo phosphorylation in vivo at the same sites affected by CK2 in vitro. In all these proteins, CK2 recognizes phosphoacceptor sites that are specified by clusters of acidic residues, the one at position *n*+3 relative to the target amino acid playing an especially crucial role *(3)*. Pleiotropicity of CK2 is further amplified by its ability to use two instead of just one phosphate donor (i.e., GTP, besides ATP) and to phosphorylate, under special circumstances, tyrosyl residues as well *(4, 5)* despite belonging to the subfamily of Ser/Thr-specific protein kinases.

There is mounting evidence that CK2 is implicated in cancer *(2, 6, 7)* and in infection by viruses *(2)* and parasites *(8)*. It is generally believed that such a pathogenic potential of CK2 reflects its lack of any form of tight regulation. The molecular features conferring constitutive activity to the isolated catalytic subunit have been revealed by the crystal structure of maize CK2α (70% identical to its human counterpart), showing extensive contacts between the N-terminal segment and the activation loop *(9)*. These unique interactions displace the activation segment into an open conformation, compatible with constitutive activity, as also confirmed by mutational studies *(10)*. This is not the case for cyclin-dependent protein kinases (CDKs) belonging to the same phylogenetic branch of CK2 *(11)* where the opposite orientation of the activation loop prevents the access to the catalytic cleft unless the activatory cyclin molecule binds to the catalytic subunit and, by doing that, it displaces the activation loop toward the "open" conformation *(12)*. The role of the functional analogue of cyclin, the β-subunit of CK2 which associates with the catalytic (α and/or α′) subunits to give stable heterotetrameric holoenzyme, is more enigmatic, first because it binds to catalytic subunits which are already active per se, second because the binding is not reversible, and third because the functional consequences of β-subunit binding are elusive and to some extent contradictory. On one hand, in fact the β-subunit protects the holoenzyme against thermal inactivation and increases catalytic activity measurable with peptide substrates; on the other hand, it displays an intrinsic down-regulation, which is especially evident with some protein substrates (notably with calmodulin) and which is reversed by polybasic effectors, e.g., histones and protamine *(13)*.

Another feature of the β-subunit is its tendency to form homodimers which then associate with the monomeric catalytic subunits to give rise to the heterotetrameric holoenzyme. Mutational analysis *(13−15)* in conjunction with

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[1] Abbreviations: CK2, protein kinase CK2; CDK, cyclin-dependent kinase; Fmoc, 9-fluorenylmethyloxycarbonyl; NMP, *N*-methylpyrrolidone; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; RP-HPLC, reversed-phase high-performance liquid chromatography; MALDI, matrix-assisted laser desorption ionization.

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usage of synthetic fragments of the β-subunit *(16)* and solution of the crystal structure of a truncated form of the β-subunit lacking its C-terminal region *(17)* have provided substantial information about the localization of its functional domains (see Figure 1). While a central region spanning residues 105−146 forms a zinc finger which is essential for homodimerization *(17)*, a pseudosubstrate acidic stretch in the N-terminal part of the molecule, possibly in association with the autophosphorylation site (Ser2/Ser3), is mostly responsible for down-regulation *(13, 14, 18)*. This acidic region is also the target of polycationic effectors *(13, 19)*. In contrast, high-affinity binding to the α-subunit, up-regulation of catalytic activity, and protection against denaturation are mediated by structural elements present in the C-terminal domain. Consequently, a truncated form of CK2β, lacking the C-terminal domain ($\beta\Delta$171−215), is still able to dimerize but is defective in regulatory properties *(20)* as well as in autophosphorylation *(21)*. Here we show that these functions can be restored by complementing $\beta\Delta$171−215 with a peptide spanning the deleted segment, 170−215, and we localize within this segment a key element with structural similarity to the region of cyclin A interacting with the PSTAIRE motif of CDK2, which plays a crucial role in the activation of cyclin-dependent kinases.

MATERIALS AND METHODS

Expression and Purification of Recombinant Subunits. Expression and purification of human recombinant subunits α, β, and $\beta\Delta$171−215 of protein kinase CK2α and of Zea mays α were performed as described in detail previously *(22)*. Murine CK2α′ was cloned as previously described *(7)*, introduced into pGEX (Pharmacia) and expressed as in (*22*). For CK2α′ purification, 2 g of bacteria pellet was resuspended in 20 mL of buffer A (PBS: 0.14 M NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.3, and 7 mM 2-mercaptoethanol) and sonicated (6 × 20 s) in ice. The bacteria extract was centrifuged for 10 min at 80000*g* and then loaded on 2 mL of glutathione Sepharose 4B previously equilibrated with 20 mL of buffer A. The supernatants were continuously loaded for 2 h, and then the column was washed with 50 mL of buffer A. Cleavage of fusion protein bound to column matrix was performed by incubating 2 mL of buffer A containing 20 cleavage units at 4 °C for 16 h. Cleavage protein was eluted with 15 mL of buffer A, and removal of thrombin from the sample was by a gel filtration chromatography step (Superdex 75 HiLoad 26/60, Pharmacia). The column was eluted with buffer A + 0.5 M NaCl, and the fractions were analyzed by 12% SDS−PAGE. The fractions containing α′ were pooled and stored at −20 °C.

Synthetic Peptides. Peptides β155−215 and β170−215 and the shorter derivatives β170−192 and β206−215 were synthesized as previously described *(16)*. Peptide reproducing the central helix of calmodulin (CaM 68−92) was prepared as described in *(23)*. The peptides AcNQFVPRLYGFKIHPMAYQLQLQA, AcNQFVPRLYGFKAHPMAAQAQLQA, AcNQFVPRLYGFKDHPMAAQDQLQA, and FVPRLYGFKIHPMAYQLQ were synthesized by using a peptide synthesizer (model 431-A, Applied Biosystems) and Fmoc strategy *(24)*. Chain assembly of peptides was performed on 0.1 mmol of Wang resin (0.96 mmol/g) using HBTU activation chemistry according to the manufacturer's protocol. The following amino acid side-chain protection was used: Fmoc-Arg(Pmc)OH, FmocAsn(Trt)OH, FmocGln(Trt)OH, FmocTyr(tBut)OH, FmocLys(Boc)OH, FmocHis(Trt)OH.

Acetylation of N-terminal amine was performed manually by treating the peptide-resin with 10% acetic anhydride in NMP for 10 min. After cleavage, peptides were purified to homogeneity by RP-HPLC with a 0−60% gradient of acetonitrile in aqueous trifluoroacetic acid and characterized by MALDI mass spectrometry.

Phosphorylation Assay. Phosphorylation experiements were performed at 37 °C for 10 min in a medium (25 μL final volume) containing 50 mM Tris-HCl, pH 7.5, 12 mM $MgCl_2$, 100 mM NaCl. Concentrations of CK2 and of the phosphorylatable substrates are detailed in figures and tables. After 10 min preincubation in ice, to allow association of the α-subunit with the β-subunit or its fragments, the reaction was started by addition of 20 μM [γ-^{32}P]ATP with a specific radioactivity of 500−1000 cpm/pmol and was stopped by cooling in ice. The evaluation of phosphate incorporated was routinally done by subjecting samples to PAGE−SDS according to Laemmli *(25)*, followed by staining with Coomassie Brilliant Blue and either autoradiography or direct scanning on an Instant Imager (Canberra Packard). With synthetic peptide as phosphoacceptor substrate, the phosphocellulose paper procedure *(26)* was adopted.

Surface Plasmon Resonance (SRP) Analysis. The molecular interactions of β-peptides reproducing regions of β-subunit with CK2α were analyzed with a Biacore X system exploiting surface plasmon resonance (SRP) phenomena *(27)*. CK2α immobilization was performed as previously described *(28)*. Injection of 35 μL β-peptide solutions (100 nM−100 μM) over the surface was performed with a flow rate of 10 μL/min in running buffer HBS (10 mM Hepes, pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v surfactant P20), and after injection, running buffer replaced the analytes. Surface was regenerated by injection of 10 mM NaOH (10 μL, flow rate of 10 μL/min). The SRP response is expressed as resonance units (RU). The association and the following dissociation reactions were detected in real time considering the resonance units. Each sensorgram (time course of the SRP signal) was subtracted from the response obtained from the surface devoid of immobilized protein.

RESULTS

A schematic representation of CK2 β-subunit and of its fragments, either recombinant or synthetic, used in the present study, is provided in Figure 1. C-Terminal truncation of the β-subunit of protein kinase CK2 gives rise to a deleted mutant, $\beta\Delta$171−215, which, in the presence of physiological salt concentration, has lost its ability to stably associate with the α-subunit as judged by ultracentrifugation analysis *(14)*. This mutant still includes the homodimerization domain *(17)*, and consequently it exists as a dimer, as judged from gel filtration experiments (not shown). However, it is defective in most of the other functions of the β-subunit, not only those which reside in the deleted region, namely, up-regulation of catalytic activity and protection against thermal denaturation, but even those which are intrinsic to its 1−170 sequence, notably inhibition of calmodulin phosphorylation (which reflects the down-regulatory potential of the N-terminal domain), autophosphorylation, and responsiveness to poly-

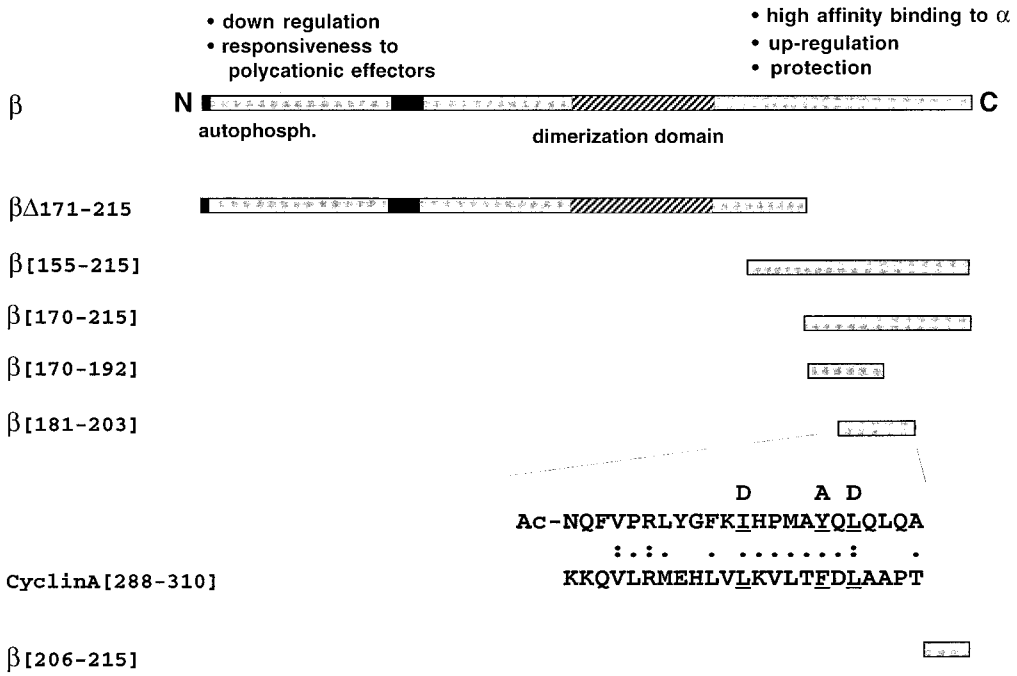

FIGURE 1: Schematic representation of CK2 β-subunit and of its recombinant and synthetic fragments utilized in this work. Functional domains are indicated. Alignment of CK2 β181−203 sequence with cyclin A domain (288−310) shows identical (:) and conserved (.) amino acids. The residues of cyclin A crucial for interaction with the PSTAIRE region of CDK2 *(12)* and their homologues in the β181−203 peptide are underlined. A derivative of β181−203 in which these residues were replaced by Asp, Ala, and Asp, respectively, was also utilized (β181−203DAD).

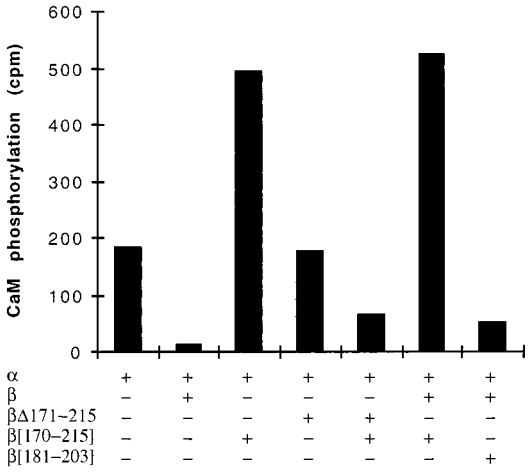

FIGURE 2: Phosphorylation of calmodulin by CK2α: variable effect of fragments of the β-subunit. Calmodulin (5 μM) was phosphorylated under the conditions described under Materials and Methods by CK2 α-subunit (5 pmol) either alone or previously mixed (and stored 10 min in ice) with equimolar amounts of full-length or truncated ($\beta\Delta$171−215) β-subunit. C-Terminal synthetic fragments were added, when indicated, in 50-fold molar excess with respect to α-subunit. The data are the mean of 4 independent determinations with a standard error not exceeding ±15%.

basic effectors. To check whether and to what extent such a loss of functions could be complemented by the deleted domain, the peptide encompassing residues 170−215 has been assayed for its ability to restore the properties which are lost in $\beta\Delta$171−215.

As shown in Figure 2 and consistent with previous studies *(29)*, the peptide β170−215 on its own stimulates, instead of inhibiting, calmodulin phosphorylation by CK2α; however, if added together with truncated $\beta\Delta$171−215 (which by itself is ineffective), it promotes *inhibition* of calmodulin phosphorylation, almost as efficiently as full size β-subunit.

Table 1: Effect of Polylysine (PL) on the Phosphorylation of Calmodulin by Variably Assembled CK2

phosphorylating enzyme	phosphate incorporated (%)		stimulation (*x*-fold)
	−PL	+PL	
α	100	104	1.04
α + β	22	2,176	98.90
α + β[170−215]	268	255	0.95
α + $\beta\Delta$171−215	95	97	1.02
α + $\beta\Delta$171−215 + β[170−215]	35	549	15.68

[a] Phosphorylation conditions and evaluation of phosphate incorporated into calmodulin were performed as described under Materials and Methods. [Polylysine] was 336 nM. Values are obtained by quantitation of the radioactivity incorporated into the calmodulin band on the autoradiogram. The data relative to calmodulin phosphorylation by CK2α alone (=100) are the mean of values obtained in triplicate with an SD not exceeding 15%.

The concept that simultaneous addition of the two complementary fragments of β, 1−170 and 170−215, mimicks the effects of full size β is corroborated by experiments with polylysyine, which is known to overcome the negative effect of β-subunit and to trigger calmodulin phosphorylation by CK2 holoenzyme *(30)*: as shown in Table 1 the stimulatory effect of polylysine, albeit much less pronounced, is also evident if the reconstituted $\alpha_2\beta_2$ holoenzyme is replaced by a mixture of α, $\beta\Delta$171−215, and β-[170−215] in 1:1:50 molar ratio.

Another prominent feature of the β-subunit, which, in the presence of salts, is almost entirely lost upon C-terminal truncation, is autophosphorylation at its N-terminal residues, Ser-2 and Ser-3 *(31)*: this normally takes place very rapidly through an intramolecular mechanism which is counteracted by polylysine *(32)*. In contrast, "trans" phosphorylation of

Table 2: Synopsis of the Functional Properties of Full-Length β-Subunit, of Its Truncated Mutant $\beta\Delta171-215$ ($\beta\Delta C$), and of in Vitro Reconstituted Complex with the Complementary Peptide $\beta170-215$[a]

	β homodimerization	α protection	stimulation of peptide phosphorylation	inhibition of CaM phosphorylation	β Ser2/Ser3 autophosphorylation	responsiveness to polylysine
β	+++	+++	+++	+++	+++	+++
$\beta\Delta C$	+++	−	−	−	−+	−
$\beta170-215$	−	++	++	−	−	−
$\beta\Delta C + \beta170-215$	+++	+++	+++	++	+++	+

[a] Drawn from data presented here and elsewhere *(34)*.

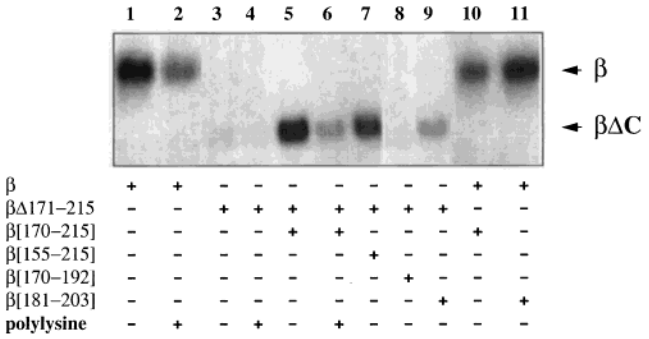

FIGURE 3: Phosphorylation of full size and truncated β-subunit by CK2α. Effect of β fragments. Phosphorylation conditions are described under Materials and Methods. CK2 α-subunit (5 pmol) was previously mixed and stored for 10 min in ice with equimolar amounts of full-length β or of its truncated $\beta\Delta171-215$ derivative ($\beta\Delta C$) either in the absence or in the presence of 50-fold molar excess of the indicated synthetic peptides. Polylysine, where present, was 336 nM. The positions of β and of $\beta\Delta171-215$ ($\beta\Delta C$) are indicated. The autoradiography by PAGE−SDS is shown.

the same residues, when the β-subunit is not assembled into the holoenzyme, takes place slowly and is stimulated by polylysine *(21)*. As shown in Figure 3 phosphorylation of $\beta\Delta171-215$ is negligible as compared to full size β (compare lanes 3 and 1); it is restored to normal level, however, if the peptide 170−215 is also added (compare lanes 5 and 1). The phosphorylation occurring under these conditions moreover is inhibited, instead of being enhanced, by polylysine (compare lanes 6 and 5), whereas the modest phosphorylation of $\beta\Delta171-215$ alone by CK2 holoenzyme is increased by it (not shown).

Interestingly, a β-peptide longer than $\beta170-215$, encompassing residues 155−215, is less effective in restoring the regular autophosphorylation of the truncated mutant $\beta\Delta171-215$ (see Figure 3, lane 7) as well as in promoting inhibition of calmodulin phosphorylation (not shown). A possible explanation would be that the peptide $\beta155-215$ destabilizes the regular binding of $\beta\Delta171-215$ to the α-subunit through a competitive mechanism, mediated by its part of sequence (155−170) that overlaps that of truncated β. This point of view is corroborated by the finding that the peptide 170−215, which restores the functional properties of truncated $\beta\Delta171-215$, counteracts the same properties when they are displayed by full size β-subunit. This is shown in Figure 2 and Figure 3 as far as inhibition of calmodulin phosphorylation and autophosphorylation are concerned, respectively.

A synopsis of the data above, shown in Table 2, highlights the ability of the peptide $\beta170-215$ to restore a number of functions which are inherent to in the N-terminal moiety of β but nevertheless are either missing or severely impaired in the deletion mutant $\beta\Delta171-215$. It can be concluded from these data that the C-terminal domain of β not only plays an essential role in up-regulation and stabilization of α, but also renders the catalytic subunit more amenable to modulation by the rest of the β-subunit. Apparently this applies to both the α- and α′-subunits since the most relevant experiments run with α (Figure 2, Table 1 and Figure 3) were also repeated with α′, providing similar results (not shown).

In an attempt to map within the 170−215 sequence the structural elements responsible for α up-regulation, three peptides variably encompassing this sequence, 170−192, 181−203, and 206−215 (see Figure 1), have been assayed for their ability to stimulate calmodulin phosphorylation. As shown in Figure 4A, left panel, the peptide 181−203 is as effective as the whole C-terminal fragment, 170−215, as a stimulator of calmodulin phosphorylation when added in 10-fold molar excess (compare lanes 3 and 5). In contrast, the peptide 170−192 (lane 4) is almost ineffective, and the peptide 206−215 (lane 6) has no detectable effect. Surprisingly, a slightly shortened derivative of the peptide $\beta181-203$, encompassing residues 183−200, is significantly more stimulatory than $\beta181-203$ itself (compare lanes 7 and 5). Two other catalytic subunits of CK2, human α′ and Zea mays α, displaying about 70% identity with human CK2α, but both lacking its C-terminal region, are also susceptible to stimulation by $\beta181-203$ when calmodulin is the phosphoacceptor substrate (see Figure 4A, right panel).

Similar results were obtained if CK2α activity was monitored using a synthetic peptide substrate (reproducing the central helix of calmodulin), whose phosphorylation is increased, instead of being inhibited, by full size β-subunit. As shown in Figure 4B, a stimulatory effect, comparable to that of β full size, is displayed by the C-terminal fragments 170−215, 181−203, and 183−200, when added to CK2α with a molar ratio of just 2:1, comparable to that of full-length β. In contrast, the β-peptides encompassing the sequences 170−192 and 206−215 proved ineffective, and the truncated mutant of β with the $\Delta171-215$ deletion displayed a slight but reproducible inhibition.

Interestingly, the β segment reproduced by peptide $\beta181-203$ displays significant sequence similarity with a region of cyclin A which interacts with and activates CDK2 *(12)* (see Figure 1). In particular, three cyclin A nonpolar residues which play a prominent role in making hydrophobic contacts with the PSTAIRE motif of CDK2 are also present in the β-subunit. If these β residues, I-192, Y197, and L199, were replaced by Asp, Ala, and Asp, respectively, the resulting derivative of peptide $\beta181-203$ ($\beta181-203$DAD) has lost the ability to physically interact with the α-subunit as judged by plasmon resonance (BIAcore) analysis (Figure 5). The substituted peptide is also unable to stimulate the catalytic activity of CK2α toward calmodulin (Figure 4A, compare lanes 5 and 6) and the calmodulin peptide (Figure 4B).

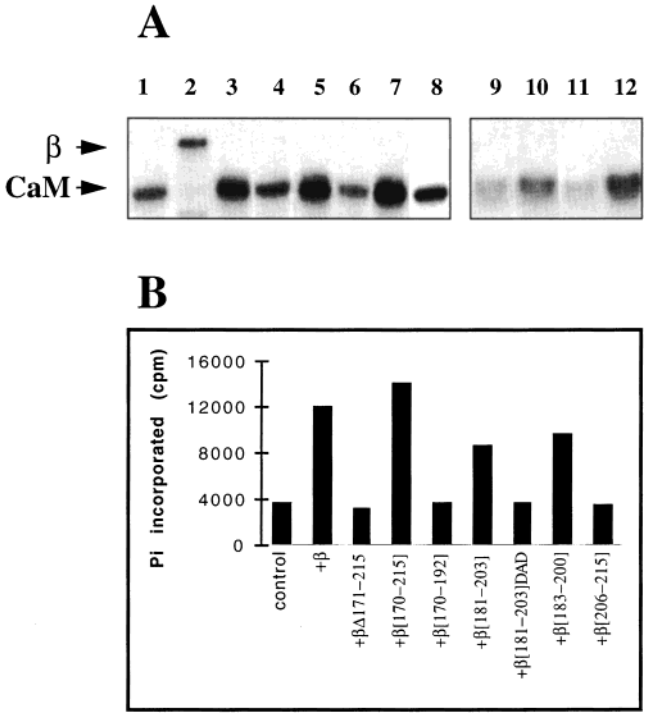

FIGURE 4: Effect of C-terminal fragments of the β-subunit on the CK2α-mediated phosphorylation of calmodulin (A) and of a peptide reproducing the central helix (68−92) of calmodulin (B). Phosphorylation conditions and evaluation of phosphate incorporated are described under Materials and Methods. (A) Calmodulin (5 μM) was phosphorylated by 5 pmol of CK2α either alone (lane 1) or in the presence of an equimolar amount of full-length β (lane 2) or a 10-fold excess of β170−215 (lane 3), β170−192 (lane 4), β181−203 (lane 5), β181−203DAD (lane 6), β183−200 (lane 7), and β206−215 (lane 8) preincubated with α for 10 min in ice prior to being added to the assay. Parallel experiments were performed with recombinant α′ (lanes 9 and 10) and α from Zea mays (lanes 11 and 12) in the absence (lanes 9, 11) and in the presence (lanes 10, 12) of β181−203. The autoradiogram of PAGE−SDS is shown. The arrows indicate the positions of calmodulin and β-subunit. (B) The synthetic peptide CaM68−92 (20 μM) was phosphorylated as in (A) in the absence and in the presence of a 2-fold molar excess of β-derived fragments, as indicated.

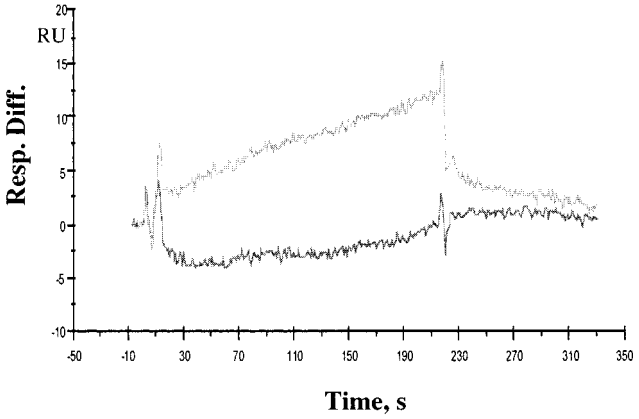

FIGURE 5: Plasmon resonance analysis of the interaction of peptides β181−203 (upper trace) and β181−203DAD (lower trace) with CK2 α-subunit. The occurrence of interaction was measured with a SPR BIAcoreX sensor system. 35 μL of 100 μM β peptides was spread over the sensor chip containing 5100 RU of immobilized CK2α at a flow rate of 10 μL/min. From the sensorgrams shown, the response of the control sensor surface (without CK2α) was subtracted. Resp. Diff.: response difference. RU: resonance units. Further details are given under Results and Materials and Methods.

DISCUSSION

Two outcomes of this study shed new light on the elusive mechanism by which CK2 undergoes regulation by its noncatalytic β-subunit. First, our data clearly show that the C-terminal region of the β-subunit, from residues 170 to the end, already known to be required for up-regulation of CK2 activity and protection against thermal denaturation *(14, 29)*, is also essential for the displaying of functions which are mediated by the N-terminal moiety of the molecule. Second, we have mapped within the 183−200 sequence a structural element which accounts for the binding and stimulatory properties of the C-terminal β domain, and displays sequence similarity to a region of cyclin A which plays an important role in the activation of cyclin-dependent protein kinases.

The concept that the C-terminal domain of β is also required to mediate functional effects of elements residing in the N-terminal region stems from a number of experiments where a synthetic peptide encompassing the 170−215 sequence proved able to complement the loss of function of a truncated form of β, $\beta\Delta$171−215, which is defective in several properties inherent to its N-terminal moiety. Especially remarkable in this respect is the ability of β170−215 to restore inhibition of calmodulin phosphorylation: although this effect is mediated by an acidic region close to the N-terminus, it is almost entirely lost upon truncation of the C-terminal domain; we have shown here that by combining two fragments of β which by themselves are either ineffective on calmodulin phosphorylation by CK2α ($\beta\Delta$171−215) or actually *stimulatory* (β170−215), the paradoxical outcome is *inhibition* of calmodulin phosphorylation comparable to that observed with full size β. Another example of the cooperative effect between these two fragments of β is autophosphorylation, which takes place at serines 2 and 3 of full size β, being nevertheless dramatically impaired by its C-terminal truncation. Also in this case addition of the C-terminal fragment restores autophosphorylation to the normal level. To a lesser extent also responsiveness to polycationic stimulators is restored by the C-terminal fragment, while a number of β fragments tested individually or in combination proved unable to mediate up-regulation by polylysine *(33)*. Significant stimulation by polylysine takes place if the two complementary fragments of β, 1−170 (i.e., $\beta\Delta$171−215) and 170−215, are simultaneously added.

The second relevant outcome of this work is the identification, within the C-terminal domain, of a sequence of 20 amino acids or possibly even less which, on the one hand, is able to bind to α and to up-regulate its catalytic activity while, on the other hand, it displays intriguing similarity to that region of cyclin A which binds to and activates the catalytic subunit of cyclin-dependent protein kinases. That this parallelism may be not a mere coincidence but could reflect functional similarities between CK2 and CDKs is supported by the finding that the binding and the stimulatory properties of the peptide encompassing the 181−203 sequence are lost upon replacement of three nonpolar residues which are homologous to those which in cyclin A are mainly responsible for the hydrophobic interaction with the catalytic subunit of CDK2 *(12)*.

The crucial relevance of one of these residues, Ile-192, in making contacts with the α-subunits of CK2 has been recently corroborated by crystallographic analysis of a complex between maize CK2α (>70% identical to its human

counterpart) and the human β181−203 peptide utilized in this work (*35*). At variance with the equivalent residue of cyclin A, Leu-299 (see Figure 1), which interacts with the PSTAIRE motif of an individual catalytic subunit (*12*), Ile-192 is buried in a large hydrophobic pocket formed by juxtaposition of two α-subunits (*35*). All the residues of maize CK2α involved in this interaction are also conserved in human CK2α, suggesting that a similar complex is also formed in our case. This picture may account for some unique features of CK2β, whose regulatory role appears to be more faceted than that of cyclins and whose interaction with two catalytic subunits gives rise to a tetrameric holoenzyme composed of two α- and two β-subunits, whereas the active form of CDK is a dimer composed of one catalytic subunit and one cyclin molecule.

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