Binding of Polyamines to an Autonomous Domain of the Regulatory Subunit of Protein Kinase CK2 Induces a Conformational Change in the Holoenzyme

A PROPOSED ROLE FOR THE KINASE STIMULATION*

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The means by which the cell regulates protein kinase CK2 remain obscure. However, natural polyamines, cellular compounds required for cell proliferation, have been reported to strongly stimulate CK2-mediated phosphorylation of a number of substrates. Using spermine analogs, we have shown that polyamines directly interact with the CK2 β subunit, and the chemical features of the highly acidic binding site (Asp91-Tyr96) have been determined. In the present study, we show that the isolated β subunit region extending from residue Asp91 to Pro110 exhibits a specific and efficient polyamine binding activity similar to that of the entire β subunit. Moreover, the replacement of Glu89, Glu91, and Glu93 of the β subunit by 3 alanine residues leads to a loss of the spermine-induced stimulation of CK2 activity which correlates with a decrease in spermine binding affinity. Thermal stability studies indicate that the binding of spermine induces a 4 °C decrease of the Tm value for the holoenzyme. This was confirmed by circular dichroism analyses, which show that the 6 °C negative shift of the CK2 Tm value provoked by spermine binding, reflects a conformational change in the kinase. Together, these observations strongly suggest that this newly defined polyamine-binding domain is involved in the intrasteric regulation of CK2 activity.

Protein kinase CK2 (CK2)1 is a serine/threonine protein kinase present in the cell cytoplasm and nucleus of eukaryotic organisms from yeast to man (1, 2). The kinase is made of the association of three dissimilar subunits, i.e., the catalytic subunits α and α′ of 35–44 kDa (3) and the β subunit of 24–29 kDa, to generate native structures exhibiting the stoichiometry α2ββ′2, α′αβ′2, and αα′ββ′. The β subunit contains two autophosphorylation sites located at Ser9 and Ser3 and is termed the regulatory subunit because of its potential to stimulate the activity of the α subunit in the tetramer by 5–10-fold (4–6). Among the numerous substrates of CK2, a large number corresponds to transcription factors and oncoproteins such as Myc (7), Myb (2, 8), fos (9), and the anti-oncogene p53 (10). Other proteins localized in the cytoplasm or associated to membranes were also identified as CK2 substrates. The disruption of the genes encoding the CK2 catalytic subunits α and α′ led to a lethal phenotype in yeasts, underscoring CK2’s essential role in cell proliferation (11).

To date, no intracellular messenger has been characterized as a crucial regulator of CK2. However, in vitro experiments that show significant stimulation of the CK2 catalytic activity in the presence of naturally occurring polyamines (12), provides strong evidence that such regulation exists. The stimulation by polyamines is preferentially observed with selected substrates, such as casein or the transcription factor MyoD, and is strictly dependent on the presence of the β subunit (13) and on low magnesium concentrations (14). Further evidence supporting a role for polyamines as physiological CK2 stimulators includes the observation that extraphysiological magnesium concentrations (20–30 mM) are required for maximal CK2 catalytic activity. Indeed, most of the protein kinases, including the CK2 α subunit alone, are fully active in the presence of 4–5 times lower magnesium concentrations. It is thought that polyamines may act in synergy with this physiological magnesium concentration to confer a maximal activity to the tetrameric form of CK2. However, the precise molecular mechanism leading to the catalytic stimulation remains to be determined.

Polyamines are ubiquitous cellular components that are indispensable for cell proliferation and differentiation (15, 16). Polybasic compounds including natural polyamines have been shown to modulate the catalytic activities of a broad range of proteins such as insulin receptor, vitamin D receptor, GTPase activity of G proteins, protein phosphatase 2A, and DNA-topoisomerases I and II (17–22). Considering the crucial roles played in the cell by most of these polyamine-stimulated enzymes, the understanding of the mechanism by which polyamines exert their effects is urgently required.

We have previously described a partial characterization of the mechanism by which polyamines stimulate CK2 activity. It has been initially observed that the kinase binds different polyamines and that the binding activity is localized to the β subunit (13). Subsequently, application of a photoaffinity labeling strategy, with a photoactivated analog of spermine, led to the identification of the polyamine-binding site within a highly acidic stretch situated between amino acid residues Asp91 and Tyr80 of the CK2 β subunit (23). The characterization of the
polypeptide-binding site strongly suggested the involvement of four acidic amino acid residues in the interaction of the kinase with a spermine molecule. Furthermore, from the study of the interaction of several spermine analogs with the kinase, it has been hypothesized that the CK2 polypeptide-binding site could fold into a pocket shape.²

Overall, these in vitro studies point to the likelihood that the interaction of polypeptides with this polypeptide-binding site may induce conformational changes responsible for the stimulation of CK2 activity. A detailed analysis of this binding site is presented in this study, along with an evaluation of a potential model for a general mechanism of CK2 stimulation by polybasic ligands. In particular, we ask the following questions: what are the amino acid residues involved in the CK2 polypeptide-binding site? Does this binding site behave like an autonomous and functional polypeptide-binding domain when expressed alone? To what extent does the binding of polypeptides to this domain provoke conformational changes in the CK2 holoenzyme?

**EXPERIMENTAL PROCEDURES**

**Mutagenesis of the CK2 β Subunit**

The mutagenesis of the CK2 β subunit was performed according to the rapid for site-directed mutagenesis described in Ref. 24. The plasmid pSG5βHA, containing the cDNA of the chicken β subunit fused to the nucleic sequence coding for the HA epitope, was used as template for the PCR reactions performed with the following oligonucleotides: 1, TGGAAATCTGGAGCCCTCACTATGCTTTCGGAGAGGTGTCGGTCTGCTGCTGCAG; 2, ATGGATCCCTAGCTAAGCATAACATCTGAGAACTCTATGAGACAGCATGATCCTCAGGACGTATGAGTCTCAAGAGCACATGATCCTCAGGACGTATGAGTCTCAAGAGCACTGAGGATGCAGTG; M, CAGGCCGCGCTCAGCGGATGGTCTTCACCG; M, CAGGCCGCGCTCAGCGGATGGTCTTCACCG. The M oligonucleotide confers the replacement of the β subunit residues Glu60, Glu61, and Glu63 by 3-alanine residues. The mutated PCR product was digested by endonuclease BamHI and cloned in the corresponding site of the plasmid pSG5 poly linker. The resulting recombinant plasmid pSG5β3HA was used in transient transfections to express the mutated subunit βA60, A61, A63HA.

**Expression of the CK2 α and β Subunits in COS Cells**

COS 7 cells were cotransfected according to the DEAE-dextran method by the vectors pSG5α and pSG5βHA or pSG5α and pSG5β3HA (20 μg each) allowing the coexpression of the wild type α and βHA subunits or the coexpression of the wild type α and the mutated βA60, A61, A63HA subunits, respectively. Transfection of COS 7 cells by the vector pSG5 was performed with control. The transfected cells were grown in Dulbecco’s modified Eagles medium in 6 cm diameter dishes for 24 h before being added to the phosphorylation medium of the kinase assay at a final concentration of 4.8 nM. The kinase reaction was stopped by addition of 2 ml of 12.5% trichloroacetic acid. The following steps of the assay were performed as described in Ref. 25. An immunoprecipitation performed with the lysate of COS cells transfected with the vector pSG5 was also analyzed for CK2 activity and was used for blank values of the kinase assay.

The polyamine binding assay was achieved by the incubation at 4 °C for 5 min of the immunocomplexes with buffer B (10 mM Tris–HCl, pH 7.4) containing 0.4 μM [3H] spermine (0.5 μCi/assay) in the absence or presence of increasing spermine concentrations in a final volume of 80 μl. The final NaCl concentration was adjusted to 25 mM. The immunocomplexes were then washed three times with buffer B and the binding of [3H] spermine was analyzed by liquid scintillation counting. Polyamine binding assays performed on the immunoprecipitates prepared from cell lysates of COS cells transfected with the vector pSG5 were used for blank values.

**Genetic Construction, Expression, and Purification of Fusion Proteins**

The β subunit domain encompassing amino acid residues Asp31 to Pro110 and the entire β subunit were fused to the maltose-binding protein (26). The nucleotide sequences encoding the β subunit domain or the entire β subunit were amplified by PCR from the chicken β cDNA (a generous gift of Dr E. Nigg), respectively, with the following oligonucleotides: β domain, 5′-CGGATCCGTCGACATGATCCTCGACCTC-3′ and 5′-ATGGATCCATGACAGCTGGAGTCTCAAGAGCACATGATCCTCAGGACGTATGAGTCTCAAGAGCACTGAGGATGCAGTG; β domain, 5′-CAAGCTTCGACATGATCCTCGACCTC-3′ and 5′-ATGGATCCATGACAGCTGGAGTCTCAAGAGCACATGATCCTCAGGACGTATGAGTCTCAAGAGCACTGAGGATGCAGTG. The β subunit domain was amplified from the recombinant plasmid pSG5βHA with the oligonucleotides β 5′ and B 3′. The PCR amplifications led to fragments carrying a BamHI site located at their 5′ extremities and an in-frame stop codon following the HA epitope. Following digestion of the fragments by the corresponding endonucleases, PCR products were cloned in the poly linker of the pMal C2 vector (protein fusion and purification system, New England BioLabs). The resulting recombinant vectors were used to transform Escherichia coli strain BL21. The cultures were induced during 2 h with 0.3 mM isopropyl thigalactosidase. The cell pellets were resuspended in cold lysis buffer (10 mM sodium phosphate, 30 mM NaCl, 0.25% Tween 20, 10 mM EDTA, 10 mM EGTA, pH 7.0). After a thermal shock (~70 °C to +20 °C) and a 3 × 2 min sonication, the lysates were adjusted to 0.5 mM NaCl and subjected to centrifugation at 9,000 × g for 20 min. The supernatants were mixed with an auxose resin (New England BioLabs) at 4 °C during 1 h and the fusion proteins were eluted with 10 mM molar added to the column buffer (10 mM phosphate, 0.5 mM NaCl, 1 mM sodium azide, 1 mM EGTA, pH 7.0). The recombinant proteins MBPβ31–110, MBPβ1–215, and MBPβ3 were finally concentrated on a Centricon cell up to 2 mg/ml.

**Binding of Spermine**

Recombinant proteins (0.5–2 μg of CK2 or the fusion proteins MBPβ31–110, MBPβ1–215) were incubated at 4 °C for 5 min with 0.5 μM [3H] spermine (106 cpm) in the absence or presence of different concentrations of non radioactive polyamines in a final assay volume of 80 μl of assay buffer II (pH 7.4). The NaCl concentration was settled at 25 mM in the binding experiments. The mixtures were then rapidly centrifuged at 4 °C, according to Penefsky (27), through small Sephadex G-50 superfine column previously equilibrated in Tris buffer containing 1 mg/ml BSA. Bound [3H] spermine was determined by radioactivity counting of the eluted volume, after subtraction of the blank volume obtained in the absence of recombinant protein. Blank values represent less than 0.2% of the input radioactivity.

**Analysis of the Thermal Denaturation of CK2**

**CK2 Activity Assay**—CK2 was incubated for 10 min in the absence or presence of 500 μM spermine or 20 mM MgCl₂ at different temperatures ranging from 30 to 65 °C in a test tube heater (Stuart Scientific) before being added to the phosphorylation medium of the kinase assay at a final concentration of 4.8 μg/ml. The CK2 activity assay was performed as described (6) by monitoring the phosphorylated peptide was determined by liquid scintillation counting.

**Polyamine Binding Assay**—CK2 was incubated for 10 min at different temperatures ranging from 30 to 70 °C in a test tube heater (Stuart Scientific) before being added to the medium of the polyamine binding assay at a final concentration of 71 μg/ml. The assay was performed as described above and the amount of [3H] spermine bound to CK2 was determined by liquid scintillation counting.

**Circular Dichroism Analyses**—CK2 (2.14 μM) was incubated at different temperatures for 5 min in TDBG buffer (10 mM Tris-HCl, pH 7.4, 1 mM dithiothreitol, 2% glycerol) in the absence or presence of 500 μM spermine in a final volume of 100 μl. CD analyses were performed on a CD6 dichrograph (Jobin Yvon, Lyon, France). The temperature of the quartz cell was maintained by a water bath monitored through a Spec.
and reached a plateau at 250 μm spermine. The resulting stimulation factor calculated as the ratio between the CK2 activities measured in the absence or presence of 500 μm spermine, was 4 times weaker for the mutated kinase.

When the immunoprecipitates of both wild type and mutated CK2 were incubated with tritiated spermine, the total binding of polyamine was 2.3 times lower with the mutated CK2, as compared with the wild type enzyme (data not shown). Scatchard plot analyses showed that both CK2 forms exhibit two binding systems (Fig. 1, C and D). Calculation of the affinity constants for the highest affinity system gave a $K_d$ of 1.4 μm for the wild type CK2 (Fig. 1C) and 4.3 μm for the mutated kinase (Fig. 1D). For both forms of CK2, the stoichiometries of bound spermine were equivalent. Thus, the 3-fold lower affinity of the mutated CK2 for spermine strongly suggests the involvement of at least one of the three mutated glutamic acids in the CK2 polyamine-binding site.

We have already observed the apparent divergence between the concentration of spermine required to stimulate kinase activity by 50% and the calculated $K_d$ values for the binding of spermine to CK2 (28). Such feature was attributed to nonspecific interactions between polyamines and casein in the activity assay (29).

**Determination of an Autonomous and Functional Polyamine-binding Domain in the CK2 β Subunit**—Previous experiments using a photoactivable spermine analog have shown the labeling of two β subunit peptides at amino acid residues Thr$^{72}$ and His$^{108}$ (23). In addition, the mutational analysis described above suggests the involvement of at least one of the three glutamic acid residues 60, 61, and 63, located in the polyacidic region of the CK2 β subunit, in polyamine binding. Together, these observations define a domain lying from Asp$^{51}$, the first amino acid residue of the β polyacidic region, to Pro$^{110}$, the first secondary structure breaker following His$^{108}$. The corresponding sequence was fused to the maltose-binding protein and the overexpressed fusion protein MBP β51–110 was purified in one step on amylose resin to roughly 90% purity. The entire β subunit was fused to MBP and was purified similarly (Fig. 2).

Spermine binding analyses were performed using the fusion proteins MBP β51–110, MBP β3–215, and MBPΔlacZ as control. No spermine binding activity could be detected with the fusion protein MBPΔlacZ (data not shown). In contrast, a spermine binding activity was observed for the MBP β51–110 and MBP β3–215 fusion proteins and half-maximal binding values were, respectively, 1.7 and 1.0 μm. The data analyzed by Scatchard plot showed the presence of one single binding system exhibiting affinity constants of 0.5 and 0.6 μm for the fusion proteins MBP β51–110 and MBP β3–215, respectively (Fig. 2, A and B). From the same plots, it was calculated that 0.8 and 0.6 mol of spermine were bound per mol of fusion proteins MBP β51–110 and MBP β3–215, respectively. The calculated binding affinities fit with the values obtained in the experiments described in Fig. 1 using recombinant wild type CK2.

The ligand binding specificities of both fusion proteins were analyzed and compared with those of the recombinant wild type CK2. Recombinant CK2 or the fusion proteins were incubated with [3H]spermine in the presence of spermine, spermidine, putrescine, polylysine (10 μm), and magnesium (10 mM). Spermine was found to be the strongest competitor for the interaction, since the presence of 10 μm spermine decreases the bound [3H]spermine by 60 and 80%, for CK2 and the fusion proteins MBP β51–110 and MBP β3–215, respectively (Fig. 3). A 30–50% loss of spermine binding was detected for all three proteins in the presence of spermidine. Putrescine was found to be the weakest competitor decreasing the interaction of spermine by only 10–20%. Polylysine is much less efficient at com-
peting with spermine for binding full-length CK2 (20% displacement) while on both fusion proteins polylysine provokes a 60–80% displacement. Finally, magnesium was shown to antagonize the binding of spermine by 40–70% on all three proteins. Thus, we conclude that the fusion proteins MBPβ1–110, MBPβ1–215, and the oligomeric CK2 exhibit comparable ligand binding specificities.

Effect of Spermine on the Thermal Stability of CK2—The thermal stability of CK2 was analyzed by the measurement of CK2 activity after preincubation of the enzyme at different temperatures in the absence or presence of 500 μM spermine or 20 mM magnesium. As shown on Fig. 4A, a maximal activity of CK2 alone was determined for preincubation temperatures ranging between 30 and 45 °C. A striking decrease of CK2 activity was detected after preincubation of the enzyme at temperatures between 45 and 55 °C, and the catalytic activity was completely lost at temperatures above 55 °C. Half-maximal activity was obtained at 48 °C. In the presence of 500 μM spermine or 20 mM magnesium, a maximal CK2 activity was detected for preincubation temperatures ranging between 30 and 40 °C. This activity strongly decreased between 40 and 50 °C. Preincubation temperatures higher than 50 °C led to a total loss of CK2 activity. Under these conditions, half-maximal activities were obtained at 44 °C. A small variation in ionic strength of the buffer (equivalent to >50 mM NaCl) was observed after inclusion of 20 mM MgCl₂. However, this variation could not account for the change in the thermal stability of the kinase. Similarly, resistivity determination indicated that the spermine-induced change in the thermal stability of CK2 could not be attributed to variation in the ionic strength of the medium. Therefore, the data provide strong evidence for a specific role of spermine in the change of CK2 thermed stability.

The spermine binding activity of CK2 preincubated at different temperatures in the absence or presence of 500 μM spermine or 20 mM magnesium. As shown on Fig. 4B, a maximal activity of CK2 alone was determined for preincubation temperatures ranging between 30 and 45 °C. A striking decrease of CK2 activity was detected after preincubation of the enzyme at temperatures between 45 and 55 °C, and the catalytic activity was completely lost at temperatures above 55 °C. Half-maximal activity was obtained at 48 °C. In the presence of 500 μM spermine or 20 mM magnesium, a maximal CK2 activity was detected for preincubation temperatures ranging between 30 and 40 °C. This activity strongly decreased between 40 and 50 °C. Preincubation temperatures higher than 50 °C led to a total loss of CK2 activity. Under these conditions, half-maximal activities were obtained at 44 °C. A small variation in ionic strength of the buffer (equivalent to >50 mM NaCl) was observed after inclusion of 20 mM MgCl₂. However, this variation could not account for the change in the thermal stability of the kinase. Similarly, resistivity determination indicated that the spermine-induced change in the thermal stability of CK2 could not be attributed to variation in the ionic strength of the medium. Therefore, the data provide strong evidence for a specific role of spermine in the change of CK2 thermed stability.

The spermine binding activity of CK2 preincubated at different temperatures was also measured as described under “Experimental Procedures.” A maximal binding of spermine (10.0–13.0 pmol) was obtained for CK2 preincubated between 30 and 45 °C (Fig. 4B). A strong decrease of the spermine binding efficiency was observed between 45 and 55 °C and a residual binding activity (15%) was observed after preincubation of the
enzyme at temperatures above 55 °C. Half-maximal binding of spermine was reached at 48 °C.

Changes in CK2 Conformation upon Spermine Binding—To further investigate the effects of spermine on CK2 conformation, a spectroscopic method was used to reliably monitor the structural modifications that accompany the binding of this polyamine to the CK2 holoenzyme. Far UV circular dichroism spectra were recorded for CK2 at different temperatures in the absence or presence of 500 μM spermine. Fig. 5A shows primary and secondary maxima of the ellipticity at 209 and 223 nm, respectively. A loss of ellipticity was observed as temperatures increased from 20 to 90 °C. This observation is in agreement with a modulation of the CK2 conformation from a well defined structural state into a random conformation. Addition of 500 μM spermine led to a decrease of ellipticity as compared with the corresponding conditions in the absence of spermine. Changes in the spectrum were observed primarily in the ellipticity at 209 nm and secondary at 221 nm (Fig. 5B).

To compare the thermal stability of CK2 in the absence and presence of 500 μM spermine, the maximal ellipticities measured at 209 nm were represented as a function of the temperature (Fig. 6). Both sigmoidal curves were characterized by a maximal ellipticity corresponding to the CK2 folded state and a minimal ellipticity, which was attributed to the random conformation of the denatured CK2. Half-denaturation constants of 42 and 48 °C were determined for CK2 incubated in the presence and absence of spermine, respectively. Thus the binding of spermine induced a significant difference (6 °C) in the thermal stability of CK2, which may reflect a fundamental change in the conformation of the enzyme.

Thermal stability of wild type CK2 and spermine binding-deficient mutant (βAla60,Ala61,Ala63) was also compared at different temperatures (Fig. 7). A maximal CK2 activity was
obtained for CK2 preincubated between 30 and 40 °C. A decrease of CK2 activity was detected after preincubation of the enzyme at temperatures between 40 and 55 °C, and the catalytic activity was completely lost at temperatures above 55 °C. Half-maximal activity was obtained at 47 °C. In contrast, a sharp and constant decrease of the catalytic activity of the mutant kinase was observed for temperatures above 30 °C. Under these conditions, half-maximal activities were obtained at 41 °C. Thus the mutant kinase exhibits a much lower thermal stability and the data provide additional evidence for the implication of the acidic region of the β subunit in the polyamine-induced change of CK2 conformation.

**DISCUSSION**

The biochemical analyses, previously used to demonstrate an interaction of CK2 with polyamines, were expanded in this work to investigate the mechanism by which these ligands stimulate the kinase. Particular attention was paid to the delineation of a polyamine-binding domain and to the detection of conformational changes induced upon binding of polyamines to this domain. We have shown that site-directed mutagenesis of the three glutamic acid residues (Glu60, Glu61, and Glu63) in the acidic stretch of the CK2 β subunit lead to a decrease in the spermine binding affinity of the free β subunit. These results are in fair agreement with the observations of Boldyreff et al. (30, 31) who showed an hyperactivated form of a CK2 holoenzyme containing mutations of acidic residues 55–57 and 59–64 of the β subunit. In our study, the effect of spermine on the CK2 activity was analyzed at physiological magnesium concentration (1 mM). Under these conditions, the basal activity of the CK2 was found 4 times higher than the activity of the wild type enzyme. Consequently, spermine is able to stimulate the basal activity of the wild type enzyme by 8-fold, while the mutated kinase was stimulated only 2-fold. This difference correlates with the lowered spermine binding affinity observed for the mutant CK2. Interestingly, for spermine concentrations above 50 μM, the kinase activity of the wild type or mutated CK2 were similar. These data support the notion that mutations of specific residues in the acidic region of the CK2 β subunit eliminate a catalytic autoinhibition which is responsible, in physiological conditions, for the low basal activity of the wild type CK2. Thus, this effect would represent an intrasteric regulation of CK2 involving its β subunit.

Previous observations have shown that a photoactivatable spermine analog interacts with specific residues in a highly acidic stretch lying between amino acid residues 51 and 80. These data together with the observation that mutations of acidic residues in this region affect both the polyamine binding affinity and the polyamine-induced stimulation of the enzyme point to the likelihood that this region represents an important CK2 regulatory domain. This contention is supported by the experiments showing that a fusion protein involving the β subunit region Asp51 to Pro110 exhibits an efficient and specific interaction of CK2 with polyamines. A possible interpretation for this difference would be that the conformation of the β subunit changes when it is associated with the catalytic subunit. The second interpretation which is not exclusive of the first, is that the higher polyamine binding affinity of the free β subunit is due to the absence of interactions between its spermine-binding domain and the α subunit catalytic site (see below). Thus the polyamine-binding domain of the CK2 β subunit is still functional when it is isolated from the full-length protein, a situa-
tion already described for the well known SH2, SH3, and PH domains. For instance, SH2 and SH3 domains isolated from the proteins phosphatidylinositol 3'-kinase-associated protein p85 and phosphatidylinositol 3'-kinase exhibit full binding efficiencies for tyrosine-phosphorylated peptides and proline-rich peptides, respectively (32, 33). Similarly, pleckstrin homology (PH) domains isolated from the proteins phospholipase C-81 and pleckstrin were shown to specifically bind inositol phosphates (34).

In a series of experiments designed to test the possibility that the binding of polyamines could induce changes in the CK2 conformation, it was observed that the thermal stability of the enzyme was affected by the presence of polyamines and magnesium. A difference of 4 °C in the \( T_m \) values was observed for the holoenzyme incubated in the absence or presence of spermine or magnesium. This is large enough to correspond to a loss of CK2 stability upon spermine or magnesium binding. By analogy, a shift of 7 °C was recorded for the \( T_m \) of the T4 lysozyme containing an engineered disulfide bond as compared with the \( T_m \) of the wild type T4 lysozyme (35, 36). In this study, the difference in thermal stability was interpreted as reflecting a change in the conformation of the mutated protein, providing a protection against a conformationally-related irreversible inactivation.

The effect of spermine on the thermal stability of CK2 was also readily monitored over a wide range of temperatures by using far UV circular dichroism analyses. Raising the temperature induces a decrease in the ellipticity at 209 and 221 nm. Upon spermine binding a striking change in the ellipticity recorded at 209 nm was observed and a difference of 6 °C in the half-denaturation constants for CK2 incubated in the absence or presence of spermine was determined. Again this difference, which fits with the change in the \( T_m \) values obtained in the study of the thermal inactivation of CK2 activity, is a strong indication that the observed loss of CK2 stability reflects a spermine-induced change in the enzyme conformation. The modifications in the circular dichroism spectra observed in the presence of spermine may arise from a decrease of the helical feature of the polyamine-binding domain complexed with spermine.

Based on enzymatic and spectroscopic data, we propose a possible mechanism for the regulation of CK2 activity by polyamines. The CK2 holoenzyme is drawn as a globular sphere representing the association of the \( \alpha \) catalytic subunit with the \( \beta \) regulatory subunit (Fig. 8). In addition to the C-terminal domain, which is involved in the tight interaction between the two subunits (30), the complex is stabilized by four electrostatic interactions between the positively charged region of the \( \alpha \) catalytic site and the highly negative stretch of the polyamine-binding domain of the \( \beta \) subunit. The resulting closed conformation is maintained in the presence of 1 mM magnesium and is responsible for the steric obstruction of the catalytic site and the limited access of protein substrates. Addition of spermine or increasing the magnesium concentration leads to the release of the polyamine-binding domain, resulting in an open conformation. The efficiency of the substrate binding to the catalytic pocket is consequently enhanced and the overall kinase activity is stimulated. This model takes into account the thermal denaturation studies, which show that, in the absence of spermine or high magnesium concentrations, the enzyme exists in a stable form (corresponding to the closed conformation), whereas, upon spermine or magnesium binding, the kinase adopts a less stable form possibly reflecting the open conformation.

This model is also supported by the experiments performed on a CK2 holoenzyme containing specific mutations in the polyamine-binding domain. The prediction is that a decrease in the number of electrostatic interactions between the \( \beta \) subunit polyamine-binding domain and the \( \alpha \) subunit catalytic site would lead to a weaker interaction resulting in a more efficient conversion to the open conformation, and an increased catalytic activity at low magnesium concentrations. As a result, the mutant was found to have a lowered thermal stability and a difference of 5 °C in the half-denaturation constants was observed for the wild type and mutant kinase. The polyamine-induced change in the CK2 conformation is also supported by our previous observations that both the maximal velocity of the catalytic reaction and the affinity of the kinase for its substrate increase in the presence of spermine (37). According to our model, the accessibility of the catalytic site, which depends greatly on the kinase conformation, should be also influenced by the size of the protein substrate. This is in keeping with our observations that the optimal phosphorylation of a substrate such as casein by either the free catalytic subunit or the CK2 holoenzyme requires very different magnesium concentrations (7 and 20 mM, respectively). In contrast, an optimal phosphorylation of the small peptide substrate RRRDDDSEEE by both forms of CK2 is observed at the same magnesium concentration (7 mM). Moreover, the phosphorylation of this peptide substrate

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**FIG. 8. Schematic representation of the mechanism proposed for the stimulation of CK2 activity by polyamines.** The catalytic site of the CK2 \( \alpha \) subunit is flanked by positive charges and is partially occupied by the polyamine-binding domain of the CK2 \( \beta \) subunit. Upon spermine binding, the catalytic site which becomes free to receive a protein substrate.
by the CK2 holoenzyme was found to be totally insensitive to polyamines at physiological magnesium concentrations.\footnote{D. Leroy and C. Cochet, unpublished results.}

In summary, CK2 appears to be the target of an intrasteric regulation that involves an autoinhibition of its catalytic subunit by the β regulatory subunit. The driving force for this inhibition requires the acidic region of the β subunit, which is able to bind polyamines. The binding of polybasic ligands to this domain releases an intrasteric inhibition and allows access to the catalytic cleft to large protein substrates. These experimental results predict that the region of the subunit, which is catalytically active to bind polyamines. The binding of polybasic ligands to this domain releases an intrasteric inhibition and allows access to the catalytic cleft to large protein substrates. These experimental results predict that the region of the β subunit that blocks the catalytic site has a relatively large freedom of movement. In this respect, CK2 belongs to the growing list of modular protein kinases such as protein kinase C, myosin light chain kinase, and calmodulin-dependent protein kinase which catalytic sites are autoinhibited by regulatory domains (38–40). Whether the intrasteric regulation of CK2 is relevant to the enzyme in vivo remains to be explored.

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