DNA Inhibits the Catalytic Activity of the α Subunit of Protein Kinase CK2†

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ABSTRACT: The recombinant α subunit of protein kinase CK2 (casein kinase 2) from *Xenopus laevis* is inhibited by the addition of single stranded or double stranded DNA. This inhibition is competitive with the casein substrate, having an apparent Ki of 160 nM for an 86 bp DNA fragment. Assays with a fragment containing the putative promoter of the human CK2β gene indicated that the affinity of CK2 for this fragment was not greater than that of other unrelated DNA. The inhibitory capacity of DNA toward the protein phosphorylating activity of CK2α is greatly reduced by the presence of the β subunit which can completely reverse the inhibition. The interaction of CK2α with DNA can also be assayed by the nitrocellulose filter binding assay. This assay demonstrates that the interaction of CK2α with the tested DNAs is not sequence specific and that the β subunit can also greatly diminish the binding of CK2α to DNA. Casein at substrate concentrations also is inhibitory to CK2α DNA binding. Likewise, polyanionic inhibitors of the CK2 catalytic activity, such as heparin, poly(U), and copoly(Glu:Tyr) polypeptides, can compete for and inhibit the binding of DNA to CK2α. However, quercetin, which also inhibits CK2 phosphorylation activity, and ATP do not affect DNA binding. A mutant CK2α in which glutamic acids replace two lysine residues in positions 75 and 76 of the α peptide chain is less susceptible to DNA inhibition, indicating that this basic region of the molecule is involved in its interaction with DNA.

Protein kinase CK2 (also known as casein kinase 2) is a Ser/Thr protein kinase that has been found in all eukaryotic cells and that is located preponderantly in the nucleus although it is also present in the cytoplasm.

CK2 is responsible for the phosphorylation of many proteins that are involved in nucleic acid synthesis and in the control of cell proliferation such as RNA and DNA polymerases, DNA ligase and topoisomerases, the oncogenes myc, myb, and jun, and the tumor suppressor proteins Rb and p53 (Tuazon & Traugh, 1991; Pinna, 1990; Issinger, 1993). The mechanism of the physiological regulation of the activity of this enzyme is not known. Naturally occurring polyamines such as spermine and spermidine can activate CK2. Polylysine can also activate CK2, and with some substrates, such as calmodulin, the addition of this polycationic peptide is an absolute requirement for the phosphorylation to occur (Meggio et al., 1994).

It has been determined that CK2 phosphorylates acidic peptides that contain serine or threonine followed toward the carboxyl end by clusters of glutamic or aspartic acids or by phosphorylated amino acids (Meggio et al., 1984). Heparin is a very potent inhibitor of CK2, while other polyanions such as peptides rich in glutamic or aspartic acid (Marchiori et al., 1988; Téllez et al., 1990), polynucleotides (Gatica et al., 1989), or folylpolyglutamates (Téllez et al., 1992) are also inhibitory. Recent studies with mutated recombinant subunits have demonstrated that a highly basic region of the α subunit (amino acids 68−80) is involved in the interaction of the enzyme with polyanionic inhibitors (Hu & Rubin, 1990; Gatica et al., 1994).

Our laboratory has been studying the activity of recombinant CK2 from *Xenopus laevis*. The cDNA coding α and β subunits of this enzyme have been cloned, expressed in *Escherichia coli*, and reconstituted to form a fully active holoenzyme (Jedlicki et al., 1992; Hinrichs et al., 1993).

It has been reported that the α subunit of human CK2 can bind specifically to a promoter-type sequence upstream of the human gene coding for the CK2β subunit (Robitzky et al., 1993). This observation has encouraged us to look at the interaction of the recombinant *X. laevis* α subunit of CK2 with DNA and to study the effect of DNA on the catalytic activity of both the isolated α subunit and on the reconstituted holoenzyme. In this paper it is shown that the α subunit of CK2 can indeed interact with DNA and that this interaction inhibits its catalytic activity. The addition of CK2β, which itself does not interact with DNA, greatly decreases the inhibition caused by DNA. The inhibitory effect has been observed with both single and double stranded DNA of different sequences. However, no specific interaction with the putative promoter of the human CK2β gene was observed. Separate experiments measuring the binding of DNA to the α subunit by a nitrocellulose filter retention assay also demonstrate that CK2α binds to DNA and that the presence of β can drastically decrease this interaction.

MATERIALS AND METHODS

Preparation of the Recombinant X. laevis CK2 α and β. The α and β subunits of *X. laevis* CK2 coded by their cloned cDNAs were expressed in *E. coli* exactly as described previously (Hinrichs et al., 1993) using expression vector pT7-7 for CK2α and pGEX-2T for CK2β. The two protein subunits were also purified to apparent homogeneity as described in the same publication. The CK2α$E^{75}E^{76}$ mutant was prepared and expressed as described previously (Gatica et al., 1994).

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Assay of CK2 Phosphorylating Activity. The catalytic activity of CK2 reconstituted holoenzyme measured by the phosphorylation of β-casein was assayed as previously described (Gatica et al., 1989) using an incubation mixture of 30 μL containing 50 mM Hepes, pH 7.8, 135 mM KCl, 7 mM $MgCl_2$, 0.5 mM dithiothreitol, and 50 μM [α-^{32}P]-ATP (500−1000 cpm/pmol). The activity of subunit α was determined under the same conditions except that 100 mM KCl was used. The assays also contained CK2 subunit(s) as specified in each experiment and 0.5 mg/mL dephosphocasein. It should be noted that this level of casein substrate is subsaturating (Lin et al., 1991) and is employed in order to better appreciate the inhibitory effect of added DNA.

The reaction was started by the addition of the enzyme, and the incubation was carried out for 10 min at 30 °C. Aliquots were spotted on a 2 × 1 cm Whatman P81 phosphocellulose paper, which was then immersed in 75 mM phosphoric acid. The paper was washed 3 times in the same phosphoric acid solution, dried, and counted. Values reported have been corrected for controls run in the presence of heat-denatured enzyme. All assays were performed in duplicate and are representative of two to four experiments.

Nitrocellulose Retention Assay of DNA Binding. The binding of DNA by CK2α was assayed by the retention of ^{32}P-labeled DNA on 25 mm nitrocellulose filters (HA 0.45 μm pore). Incubations were carried out in 35 μL containing the following: assay buffer (40 mM Hepes, pH 7.8, 50 mM KCl, 5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM dithiothreitol), 1−4 pmol of recombinant purified CK2α, and [^{32}P]DNA (50 000−200 000 cpm) at the concentrations given in each experiment.

The reaction was started by the addition of the enzyme, followed by incubation for 5 min at 0 °C. The reaction was stopped by dilution with 1 mL of ice cold assay buffer and immediately filtered through Millipore nitrocellulose filters presoaked in assay buffer. The filters were washed two times with 1 mL of the same buffer, dried, and counted. When the effect of CK2β was studied, CK2α and -β were preincubated together for 1 h at 0 °C in order to allow interaction of these subunits prior to their addition to the standard incubation system.

Analyses were performed in duplicate, and corrections were made for radioactivity observed in control experiments where no CK2α was added, for each level of radioactive DNA used. Binding of [^{32}P]DNA in the absence of CK2α is usually 1−3% of the total input radioactivity. No DNA is retained in the presence of up to 36 pmol of β subunit in the absence of CK2α. CK2-dependent binding is sensitive to ionic strength, 60% less DNA being retained at 200 mM KCl as compared to 100 mM. Optimal $MgCl_2$ concentration range is 4−7 mM.

Preparation of DNA Fragments. The 568 bp DNA fragment corresponding to the putative promoter of the human CK2β gene (Robitzky et al., 1993) was prepared by PCR amplification of human genomic DNA, using as oligonucleotide primers:

upstream primer: 5′-GATCTGTCGGTTGGGGTCC-3′

downstream primer:
5′-GGATCTCTTTCCGCAGCTCTCC-3′

The resulting 568 bp fragment (referred to as P568) was purified through microcolumns WIZARD PCR PREPS DNA purification system of Promega. The radioactive promoter fragment was prepared by using [α-^{32}P]dATP in the PCR reaction. For verification of the published sequence of this fragment, it was cloned in the pGEX2T vector and the nucleotide sequence obtained by the dideoxy method (Sanger et al., 1977). The sequence obtained corresponded to the sequence published by Robitzky et al. (1993).

The two complementary strands of 86 bp oligonucleotide fragment (86-mer), which included a 70 bp nucleotide sequence of the human CK2β promoter protected from nuclease attack by human CK2α (Robitzky et al., 1993), were synthesized. The sequence of the coding strand (for human CK2β region) was 5′-ATAAGCTTTTCGCACTAGGGGC-CCAACAGGCAATAAGGACCCAGCGGATTG-GCCGAGGATAGGCCAGTCCCCTGGGCAAAGCTTAC-3′. Eight bases were added in each terminus to include restriction sites for *Hin*dIII for cloning. The two complementary strands were annealed, and the two stranded product was found to correspond to the correct size on agarose gels.

A fragment of 588 bp muscarinic receptor of *X. laevis* was prepared by PCR amplification of a *X. laevis* oocytes cDNA library (in a λgt10 vector, donated by D. Melton) using as follows:

upstream primer:
5′-CTGGCTCTGGACTATGTGGTG-3′

downstream primer:
5′-GTTGTGAGATAGGCTGGC-3′

This amplification yielded a 588 bp DNA fragment (R588) which corresponded to part of a cDNA coding for a muscarinic receptor of *X. laevis* which has been cloned and sequenced in our department (Herrera et al., in press).

A 435 bp fragment corresponding to DNA of *Thiobacillus ferrooxidans* was prepared by PCR amplification of *T. ferrooxidans* DNA using as oligonucleotide primers:

upstream primer:
5′-ATTTATGACTATGAAGCATCAGG-3′

downstream primer:
5′-GCGGCAATCTGGATCAGCTCC-3′

This fragment (T435) corresponds to the amino-terminal end of a gene coding for tyrosyl-tRNA synthetase which was cloned and sequenced by Salazar et al. (1994).

Reagents. Radioactive nucleotides were from ICN. Salmon sperm DNA, poly(U) (100 000 molecular weight average), poly(Glu:Tyr) 4:1 (46,000 molecular weight average), heparin (166 USP units/mg), dephosphorylated β-casein, quercetin, dithiothreitol, buffers, and salts were obtained from Sigma Chemical Co. Oligonucleotide primers were synthesized in an Applied Biosystems instrument by OLIGO PEPTIDO−Chile, the core facility of the University of Chile. Rabbit anti-CK2α antiserum was prepared by using a synthetic peptide designed on the basis of the *X. laevis* α subunit amino acid sequence (ILGRHSRKRWERF). Nitrocellulose filters (HA 0.45 μm) were obtained from Millipore.

RESULTS

Inhibition of the Catalytic Activity of the CK2α Subunit by DNA. The catalytic activity of the recombinant α subunit of CK2 can be inhibited by the addition of nucleic acids. Figure 1 shows the inhibition of casein phosphorylation by CK2α by increasing concentrations of bulk salmon sperm

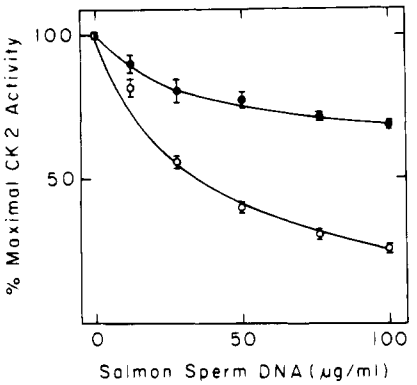

FIGURE 1: Inhibition of protein kinase CK2 by DNA. The effect of increasing concentrations of total salmon sperm DNA on the activity of 2.1 pmol of recombinant α catalytic subunit (○) or of the reconstituted holoenzyme formed from 2.1 pmol of α subunit and 7 pmol of β subunit (●) was measured using the standard assay described in Materials and Methods.

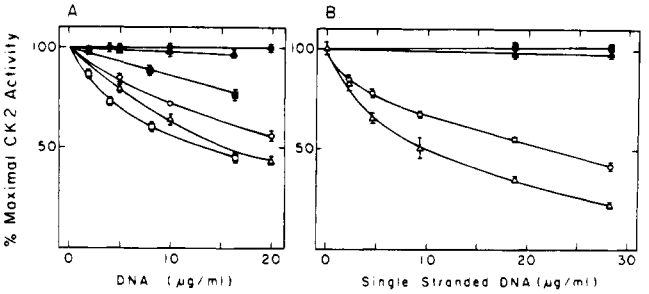

FIGURE 2: Inhibition of CK2 by sequence-specific DNA. (A) Recombinant CK2α (2.9 pmol) was assayed in the presence of varying concentrations of specific double stranded DNAs in the absence (open symbols) or presence (closed symbols) of CK2β subunit (7 pmol) under standard conditions. DNA fragments used were P568 (△, ▲), R588 (□, ■), and 86-mer (○, ●). (B) CK2α (open symbols) or CK2 holoenzyme (closed symbols) were assayed as described in part A with different concentrations of single stranded 86-mer coding strand (○, ●) or its complementary strand (△, ▲).

DNA. The reconstituted holoenzyme is notably less sensitive to DNA inhibition.

Robitzky et al. (1993) reported that CK2α binds specifically to a 568 bp promoter region upstream of the human CK2β gene, and footprinting analysis showed that CK2α protected from nuclease attack a 70 base pair region within that putative promotor. We have prepared these same DNA fragments (designated P568 and 86-mer, respectively) as detailed in Materials and Methods and tested them as inhibitors of CK2α catalytic activity. An unrelated 588 bp DNA fragment (R588) corresponding to the coding region of an *X. laevis* gene for a muscarinic receptor (Herrera et al., in press) was also tested. Figure 2A shows the effect observed on the catalytic activity of CK2α by the addition of increasing amounts of these DNA fragments and also their effect on the reconstituted holoenzyme. Inhibition of CK2α (open symbols) is observed with the three different DNA fragments. The promoter fragment of human CK2β gene (P568) is somewhat less efficient than the nonrelated muscarinic receptor fragment (R588). Comparison of the inhibition by these fragments on molar basis gives I_{50} values of 0.04, 0.05, and 0.4 μM for R588, P568, and 86-mer, respectively, suggesting that the inhibitory capacity is roughly proportional to the molecular weight of the DNAs. It is also evident that the inhibitory potency of these DNAs is not very different from that observed with the unfractionated salmon

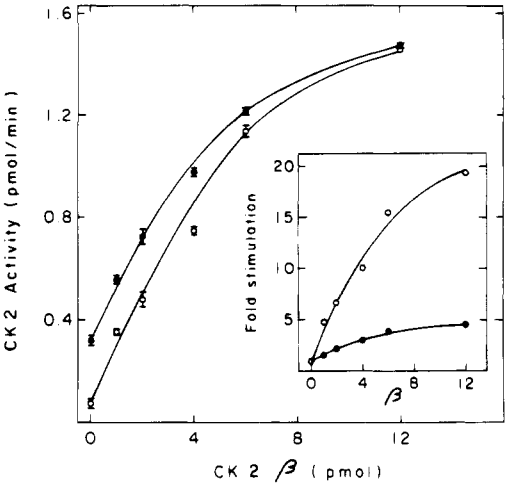

FIGURE 3: Inhibition of CK2α catalytic subunit by DNA is reversed by CK2β subunit. 4 pmol of CK2α was assayed under standard conditions in the absence (●) or presence (○) of salmon sperm DNA (140 μg/mL) and increasing amounts of CK2β. The insert shows the fold stimulation of CK2α by β subunit for each condition, (●) without or (○) with DNA.

sperm DNA since the I_{50} values range from 14 to 22 ng/mL for the specific sequence DNAs as against 35 ng/mL for the bulk salmon sperm DNA. These results indicate that none of the DNA fragments tested contain specific sequences that are particularly efficient in the inhibition of the recombinant *Xenopus* CK2α. Figure 2A also shows that the presence of the β subunit to form the holoenzyme essentially eliminates the inhibitory effects of the two putative promoter fragments and greatly reduces the effect of the muscarinic receptor DNA sequence (solid symbols).

Figure 2B shows that addition of single stranded DNAs that were synthesized to constitute the short CK2β promoter fragment (86-mer) also causes inhibition of the CK2α activity, without affecting significantly the activity of the $\alpha_2\beta_2$ holoenzyme that is formed when β is present. The I_{50} values are 0.85 and 0.40 μM for the coding and noncoding strands, respectively, with CK2α.

Kinetic analysis of the inhibition observed with human CK2β promoter 86-mer fragment was carried out with four levels of casein (between 0.25 and 2 mg/mL) as substrate and different concentrations of the inhibiting 86-mer double stranded DNA. Analysis of these results (not shown) indicates that the inhibition best fits a competitive inhibitory mechanism with a calculated apparent K_i of 160 nM.

The effect of the addition of the β subunit to form the $\alpha_2\beta_2$ holoenzyme on the inhibition of α caused by DNA was examined further. Figure 3 shows the catalytic activity of CK2α in the presence and absence of salmon sperm DNA with increasing amounts of β. In the absence of CK2β, the activity of α subunit is 80% inhibited by the presence of DNA. Addition of CK2β increases the activity of α subunit both in the presence and in the absence of DNA, but as the amount of added β increases, the inhibition caused by DNA decreases, and essentially no inhibition is seen at the highest concentrations of β used. In the insert of Figure 3 the fold stimulation attained by the addition of subunit β is shown. In the absence of DNA, CK2β stimulates α subunit about 5-fold, as has been previously observed (Hinrichs et al., 1993). In the presence of DNA, however, the stimulation caused by β is approximately 20-fold, because of the combined effect of the reversal of the inhibition by DNA and the formation of the holoenzyme.

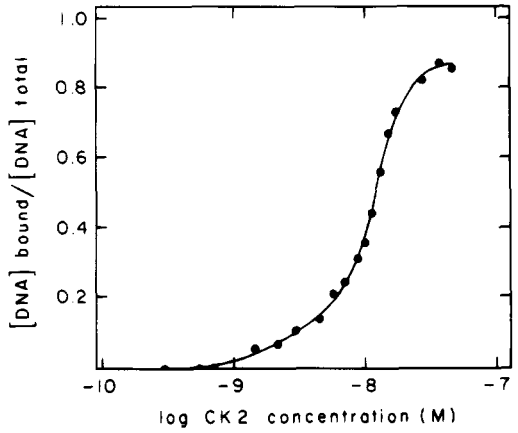

FIGURE 4: The retention of [^{32}P]DNA on nitrocellulose filters as a function of CK2α concentration. A constant concentration (2.6 nM) of P568 [^{32}P]DNA was incubated under standard conditions described in Materials and Methods in the presence of increasing concentrations of CK2α. Samples were filtered through nitrocellulose membranes, and radioactivity was determined by scintillation counting.

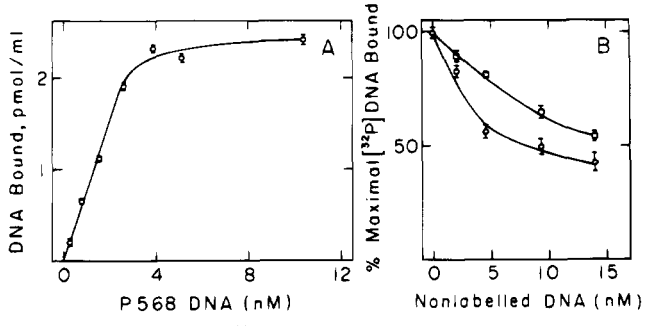

FIGURE 5: Binding of [^{32}P]DNA as a function of DNA concentration. (A) Increasing concentrations of [^{32}P]P568 DNA were incubated in the presence of 80 nM CK2α, and radioactivity was measured in the standard binding assay. (B) A constant concentration (5 nM) of [^{32}P]P568 DNA and 80 nM CK2α were incubated with increasing concentrations of unlabeled P568 (□) or R588 (○) DNA and assayed in 100 μL final volume and otherwise in the standard binding conditions.

The effect of the addition of CK2β on the time course of the phosphorylation reaction was studied in the presence and absence of DNA. The addition of subunit β 5 min after the initiation of the reaction causes an immediate acceleration of the velocity in both cases, showing that the presence of DNA does not cause a measurable lag in the interaction of α with β (not shown).

Binding of CK2α to DNA. In addition to the studies on the effect of DNA on the catalytic activity of CK2α, experiments were performed to demonstrate the physical interaction of this protein with DNA. For this purpose the protein-dependent retention of labeled DNA on nitrocellulose filters was analyzed. Figure 4 shows that addition of increasing amounts of recombinant CK2α leads to a proportional increase in the retention of ^{32}P labeled P568 DNA (human promoter fragment) until 85% of the labeled DNA is detected as a complex. Figure 5A shows a similar experiment but in which the amount of CK2α is maintained constant while the amount of [^{32}P]DNA is increased. Analysis of these data by estimating the half-saturation concentration of DNA (Riggs et al., 1970) gives an equilibrium dissociation constant, K_D, of approximately 1.2 nM.

Similar binding experiments have been carried out with [^{32}P]DNA corresponding to fragments of the *X. laevis*

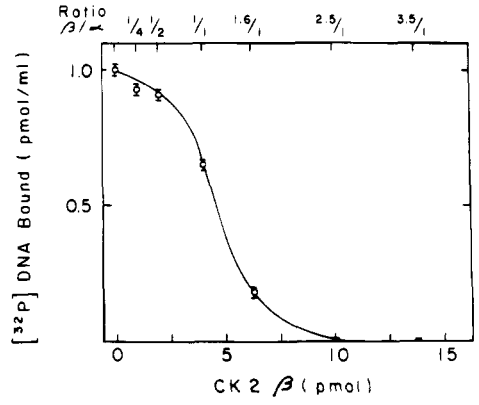

FIGURE 6: Effect of the regulatory subunit CK2β on α subunit-dependent DNA binding. The binding of DNA was measured under standard conditions using 5 nM [^{32}P]P568 DNA and 4 pmol (114 nM) of CK2α in the presence of increasing amounts of CK2β.

muscarinic receptor R588 and of *T. ferrooxidans* tyrosyl-tRNA synthetase, T435. The results obtained (not shown) with these DNAs of different sequence were very similar to those described above, indicating that CK2α has essentially the same affinity for these fragments.

Figure 5B shows the competition of two unlabeled DNAs with the labeled P568 promoter fragment measured by the incubation of labeled DNA and increasing amounts of nonlabeled fragments. The muscarinic receptor fragment is a somewhat better competitor than the homologous non-radioactive nucleic acid, in agreement with the data presented in Figure 2A showing that this specific DNA is also a more efficient inhibitor of the catalytic activity.

The effect of the β subunit on the retention of labeled DNA by CK2α is observed in Figure 6. It is evident that β subunit greatly reduces the binding of DNA to α and that a ratio of 2:1 of β/α completely eliminates the interaction of the CK2α subunit with DNA as measured by this assay.

A mutant form of subunit α in which lysine residues 75 and 76 in the sequence ^{71}KPVKKKKIKR^{80} are substituted by glutamic acids (αE^{75}E^{76}) was also analyzed for both catalytic activity in the presence of DNA and DNA binding. The catalytic activity of this mutant is much less sensitive to DNA, only 20% inhibition of the activity being observed at 100 μg of salmon sperm DNA/mL (not shown) while the wild type α subunit is 75% inhibited at this level of DNA. No inhibition is observed at this DNA concentration when reconstituted mutant holoenzyme is used.

In contrast to this drastic loss of sensitivity of the αE^{75}E^{76} mutant to DNA at the level of catalytic activity, the binding of DNA to this mutant is only partially decreased. As seen in Table 1, 81% of labeled DNA is filter-bound in the presence of the mutant CK2α, as compared to that observed with an equivalent amount of wild type subunit. A range of αE^{75}E^{76} from 50 to 200 nM was studied and gave similar results; that is, between 70% and 80% of the binding capacity of wild type α was observed.

Table 1 also demonstrates that quercetin, a non-polyanionic inhibitor, at a concentration that completely inhibits the CK2α catalytic activity, does not affect the retention of DNA mediated by this subunit. The addition of substrate ATP at a concentration used for the phosphorylation assays also does not alter the binding of DNA. The presence of substrate casein does cause inhibition of DNA retention. This observation is coherent with the fact that DNA inhibition of CK2α activity is competitive with the casein substrate. The

Table 1: Effect of Added Inhibitors and Substrates of CK2 on the Retention of [^{32}P]DNA on Nitrocellulose Filters[a]

	CK2 subunit	addition	[^{32}P]DNA retained pmol/mL	%
expt 1	α^{WT}		3.90	100
	$\alpha E^{75}E^{76}$		3.16	81
expt 2	α^{WT}		2.38	100
	α^{WT}	quercetin (10 μM)	2.32	97
	α^{WT}	ATP (50 μM)	2.27	95
	α^{WT}	casein (1.4 mg/mL)	0.57	24
	α^{WT}	heparin (3 μg/mL)	0.40	17
	α^{WT}	poly(U) (100 μg/mL)	0.05	2
	α^{WT}	poly(Glu:Tyr) (5 μM)	0.59	25
	α^{WT}	anti-CK2α	2.44	102
	heated α^{WT}		0.06	2.5

[a] CK2α subunits were added at 100 nM. Heated α^{WT} was treated at 60 °C for 15 min prior to assay. Anti-CK2α antiserum was added at a 1:100 dilution. In expt 1, 8 nM [^{32}P]DNA (190 000 dpm/pmol) and, in expt 2, 7 nM [^{32}P]DNA (190 000 dpm/pmol) were added, using the standard binding assay described in the Materials and Methods.

presence of polyanionic inhibitors such as heparin, poly(U), or copoly(Glu:Tyr) (4:1) greatly reduces the binding of DNA. At the concentrations used in these binding assays, these same inhibitors cause a decrease of more than 90% of the CK2α catalytic activity. Anti-CK2α antiserum does not alter DNA binding; however, heat inactivation of the enzyme completely eliminates the capacity of CK2α to bind DNA. Heat also destroys the capacity of CK2β to revert inhibition by DNA (not shown).

DISCUSSION

The results presented above demonstrate that DNA can interact with the α subunit of CK2 and that this interaction inhibits the protein phosphorylating activity of this subunit.

Both single stranded and double stranded DNAs interact with and inhibit CK2α, and no great differences in affinity were observed with the various DNAs assayed, although they contained different sequences. Comparison of the effects of DNAs of different length, for instance, the 86-mer and the P568 fragment, indicated that increase in affinity on a molar basis is proportional to the length of the DNA molecule. This evidence would argue that CK2α is able to bind DNA in a non-sequence-specific manner. Our experiments, however, do not rule out the possibility that CK2α may recognize a particular specific DNA sequence with much higher affinity. It is well-known that most proteins that recognize specific DNA sequences also bind to DNA in a nonspecific manner, albeit with lower affinities.

No specific binding was observed when the recombinant *X. laevis* CK2α protein was assayed with fragments containing the putative promoter of the human CK2β gene. Robitzky et al. (1993) have reported that the human CK2α binds to the same 568 bp fragment of this promoter as used in these experiments and that the human α subunit protects from nuclease attack a 70 bp segment within this promoter region. There are several possible explanations for this apparent discrepancy. One of them involves the fact that we are using the recombinant *X. laevis* CK2α. Although the CK2α subunits from human and *X. laevis* are nearly identical throughout their catalytic domain, the human α subunit has 39 additional amino acids at its carboxyl end that are absent from the *X. laevis* protein (Jedlicki et al., 1992). In addition, the recombinant *X. laevis* enzyme has 15 extra amino acids in its amino end, originating from the pT7-7 cloning expression vector. Also, different assays were employed by the two laboratories in studying the CK2α and DNA interaction, since Robitsky et al. did not assay the effects of DNA on enzyme activity and used exclusively the gel retardation assay. It may be pertinent, however, to note that the upstream region of the mouse CK2β gene does not contain a sequence similar to that of the corresponding human gene observed to interact with CK2α (Boldyreff and Issinger, personal communication). This would suggest that the binding of CK2α to the β promoter would not be a general regulatory mechanism for the coordinate expression of the two subunits.

Our results, however, agree with those of Robitzky et al. (1993) in an important aspect, and that is the negative effect of CK2β on the interaction of the α subunit with DNA. The presence of β greatly reduces the effect of DNA on α, reversing the inhibition that the nucleic acid causes in its catalytic activity. This effect is also observed in the DNA binding assay, in which the addition of β titrates away the radioactivity of the DNA retained on the nitrocellulose filters.

With this assay it is also possible to study the relative affinities of the different DNAs for α through their capacity to compete for the protein subunit. These experiments again demonstrated that the human promoter segment of the CK2β is not binding preferentially to this subunit.

In the present studies, it has not been determined how many molecules of CK2α are necessary to complex to the DNA in order to be detected in the filter retention assay. This number is variable in other DNA binding systems, depending on the nature of the protein molecule (Riggs et al., 1970). What is clear from our results, however, is that while 85% of the input DNA can be retained in the presence of excess CK2α, a large molar excess of α (between 30- and 40-fold) is required to yield the retention of 1 mol of DNA. A possible explanation of the molar excess of CK2α required would be that a large proportion of the enzyme is inactive with regard to DNA binding. This seems unlikely because the addition of β subunit in stoichiometric amounts results in both full catalytic activation of CK2α (Hinrichs et al., 1993) and the elimination of DNA binding (Figure 6). A second possibility may be the requirement for several subunit molecules to bind one DNA fragment. Cooperative binding of protein molecules to DNA has been observed, and in some cases protein oligomerization induced by DNA binding has been accepted (von Kries et al., 1991). Self-aggregation and filament formation have been reported to occur with the holoenzyme CK2 at low salt (Glover, 1986), but no evidence of such aggregation has been reported for the recombinant CK2α subunit (Issinger, 1993). A more detailed study of CK2α binding to DNA is necessary to determine whether there are specific sequences with high affinity for CK2α and the stoichiometry of this interaction.

It also has been possible to demonstrate that DNA binds to the CK2α subunit in a manner similar to that of other polyanionic inhibitors. This has been shown by the fact that several of them, including heparin, a random polypeptide composed of glutamic acid and tyrosine (4:1), and poly(U), are able to compete and block the binding of DNA to α, while a different type of inhibitor, such as quercetin, does not affect this interaction. The fact that DNA inhibition appears to be competitive with the acidic protein substrate casein also is corroborated by the finding that casein diminishes DNA retention mediated by CK2α.

In addition, it would be supposed that the highly basic region in the sequence of CK2α extending from amino acids

71 to 80 is involved in the binding of DNA to this subunit. This is demonstrated by the fact that the catalytic activity of the CK2αE^{75}E^{76} mutant (Gatica et al., 1994) is considerably less susceptible to inhibition by DNA. On the other hand, the efficiency of this mutant α subunit in promoting DNA retention on nitrocellulose filters is not greatly reduced, indicating the difference in the two assays, since in the assay of the catalytic activity, for instance, casein is present both as a substrate and as a competitor with DNA for its binding to the protein.

It is not too surprising that DNA can interact with and inhibit CK2α, since many other polyanions, including polynucleotides, have been shown to have similar effects on CK2 holoenzyme. What is particularly interesting is the finding presented in this study that subunit β greatly affects this interaction. The presence of β also reduces inhibition of the catalytic subunit by the ribopolynucleotide poly(U) (not shown). In the case of heparin, there are some controversial results. While Hu and Rubin (1990) and Lin et al. (1991) did not observe significant differences in the inhibition of the CK2 holoenzyme and CK2α by heparin, Boldyreff et al. (1993) present evidence that indicates that the presence of CK2β reduces the sensitivity of CK2α to heparin inhibition. With these other polyanions, however, it has not been shown that excess β can completely reverse the inhibition or eliminate the interaction as has been demonstrated in the case of DNA in this report.

Another interesting example in which the β subunit greatly affects the recognition of α for another macromolecule is that of calmodulin. This protein is a very good substrate for the isolated CK2α subunit, but it is not phosphorylated if CK2β is present to form the holoenzyme tetramer (Meggio et al., 1994). It is interesting that polylysine can alter this situation and permit the phosphorylation of calmodulin by the holoenzyme.

It is reasonable to postulate that the interaction of the CK2α subunit with DNA may have physiological importance. Interactions with RNA, not analyzed in detail in this work, may also be significant. Obviously, the fact that CK2 is predominantly in the cell nucleus places both components in the same organelle and may allow these interactions to take place. The preferential binding of DNA to CK2α rather than to the holoenzyme could mean that DNA may serve to cause dissociation of the subunits of CK2, a fact that might be important in the regulation of its activity. Considering the fact that eukaryotic DNA is complexed to both histone and non-histone proteins, the interaction DNA−CK2α may involve specific structural restraints. It is relevant that Stigare et al. (1993) have recently reported that differential salt extraction of nuclei demonstrates that CK2α, free of CK2β, is bound to chromosomal components. Lüscher and Litchfield (1994) also show that a small fraction of nuclear CK2α is very tightly bound, being solubilized only after detergent treatment.

The question of whether the activity of CK2 is post-translationally regulated is controversial because no clear mechanism that can carry out this regulation has been convincingly demonstrated (Pinna, 1990; Issinger, 1993; Allende & Allende, in press). The existence of free CK2α subunit or of mechanisms that tend to dissociate the α and β subunits from the holoenzyme would provide for such a mechanism, since then the CK2β subunit would play a truly regulatory role, since it can greatly activate the catalytic activity of CK2α and change its substrate specificity. If this were the case, the CK2β subunit may function as cyclin does for the family of the cdks. This analogy has been strengthened by the realization that amino acids 47−55 of most CK2β subunits contain a very similar sequence to the "destruction box" of cyclins (Allende & Allende, in press; Glotzer et al., 1991) and by the findings of Lüscher and Litchfield (1994) that indicate that CK2β is degraded much more rapidly than CK2α by a nonlysosomal system that requires low amounts of ATP.

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