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Dual Effect of Lysine-Rich Polypeptides on the Activity of Protein Kinase CK2

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Abstract Protein kinase CK2 (casein kinase II) is normally a heterotetramer composed of catalytic (α, α′) and regulatory subunits (β). CK2 is able to phosphorylate a large number of protein substrates but the physiological mechanisms of its regulation are still unresolved. Lysine-rich peptides such as polylysine and histone H1 are known to stimulate the catalytic activity of the holoenzyme. This activation is mediated through the CK2β regulatory subunit. In this communication, we report that the same concentrations of lysine-rich peptides or proteins that activate the holoenzyme cause strong inhibition of the phosphorylation of proteins catalyzed by the free catalytic CK2α subunit. The inhibitory effect of polylysine and histone H1 is observed with several protein substrates of CK2α (casein, adeno E1A, transcription factor II A, and CK2β itself). With calmodulin, however, the inhibition of CK2α phosphorylation caused by polylysine is much lower while with a model peptide substrate of CK2 the inhibition caused by this polycation is negligible. The inhibition of CK2α by polylysine is observed only at limiting concentrations of the target substrate proteins. The dual effect of polylysine and of histone H1, which results in the inhibition of CK2α and stimulation of the CK2 $\alpha_2\beta_2$ tetrameric holoenzyme, has the consequence that the addition of the CK2β, in the presence of polylysine and low concentrations of substrate protein, can cause a 242-fold stimulation of the activity of CK2α. Other polycationic compounds such as polyarginine and spermine do not inhibit the phosphorylation of casein by CK2α, indicating that the effect is specific for lysine-rich peptides. Since there is evidence that there may be free CK2α subunits in the nuclei of cells, where there is abundant histone H1, the inhibition of CK2α by this lysine-rich protein may have physiological relevance. J. Cell. Biochem. 89: 348–355, 2003.

Key words: casein kinase II; CK2 inhibition; polylysine; histone H1; catalytic subunit CK2α

Protein kinase CK2 (also known as casein kinase II) is ubiquitous in eukaryotes and is responsible for the phosphorylation of a large number of protein substrates [Allende and Allende, 1995; Pinna and Meggio, 1997; Guerra and Issinger, 1999]. The holoenzyme is composed of catalytic (α and α′) and regulatory subunits (β) and is usually found conforming stable tetramers with the composition $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, and $\alpha'_2\beta_2$. The α and α′ catalytic subunits are active by themselves and are coded by different but highly homologous genes. In some species (*Z. mays* and *D. discoideum*) the CK2 activity has been found to reside in structures lacking regulatory subunits [Dobrowolska et al., 1991; Kikkawa et al., 1992] and there are reports of free catalytic subunits in the nuclei of some cells [Stigare et al., 1993]. The regulatory subunit has several effects upon binding to the catalytic subunit of CK2. With the majority of protein substrates, the CK2β subunit causes a significant stimulation of the phosphorylating activity of CK2α (varying from 4- to 10-fold). However, with a few substrates (calmodulin, MDM-2), CK2β is a potent inhibitor of the phosphorylation catalyzed by CK2α [Meggio et al., 1994]. In addition, CK2β interaction causes an important increase in the heat stability of CK2α and also results in a stabilization of the catalytic subunit to proteolysis [Guerra and Issinger, 1999]. CK2β also causes a significant upward shift in the optimal ionic strength required for

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protein phosphorylation by CK2α. The optimal salt concentration for CK2α activity is 50 mM while for the $\alpha_2\beta_2$ holoenzyme the optimum is 125 mM [Antonelli et al., 1996].

Studies carried out with replacement mutants and deletions have established the effects of different regions of CK2β on CK2α. It is clear that the carboxyl half of CK2β (residues 127–205), is responsible for the main interaction and activation of the phosphorylation activity of CK2α [Kusk et al., 1999]. The region for dimerization of CK2β, clearly defined by the solution of the CK2β structure by X-ray diffraction [Chantalat et al., 1999] involves a "zinc finger" structure situated within residues 110–150. Recently, the structure of the CK2 holoenzyme tetramer was solved by X-ray crystallography [Niefind et al., 2001]. This structure tells us that the CK2β dimer interacts with the amino terminal regions of the catalytic subunits. The structure also demonstrates that $\alpha_2\beta_2$ tetramer formation does not change greatly the overall conformation of CK2α as compared to that observed in the isolated CK2α that was determined for the *Z. mays* enzyme [Niefind et al., 1998]. In the amino half of CK2β, there is a cluster of acidic residues (amino acids 55–64). Mutation analysis of this acidic cluster has shown that it has an inhibitory effect on the catalytic activity of CK2α [Boldyreff et al., 1993; Hinrichs et al., 1995]. Surprisingly, this acidic cluster of CK2β that influences the phosphorylating activity of the enzyme is located in a region that is not in direct contact with the catalytic domain of the tetramer, as seen in the crystals of the holoenzyme.

Little is known about the regulation of CK2 activity in vivo. Transcriptional regulation probably accounts for the increase of CK2 activity observed in actively dividing cells of embryos or tumors [Münstermann et al., 1990]. Cell localization and targeting through "docking sites" may be other mechanisms that regulate the availability of CK2 [Holland and Cooper, 1999]. Recent reports indicate that the p38 MAP kinase [Sayed et al., 2000] or the APC protein involved in colon cancers [Homma et al., 2002] may bind CK2 and regulate its activity.

On the other hand, much evidence has been gathered that indicates that naturally occurring polyamines such as spermine and proteins rich in lysine such as histones and synthetic polylysine can activate the phosphorylating activity of CK2 [Mäenpää, 1977; Meggio et al., 1992, 1994]. This evidence indicates that this activation is mediated through the interaction of the polyamines with the acidic cluster of CK2β (amino acids 55–64) since CK2α in the absence of CK2β or in the presence of CK2β that has been mutated in those acidic residues is no longer stimulated by these polycations [Meggio et al., 1994]. In this study, it is also observed that polylysine is able to reverse the inhibitory effect caused by CK2β on the phosphorylation of calmodulin.

Since polyamines are abundant in proliferating cells and lysine-rich histones are present at high concentrations in the nuclei of cells, where CK2 is preferentially located in dividing cells, it is possible that the positive effect of these compounds on CK2 activity may be physiologically relevant.

While studying the phosphorylation of several recombinant proteins by CK2, we have found a different effect of polylysine on CK2 activity. In this communication, we present data that demonstrates that at limiting concentrations of several protein substrates, polylysine, and histone H1 can drastically inhibit the phosphorylation of these proteins by the isolated catalytic subunit CK2α. Under similar conditions and concentrations of polylysine and substrate, the holoenzyme is significantly stimulated by these polycations. The inhibitory effect of polylysine and histone H1 on the phosphorylating activity of CK2α is decreased as the concentration of the protein substrate is increased. This inhibition is negligible when calmodulin or a model peptide are used as substrates. Other polycations such as polyarginine or spermine do not cause an inhibition of CK2α phosphorylation of protein substrates that are inhibited by polylysine. With these substrates polyarginine actually stimulates casein phosphorylation while spermine has no effect.

MATERIALS AND METHODS

Polylysine (average molecular mass of 29 kDa), polyarginine, spermine, histone H1, calmodulin, and dephosphocasein were acquired from Sigma Chemical Co. (St. Louis, Mo).

The Ni^{+2} agarose resin was obtained from Invitrogen (Carlsbad, CA).

The specific peptide substrate, R R R A D D S D D D D D, was synthesized by Oligopeptido, the core facility of the University of Chile.

Recombinant *Xenopus laevis* CK2α and CK2β

The expression of recombinant *X. laevis* CK2α and CK2β in *Escherichia coli* has been previously reported [Hinrichs et al., 1993]. These subunits were expressed with $(His)_6$ tags in *E. coli* BL-21 as has been described [Cosmelli et al., 1997]. The recombinant proteins were purified using Ni^{+2}–NTA-agarose columns.

Protein Kinase Assay

The standard assay mixture contains in a total volume of 30 μl: 50 mM HEPES pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 1.7 μM [γ^{32}P] ATP 130,000 cpm/pmole (unless otherwise stated), protein substrates, and the CK2 subunits at the concentrations given in each case. Incubations were carried out for 10 min at 30°C. Reactions were stopped by the transfer of the incubation to P81 phosphocellulose and washing performed in 75 mM phosphoric acid as described [Hinrichs et al., 1993].

Where specified, the effect of lysine-rich peptides on the CK2 catalyzed phosphorylation of protein substrates was assayed using these same incubation conditions followed by autoradiography of the resolved proteins on 12% SDS–polyacrylamide gels (SDS–PAGE). In these experiments, the reaction was stopped by addition of the SDS-gel loading buffer and heating to 95°C for 5 min as described by Laemmli [1970].

Recombinant adenovirus protein E1A, cloned in the expression vector pGEX-2T was supplied by Dr. Lawrence Banks (Trieste). GST-EIA was purified on GSH-agarose. Purified recombinant transcription factor II A was made available by Dr. E. Maldonado (ICBM, Santiago).

RESULTS

Polylysine can Inhibit the CK2α Phosphorylation of Low Concentrations of Casein

The addition of polylysine can drastically inhibit the phosphorylation of casein by CK2α (Fig. 1). This inhibition depends on the concentration of polylysine, reaching levels of 80% inhibition with 15 pmole of added polypeptide. The inhibition observed with CK2α and polylysine as seen in Figure 1 was obtained with a low concentration of casein (166 pmole in the assay, which is equivalent to 0.17 mg/ml). An experiment under identical concentrations of casein and of polylysine but using recombinant zebrafish protein kinase CK1α [Burzio et al., 2002] was carried out to show whether the polylysine blocked the availability of this protein substrate to another kinase. CK1α (also known as casein kinase 1α) is similar to CK2 in that it targets acidic or phosphate-primed regions of casein and other substrates [Burzio et al., 2002]. No effect of polylysine on the phosphorylation of casein by CK1α was observed (not shown).

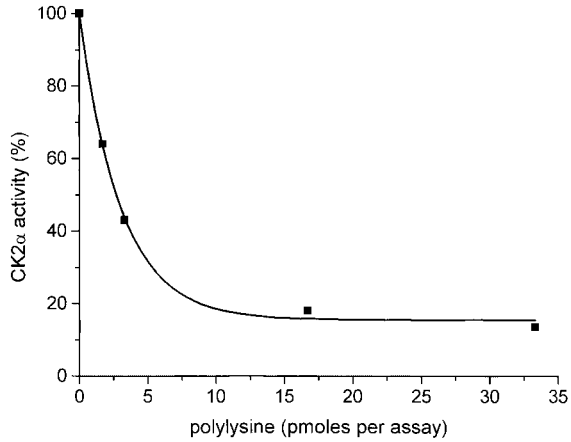

Fig. 1. Inhibitory effect of different concentrations of polylysine on the phosphorylation of casein by catalytic subunit casein kinase II alpha (CK2α). The assay for the incorporation of [^{32}P] into casein was as described in Materials and Methods using 166 pmole (5 μg) of casein and 1.4 pmole of protein kinase CK2α. The indicated amount of polylysine was added to each 30-μl assay. The value obtained in the absence of polylysine, 0.014 pmole of [^{32}P] incorporated per minute was used to set 100% activity.

As seen in Figure 2, the extent of the inhibition caused by 33 pmole of polylysine depends also on the concentration of the casein substrate. As the amount of protein substrate is increased from 166 to 830 pmole in the assay, the inhibition decreases from 80% to 0. At higher concentrations of casein, polylysine causes a stimulation of approximately 40%.

It seemed interesting to analyze the effect of the presence of polylysine on the determination of the apparent Km for casein using the CK2α subunit. Figure 3 shows the results obtained in a double-reciprocal plot of a similar experiment carried out with different concentrations of casein in the presence (■) or absence (△) of 33 pmole of polylysine in the assay. In the absence of polylysine, the plot yields a very good fit of the data with an apparent Km for casein of 2.1 mg/ml, which is within the limits of values found in other studies [Antonelli et al., 1996]. In

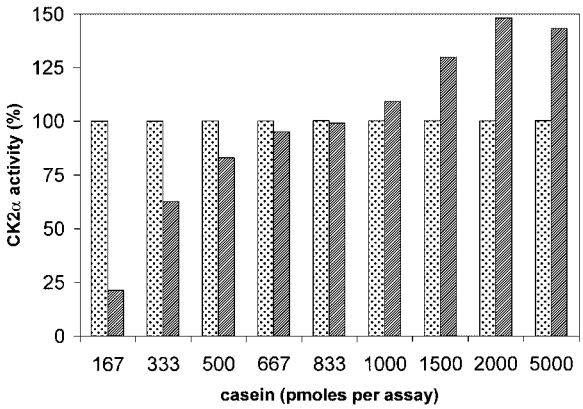

Fig. 2. The effect of increasing concentrations of casein on the inhibition of subunit CK2α by polylysine. The incorporation of [^{32}P] into casein was measured in the presence of 1.4 pmole CK2α by the assay described in Methods. The different amounts of casein indicated were present in each assay (30 μl). The stippled bars (⁘) indicate the values obtained without polylysine which were set at 100% activity, while the cross-hatched bars (▨) indicate the relative values obtained in the presence of 33 pmole polylysine.

the presence of polylysine (■), the data obtained does not fit the expected straight line due to the inhibition observed at low concentrations of the protein substrate and the stimulation caused by the polylysine peptide at higher concentrations of casein. Although, the inhibition is eliminated by higher concentrations of substrate, polylysine does not behave as a classical competitive inhibitor due to its dual role of inhibitor and activator.

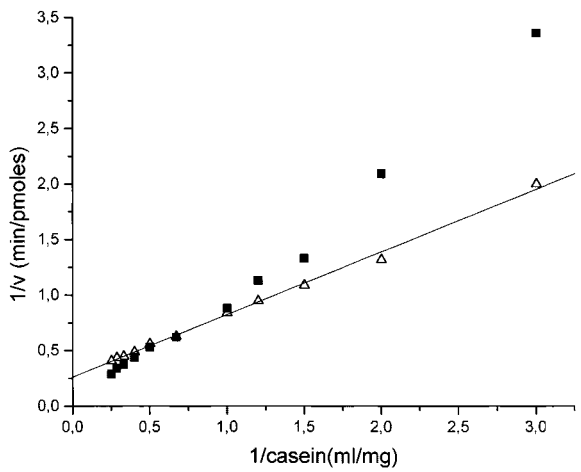

Fig. 3. Double reciprocal plot of casein substrate concentration against phosphorylating velocity catalyzed by CK2α in the presence of absence of polylysine. In this experiment, the activity was assayed with varying concentrations of casein as specified in Methods except that [^{32}P]γ ATP concentrations was 100 μM (3,000 cpm/pmole) 1.1 pmol of CK2α was used. The assays were carried out in the presence of 33 pmole of polylsine (■) and in the absence of this basic polypeptide (△).

Reversal of the Inhibitory Effect of Polylysine by the Addition of the CK2β Subunit

Using limiting concentrations of casein, the effect of polylysine was tested as increasing concentrations of CK2β, the regulatory subunit of CK2, were added.

Figure 4 shows the results obtained. The values obtained without polylysine (◆) and with limiting concentrations of the casein substrate, demonstrate that the addition of CK2β causes a 5.7-fold stimulation of the phosphorylating activity of CK2α. This stimulation is near maximal at equimolar concentrations of the regulatory CK2β subunit (1 pmole) with respect to the CK2α catalytic subunit and is within the known range observed by the addition of CK2β when casein concentration is saturating [Antonelli et al., 1996]. Parallel assays were carried out in the presence of 33 pmole of polylysine (●). In the presence of polylysine, the effect of CK2β is much more dramatic. In the table inserted in the figure, it can be seen that, congruent with the previous results in this communication, in the absence of CK2β, polylysine causes a strong inhibition (80% inhibition). In the presence of CK2β, the situation is reversed since polylysine causes a very high stimulation. This reversal in the sign of the effect of polylysine on CK2 activity explains the capacity of CK2α to cause a maximal stimulation of 242-fold, an exceptionally high

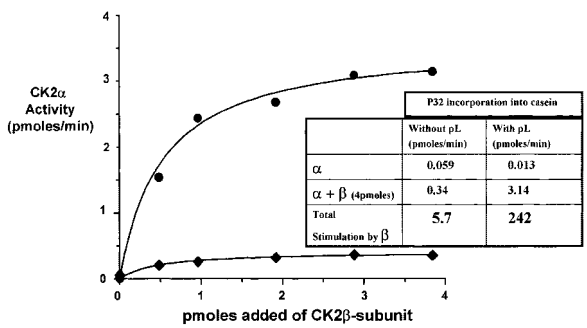

Fig. 4. The effect of the addition of CK2β on the phosphorylation of casein by CK2α in the presence or absence of polylysine. Reactions for the assay of the incorporation of [^{32}P] into casein were carried out as described in Materials and Methods using 1 pmole of CK2α and 166 pmole (5 μg) of casein. (●) incubations contained 33.3 pmole of polylysine; (◆) no polylysine peptide was added. CK2β was added as indicated. In the insert, the table provides the value of [^{32}P] incorporation into casein in the presence and absence of polylysine in the assays containing CK2α (1 pmole) with and without CK2β (4 pmole).

value. Under these conditions, the stimulation caused by polylysine in the presence of CK2β, estimated by comparing the maximum values of the two curves, is approximately eightfold which is higher than the stimulation observed with saturating concentrations of casein (threefold) in other studies (not shown).

Inhibition of CK2α Phosphorylation by Polylysines and Histone H1 is Observed With Other Protein Substrates but not With a Model Peptide

The effect of polylysine and of histone H1, which contains 34% of lysine residues, was tested with several other protein substrates of CK2α, using autoradiography of proteins fractioned by SDS–PAGE electrophoresis.

As can be observed in Figure 5, both polylysine (lane 2) and histone H1 (lane 3) are inhibitors of the phosphorylation of transcription factor II A (A) and adenovirus E1A (D) as compared to the incubations without inhibitor (lane 1). Histone H1 is a weaker inhibitor of TF IIA phosphorylation as compared with polylysine. The inhibition of these two proteins caused by polylysine is comparable to that shown by heparin (5 μg/ml) (lane 4) a known strong inhibitor of CK2. The results obtained with calmodulin seen in 4B contrast with the above observations. This atypical substrate is much less inhibited by polylysine and histone H1. Densitometric quantification indicates that the inhibition is of the order of 40% in the presence of polylysine and only 25% with histone H1. Panel C of Figure 5 shows the results on the autophosphorylation of CK2β. Both histone H1 and polylysine cause a significant inhibition of CK2β phosphorylation. The inhibitory effect of polylysine on CK2β auto-phosphorylation has been previously described [Meggio et al., 1992].

The peptide RRRADDSDDDDD is a well-known model peptide, which is specific for CK2 phosphorylation [Sarno et al., 2002]. Phosphorylation by CK2α for this peptide was used to test the effect of polylysine (Fig. 6). Although, a wide range of concentrations considerably lower than the apparent Km of 185 μM (or 5,500 pmole/assay) for this substrate was used, no significant inhibition of polylysine on its phosphorylation was observed. At higher concentrations of the peptide, a slight stimulation of the CK2α activity is observed.

Inhibition is not Observed With Other Polyamines

The question arises as to the specificity of the inhibition observed with polylysine and histone H1. This was studied through experiments using polyarginine and spermine. Under the conditions of casein concentration that permit a strong inhibition by polylysine on the phosphorylation caused by CK2α, polyarginine had no effect at concentrations below 20 pmole per assay while at higher concentrations this polypeptide causes a significant stimulation reaching a value of 3.8-fold with 33 pmole of polyarginine (not shown). Spermine, tested by adding 10–50 pmole in the assay, did not cause a significant effect on the phosphorylation of casein by CK2α (not shown). These results demonstrate that the inhibitory effects observed

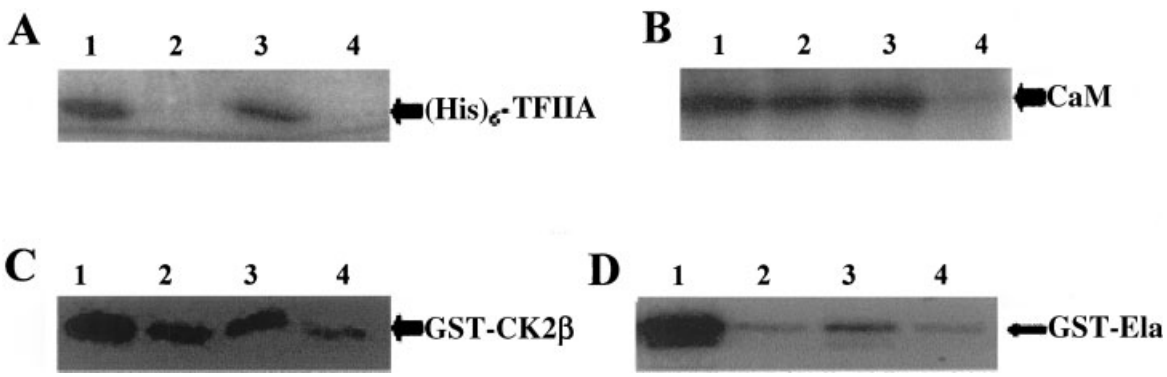

Fig. 5. The effect of polylysine and histone H1 on the phosphorylation of several protein substrates by CK2α. The effect of polylysine and histone H1 was tested by incubating the indicated substrate proteins under the assay conditions specified in Materials and Methods, with the different inhibitors and at the end of the 10 min incubation, proteins were fractionated on SDS–polyacrylamide gels (SDS–PAGE) and analyzed by autoradiography. In all experiments, 8.5 pmole of CK2α were used and additions were as follows: **lane 1**, without inhibitor; **lane 2**, 33 pmole of polylysine were added; **lane 3**, 80 pmole of histone H1 were added and in the fourth lane, 5 μg/ml of heparin were present. In (**A**), 3 pmole of transcription factor II A were added; in (**B**), 40 pmole of camodulin; in (**C**), 0.9 pmole of CK2β; and in (**D**), 0.4 pmole of adenovirus GST-E1A was used as protein substrate.

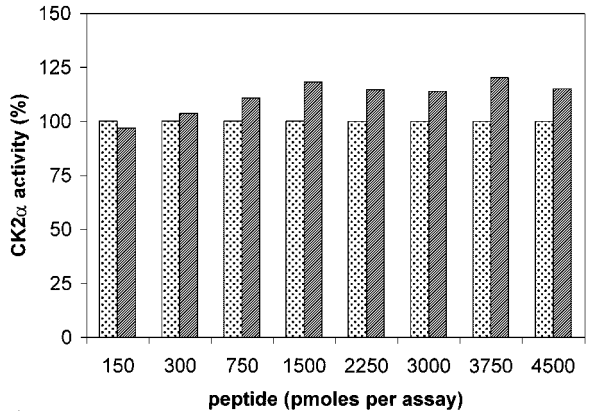

Fig. 6. The effect of polylysine on the phosphorylation of a CK2 model peptide substrate. The assays of CK2α phosphorylating activity were carried out as specified in Methods using 1.1 pmol of CK2α. The values obtained in the absence of polylysine (stippled bars, ⁘) were set at 100% activity, while the values obtained in the assays containing 67 pmole of polylysine (cross-hatched bars, ▨) were expressed as the relative percentage of those found in its absence.

with lysine-rich peptides are not simply due to the polycationic nature of the compounds studied.

DISCUSSION

The regulation of CK2 activity in the cell is still an unsolved problem. One of the key pending questions is the role played by the regulatory subunit (CK2β) in this modulation. The generally held view that CK2 is always present in the cell as a heterotetrameric holoenzyme has been challenged by several observations that indicate that there may be some free catalytic or regulatory subunits. One of the indications supporting the possibility of the existence of free subunits in vivo is the finding by several laboratories that transfection of cells with CK2β and the ensuing over-expression of this subunit results is a significant increase of CK2 activity in the extracts of these cells [Heller-Harrison and Czech, 1991; Korn et al., 2001]. The simplest explanation for this observation would be the existence of a pool of free CK2α subunits that could bind the ectopically produced CK2β. Also supporting this concept is the fact that crystallographic studies indicate that the α/β subunit surface contact area is inferior to that seen in stable multiprotein complexes [Niefind et al., 2001]. Recently, experiments carried out by Cochet and his collaborators [Martel et al., 2001] indicate that the α and β subunits of CK2 enter the nucleus separately. This observation agrees with an older report of the presence of free CK2α bound to the chromatin fractions [Stigare et al., 1993]. In *D. discoideum* and in some plant tissues, CK2 activity has been found to correspond to catalytic subunits independent of regulatory proteins [Dobrowolska et al., 1991; Kikkawa et al., 1992].

These observations provide relevance to studies such as the one presented here that describe properties of the free CK2α subunit that are very different from those observed with the holoenzyme. With free CK2α subunit and low concentrations of several protein substrates, polylysine has been found to be a potent inhibitor. Histone H1, which is rich in lysine, has a similar but less pronounced effect. Under similar conditions, the holoenzyme is very significantly stimulated by these two polypeptides. As seen in Figure 4, the summation of these two effects can mean that, in the presence of polylysine or histone H1, the activity of CK2α can be almost totally dependent on the presence of CK2β. A stimulation of 242-fold of the activity of CK2α by CK2β is much greater than the 4- to 10-fold stimulation normally observed in the test tube by the addition of CK2β in the absence of the polycation. The extrapolation of this observation to a physiological situation is obviously hypothetical. However, there apparently could be free CK2α in the nucleus, where histone H1 or other lysine-rich proteins are abundant.

In this study, we have observed that the inhibitory effect of polylysine with CK2α is only detected when the protein substrates are present at low concentrations. Since CK2 phosphorylates many proteins, it is quite probable that some substrates may be in the cell at limiting concentrations, far below the apparent Km values observed in vitro. This is especially true for the many transcription factors that are known to be phosphorylated by CK2 and which are expressed at low concentrations in the cell [Allende and Allende, 1995].

The relation between the concentration of substrate and the inhibition by polylysine and histone H1 needs further analysis. Since the substrate sites for CK2 are acidic rich regions, the inhibition caused by these two polycations could be thought to be due to blocking of the substrate sites by ionic interactions. This simple explanation does not hold because CK2 holoenzyme and CK1α, a totally different protein kinase, which has similar substrate

preferences, can act on the same substrate in the presence of polylysine without any interference. CK1α, like CK2, recognizes acidic clusters in protein substrates [Pulgar et al., 1999]. In addition, the fact that other polycations such as polyarginine and spermidine do not show a similar behavior, argues against a mere interaction of ionic charges.

Since the inhibitory effect is only observed with free CK2α, it seems most probable that the interaction of polylysine could be with the catalytic enzyme subunit. On the other hand, excess substrate might capture the polycationic inhibitor and compete for its interaction with the enzyme, explaining the lack of inhibition at high substrate concentrations. This explanation is again simplistic because it does not account for the variations observed with other substrates such as calmodulin which is weakly inhibited by polylysine and the model peptide substrate which is not inhibited. As mentioned previously, calmodulin is atypical as a substrate since its phosphorylation by CK2α is inhibited by CK2β and this inhibition is reversed by polylysine [Meggio et al., 1994]. The autophosphorylation of CK2β itself is also partially inhibited by polylysine as had been previously reported [Meggio et al., 1992]. This inhibition is atypical since polylysine stimulates the phosphorylation activity of the holoenzyme in general. The tridimensional structure of the holoenzyme tetramer published recently by Niefind et al. [Niefind et al., 2001], clearly demonstrates that the CK2β interaction does not greatly change the structure of the CK2α catalytic subunit. Since the tetrameric structure was not determined in the presence of a peptide or protein substrate, this structure does not explain how the regulatory subunit is able to modify the phosphorylating activity of the catalytic CK2α or how the substrates are recognized.

Sarno et al. [2002] have recently uncovered an interesting difference in the structure/function relation between CK2α and the $CK2\alpha_2\beta_2$ tetramer conforming the CK2 holoenzyme. In the isolated CK2α, its amino terminal plays an essential role in maintaining the catalytic pocket in an open conformation through its interaction with the activation segment of this kinase. On the other hand, in the holoenzyme the amino terminus of CK2α can be dispensed with because CK2β interacts with CK2α in a way that fixes the activation segment in an active conformation. On this basis, one may try to explain the inhibitory effect of polylysine on CK2α by the proposal that this polycation might interfere with the interaction between the N-terminal fragment of the free subunit and the activation segment of its catalytic center. Since the addition of CK2β eliminates this function of the amino end of CK2α, the inhibitory effect of polylysine would not be observed in the holoenzyme. As a matter of fact, the amino end of CK2α has a region (E^{22} Y W D Y E S H V V E W G N Q D D Y^{40}) which is rich in acidic and hydrophobic residues and which is clearly involved in the interaction of this region with the activation segment and with CK2β [Sarno et al., 2002]. It is possible that this region might interact with polylysine. This hypothesis, however, does not explain why the CK2 model peptide and the atypical substrate, calmodulin, are not inhibited by polylysine in their phosphorylation by CK2α.

In the last few years, it has become clear that protein kinases are aided in selecting their substrates through "docking sites" that are separate from their catalytic centers and that bind regions of the substrate that are different from the direct targets of phosphorylation [Holland and Cooper, 1999]. The binding of substrates through these docking sites results in a functional increase of the concentration of the substrates in the vicinity of the catalytic centers of the enzymes, favoring their phosphorylation. There have not been detailed studies about possible docking sites in the CK2 subunits and substrates, however, there are some indications that CK2β may be contributing docking sites for some substrates since it is known to form complexes with several proteins phosphorylated by CK2 [Meggio et al., 2001]. The effects of polylysine and histone H1 presented in this report, may be related to docking sites existing in CK2α that might be selectively antagonized by these polycations. The model peptide would lack these secondary interacting sites, which may explain its insensitivity to polylysine inhibition. The holoenzyme would have different docking sites supplied by the regulatory subunit and the stimulatory effect of polylysine would be related to its known interaction with the acidic cluster present in CK2β.

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