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Modulation of the Protein Kinase CK2 Activity by a Synthetic Peptide Corresponding to the N-Terminus of Its β Regulatory Subunit

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Using a synthetic peptide corresponding to the sixteen amino-acid N-terminus of the β subunit, the structure-activity relationship study of casein kinase 2 (CK2) was performed with regard to its previously reported property to polymerize and oligomerize in vitro. Velocity sedimentation experiments show that the peptide β 1-16 prevents the thick filament formation and stabilizes the ring-like structure of the kinase. Furthermore, the peptide β 1-16 stimulates the kinase activity by 3-fold toward exogenous substrates as well as the intrinsic autophosphorylation of the kinase. Such observations are in agreement with the proposed model of an activated state of CK2 when the ring-like structure is adopted. Comparison of the effects of spermine and peptide β 1-16 on CK2 structure and activity suggests that these two activating molecules may function in a different way. Our study suggests that the N-terminal region of the β subunit of CK2 could regulate the kinase activity by controling the quaternary structure of the enzyme.

Protein kinase CK2 is an ubiquitously expressed serine threonine kinase. The enzyme purified from most sources has been characterized as a heterotetramer composed of two dissimilar subunits, ie α subunit of 35-44 kDa and β subunit of 24-29 kDa. The subunits associate to form a native $\alpha_2\beta_2$ structure. Earlier studies have shown that the α subunit bears the catalytic site of the enzyme (1-4). The β subunit which is the target of the kinase self-phosphorylation behaves as a regulatory moiety that greatly stimulates the catalytic activity of the α subunit (1). It is generally accepted that in living cells a number of protein kinase activies are under the control of specific intracellular messenger(s). Despite many reports on its potential role in proliferation and tumorigenesis, the mechanism by which CK2 is regulated in living cells is poorly understood and the enzyme is commonly classified as a messenger-independent protein kinase. Nevertheless, a number of compounds which activate or inhibit enzyme activity in vitro have been identified. Most inhibitors such as heparin, polyglutamate are polyanions which are competitive toward the protein substrate (5-6), whereas most activators including spermine, protamine, polylysine and polyarginine are polycations (7-8). Moreover the kinase needs high concentrations of magnesium (20mM) for optimal catalysis (9).

We have previously shown that Drosophila recombinant CK2 can undergo a self polymerization generating three distinct molecular forms in aqueous solution: ring-like structure, thin and thick filaments (10). Structure-activity relationship studies have shown that the ring-like structure exibits the optimal specific activity of the enzyme, and that the others molecular structures probably correspond to inactive forms of CK2. Although the different polymeric forms of CK2 have not been yet detected in intact cells, the fact that they are observed at physiological values of the ionic strength and protein concentration (10) raises the possibility that their formation may also take place in vivo. Thus, we have proposed that CK2 may be considered as an associating-dissociating enzyme, and that the regulation of the quaternary structure of this protein kinase could be one of the mecanisms controling its activity.

Based on the generally accepted model for the regulation of the cAMP-dependent protein kinase (11-15), one possible model to explain the CK2 inactivation observed upon thick filament formation is as follow: the association of ring-like structures in thick filament involves a substrate analogue sites of one ring-like structure which interact with catalytic sites on the α subunit of the next, thereby sterically hindering the approach

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Abbreviations: CK2, protein kinase CK2; BSA, bovine serum albumin; DTT, dithiothreitol.

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of external substrates. Indeed, the N-terminus region of the β subunit bears the autophosphorylation site of the enzyme and thus may be a good candidate to act as a pseudosubstrate during the process of thick filament formation.

The present study reports that a synthetic peptide corresponding to the sixteen amino-acid N-terminus of the β subunit, ie peptide β 1-16, influences both the structure and the activity of CK2. It prevents the thick filament formation, stabilizes the ring-like structure and stimulates the kinase activity by 3-fold toward exogenous substrates as well as the CK2 intrinsic autophosphorylation. This activation reflects a conformationnal change of the kinase. However peptide β 1-16 does not act as a competitor toward substrate since it induces only a slight decrease of the Km value, suggesting that it might function as a tethered ligand, binding to an as yet unidentified domain which is different from the catalytic pocket.

MATERIALS AND METHODS

Materials. (γ-32P)ATP (3,000 Ci/mmol) was purchased from Amersham. The peptide substrate (RRREEETEEE) for CK2 and the peptide β 1-16 (MSSSEEVSWVTWFCGL), β1-13 peptide (MSSSEEVSWVTWF), β7-16 peptide (VSWVTWFCGL), were synthesised by Neosystem Laboratory (Strasbourg, France). β8-13 peptide (SWVTWF) was kindly provided by Dr. Gerard Arlaud. Spermine was obtained from Sigma (St Louis, U.S.A.).

Preparation of recombinant CK2. Recombinant oligomeric CK2 from Drosophila melanogaster was overexpressed in Sf9 cells, purified to homogeneity as previously described (16) and stored at −80°C in 10 mM Tris HCl pH 7.5, 1 mM DTT, 2% glycerol, 0.1% Triton X-100 and 1 M NaCl until used.

Velocity sedimentation. Linear 5%-25% (w/v) sucrose gradients were prepared in TD buffer (10 mM Tris HCl pH 7.5, 1 mM DTT). For each experiment, samples (1.5 μg) of purified recombinant CK2 were preincubated at 4°C in 0.1 ml TD buffer for 2 h prior to loading. Gradients were centrifuged at 4°C for 3.5 hours at 200,000 × g and fractionated in 200 ml aliquots. For each ionic condition identical gradients were run using, aldolase (8S), catalase (11.2S) and α_2-Macroglobulin (19S) as sedimentation standards. Standards were localized by the Bradford protein assay.

CK2 assay. CK2 activity was assayed using casein as previously described (9).

CK2 autophosphorylation. 520 ng of purified recombinant Drosophila CK2 was preincubated 30 min at 4°C in 10 mM Tris/HCl, pH 7.5, 100 mM NaCl, and increasing amount of peptide β 1-16, ie CK2: peptide β 1-16 ratio of 1:0, 1:100, 1:500, 1:1000, 1:2000, 1:5000. Autophosphorylation assay was realised at room temperature for 4 min, in the presence of 1 mM MgCl2, 1 mM (γ-32P)ATP. The reaction was terminated by adding 5 ml of 5× SDS sample buffer. It was analysed by 12% SDS-polyacrylamide gel electrophoresis, followed by a Coomassie Blue staining and autoradiography. The 32P incorporated in the β subunit was measured with a Phosphorimager.

RESULTS

1. Effect of the β1-16 synthetic peptide on CK2 polymerization. We analysed the sedimentation behavior of CK2 in the absence or the presence of the synthetic peptide corresponding to the sixteen amino-acid N-terminus of the β subunit (ie peptide β 1-16). As previously described (10), in 0.1 M NaCl, CK2 sediments as a mixture of various interconverting oligomers (Fig. 1): the ring-like structure $(\alpha_2\beta_2)_4$ (13.6S), the thick filaments $((\alpha_2\beta_2)_4)_n$ (from 15 to 44S) which may result from the linear association of ring-like structures and the thin filaments $(\alpha_2\beta_2)_n$ (> 44S) which correspond to the linear polymerization of protomers. In contrast, in the presence of the peptide β 1-16 the population of the thick filaments is dramatically reduced to the advantage of the ring-like structure, which becomes the main molecular form. We conclude that the peptide β 1-16 prevents the thick filament formation and stabilizes the enzyme under its ring-like structure. This prompted us to examine the effect of this peptide on CK2 activity.

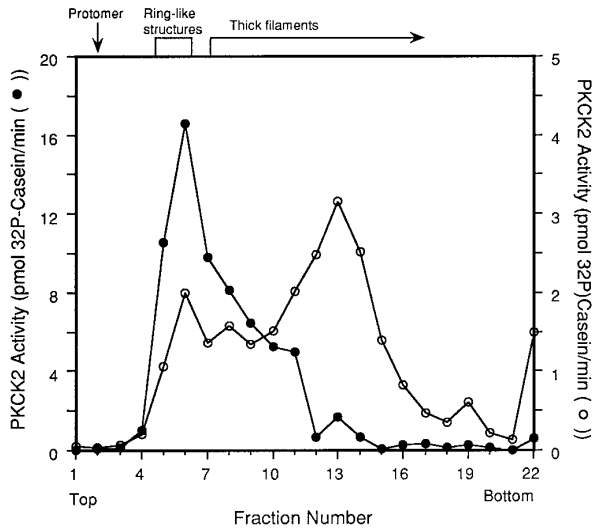

FIG. 1. Effect of the peptide β 1-16 on the oligomeric state of CK2. Purified recombinant Drosophila CK2 was preincubated for 2h at 4°C in 100 μl of 10 mM Tris/HCl, pH 7.5, 1 mM DTT, 0.1 M NaCl, in the absence (○) or the presence (●) of peptide β 1-16 with a CK2:peptide ratio = 1:2000 and sedimented through 5-25% sucrose gradients under the same salt condition and containing the same amount of peptide β 1-16. Gradients were fractionated and CK2 activity was determined in each fraction.

2. Effect of the peptide β 1-16 on CK2 activity. Recombinant Drosophila CK2 was preincubated with increasing concentrations of the peptide β 1-16 and the kinase activity was assayed toward casein. The kinase assay was performed in the presence of 1 mM $MgCl_2$ as we previously showed that high concentrations of magnesium affect the quaternary structure and the activity of CK2 (10). The synthetic peptide stimulated the phosphotransferase activity of CK2. For a CK2/peptide ratio of 1/3900 (peptide concentration of 20 mM), the CK2 activity was increased by 3-fold. Control experiments disclosed that under these conditions, the peptide β 1-16 was not phosphorylated by CK2 therefore indicating that the capacity

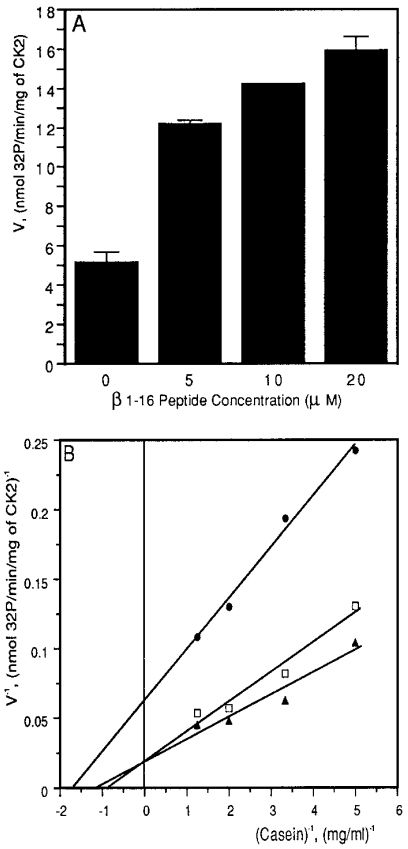

FIG. 2. Effect of the peptide β 1-16 on the phosphotransferase activity of CK2. (A) Recombinant Drosophila CK2 was assayed for phosphotransferase activity toward casein as described under experimental procedures in the presence of increasing concentrations of peptide β 1-16. (B) Lineweaver-Burk plots of the results obtained in the presence of different concentrations of the peptide β 1-16, control (●), 5 mM (□), and 20 mM (▲). Values are the means of at least two determinations. Similar results were obtained in at least three independent experiments.

of this peptide to stimulate the kinase activity is unrelated to its ability to act as a substrate. The peptide β1-16 stimulated CK2 activity to the same extend when the kinase activity was assayed with the synthetic peptide RRREEETEEE as a substrate (not shown). In contrast, no stimulation of the phosphotransferase activity of the free α subunit toward casein was observed upon addition of the peptide β 1-16 (not shown).

To further explore the mechanism by which this peptide stimulates the kinase activity, we examined the effect of the peptide β 1-16 on the phosphorylation by CK2 of different concentrations of casein. The data are illustrated in Fig 2B and Table I. The apparent Km value was only slightly modified by the presence of the peptide β 1-16 (ie 0.83 (mg/ml)-1 in the absence versus 0.61 (mg/ml)-1 in the presence of the peptide). However the Vm value of CK2 in the presence of the peptide β 1-16 (CK2/peptide ratio: 1/3250) was more than 3-fold higher than that in its absence (52.5 nmol 32P/min/mg of CK2 versus 16.4 nmol 32P/min/mg of CK2 respectively). We conclude that the stimulation of CK2 kinase activity by the peptide β 1-16 may reflect a conformationnal change of the kinase.

TABLE I

Effect of the Peptide β 1-16 on Kinetic Constants of the CK2 Holoenzyme

Peptide β1-16 (μM)	Vm (nmol ^{32}P/min/mg of CK2)	Km (mg/ml)	Efficiency Vm/Km
0	16.45	0.61	27
5	52.54	1.12	47
20	52.54	0.83	63.3

Kinetic constants derived from Lineweaver-Burk plots.

3. Effect of the peptide β 1-16 on CK2 autophosphorylation. Figure 3 shows the autophosphorylation of the β subunit in the presence of increasing concentrations of the peptide β 1-16. A 3.5 fold stimulation of the autophosphorylation was obtained for a CK2/peptide ratio of 1:5000. This result confirms that although peptide β 1-16 contains the autophosphorylation sites, the autophosphorylation reaction is not competed away by the peptide. Moreover, it demonstrates that the peptide β 1-16 stimulates the phosphorylation by CK2 of exogenous substrates as well as the autophosphorylation of the kinase.

4. Comparison of CK2 activity stimulation by the peptide β 1-16 and spermine. Naturally occuring poly-

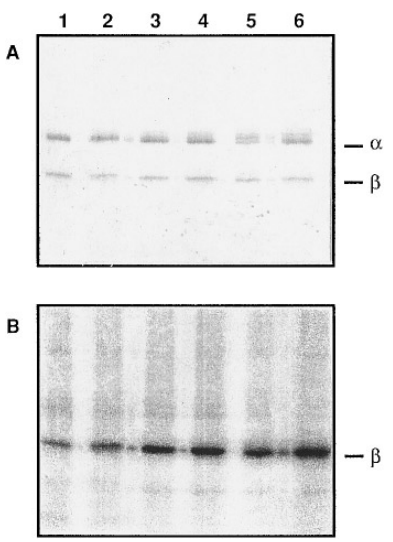

FIG. 3. Effect of peptide β 1-16 on the autophosphorylation of CK2 holoenzyme. CK2 was autophosphorylated as described in experimental procedures in the presence of increasing concentrations of peptide β 1-16. Samples were electrophoresed in 12% SDS-polyacrylamide gels, stained with Coomassie blue (A), and autoradiographed (B). Lanes 1-6, CK2:peptide β 1-16 ratio = 1/0, 1/100, 1/500, 1/1000, 1/2000, and 1/5000.

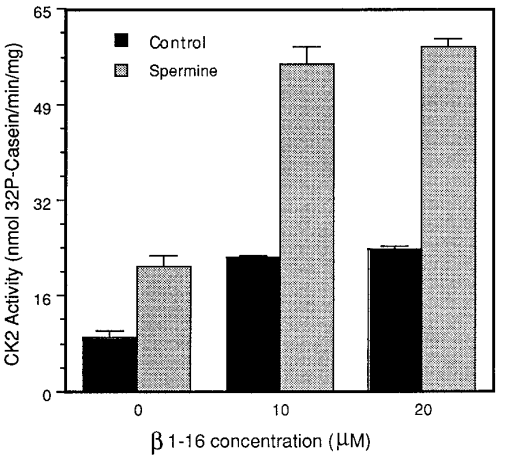

FIG. 4. Comparison of CK2 activation by peptide β 1-16 and spermine. Purified recombinant Drosophila CK2 (40 ng) was preincubated for 2h at 4°C with increasing concentration of peptide β 1-16 (ie 0 mM, 10 mM, 20 mM), in the absence or the presence of 1 mM spermine. Each assay was initiated by the addition of a solution containing substrates and cofactors of the enzyme. Protein kinase assay was performed at 20°C for 5 min in 10 mM Tris/HCl, pH 7.5, 0.1 M NaCl, 1 mM MgCl2, 10 μM ATP, and 0.3 mg/ml casein.

amines such as spermine are potent activators of CK2 (7). We previously showed that like peptide β 1-16, spermine prevents the thick filament formation and stabilizes CK2 under its ring-like structure (10). Figure 4 illustrates the effect of spermine on the stimulation of CK2 activity in the presence of increasing concentrations of the peptide β 1-16. It was observed that the extend of stimulation of CK2 activity by spermine (2.3-fold) was not affected by the presence of increasing concentrations of the peptide β 1-16. The data indicate that the stimulation of CK2 activity by the peptide β 1-16 and spermine are additive.

5. Stimulation of CK2 activity by β1-16 related peptides. In order to determine which region of the peptide β 1-16 was necessary for CK2 activation, different related peptides illustrated in Fig 5A were tested on CK2 activity. Figure 5B shows the stimulation of CK2 by the different peptides. Peptide β1-13 peptide was as efficient as peptide β1-16 in stimulating CK2 activity. Conversely, β7-16 peptide and β8-13 peptide showed a weaker stimulating effect. These results suggest that the N-terminal region of the peptide is important for its full stimulating effect whereas its C-terminal region is more dispensable.

DISCUSSION

Until now, no intracellular messenger involved in CK2 regulation has been identified. Using recombinant Drosophila melanogaster CK2, we have investigated the effect on the CK2 polymerization and CK2 activity by a synthetic peptide corresponding to the sixteen amino-acid N-terminus of the β subunit. Velocity sedimentation experiments show that the peptide β 1-16, like polyamines (10), prevents the thick filament formation and stabilizes the ring-like structure of the enzyme. This observation is in line with the unability of the catalytic subunit to polymerize without β subunit (10).Two simple models can explain this result: first, the peptide β 1-16 could compete with the N-terminus of the β subunit toward an interaction site involved in the formation of the thick filament. Second, the peptide could bind to CK2 inducing a conformationnal change of the kinase under which the ring-like structures are not able to associate in thick filaments. Experimentals evidence in favor of one of this two models are still lacking, and both models are not exclusive.

The peptide β 1-16 induces a stimulation of CK2 activity toward either casein or the CK2 specific peptide substrate (RRREEETEEE). With casein as the substrate, a maximal stimulation of 3-fold was reached for peptide concentration of 20 mM. The high peptide concentrations required to observed a CK2 stimulation (CK2/peptide ratio equal to 1:3900) could be explained if one assumes that the peptide adopts only transitorily its optimal conformation. Under the same conditions, it was previously shown that polyamines stimulate CK2 activity towards casein, but are ineffective to enhance the phosphorylation of the peptide substrate (17). This result is in line with the proposed model of an activating state of CK2 when the enzyme is under its ring-like structure (10) since it was observed that the peptide β 1-16 stabilises this molecular form.

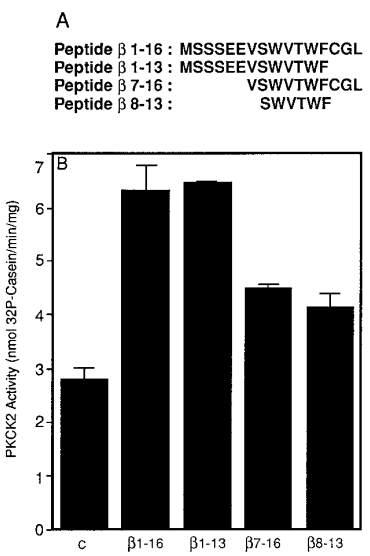

FIG. 5. Comparaison of different β1-16 related peptides on CK2 activity. Recombinant Drosophila CK2 (40 ng) was preincubated for 2h at 4°C with 20 μM peptide β1-16, β1-13, β7-16, β1-16, or β8-13. Each assay was initiated by the addition of a solution containing substrates and cofactors of the enzyme. Protein kinase assay was performed 20°C for 5 min with casein as a substrate.

Analysis of the kinetic constants of the phosphorylation of casein showed that the Km values slightly decreased in the presence of the peptide β 1-16 (i.e. 0.83 (mg/ml)-1 versus 0.61 (mg/ml)-1), reflecting that the peptide does not act as a competitive inhibitor toward casein nor it interacts with the catalytic pocket of the kinase. Moreover, in the presence of optimal amount of peptide β 1-16 the Vmax of the kinase increases by greater than 3-fold, sugesting that stimulation of CK2 by this synthetic peptide reflects a conformationnal change of the enzyme.

We have observed that the peptide β 1-16 was also able to enhance by more than 3-fold the extend of autophosphorylation of the β subunit in the holoenzyme. Thus, like for CK2 activity on exogeneous substrates, there is also a correlation between a high CK2 β subunit autophosphorylation in the presence of peptide β 1-16, and the ring-like structure conformation. Comparatively, it was previously observed that unlike the peptide β 1-16 the binding of polyamines did not affect the autophosphorylation of the CK2 β subunit (17).

Comparison of different β1-16 related peptides on CK2 activity revealed that the C-terminal part of the peptide is not necessary for its CK2 activating property, whereas the N-terminal region is important for its full activity. It is worthnoting that this region bears the autophosphorylation site of the kinase, and one may speculate on the possible regulation of CK2 polymerisation by its autophosphorylation. Bodenbach and al (19) demonstrated that point mutations on the autophosphorylation sites of the β subunit of CK2 does not change the specific activity of the kinase.

The fact that the peptide β 1-16 did not activate the free catalytic subunit suggests that this peptide may release an inhibitory constrainst imposed by the presence of the β subunit in the holoenzyme. By analogy Boldyreff and al (18) demonstrated that the acidic stretch (amino acid 55-64) localised in the β subunit is probably responsible for a negative control of CK2 activity since mutations of acidic amino acids within this strech to alanine lead to an hyperactive CK2 holoenzyme.

We have observed that like spermine (10), the peptide β 1-16 prevents the formation of CK2 thick filaments, stabilizes the ring-like structure and consequently stimulates CK2 activity towards casein. Since the thick filaments of CK2 represent poorly active forms of the enzyme, it is tempting to propose that like the acidic strech, the N-terminal region of the β subunit may also participate in the thick filament formation. However, our study demonstrated that this peptide and spermine may function in a different way for the following reasons: 1) unlike the peptide β 1-16 the polyamines did not affect the autophosphorylation of the CK2 β subunit (17) and the phosphorylation of the specific peptide substrate (RRREEETEEE) (17), 2) when tested in the same assay, the two molecules have strickly additive stimulating effect on CK2 activity.

Obviously, the final demonstration for the involvement of the N-terminal region of the β subunit in the control of CK2 activity will require site-directed point mutagenesis in the region. This study is in progress and it is hoped that it will shed new light on the control of the oligomeric organization of this protein kinase.

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