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A novel CK2-mediated activation of type II cAMP-dependent protein kinase through specific phosphorylation of its regulatory subunit (RIIα) in vitro ☆

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Abstract

The physiological significance of the casein kinase 2 (CK2)-mediated phosphorylation of type II cAMP-dependent protein kinase (PKAIIα) and free type II regulatory (R) subunit (RIIα) on their activities was mainly investigated in vitro. In these experiments, [γ-^{32}P]GTP was used as a phosphate donor for the CK2-mediated phosphorylation of free RIIα and PKAIIα (bovine heart) in vitro. It was found that: (i) CK2 phosphorylated only threonine (Thr)-residues of free RIIα and phosphorylated preferentially Thr-residues of the R subunit (RIIα) of PKAIIα (PKA RIIα) in vitro; (ii) this phosphorylation was selectively inhibited by quercetin (an CK2 inhibitor); and (iii) the phosphorylation of free RIIα by CK2 resulted in the reduction of its suppressive effect on the activity (phosphorylation of histone H2B) of the catalytic (C) subunit and in the reduction of its ability to form a complex with the C subunit in vitro. As expected, the activity of PKAIIα was approx. 3.5-fold enhanced after its R subunit was fully phosphorylated by CK2 in vitro. cAMP synergistically stimulated the activity of PKAIIα phosphorylated by CK2 in vitro. These results strongly suggest that CK2 may be a protein kinase responsible for the activation of PKAIIα through specific phosphorylation of its R subunit at the cellular level.

Keywords: Type II cAMP-dependent protein kinase; Casein kinase 2; Specific phosphorylation of regulatory subunit; Activation of PKAIIα

It is well known that CK2, a cAMP-, cGMP-, and Ca^{2+}/phospholipid-independent serine (Ser)/threonine (Thr)-protein kinase, plays important roles in the regulation of DNA replication, transcription, and cell proliferation through specific phosphorylation of their regulatory mediators in vitro and in vivo [1,2]. We have reported that: (i) CK2 mediates the stimulation of the activities of several glycyrrhizin (GL)-binding enzymes, such as soybean lipoxygenase 3 [3], secretory type IIA phospholipases A_2 purified from the synovial fluids of patients with rheumatoid arthritis [4], and two HIV-1 enzymes (reverse transcriptase [5] and protease [6]) in vitro and (ii) the CK2-mediated extreme phosphorylation of a 98 kDa nucleolin-like DNA-binding protein (p98) is involved in the initial stages of proliferation of T cells induced by interleukin 2 [7] and in the fertilization of sea urchin eggs [8,9].

Furthermore, it has been reported that CK2 can phosphorylate the regulatory (R) subunit of PKAII [10–12], p34^{cdc-2}-kinase [13], insulin receptor tyrosine (Tyr)-kinase [14], insulin growth factor-II (IGF-II) receptor Tyr-kinase [15], and cyclin-dependent protein kinase II [16] in vitro and in vivo. The PKA holoenzyme (R_2C_2) is a tetramer consisting of a dimer R subunit and two monomeric C subunits. The R subunit of PKA can be classified as type I or II based on its amino acid sequence [17]. Functionally, type I and II PKAs (PKAI and

☆ *Abbreviations:* PKA, cAMP-dependent protein kinase; C subunit, catalytic subunit; CK2, casein kinase 2; DTT, dithiothreitol; PKAI, type I PKA; PKAII, type II PKA; R subunit, regulatory subunit; RIα, α-isoform of the type I R subunit; RIIα, α-isoform of the type II R subunit; RIIβ, β-isoform of the type II R subunit; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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PKAII) can be distinguished readily on the basis of their potentials for autophosphorylation: PKAII contains autophosphorylation sites on its R subunit (RII), while PKAI is not autophosphorylated [17]. There are four R subunit genes (*RIα*, *RIβ*, *RIIα*, and *RIIβ*) and three C subunit genes (*Cα*, *Cβ*, and *Cγ*) in mammals [18,19]. The *RIα* gene is expressed in most tissues [20] and the RIIα isoform comprises more than 90% of the protein kinase and is found as a soluble enzyme in bovine tissues, such as heart, liver, and skeletal muscle [21,22]. Although it has been reported that CK2 phosphorylates only Ser-residues (Ser-74 and Ser-76) on the R subunit (RIIα) of PKAIIα (PKA RIIα) in vitro [10,11], the physiological significance of this phosphorylation on the activity of PKAIIα remains to be elucidated.

In this paper, we describe: (i) the phosphorylation kinetics of free RIIα and the RIIα of PKAIIα (PKA RIIα) by CK2 in vitro; (ii) characterization of quercetin as a potent selective inhibitor for the CK2-mediated phosphorylation of free RIIα in vitro; and (iii) the physiological significance of the CK2-mediated phosphorylation of free RIIα and PKAIIα on their activities in vitro.

Materials and methods

Chemicals. [γ-^{32}P]GTP (3000 Ci/mmol) and [γ-^{32}P]ATP (3000 Ci/mmol) were obtained from Amersham–Pharmacia Biotech. (Arlington Heights, USA); dithiothreitol (DTT) and quercetin (3,3′,4′,5,7-pentahydroxyflavone) were from Sigma Chemical (St. Louis, USA); anti-PKA C subunit and anti-RIIα sera were obtained from BD Bioscience (New York, USA).

Protein kinases. Purified PKA C subunit (specific activity: 83 U/μg protein) and free RIIα (inhibitory activity: 83 U/μg protein) were obtained from Sigma Chemical (St. Louis, USA). Recombinant human CK2 (rhCK2) [a heterodimer of $\alpha_2\beta_2$; specific activity: 400 kU/mg protein using a peptide substrate (RRREEETEEE) per minute at 30 °C at pH 7.5] was obtained from Biomol Research Laboratories (Plymouth Meeting, PA, USA). Both PKAIα and PKAIIα were further purified from the commercial PKA preparation (bovine heart muscle) using a Mono Q HPLC column, as previously reported [23].

Phosphorylation of free RIIα, PKAIα, and PKAIIα by CK2 in vitro. To phosphorylate free RIIα and two PKAs (PKAIα and PKAIIα) by CK2 in vitro, they (approx. 3 μg each) were separately incubated for 30 min at 30 °C in reaction mixtures comprising 40 mM Tris–HCl (pH 7.4), CK2 (approx. 50 ng), 1 mM DTT, 3 mM Mn^{2+}, and 5 μM [γ-^{32}P]GTP (500 cpm/pmol). ^{32}P-Labeled RIIα (p55) or two distinct R subunits (RIα and RIIα) of PKAIα and PKAIIα in the reaction mixtures were detected by autoradiography after SDS–PAGE, as reported previously [3–6,23].

Assay for the activities of PKAIα, PKAIIα, and the C subunit of PKA in vitro. The activities (phosphorylation of histone H2B) of PKAIα, PKAIIα, and the free C subunit of PKA (bovine heart muscle) were separately assayed at 30 °C in reaction mixtures comprising 40 mM Tris–HCl (pH 7.4), either PKAIα, PKAIIα or the C subunit (approx. 20 ng each), 5 μM [γ-^{32}P]ATP (500 cpm/pmol), 1 mM DTT, 10 mM Mg^{2+}, and 3 μg histone H2B (substrate). ^{32}P-Labeled histone H2B in the reaction mixtures was detected by autoradiography after SDS–PAGE, as reported previously [23]. The activities of both PKAIα and PKAIIα were separately measured in the absence of cAMP.

Results

Phosphorylation of free RIIα and PKA RIIα by CK2 in vitro

To confirm the phosphorylation of free RIIα and the RIIα of PKAIIα (PKA RIIα) by CK2 in vitro, either purified RIIα or PKAIIα (bovine heart) was incubated with CK2 and 5 μM [γ-^{32}P]GTP (500 cpm/pmol) in the presence or absence of 50 μM ATP, heparin (a CK2 inhibitor) or poly-Arg (a CK2 activator) in vitro. Both RIIα (lane 3) and PKA RIIα (lane 4, Fig. 1A) were phosphorylated by CK2 in vitro, as it has been reported [10–12]. The CK2-mediated phosphorylation of free RIIα was completely inhibited by 50 μM ATP (lane 2), heparin (lane 3) or poly-Arg (lane 4, Fig. 1B). Under the given experimental conditions, CK2 phosphorylated only the Thr-residues on RIIα (lane 1) and phosphorylated Thr in preference to Ser in PKA RIIα (lane 2, Fig. 1C).

The CK2-mediated phosphorylation of free RIIα increased time-dependently up to 60 min at 30 °C and

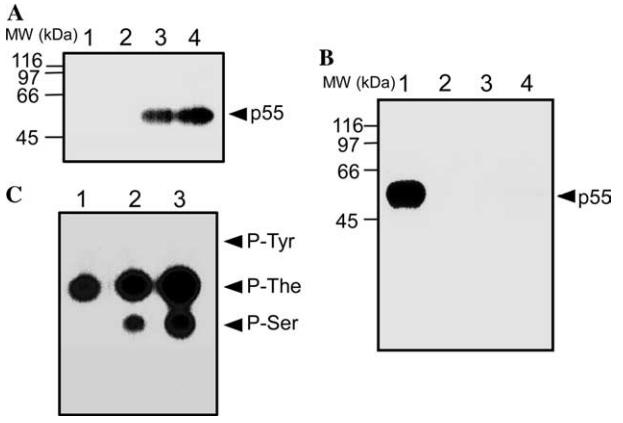

Fig. 1. Phosphorylation of free RIIα and the RIIα of PKAIIα by CK2 in vitro. (A) Purified bovine heart RIIα or PKAIIα (approx. 3 μg each) was incubated with CK2 (approx. 50 ng) in reaction mixtures comprising 40 mM Tris–HCl (pH 7.8), 5 μM [γ-^{32}P]GTP (500 cpm/pmol), 3 mM Mn^{2+}, and 1 mM DTT. After incubation for 30 min at 30 °C, ^{32}P-labeled RIIα (p55) or RIIα (p55) of PKAIIα in the reaction mixtures was detected by autoradiography after SDS–PAGE. Lane 1, RIIα alone; lane 2, PKAIIα alone; lane 3, RIIα + CK2; and lane 4, PKAIIα + CK2. (B) Purified RIIα (approx. 5 U) was incubated with CK2 (approx. 50 ng) in reaction mixtures comprising 40 mM Tris–HCl (pH 7.8), 5 μM [γ-^{32}P]GTP (500 cpm/pmol), 3 mM Mn^{2+}, and 1 mM DTT. After incubation for 30 min at 30 °C in the presence (lane 2) or absence (lane 1) of 50 μM ATP, poly-Arg (0.2 μg, lane 3) or heparin (0.2 μg, lane 4), ^{32}P-labeled RIIα (p55) in the reaction mixtures was detected by autoradiography after SDS–PAGE. (C) ^{32}P-Labeled RIIα, PKAIIα, and α-casein were separately hydrolyzed in 6 N HCl at 110 °C for 120 min. After the mixtures were applied on a cellulose TLC-plate, ^{32}P-labeled amino acids on the plate were separated by TLC and detected by autoradiography. Lane 1, RIIα; lane 2, PKAIIα; and lane 3, α-casein. Arrows indicate the positions of phosphotyrosine (P-Tyr), phosphothreonine (P-Thr), and phosphoserine (P-Ser), visualized by ninhydrin staining.

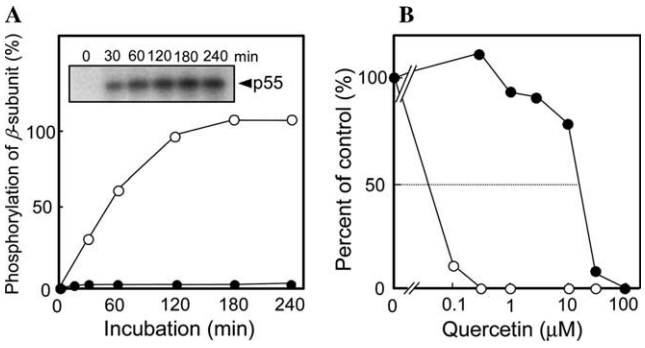

Fig. 2. (A) The kinetics of the CK2-mediated phosphorylation of free RIIα in vitro. Purified RIIα (approx. 5 U) was incubated with CK2 (approx. 50 ng) in reaction mixtures, as described in Fig. 1A. (Inset) After incubation for the indicated periods (0–240 min) at 30 °C, ^{32}P-labeled RIIα (p55) in the reaction mixtures was detected by SDS–PAGE followed by autoradiography. The autoradiogram was scanned with a spectrophotometer. 100% represents the phosphorylation of free RIIα by CK2 for 180 min. RIIα incubated with (○) and without CK2 (●). (B) The inhibitory effect of quercetin on the CK2-mediated phosphorylation of RIIα and on the activity of the C subunit in vitro. The inhibitory effect of quercetin on the CK2-mediated phosphorylation of free RIIα and on the activity of the C subunit in vitro was separately determined. After incubation (30 min at 30 °C) in the presence of the indicated doses of quercetin, ^{32}P-labeled RIIα (p55) in the reaction mixtures was determined, as described in B. The autoradiogram was scanned with a spectrophotometer. 100% represents the rate of CK2-mediated phosphorylation of RIIα determined in the absence of quercetin. (●) Effect of quercetin on the PKAII activity and (○) effect of quercetin on the phosphorylation of RIIα by CK2.

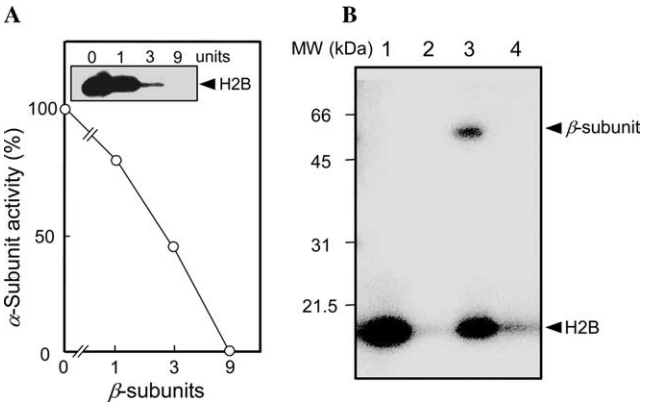

Fig. 3. (A) The suppressive effect of RIIα on the activity of the C subunit in vitro. The suppressive effect of RIIα on the activity (phosphorylation of histone H2B) of the C subunit was determined after incubation (20 min at 30 °C) of the C subunit with the indicated doses of RIIα. Lane 1, C subunit (control); and the C subunit incubated with the indicated units of RIIα. The autoradiogram was scanned with a spectrophotometer. 100% represents the activity of the C subunit incubated without RIIα. (B) The effect of the CK2-mediated phosphorylation of free RIIα on the activity of the C subunit in vitro. To determine the effect of the CK2-mediated phosphorylation of free RIIα on the activity of the C subunit, initial reaction mixtures comprising 40 mM Tris–HCl (pH 7.8), CK2 (approx. 50 ng), 5 μM [γ-^{32}P]GTP (500 cpm/pmol), 3 mM Mn^{2+}, and 1 mM DTT were incubated for 120 min at 30 °C. Thereafter, histone H2B (4 μg) and 5 μM [γ-^{32}P]ATP (500 cpm/pmol) were added to the reaction mixtures, which were incubated for a further 15 min at 30 °C. ^{32}P-Labeled histone H2B in the reaction mixtures was determined by autoradiography after SDS–PAGE. Lane 1, C subunit (approx. 5 U) incubated without CK2 or RIIα; lane 2, RIIα (approx. 9 U) without CK2 + C subunit; lane 3, RIIα incubated with CK2 + C subunit; and lane 4, RIIα incubated with CK2, in the presence of 0.3 μM quercetin, + C subunit.

reached a plateau within 180 min (Fig. 2A). The CK2-mediated phosphorylation of RIIα was inhibited by quercetin (ID_{50} = approx. 30 nM), but a high dose (ID_{50} = approx. 20 μM) was required to inhibit the activity of the C subunit in vitro (Fig. 2B). A similar inhibitory effect of quercetin on the activity of PKAIIα was observed in vitro (data not shown).

Effect of the CK2-mediated phosphorylation of free RIIα on the activity of the C subunit in vitro

As reported previously [23], free RIIα suppressed the activity (phosphorylation of histone H2B) of the C subunit in a dose-dependent manner (Fig. 3A). On the basis of the above two experimental results (Figs. 2B and 3A), the physiological effect of the CK2-mediated phosphorylation of free RIIα on the activity of the C subunit was determined in vitro. The phosphorylation of histone H2B by the C subunit or by the PKAIIα was quantitatively determined using [γ-^{32}P]ATP, since CK2 does not phosphorylate histone H2B and PKAIIα can utilize only [γ-^{32}P]ATP as a phosphate donor in vitro. The activity of the C subunit was highly reduced when it was incubated with nonphosphorylated RIIα (lane 2, Fig. 3B). After the incubation (150 min at 30 °C) of free RIIα with CK2 and 5 μM cold GTP in vitro, the effect of phosphorylated RIIα on the activity of the C subunit was determined. The phosphorylation of RIIα by CK2 resulted in a significant reduction of its suppressive effect on the activity of the C subunit (lane 3, Fig. 3B). This lowered suppression was completely prevented by including 0.3 μM quercetin in the CK2 incubation step (lane 4, Fig. 3B).

Reduction of the ability of RIIα to form a complex with the C subunit after its specific phosphorylation by CK2 in vitro

The ability of RIIα to form a complex with the C subunit was examined after its full phosphorylation by CK2 in vitro. After the preincubation (150 min at 30 °C) of RIIα (approx. 2 U) with CK2 and 5 μM [γ-^{32}P]GTP (500 cpm/pmol) in vitro, the C subunit (approx. 1 U) was added to the reaction mixtures and then immunoprecipitated with anti-RIIα serum in the presence of protein A. After centrifugation to separate the immunoprecipitates, the amounts of ^{32}P-labeled RIIα (p55) in the supernatant and precipitated fractions were separately determined. The amount of ^{32}P-labeled RIIα in the supernatants (lanes 1 and 3) was reduced, as compared with the unphosphorylated control (lanes 3 and 4,

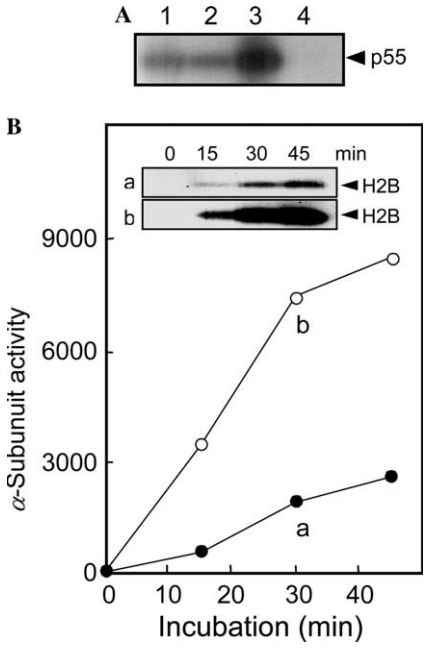

Fig. 4. The effect of the CK2-mediated phosphorylation of free RIIα on its ability to form a complex with the C subunit in vitro. (A) Purified RIIα (approx. 2 U) was incubated for 120 min at 30 °C with CK2 (approx. 50 ng) in reaction mixtures, as described in Fig. 1A. The C subunit (approx. 4 U) was then added to the reaction mixtures, which were further incubated for 60 min at 30 °C. Anti-RIIα serum and protein A were added to immunoprecipitate free RIIα in the reaction mixtures containing 1.0 M NaCl. After centrifugation (7000 rpm for 5 min, five times), ^{32}P-labeled RIIα (p55) in the supernatant and immunoprecipitated fractions was detected by SDS–PAGE followed by autoradiography. ^{32}P-Labeled RIIα (p55) in the supernatant (lane 1) and precipitated (lane 2) fractions from the immunoprecipitation obtained with anti-RIIα serum; and the supernatant (lane 3) and precipitated (lane 4) fractions from the immunoprecipitation with normal serum. (B) The activity of the C subunit in the supernatants following the immunoprecipitation with anti-RIIα serum in the presence of protein A. The activity of the C subunit was determined by incubation with 5 μM [γ-^{32}P]ATP (500 cpm/pmol) and histone H2A for the indicated periods at 30 °C. (○) Supernatant from the immunoprecipitation of unphosphorylated RIIα and (●) supernatant from the immunoprecipitation of phosphorylated RIIα.

Fig. 4A). The amount of ^{32}P-labeled RIIα in the supernatant decreased dependent upon an increased incubation period of RIIα with CK2 in the presence of 5 μM [γ-^{32}P]GTP (data not shown). The activity of the C subunit remaining in the supernatant following immunoprecipitation of phosphorylated RIIα was significantly higher than that of unphosphorylated RIIα (Fig. 4B). These results show that the ability of RIIα to form a complex with the C subunit is diminished by its phosphorylation with CK2 in vitro.

The CK2-mediated activation of PKAIIα in vitro

The physiological effect of the CK2-mediated phosphorylation of PKAIIα on its activity (phosphorylation of histone H2B) was determined in vitro. The activity of

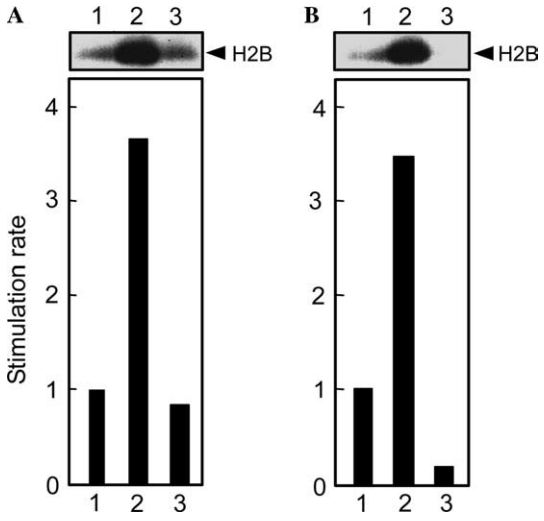

Fig. 5. The stimulation of the activities of both PKAIα and PKAIIα after their R subunits (RIIα and RIα) by CK2 in vitro. To determine the CK2-mediated stimulation of the PKAIIα activity in vitro, initial reaction mixtures comprised PKAIα or PKAIIα (approx. 3 μg) and the same components, as described in Fig. 1A. Subsequently, histone H2B (approx. 4 μg) and 5 μM [γ-^{32}P]ATP (500 cpm/pmol) were added to the reaction mixtures, which were further incubated for 15 min at 30 °C. ^{32}P-Labeled histone H2B in the reaction mixtures was determined by autoradiography after SDS–PAGE. (A) Lane 1, PKAIIα incubated without CK2; lane 2, PKAIIα incubated with CK2; and lane 3, lane 2 + 0.3 μM quercetin. (B) Lane 1, PKAIα incubated without CK2; lane 2, PKAIα incubated with CK2; and lane 3, lane 2 + 0.3 μM quercetin.

PKAIIα was enhanced approx. 3.5-fold when its R subunit was fully phosphorylated by CK2 in vitro (lane 2) and this stimulation was completely blocked by 0.3 μM quercetin (lane 3, Fig. 5A). Under the same experimental conditions, a similar CK2-mediated stimulation of PKAIα activity was observed (Fig. 5B). These results show that the full phosphorylation of at least two PKAs (PKAIα andPKAIIα) by CK2 in the absence of cAMP results in their significant activation in vitro. In addition, the effect of cAMP on the activity of PKAIIα was determined after its phosphorylation by CK2 in the presence of cold GTP in vitro. Interestingly, it was found that: (i) the cAMP-induced activation of PKAIIα was more effective than the CK2-mediated activation of PKAIIα and (ii) cAMP synergistically stimulated the activity of PKAIIα phosphorylated by CK2 (lane 5), as compared with the cAMP-induced effect on the activity of unphosphorylated PKAIIα in vitro (lane 2, Fig. 6).

Discussion

In the present study, we observed that CK2 phosphorylates only Thr-residues on free RIIα (lane 1) and preferentially phosphorylates the Thr-residues on PKA RIIα (lane 2, Fig. 1C) using [γ-^{32}P]GTP as a phosphate donor to eliminate autophosphorylation of PKAIIα

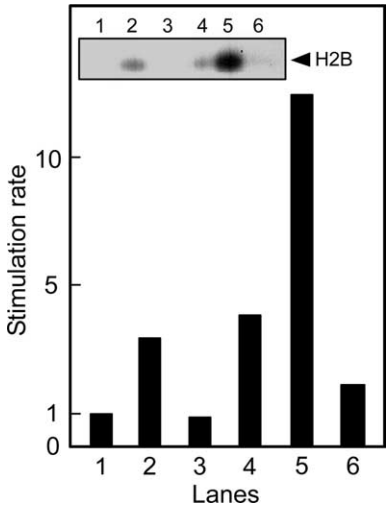

Fig. 6. The effect of cAMP on the activities of two PKAIIs after the phosphorylation of their R subunits by CK2 in vitro. The effect of cAMP on the activities of two PKAs [bovine heart PKAIα (A) and rat brain PKAIIα (B)] after the phosphorylation of their R subunits by CK2 in the presence of 5 μM GTP in vitro. The first reaction mixtures comprising 40 mM Tris–HCl (pH 7.8), PKAIα or PKAIIα (approx. 2 μg), CK2 (approx. 50 ng), 5 μM GTP, 3 mM Mn^{2+}, and 1 mM DTT were incubated for 150 min at 30 °C. The second reaction mixtures comprising 40 mM Tris–HCl (pH 7.6), 5 μM [γ-^{32}P]ATP (500 cpm/pmol), 0.5 mM Mn^{2+}, 1 mM DTT, and histone H2B (5 μg) were further incubated for a further 15 min at 30 °C. ^{32}P-Labeled histone H2B in the reaction mixtures was detected in the presence or absence of 2 μM cAMP by autoradiography after SDS–PAGE. Lane 1, PKAIIα alone; lane 2, lane 1 + CK2; lane 3, lane 2 + 0.3 μM quercetin; lane 4, lane 1 + 3 μM cAMP; lane 5, lane 4 + CK2; and lane 6, lane 5 + 0.3 μM quercetin. Values obtained from the autoradiogram (inset) by scanning with a spectrophotometer. The stimulation rate 1 represents the phosphorylation of histone H2B by PKAIIα in the absence of both cAMP and CK2 in vitro.

in vitro. Our observation (Fig. 1C) is different from earlier reports [10,11] that CK2 phosphorylates only Ser-residues (Ser-74 and Ser-76) on PKA RIIα in the presence of [γ-^{32}P]ATP in vitro. This discrepancy may be due to the choice of a phosphate donor either GTP or ATP for CK2 in vitro, because the R subunit of PKAIIα is autophosphorylated at Ser-95 upon incubation with ATP [10]. In addition, poly-Arg (a CK2 activator) effectively reduced the CK2-mediated phosphorylation of free RIIα in vitro (lane 3, Fig. 1B). This poly-Arg-induced reduction may be due to the structural character of RIIα containing Arg- and Lys-rich domains, as has been demonstrated in two HIV-I enzymes (reverse transcriptase [5] and protease [6]) and lactoferrins [24].

The physiological effect of the CK2-mediated phosphorylation of free RIIα on the activity of the C subunit was determined in vitro. It was concluded that CK2 may be a protein kinase responsible for the activation of PKAIIα through phosphorylation of its R subunit (RIIα) by CK2 in the absence of cAMP in vitro from the following observations: (i) the phosphorylation of free RIIα by CK2 results in a significant reduction of its suppressive effect on the activity of the C subunit (lane 3, Fig. 3B); (ii) the ability of RIIα to form a complex with the C subunit is diminished when the RIIα is fully phosphorylated by CK2 in vitro (Fig. 4); (iii) the activities of both PKAIα and PKAIIα are significantly enhanced through phosphorylation of their R subunits (RIα and RIIα) by CK2 in vitro (Fig. 5); and (iv) this enhancement is completely blocked by 0.3 μM quercetin (lane 3, Figs. 5A and B).

A database analysis revealed at least five potential phosphorylation sites [S/T-X-X-E/D: one Thr-residue (position 286) and four Ser-residues] for CK2 on bovine heart RIIα (400 amino acids). The Thr-286 of RIIα may be specifically phosphorylated by CK2 in the presence of GTP in vitro, since: (i) there is a potential phosphorylation Thr-residue (position 286) for CK2 on bovine heart RIIα [25]; (ii) only Thr-residue is detected as a phosphoamino acid in free RIIα phosphorylated by CK2 in the presence of 5 μM [γ-^{32}P]GTP (lane 1, Fig. 1C); and (iii) approx. 1 mol of γ-phosphate of either ATP or GTP is incorporated into a molecule of RIIα by CK2 under the given experimental conditions in vitro. Interestingly, cAMP synergistically stimulated the activity of PKAIIα fully phosphorylated by CK2 in vitro (lane 5, Fig. 6). The synergistic effect of cAMP on the PKAIα-mediated phosphorylation of histone H2B was also observed in vitro (data not shown). These results suggest that the protein phosphorylation mediated by these two PKAs (PKAIα and PKAIIα) may be highly enhanced by cAMP after the specific phosphorylation of their R subunits (RIα and RIIα) by CK2 at the cellular level.

PKA is known to be involved in the cAMP signaling pathway of endocrine cell functioning, following the action of adenylate cyclase resulting from the activation of G-protein-coupled receptors [21,22]. The cAMP-mediated activation of PKA is well explained by the binding of cAMP to specific high affinity receptor sites on the R subunit, resulting in the release of free active C subunit. Herein, we propose a novel CK2-mediated activation of PKAIIα in the absence of cAMP. This model is supported by evidence that CK2 is a protein kinase responsible for the activation of both PKAIIα and PKAIα in vitro (Fig. 5). Furthermore, it seems likely that the CK2-mediated activation of both PKAIα and PKAIIα may be closely coupled to the enhanced activation of CK2 in the initial stages of cells infected with viruses, and in fertilized eggs and malignant cells [26]. This assumption is supported by our previous observations that: (i) CK2 is highly activated in the initial stages of proliferating T cells treated with interleukin 2 [7], in the fertilization of sea urchin eggs [8,9], and in cells infected with viruses (HIV-1 [5] and vesicular stomatitis virus [27]) and (ii) the activated CK2 phosphorylates a number of functional cellular proteins, including metabolic enzymes, involved in cell proliferation [9,10] as

well as viral proteins involved in viral replication [5,27]. In addition, it has reported that an increased CK2 level is associated with increased cellular proliferation and response to stress, and loss of CK2 activity is associated with cell death [28].

To understand clearly the biological significance of the CK2-mediated activation of both PKAIα and PKAIIα in the PKA-mediated signal transduction at the cellular level, further analytical studies are required: (i) to detect the CK2-mediated specific phosphorylation of these two PKAs at the cellular level; (ii) to determine the specific phosphorylation sites (Thr) for CK2 on both RIα and RIIα in vitro and in vivo; and (iii) to biochemically characterize the CK2-mediated regulation of their substrate specificities and their physiological interaction with the PKA anchoring proteins in vitro.

Acknowledgments

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