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# High phosphorylation of HBV core protein by two α-type CK2-activated cAMP-dependent protein kinases in vitro

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**Abstract** **Two α-type CK2-activated PKAs (CK2-aPKAIα and CK2-aPKAIIα) were biochemically characterized in vitro using GST-HBV core fusion protein (GST-Hcore) and GST-Hcore157B as phosphate acceptors. It was found that (i), in the absence of cAMP, these two CK2-aPKAs phosphorylated both Ser-170 and Ser-178 on GST-Hcore and Hcore157B; (ii) this phosphorylation was approx. 4-fold higher than their phosphorylation by cAMP-activated PKAs; and (iii) suramin effectively inhibited the phosphorylation of Hcore157B by CK2-aPKAIIα through its direct binding to Hcore157B in vitro. These results suggest that high phosphorylation of HBV-CP by two CK2-aPKAs, in the absence of cAMP, may be involved in the pregenomic RNA (pgRNA) encapsidation and DNA-replication in HBV-infected cells.**


*Keywords:* cAMP-dependent protein kinase; Casein kinase 2; CK2-activated PKA; Human hepatitis B virus core protein; High phosphorylation of HBV-CP

## 1. Introduction

Previous studies have revealed that (i) hepatitis B virus (HBV) core protein (HBV-CP) is a 21 kDa phosphoprotein (185 amino acid residues) in intact cells [1]; (ii) the Ser-residues (positions 155, 162 and 170 in the strain *ayw*) of three adjacent SPRRR repeats in the arginine (Arg)-rich C-terminal region of HBV-CP are phosphoacceptor sites in vivo [2]; and (iii) the three major phosphorylation sites in the HBV-CP sequence (strain *adw*) are Ser-residues at positions 157, 164 and 172 [2]. Recently, Melegari et al. reported that specific phosphorylation of both Ser-162 and Ser-170 (strain *ayw*) on HBV-CP is implicated in promoting the sequential progression of HBV DNA-replication [3]. Several intracellular protein kinases, such as cyclin-dependent protein kinase Cdc2 [4], cAMP-dependent protein kinase (PKA) [5], $Ca^{2+}$- and phopholipid-dependent protein kinase (PKC) [5], 46-kDa serine protein kinase [6] and serine (S)/arginine (R) protein-specific kinases 1 (95 kDa SRPK1) and 2 (105 kDa SRPK2) [7], have been found to preferentially phosphorylate Ser-residues at the C-terminal region of HBV-CP in vitro. Mutational analysis strongly suggests that the preferential phosphorylation of both Ser-162 and Ser-170 on HBV-CP (in the strain *ayw*) by some intracellular protein kinases is critical for subsequent pregenomic RNA packaging [3,8].

Recently, we reported that full phosphorylation of two α-type PKAs (PKAIα and PKAIIα) by casein kinase 2 (CK2) results in their significant activation in vitro [9]. However, still remaining to be elucidated are the substrate requirements and potent inhibitors for two α-type CK2-activated PKAs (CK2-aPKAs) in vitro. Therefore, the aim of the present study was to characterize the substrate requirements of these two CK2-aPKAs using glutathione-*S*-transferase (GST)-Hcore (GST-full length HBV-CP) and GST-Hcore fusion polypeptide (Hcore157B), containing three potent phosphorylation sites (Thr-162, Ser-170 and Ser-178 in the strain *adw*) for PKA, as phosphate acceptors. This is the first report describing high phosphorylation of HBV-CP by two CK2-aPKAs in vitro.

## 2. Materials and methods

### 2.1. Materials

[γ-$^{32}$P]ATP (3000 Ci/mmol) was obtained from Amersham Pharmacia Biotech. (Arlington Heights, USA); quercetin, suramin (a polysulfonated napthylurea), histone H2B, protamine (rainbow trout sperm), PKAIα and PKAIIα (bovine heart muscle) were obtained from Sigma Chemical (St. Louis, USA). Two PKAs (PKAIα and PKAIIα) were separately purified by Mono Q column chromatography (HPLC), as previously reported [9]. Recombinant human CK2 was obtained from Biomol Research Laboratories (Plymouth Meeting, PA, USA). Galloyl pedunculagin (GP, a potent PKA inhibitor [10]) was a generous gift from Dr. T. Tanaka (Nagasaki University, Japan).

### 2.2. Preparation of GST-Hcore and GST-Hcore fusion polypeptides

GST-Hcore fusion proteins were prepared, as described by Kuroki et al. [11] using a glutathione–Sepharose column. The bound GST fusion proteins on the affinity column were eluted separately with 50 mM Tris–HCl (pH 8.0) containing 20 mM reduced glutathione. In addition, three Hcore164B variants [Hcore164B-AS (replacement of Ser-170 with Ala), Hcore164B-SA (replacement of Ser-178 with Ala), and Hcore164B-AA (replacement of both Ser-170 and Ser-178 with Ala)] were prepared.

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*Abbreviations:* cAMP-aPKA, cAMP-activated PKA; C-subunit, catalytic subunit; CK2, casein kinase 2; CK2-aPKA, CK2-activated PKA; DTT, dithiothreitol; GP, galloyl pedunculagin; GST, glutathione-*S*-transferase; HBV, hepatitis B virus; HBV-CP, HBV-core protein; PKA, cAMP-dependent protein kinase; PKC, $Ca^{2+}$- and phopholipid-dependent protein kinase; PKAI, type I PKA; PKAII, type II PKA; QCM, quartz crystal microbalance; R-subunit, regulatory subunit; RIα, α-isoform of the type I R-subunit; RIIα, α-isoform of the type II R-subunit; SDS–PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis

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*2.3. Preparation of two CK2-activated PKAs in vitro*

Purified PKAIα or PKAIIα (approx. 50 ng each) was incubated for 120 min at 30 °C in reaction mixtures comprising 40 mM Tris–HCl (pH 7.6), CK2 (approx. 50 ng), 2 mM dithiothreitol (DTT), 3 mM $Mn^{2+}$ and 10 μM GTP. The phosphorylated forms (CK2-aPKAIα and CK2-aPKAIIα) of these two PKAs were used, as previously reported [9].

*2.4. Detection of $^{32}$P-labeled GST-Hcore or GST-Hcore fusion polypeptides by SDS–PAGE followed by autoradiography*

Either GST-Hcore or GST-Hcore fusion polypeptides were added to reaction mixtures (50 μl) comprising 40 mM Tris–HCl (pH 7.6), 2 mM DTT, 3 mM $Mn^{2+}$, 5 μM [γ-$^{32}$P]ATP (500 cpm/pmol) and two PKAs or CK2-aPKAs (approx. 50 ng each). After incubation for 30 min at 30 °C, $^{32}$P-labeled GST-Hcore or GST-Hcore fusion polypeptides in the reaction mixtures were detected separately by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS–PAGE) followed by autoradiography, as previously reported [9,10]. To determine the phosphorylation kinetics, $^{32}$P-labeled substrates phosphorylated by two PKAs or CK2-aPKAs were arrested by the addition of 1.0 ml of 20% trichloroacetic acid (TCA) and 0.5 ml of 0.1 M sodium pyrophosphate containing bovine serum albumin (1 mg/ml) and 10 mM EDTA. The TCA-insoluble precipitates trapped on a glass membrane filter (Advantic GF/75, Tokyo, Japan) was measured with a liquid scintillation spectrophotometer, as previously reported [10].

## 3. Results

*3.1. Phosphorylation of GST-Hcore and GST-Hcore fusion polypeptides by PKAIα and PKAIIα in vitro*

Six distinct GST-Hcore fusion polypeptides were manufactured, as illustrated in Fig. 1. To detect the phosphorylation of GST-Hcore and these six GST-Hcore fusion polypeptides by two PKAs, they (approx. 5 μg each) were incubated separately with either PKAIα or PKAIIα and 5 μM [γ-$^{32}$P]ATP (500 cpm/pmol) in the presence or absence of 1 μM cAMP. Fig. 2A shows that (i) PKAIIα phosphorylates GST-Hcore in vitro (lane 2); and (ii) this phosphorylation is significantly stimulated by 1 μM cAMP (lane 3), but completely inhibited by 1 μM GP (lane 4, Fig. 2A). A similar phosphorylation of GST-Hcore was observed with PKAIα (Fig. 2B). Two GST-Hcore fusion polypeptides [Hcore157B (lane 2) and Hcore-164B (lane 3, Fig. 2C)], containing Ser-170 and Ser-178, were highly phosphorylated by PKAIIα in the presence of 1 μM cAMP in vitro. In contrast, the phosphorylation of both Hcore6477 (lane 5) and Hcore7077, containing Ser-170 (lane 7, Fig. 2C), by PKAIIα was about 2% of the phosphorylation of Hcore157B. No phosphorylation of recombinant GST (lane 1) and two other GST-Hcore fusion polypeptides [Hcore5769 (lane 4) and Hcore5763 (lane 6, Fig. 2C)] by PKAIIα were detected. Only phosphoserine was detected in GST-Hcore (lane 1) and Hcore157B (lane 2, Fig. 2D) phosphorylated by PKAIIα in the presence of 1 μM cAMP in vitro. Under the same experimental conditions, similar results were obtained with PKAIα instead of PKAIIα (data not shown).

Using Hcore164B and three Hcore164B variants (Hcore164B-AS, Hcore164B-SA and Hcore164B-AA), the phosphorylation sites for two PKAs (PKAIα and PKAIIα) on Hcore164B were determined in vitro. No phosphorylation was detected when Hcore164B-AA was used as a phosphate acceptor for PKAIIα (lane 6, Fig. 3). The phosphorylation level of Hcore164B-AS (lane 4) by PKAIIα was about half of the phosphorylation of Hcore164B (lane 2, Fig. 3). In contrast, Hcore164B-SA (lane 5) functioned as a poor substrate for PKAIIα, as compared with Hcore164B-AS (lane 4, Fig. 3). Under the same experimental conditions, similar results were observed in the phosphorylation of Hcore164B and three Hcore164B variants by PKAIα (data not shown). These results suggest that PKAIIα as well as PKAIα phosphorylate both Ser-170 and Ser-178 on Hcore164B in vitro.

As demonstrated in our previous report [9], it was confirmed that (i) free RIIα was phosphorylated by CK2 in vitro (lane 3, Fig. 4A); and (ii) this phosphorylation was significantly stimulated by 10mer-Arg (lane 4), but completely inhibited by 1 μM quercetin (lane 6) or cold 10 μM GTP (lane 5, Fig. 4A). Under the given experimental conditions, the CK2-mediated phosphorylation of free RIIα was approx. 3.3-fold stimulated at a low concentration (0.3 μg/ml) of GST-Hcore, but did not stimulate at concentrations higher than 10 μg/ml (Fig. 4B). The CK2-mediated phosphorylation of free RIIα was stimulated by Hcore157B as effective as 10mer-Arg up to approx. 3.8-fold in a dose-dependent manner in vitro (Fig. 4B). However, no phosphorylation of GST-Hcore and GST-Hcore fusion polypeptides by CK2 was detected.

*3.2. Characterization of CK2-aPKAIIα in vitro*

On the basis of the above results (Fig. 2), CK2-aPKAIIα was characterized in vitro using Hcore157B as a phosphate acceptor. As expected, the phosphorylation of Hcore157B by CK2-aPKAIIα, in the absence of cAMP, increased in a time-dependent manner within 20 min (Fig. 5A). However,

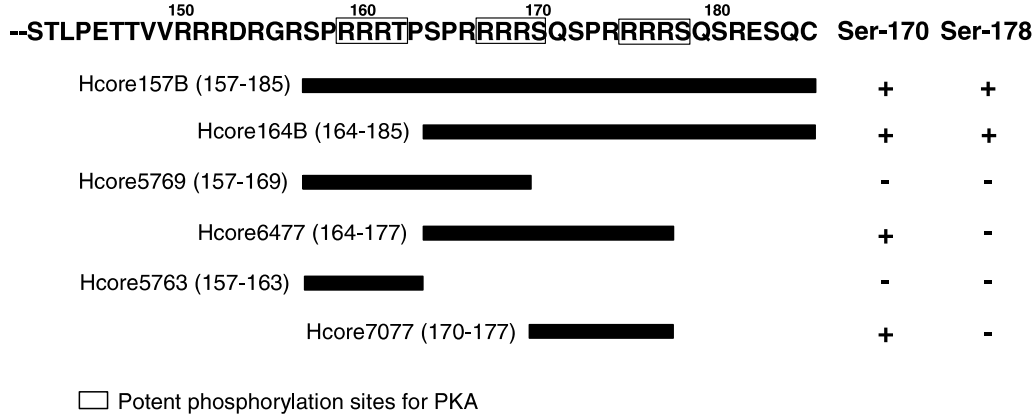


Fig. 1. Diagram of six different GST-Hcore fusion polypeptides (positions 157 and 185), containing three potent phosphorylation sites (RRXS/T: Thr-162, Ser-170 and Ser-178 in the strain *adw*) for PKA.

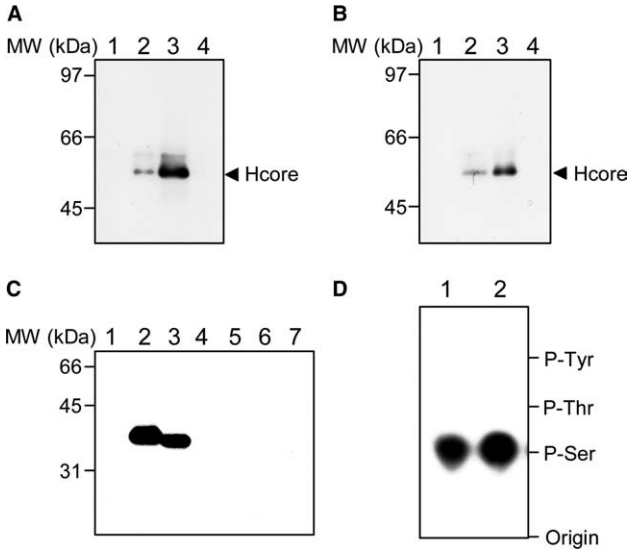


Fig. 2. Phosphorylation of GST-Hcore and six GST-Hcore fusion polypeptides by PKAIIα in vitro and analysis of their phosphoamino acids. (A) To determine the phosphorylation of GST-Hcore by PKAIIα in vitro, the reaction mixtures comprised 40 mM Tris–HCl (pH 7.6), PKAIIα (approx. 50 ng), 3 mM $Mn^{2+}$, 1 mM DTT, GST-Hcore (approx. 5 μg) and 5 μM [γ-$^{32}$P]ATP (500 cpm/pmol) were incubated for 30 min at 30 °C in the presence or absence of 1 μM cAMP. $^{32}$P-Labeled GST-Hcore in the reaction mixtures was detected by autoradiography after SDS–PAGE. Lane 1, PKAIIα alone; lane 2, PKAIIα incubated with GST-Hcore; lane 3, lane 2 + 1 μM cAMP; and lane 4, lane 3 + 1 μM GP. (B) Phosphorylation of GST-Hcore by PKAIα in vitro (protocol as described in the legend of (A)). After incubation for 30 min at 30 °C, $^{32}$P-labeled GST-Hcore in the reaction mixtures was detected by autoradiography after SDS–PAGE. Lane 1, PKAIα alone; lane 2, PKAIα incubated with GST-Hcore; lane 3, lane 2+1 μM cAMP; and lane 4, lane 3+1 μM GP. (C) Six GST-Hcore fusion polypeptides (approx. 5 μg each) were incubated separately with PKAIIα (approx. 50 ng) in reaction mixtures, as described in the legend of (A). After incubation for 30 min at 30 °C, $^{32}$P-labeled GST-Hcore fusion polypeptides in the reaction mixtures including 1 μM cAMP were detected by autoradiography after SDS–PAGE. Lane 1, GST (control); lane 2, Hcore157B; lane 3, Hcore164B; lane 4, Hcore5769; lane 5, Hcore 6477; lane 6, Hcore5763; and lane 7, Hcore7077. (D) After incubation of GST-Hcore or Hcore157B with PKAIIα and 5 μM [γ-$^{32}$P]ATP in the presence of 1 μM cAMP for 60 min at 30 °C, $^{32}$P-labeled GST-Hcore or Hcore157B were separately hydrolyzed in 6 M HCl for 2 h at 110 °C and dried in vacuo. The resulting phosphoamino acids were separated by thinlayer chromatography followed by autoradiography. Arrows indicate the positions of phosphotyrosine (P-Tyr), phosphothreonine (P-Thr) and phosphoserine (P-Ser). Lane 1, GST-Hcore; and lane 2, Hcore157B.

no significant phosphorylation of Hcore157B was detected by incubation (20 min at 30 °C) in vitro after preincubation of CK2 with PKAIIα and 10 μM GTP in the presence of 1 μM quercetin (CK2 inhibitor) for 120 min at 30 °C (line **c**, Fig. 5A). The phosphorylation of GST-Hcore or Hcore157B by CK2-aPKAIIα was found to be 13.5–14.0-fold stimulated when the RIIα subunit of PKAIIα holoenzyme was fully phosphorylated by CK2 in vitro (lane 2, Fig. 5B). No stimulation was observed when PKAIIα was preincubated with CK2 in the presence of 1 μM quercetin (lanes 3 and 6, Fig. 5B). cAMP (1 μM) significantly stimulated the phosphorylation of GST-Hcore or Hcore157B by PKAIIα (lane 4), and also slightly stimulated their phosphorylation by CK2-aPKAIIα (lane 5, Fig. 5B). Under the same experimental conditions, a similar high phosphorylation of GST-Hcore as well as Hcore157B

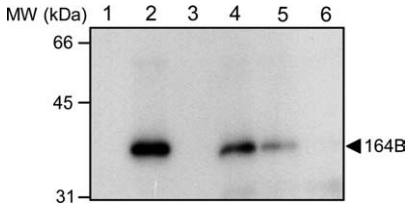


Fig. 3. Determination of the phosphorylation sites on Hcore164B by PKAIIα in vitro. Hcore164B and three Hcore164B variants (approx. 5 μg each) were incubated separately with PKAIIα (approx. 50 ng) in reaction mixtures, as described in the legend of Fig. 2A. After incubation for 30 min at 30 °C in the presence of 1 μM cAMP, $^{32}$P-labeled Hcore164B or two Hcore164B variants (Hcore164B-AS and Hcore164B-SA) in the reaction mixtures were detected by SDS–PAGE followed by autoradiography. Autoradiogram of PKAIIα alone (lane 1); lane 2, PKAIIα + Hcore164B (control); lane 3, lane 2+1 μM GP; lane 4, PKAIIα + Hcore164B-AS; lane 5, PKAIIα + Hcore164B-SA; and lane 6, PKAIIα + Hcore164B-AA.

was observed with CK2-activated PKAIα (CK2-aPKAIα) in vitro (data not shown).

The phosphorylation kinetics between CK2-aPKAIIα and cAMP-aPKAIIα were compared using Hcore157B as a phosphate acceptor in vitro. An apparent $K_m$ for Hcore157B was found to be approx. 0.11 μg/ml when it was incubated with CK2-aPKAIIα, whereas an apparent $K_m$ for Hcore157B with cAMP-aPKAIIα was approx. 0.39 μg/ml (Fig. 5C). The $V_{max}$ of CK2-aPKAIIα was approx. 0.29 nmol/μg/min and that of cAMP-aPKAIIα was approx. 0.28 nmol/μg/min (Fig. 5C). These results show that Hcore157B has a higher binding affinity for CK2-aPKAIIα than cAMP-aPKAIIα in vitro.

### *3.3. Characterization of suramin as a potent inhibitor for the phosphorylation of GST-Hcore and Hcore157B by CK2-aPKAIIα in vitro*

Since suramin is characterized as an antiviral compound [12], the inhibitory effect of suramin on the phosphorylation of GST-Hcore, Hcore157B, protamine 1B or histone H2B by CK2-aPKAIIα was compared in vitro. The phosphorylation of Hcore157B by CK2-aPKAIIα was inhibited dose-dependently by suramin ($ID_{50}$ = approx. 10 nM) (Fig. 6A). There was no difference in the suramin-induced inhibition of the CK2-aPKAIIα-mediated phosphorylation between Hcore157B and GST-Hcore (Fig. 6A). A relative high concentration of suramin was required to inhibit the CK2-aPKAIIα-mediated phosphorylation of protamine 1B and histone H2B (Fig. 6A).

The binding affinity of suramin with either GST-Hcore, Hcore157B or protamine 1B was compared in vitro by using a quartz crystal microbalance (QCM) [13]. As expected, suramin directly bound to GST-Hcore, Hcore157B and protamine 1B, but not to GST, with different affinities in vitro (Fig. 6B). Taken together, these results suggest that suramin may effectively inhibit the phosphorylation of GST-Hcore and Hcore157B by CK2-aPKAIIα through its direct binding to these substrate proteins in vitro.

## 4. Discussion

Using GST-Hcore and Hcore157B as phosphate acceptors, two CK2-aPKAs (CK2-aPKAIα and CK2-aPKAIIα) were biochemically characterized in vitro. It was found that (i) both

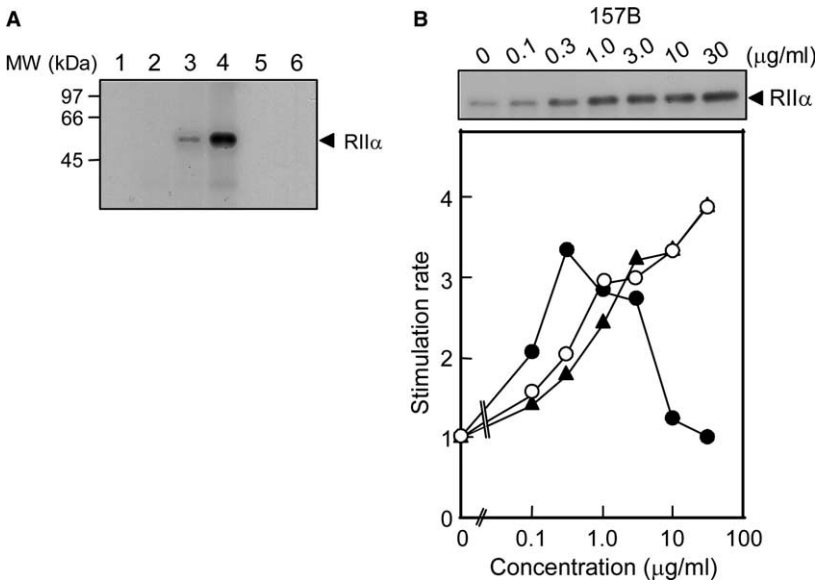


Fig. 4. (A) Characterization of the CK2-mediated phosphorylation of free RIIα in vitro. Free RIIα (approx. 5 μg) was incubated with CK2 (approx. 50 ng) in reaction mixtures comprising 40 mM Tris–HCl (pH 7.6), 5 μM [γ-$^{32}$P]ATP (500 cpm/pmol), 3 mM $Mn^{2+}$ and 1 mM DTT. After incubation for 30 min at 30 °C, $^{32}$P-labeled RIIα in the reaction mixtures was detected by autoradiography following SDS–PAGE. Lane 1, RIIα alone; lane 2, CK2 alone; lane 3, lane 1 + CK2; lane 4, lane 3 + 10mer-Arg (3 μg/ml); lane 5, lane 4 + 10 μM GTP; and lane 6, lane 4 + 1 μM quercetin. (B) The stimulatory effects of GST-Hcore, Hcore157B and 10mer-Arg on the CK2-mediated phosphorylation of free RIIα in vitro. After incubation for 15 min at 30 °C, $^{32}$P-labeled RIIα in the reaction mixtures was detected by autoradiography after SDS–PAGE. A stimulation rate of 1 represents the phosphorylation of free RIIα by CK2 in the absence of these Hcore basic polypeptides. The stimulatory effects on the CK2-mediated phosphorylation of RIIα are presented for GST-Hcore (-●-), Hcore157B (-○-) and10mer-Arg (-▲-)

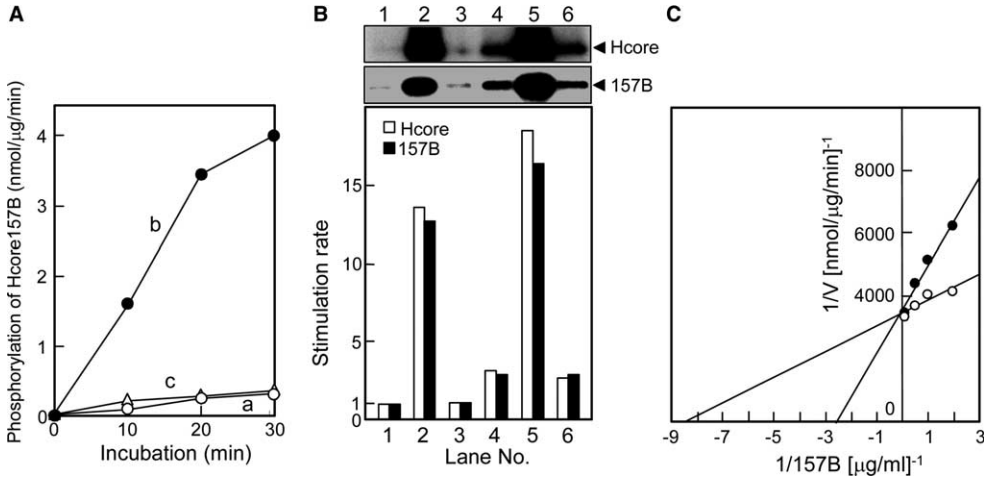


Fig. 5. Characterization of the CK2-aPKAIIα-mediated phosphorylation of GST-Hcore or Hcore157B in vitro. (A) After incubation for the indicated periods (0–30 min) at 30 °C, $^{32}$P-labeled Hcore157B in the reaction mixtures was measured by the glass membrane method [10]. Phosphorylation of Hcore157B by PKAIIα incubated without CK2 (-○-, **a**); phosphorylation of Hcore157B by PKAIIα incubated with CK2 (-●-, **b**); and phosphorylation of Hcore157B by PKAIIα incubated with CK2 in the presence of 1 μM quercetin (-△-, **c**). (B) The initial reaction mixtures comprised PKAIIα (approx. 50 ng) and the same components, as described in Fig. 2A. Subsequently, GST-Hcore or Hcore157B (approx. 5 μg) and 5 μM [γ-$^{32}$P]ATP (500 cpm/pmol) were added to the reaction mixtures, and then incubated for 20 min at 30 °C. $^{32}$P-Labeled GST-Hcore (white columns) or Hcore157B (black columns) in the reaction mixtures was determined by autoradiography after SDS–PAGE. Lane 1, PKAIIα incubated without CK2; lane 2, PKAIIα incubated with CK2; lane 3, lane 2 + 1 μM quercetin; lane 4, lane 1 + 1 μM cAMP; lane 5, lane 2 + 1 μM cAMP; and lane 6, lane 5 + 1 μM quercetin. A stimulation rate of 1 represents the phosphorylation of GST-Hcore or Hcore157B by PKAIIα or CK2-aPKAIIα. Mean values from three different experiments. (C) The phosphorylation kinetics of Hcore157B were determined by incubation for 20 min at 30 °C with the indicated concentrations of Hcore157B and 5 μM [γ-$^{32}$P]ATP in vitro. After incubation, $^{32}$P-phosphorylated Hcore157B in the reaction mixtures was measured with a liquid scintillation spectrophotometer. Lineweaver–Burk plots of Hcore157B for CK2-aPKAIIα in the absence of cAMP (-○-); and cAMP-aPKAIIα (-●-).

CK2-aPKAIα and CK2-aPKAIIα highly phosphorylated only Ser-residues on GST-Hcore as well as Hcore157B (Fig. 2); (ii) CK2-aPKAIIα phosphorylated approx. 4-fold GST-Hcore as well as Hcore157B, as compared with their phosphorylation by cAMP-aPKAIIα (Fig. 5B); (iii) Hcore157B had a higher affinity for CK2-aPKAIIα than cAMP-aPKAIIα (Fig. 5C);

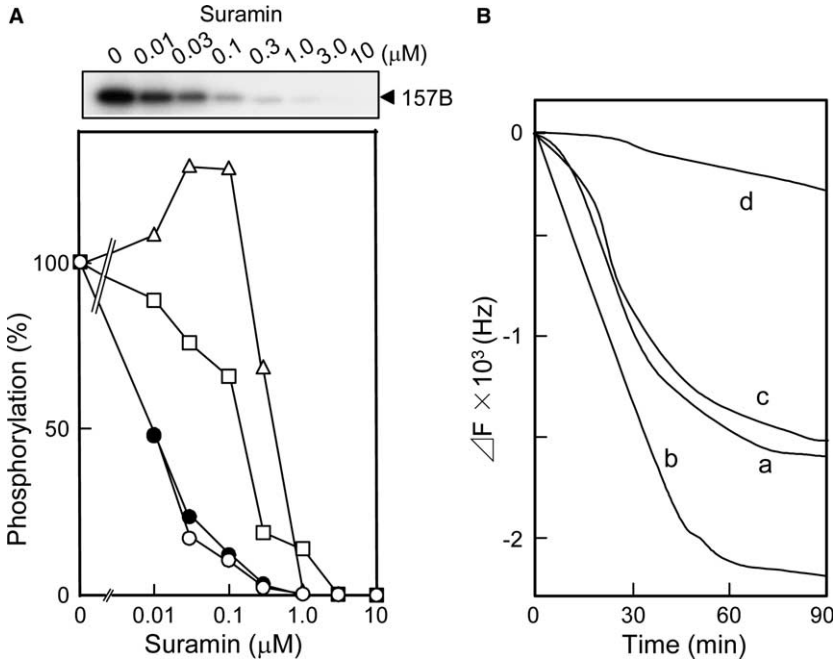


Fig. 6. (A) The inhibitory effect of suramin on the phosphorylation of GST-Hcore, Hcore157B, protamine 1B and histone H2B by PKAIIα in vitro. After incubation (20 min at 30 °C) of either GST-Hcore, Hcore157B, protamine 1B or histone H2B with CK2-aPKAIIα and 5 μM [γ-$^{32}$P]ATP in the presence of the indicated concentrations of suramin, $^{32}$P-phosphorylated GST-Hcore (-○-), Hcore157B (-●-), protamine 1B (-□-) or histone H2B (-△-) in the reaction mixtures was detected by autoradiography after SDS–PAGE. 100% represents the phosphorylation of these proteins by CK2-aPKAIIα after incubation with 5 μM [γ-$^{32}$P]ATP for 20 min at 30 °C in the absence of suramin. (B) The binding affinity of suramin with three polypeptides (approx. 2 μg each) was examined, using using a QCM (Initium, Tokyo, Japan) in 8 ml of 40 mM Tris–HCl (pH 7.6) at 25 °C. Suramin (final concentration: 1 μM) was added to equilibrated solutions containing either GST-Hcore (a), Hcore157B (b), protamine 1B (c) or GST (d).

and (iv) suramin effectively inhibited the phosphorylation of GST-Hcore as well as Hcore157B by CK2-aPKAIIα through its direct binding to these substrate proteins in vitro (Fig. 6A). There was approx. 22-fold dose-discrepancy in the suramin-induced inhibition of the CK2-aPKAIIα-mediated phosphorylation between Hcore 157B (protamine-like sequence) and protamine 1B (Fig. 6A). It seems, therefore, that the suramin-induced inhibition of the CK2-aPKA-mediated phosphorylation of HBV-CP may be involved in the antiviral action of suramin in HBV-infected cells.

Earlier studies have identified the Ser-residues at positions 157, 164 and 172 in the strain *adw* [1] and at positions 162 and 170 in the strain *ayw* [2] of three adjacent SPRRR repeats in the Arg-rich C-terminal region of HBV-CP as phosphoacceptor sites in vivo. It has also been shown that several intracellular protein kinases, such as PKA [5], PKC [5,14] and two SRPKs [7], are cellular mediators responsible for the preferential phosphorylation of HBV-CP during pregenomic RNA (pgRNA) encapsidation and DNA-replication in HBV-infected cells. These reports suggest that the Arg-rich C-terminal region, containing different phosphorylation sites for these protein kinases, may be a major targeting domain involved in the physiological function of HBV-CP in virus-infected cells. Our results that two PKAs (PKAIα and PKAIIα) phosphorylate both Ser-170 and Ser-178 on Hcore164B in vitro (Fig. 3) and two CK2-aPKAs highly phosphorylate Hcore157B as well as GST-Hcore, containing Ser-170 and Ser-178, in vitro (Fig. 5B), suggest that two α-type CK2-aPKAs, in the absence of cAMP, may be main protein kinases responsible for the preferential phosphorylation of both Ser-170 and Ser-178 on HBV-CP in virus-infected cells.

Previously, we proposed a novel CK2-mediated activation of two PKAs (PKAIα and PKAIIα) in the absence of cAMP in vitro [9]. This model is supported by evidence that CK2 may be a protein kinase responsible for the activation of these two PKAs at the cellular level. Therefore, it is possible to speculate that the CK2-mediated activation of these two PKAs may be closely coupled to the enhanced activation of CK2 in the initial stages of cells infected with DNA and RNA viruses. This speculation is supported by our previous observations that (i) CK2 is highly activated by the accumulation of Rev and Tat (viral basic proteins) in T cells infected with human immunodeficiency virus type 1 (HIV-1) [15]; and (ii) the activated CK2 effectively phosphorylates a number of functional cellular proteins, including NS protein, in the cells infected with vesicular stomatitis virus (VSV) [16]. Indeed, we observed that five other GST-Hcore polypeptides (Fig. 1) significantly stimulated the CK2-mediated phosphorylation of the R-subunits (RIα and RIIα) of two PKAs in a manner similar to that observed with Hcore157B (Fig. 4). These observations suggest that the Arg-rich fragments cleaved from the C-terminal region of HBV-CP may function as potent activators for the CK2-mediated phosphorylation of cellular functional proteins, including R-subunits of PKAIα and PKAIIα, and viral proteins, such as HIV-1 reverse transcriptase [17] and protease [18], at an increased level sufficient to activate CK2, as has been previously demonstrated in the Rev-induced stimulation of CK2 activity in HIV-1-infected cells [17,18]. Further analytical experiments to detect the generation of CK2-aPKAs and specific phosphorylation of HBV-CP by the CK2-activated PKAs in HBV-infected cells are currently under way.

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