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Biochemistry

Phosphorylation by cellular casein kinase II is essential for transcriptional activity of vesicular stomatitis virus phosphoprotein P

(protein phosphorylation/casein kinase II/transcription)

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ABSTRACT **We have previously shown that phosphorylation of vesicular stomatitis virus (VSV) phosphoprotein P by cellular protein kinase activity is an essential prerequisite for its transcriptional function. We have now purified this protein kinase by monitoring its ability to phosphorylate bacterially expressed, unphosphorylated P protein. Biochemical studies showed that the kinase is indistinguishable from casein kinase II, a ubiquitous cyclic AMP-independent protein kinase present in a wide variety of eukaryotic cells and tissues. Functional VSV transcription could be reconstituted with viral L protein, N-RNA template, and P protein phosphorylated by either purified cellular protein kinase or purified casein kinase II. The unusual role of casein kinase II in the transcription process of a nonsegmented negative-strand RNA virus would have important implications in host–virus interactions and antiviral therapy.**

The transcription complex of vesicular stomatitis virus (VSV), a prototype rhabdovirus, consists of the following: the single-stranded, nonsegmented, and negative-sense (antimessage sense) genome RNA of ≈11 kilobases, tightly wrapped with the nucleocapsid protein, N (N-RNA template); the large protein, L; and the phosphoprotein, P. The L and P proteins together constitute the viral RNA-dependent RNA polymerase that transcribes the N-RNA template to produce viral mRNAs (reviewed in ref. 1). Genetic and biochemical evidence suggested that L protein may encode the ribonucleotidyl polymerase activity, while phosphoprotein P acts as a transcription factor or a transactivator of the L protein.

The precise mechanism of transactivation by phosphoprotein and the role of its phosphorylation status in this process have been the subject of intensive research in the past few years. We have recently cloned and expressed large quantities of phosphate-free P protein in *Escherichia coli* (2). The VSV transcription reaction mixture reconstituted *in vitro* with purified L protein, N-RNA template, and unphosphorylated P protein (P_0) was found to be defective. Addition of uninfected cell extract to the transcription reaction mixture or prior phosphorylation of the P protein by the cell extract restored transcription, suggesting an essential role of cellular protein kinase-mediated phosphorylation in transcriptional activity of P protein (3). The phosphorylated product (P_1) of the cell kinase reaction was further phosphorylated by an L protein-associated kinase to produce the fully phosphorylated form (P_2). Thus, it was proposed that complete activation of P protein is mediated through a cascade phosphorylation pathway involving two protein kinase activities—the cell kinase and the L kinase—acting sequentially (3). Substrate specificity and other biochemical parameters of the two kinases revealed that they were distinct and different from each other. In this communication, we report the purification and detailed characterization of the cellular protein kinase and show that it is a single enzyme with properties identical to that of cellular casein kinase II (CKII). Phosphorylation of the phosphate-free P protein (P_0) by purified CKII and L kinase *in vitro* fully restored transcriptional activity of P protein.

MATERIALS AND METHODS

Viral (VSV, New Jersey serotype, Ogden subtype) L protein and N-RNA template and bacterially expressed P protein of the same serotype were purified as described (3). CKII, purified from bovine testis, was a kind gift from Edwin G. Krebs and David Litchfield (Howard Hughes Institute, Seattle). Oligopeptides were synthesized in a Beckman 990B automated solid-phase peptide synthesizer and purified through HPLC. Dephosphorylated casein, heparin, and protamine were purchased from Sigma. Rabbit anti-human CKII antibody (Upstate Biotechnology, Lake Placid, NY) was raised against a 23-mer synthetic peptide corresponding to residues 70–91 of the α (catalytic) subunit of human CKII coupled to keyhole limpet hemocyanin.

Purification of P_0 Kinase (PK). The P protein phosphorylating activity (PK) was purified essentially as described for CKII (4) except that the fractions were monitored by their ability to phosphorylate bacterially expressed P protein (P_0) as substrate. In brief, the postribosomal supernatant (S100) of baby hamster kidney (BHK) cell extract (10 g of protein) in buffer A [50 mM Tris·HCl, pH 7.5/0.1 mM EDTA/5% (vol/vol) glycerol/0.02% NaN_3/1 mM dithiothreitol] containing 50 mM NaCl was loaded onto a 20-ml DEAE-cellulose column. After washing with 60 ml of the same buffer, the column was developed with a linear gradient of 0.1–0.4 M NaCl in buffer A (total vol, 120 ml; collected in 60 fractions). The activity peak (at ≈0.2 M NaCl) was loaded directly onto a 3-ml phosphocellulose column, which was then washed with 6 ml of buffer A plus 0.2 M NaCl and developed with a gradient of 0.2–1.0 M NaCl in buffer A (total vol, 18 ml; collected in 60 fractions). The pooled fractions (at ≈0.6 M NaCl) were dialyzed against buffer A plus 0.2 M NaCl and rechromatographed onto a second phosphocellulose column (1 ml) in a similar manner. The pooled peak was dialyzed against buffer A plus 10 mM potassium phosphate (pH 7), chromatographed on a 0.5-ml hydroxylapatite (Bio-Rad) column, and finally concentrated by batch elution in hydroxylapatite exactly as described (4).

Protein Kinase Assay. Standard protein kinase reaction mixtures (20 μl) contained 20 ng of kinase, 1–5 μg of

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Abbreviations: PK, unphosphorylated P protein kinase; CKII, casein kinase II; VSV, vesicular stomatitis virus.

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appropriate substrate (P_0 or casein), 100 μM [γ-^{32}P]ATP (or GTP of the same concentration and specific activity, where indicated), 50 mM Tris·HCl (pH 8.0), 100 mM NaCl, 5 mM $MgCl_2$, and 1 mM dithiothreitol. Reaction mixtures were incubated at 32°C for 15 min, terminated by addition of SDS sample buffer, and analyzed by SDS/PAGE (5) followed by autoradiography. When peptide C (Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu) was used as substrate (6), 10 μl of the reaction mixture was spotted on a 2-cm^2 piece of Whatman P81, which was washed five times (≈30 min) in 150 mM H_3PO_4, dried, and assayed for ^{32}P radioactivity in a liquid scintillation counter. For peptide P (Glu-Glu-Glu-Ala-Ser-Asp-Ser-Asp-Ala-Asp), an identical procedure was followed except that the P81 paper was substituted by Whatman DE81 paper, which was carefully washed with 0.5 M Na_2HPO_4. It was necessary to use a cationic matrix for peptide P since the lack of basic residues in this peptide precluded its binding to the anionic P81 paper (data not shown). However, unreacted [γ-^{32}P]ATP also bound to DE81 and could only be removed by extensive washing with high salt, which also resulted in some loss of the bound peptide (see Table 2).

RESULTS

Purification of the Cellular Protein Kinase Phosphorylating P_0. We have previously shown that extracts of uninfected BHK-21 cells contained a potent protein kinase activity that phosphorylated bacterially expressed phosphate-free P protein (P_0) *in vitro* and that this phosphorylation was essential for transcriptional activation of P protein (3). To purify the putative protein kinase, we used an extract similar to the starting material and subjected it to chromatographic procedures. The various protein fractions were monitored by their ability to phosphorylate P_0 *in vitro* using [γ-^{32}P]ATP as phosphate donor. As shown in Fig. 1, PK activity eluted in a single peak from DEAE-cellulose and phosphocellulose columns, at about 0.2 and 0.6 M NaCl, respectively. In the final step, PK activity eluted at ≈0.2 M K_2HPO_4 from hydroxylapatite (data not shown). The elution of PK activity in a single peak from each of the three matrices strongly suggested the existence of a single protein kinase in the cell that could phosphorylate P_0. The profile of each activity peak was essentially similar when dephosphorylated casein was used as substrate. These elution parameters and our earlier demonstration that PK activity can utilize both ATP and GTP as phosphate donor (3, 4) suggested that this activity may be very similar to cellular CKII (7). We demonstrate (see below) that the properties of this preparation are indeed identical to those of CKII.

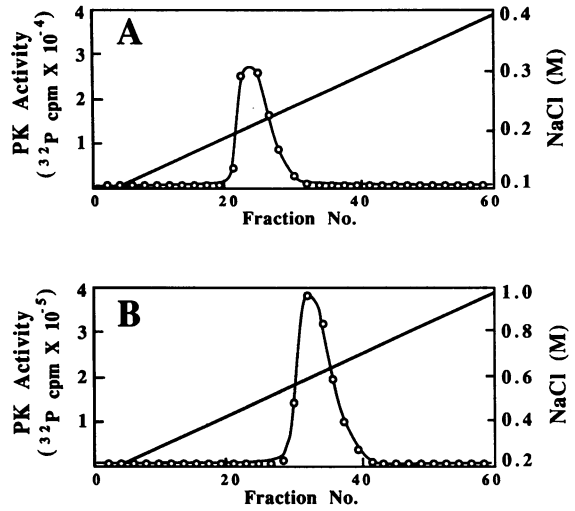

FIG. 1. Purification of PK. Elution profiles of kinase activity from DEAE-cellulose (*A*) and phosphocellulose (*B*) are shown. One microliter of each fraction was assayed for kinase activity (○) using P_0 protein as substrate in a standard reaction mixture in the presence of [γ-^{32}P]ATP. After SDS/PAGE, the P protein band was located by staining, solubilized, and quantitated by scintillation counting.

Both PK and CKII Phosphorylate P_0 and Casein. Since PK and CKII were originally purified by using P_0 and casein as substrates, respectively, it was important to determine whether the substrates and enzymes could be interchanged. Fig. 2 shows that both protein kinases can phosphorylate both the substrates, although both of them phosphorylated P_0 much more efficiently than casein. Heparin, which inhibits PK in the crude extract (data not shown), inhibited both purified PK and CKII, while protamine stimulated both enzymes ≈3-fold. Cyclic AMP had no effect on either enzyme activity (data not shown). Essentially identical results were obtained by using casein and P_0 as substrates in separate kinase reactions (data not shown). We have previously shown that unphosphorylated P_0 protein elutes from DEAE-cellulose at ≈0.17 M NaCl (3), whereas the product of its phosphorylation by the crude cell extract P_1 eluted at 0.27 M NaCl (3). Phosphorylation of P_0 by purified PK or CKII *in vitro* produced a similar chromatographic shift of P protein, indicating that phosphorylation by both enzymes produces a similar structural alteration in P protein.

Biochemical Properties of PK and CKII Are Essentially Identical. Results of a detailed study of the biochemical parameters of the two enzymes, such as Mg^{2+} optima, apparent K_m of ATP and GTP, stimulation by protamine, and inhibition by heparin and 2,3-diphosphoglyceric acid (4, 7), are listed in Table 1. It is clear that the two enzymes, purified by using different substrates, behave in an identical manner within the limits of experimental variation.

The close identity of the two enzymes was further confirmed by a comparison of their polypeptide profiles. SDS/PAGE followed by silver staining revealed that both enzymes contain two major classes of polypeptides: larger subunit(s) ($\alpha\alpha'$) in the range of ≈45 kDa, and a smaller subunit (β) of ≈25 kDa (Fig. 3*A*). When the enzymes were allowed to autophosphorylate in the absence of any added substrate, the smaller polypeptides (β) were phosphorylated in both cases (Fig. 3*B*). An antibody raised against a synthetic peptide representing the catalytic domain of the α subunit cross-reacted with the major 45-kDa doublet of both preparations but did not react with the β subunit of either (Fig. 3*C*). Taken together, these results demonstrate the immunological and catalytic similarity of PK with CKII.

Site Specificity of Phosphorylation. Proteolytic analysis (9) of ^{32}P-labeled VSV phosphoprotein showed that most of the phosphate groups are clustered between residues 35 and 70 in

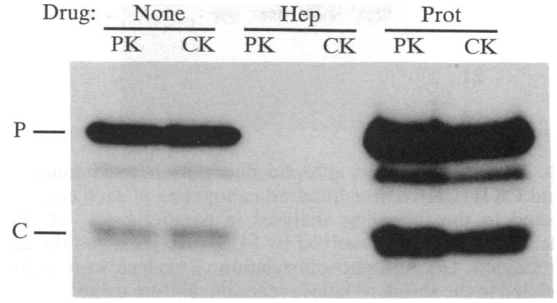

FIG. 2. Effect of protamine (Prot) and heparin (Hep) on PK and CKII (CK) activities. Twenty nanograms of either enzyme was used in a standard kinase assay with both P_0 (1 μg) and casein (2 μg) as substrate and [γ-^{32}P]ATP as phosphate donor. The drugs were used at 0.5 μg/ml. Labeled P protein (P) and casein (C) bands are indicated.

Table 1. Biochemical properties of PK and CKII

Parameter	PK	CKII
Elution		
From phosphocellulose	0.6 M NaCl	0.5–0.7 M NaCl
From DEAE-cellulose	0.2 M NaCl	0.15–0.25 M NaCl
From hydroxylapatite	0.2 M K_2HPO_4	0.18–0.31 M K_2HPO_4
Phosphate donor		
K_m of ATP	15 μM	12 μM
K_m of GTP	20 μM	25 μM
Activity at 5 mM Mg^{2+}/ activity at 0.5 mM Mg^{2+}	0.8	0.8
Substrate	P_0 >> casein	P_0 >> casein
Effect		
Of heparin (1 μg/ml)	Inhibits (20-fold)	Inhibits (20-fold)
Of DPG (2 mg/ml)	Inhibits (10-fold)	Inhibits (10-fold)
Of protamine (1 μg/ml)	Stimulates (2-fold)	Stimulates (2-fold)

Elution properties of CKII were taken from published work (4); all other properties of CKII were determined in this work and the values obtained were comparable to the published ones. Elution profiles of the enzymes were determined by using P_0 and casein as substrates for PK (this work) and CKII (4), respectively. All other parameters were measured by using both P_0 and casein as substrates (1 and 5 μg, respectively, in a standard 20-μl kinase reaction mixture) for either enzyme (e.g., as in Fig. 2); for a given enzyme, numbers obtained with the two substrates were essentially identical. All kinase reactions were performed under standard reaction conditions in the presence of [γ-^{32}P]ATP as described except that, where indicated, GTP replaced ATP. Enzyme activities were quantitated by densitometric scanning of a ^{32}P-labeled protein band in the autoradiograph obtained after SDS/PAGE analysis of the kinase reaction. P_0 >> casein indicates that, at the same concentration and under identical reaction conditions, P_0 accepted more phosphates than casein on a mol/mol basis (e.g., see Fig. 2). DPG, 2,3-diphosphoglyceric acid.

the acidic domain I (10) of P protein. In addition, phosphoamino acid analyses of viral P protein established serine as the major phosphate acceptor (11, 12). A comparison of amino acid sequences of P proteins of the New Jersey (NJ) and Indiana serotypes of VSV revealed that a total of five potential phosphate acceptor sites are conserved between residues 35 and 70; in the NJ serotype of P protein, these residues are Thr^{49}, Ser^{51}, Ser^{59}, Ser^{61}, and Thr^{63}. To determine whether these sites are in fact phosphorylated, we changed these residues to alanine by site-specific mutagenesis (20). The mutant protein P5 was expressed in *E. coli* and purified exactly as described for wild-type protein (2). The ability of PK and CKII to phosphorylate the mutant protein was then tested *in vitro*. Results (Fig. 4) showed that the P5 mutant was severely defective in phosphorylation; however, in the same reaction mixture, casein was phosphorylated normally, indicating that the P5 preparation did not contain an inhibitor of kinase activity. To exclude the possibility that it contributed a specific inhibitor of P protein phosphorylation, we included wild-type P protein along with the P5 mutant in the kinase reaction mixture; as shown, this resulted in reappearance of radioactivity in the P protein band. The phosphorylation defect of the P5 mutant strongly implicated one or a few of the five conserved residues as the site(s) of cell kinase (PK)-mediated phosphorylation in P protein. In addition, since both PK and CKII failed to phosphorylate the P5 mutant, it suggested that the two enzymes recognize and phosphorylate the same residues in P protein, which further emphasizes their similarity.

Finally, protein kinases in general are known to phosphorylate short synthetic oligopeptides corresponding to their minimal site of action (13). To further confirm the sites of phosphorylation by PK and CKII, we synthesized two decapeptides (see *Materials and Methods*), one corresponding to

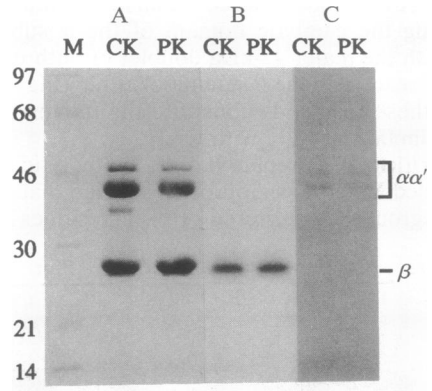

FIG. 3. Protein profile, autophosphorylation, and immunoblot of PK and CKII (CK). Three hundred nanograms of each enzyme was subjected to the following analyses in parallel lanes. (*A*) Protein profile. Enzymes were resolved by SDS/PAGE (5) and the gel was silver stained. (*B*) Autophosphorylation. Enzymes were autophosphorylated in the standard kinase reaction mixture using [γ-^{32}P]ATP; the reactions were analyzed by SDS/PAGE followed by autoradiography. (*C*) Western blot. After SDS/PAGE, the proteins were transferred to a nitrocellulose membrane by electroblotting. The membrane was first probed with a rabbit IgG against synthetic human CKII α peptide and then with peroxidase-conjugated goat anti-rabbit antibody (Boehringer Mannheim) as described (8). Lane M, protein size standards (kDa).

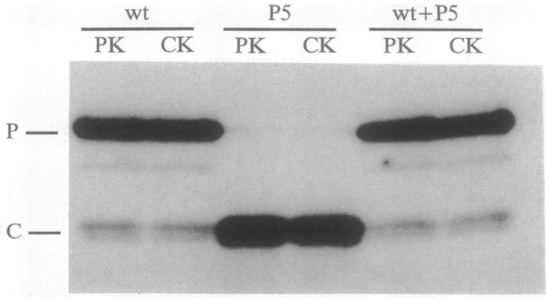

FIG. 4. P5 mutant is phosphorylation defective. Bacterially expressed wild-type P protein (wt), P5 mutant protein (P5), or a mixture of both (wt+P5) were used as substrates for 20 ng of either PK or CKII (CK) in standard kinase reaction mixtures using [γ-^{32}P]ATP. One microgram of each P protein was used. All reaction mixtures also contained 2 μg of casein as internal control. Reactions were analyzed by SDS/PAGE followed by autoradiography. Labeled P protein (P) and casein (C) bands are indicated.

the putative phosphorylated region of the P protein of NJ serotype (A. Takacs, S.B., T. Das, and A.K.B., unpublished data) and containing Ser^{59} and Ser^{61} as the only phosphate acceptor sites (peptide P), and another peptide (peptide C), which is known to serve as a highly specific substrate for CKII (6). The peptides were incubated with either PK or CKII in standard kinase reaction mixtures containing [γ-^{32}P]ATP and the ^{32}P-labeled products were quantitated as described. Results show that both peptides could be phosphorylated by either kinase (Table 2). The higher background counts and lower peptide counts obtained with peptide P are most likely due to the different paper matrix and washing procedure used. However, it is important to note that for any given peptide, the extents of phosphorylation by PK and CKII were comparable, again demonstrating the equivalence of the two enzymes.

Phosphorylation by CKII Restores the Transcriptional Activity of P Protein. We have shown earlier that phosphorylation of P protein by cellular kinase(s) is essential for its ability to function as a transcriptional activator in VSV transcription reaction mixtures reconstituted with L protein and N-RNA template (3). To test whether the purified PK or CKII could activate P protein in the same manner, essentially similar transcription reaction mixtures were reconstituted in the presence of either of these protein kinases or their phosphorylated products. Results from a series of such reactions are presented in Fig. 5. It is clearly shown that the transcription reaction mixture reconstituted with the phosphate-free P protein (P_0) was defective (lane 1); productive transcription was restored when either PK or CKII was also included in the reaction mixture (lanes 2 and 3). In another set of reactions, P_0 was first phosphorylated by PK or CKII and the phosphorylated product (P_1), purified through DEAE-cellulose chromatography, was used to reconstitute transcription. Transcription was again functional (lanes 4 and 5), demonstrating that a cell kinase-free VSV transcription can be reconstituted if the P protein has already been phosphorylated by the cell kinase (3, 14).

These conclusions were further supported by the finding that heparin, a specific inhibitor of PK and CKII (Fig. 2), also inhibited transcription reactions programmed with phosphate-free P protein and either of these kinases (Fig. 5, lanes 6 and 7). At the same concentration, heparin had little effect on L kinase, measured by the ability of the latter to phosphorylate P_1 (data not shown). As expected, heparin had no effect on transcription reactions that were independent of CKII—i.e., reconstituted with phosphorylated P protein P_1 or P_2 (lanes 8 and 9). Protamine, a specific inhibitor of L kinase-mediated phosphorylation of P_1 to P_2 (3), inhibited transcription reactions programmed with either P_0 (data not shown) or P_1 (lane 10), consistent with an essential role of L kinase in P function. Interestingly, protamine also inhibited transcription reactions programmed with P_2, the fully phosphorylated form of P protein (lane 11). This latter finding suggests that for transcription to progress the P_2-specific phosphates, which were shown to undergo a turnover during VSV transcription (3), must be continually replaced by the L kinase. Taken together, these results and the earlier ones (3, 15) demonstrate that phosphorylation of the P protein by CKII and L kinase is essential for transcriptional activation of the P protein.

Table 2. Phosphorylation of synthetic peptides by PK and CKII

Kinase	^{32}P incorporated, cpm × 10^{-2}	
	Peptide P	Peptide C
None	55	27
PK	594	2160
CKII	760	2461

Standard kinase reaction mixtures contained 1 mM peptide P or peptide C and 25 ng of either enzyme.

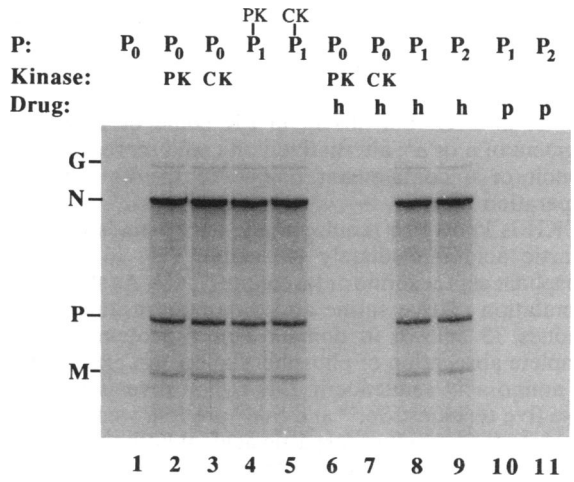

FIG. 5. Role of CKII in VSV transcription. *In vitro* VSV transcriptions (20 μl) were reconstituted by using various purified P proteins (P_0, P_1, or P_2) and host kinase-free N-RNA template and L protein exactly as described (3). Labeled transcripts were resolved in 6% polyacrylamide gel containing 50% (wt/vol) urea; an autoradiograph of the gel is shown. Where indicated, 10 ng of either protein kinase (PK, lanes 2 and 6; CKII, lanes 3 and 7) was also included in the reaction mixture. Reaction mixtures h and p received 20 ng of heparin and protamine (both from Sigma), respectively. P protein was the last component added to all reaction mixtures. Various mRNAs are indicated (G, N, P, and M). See *Results* for details.

DISCUSSION

Our studies of the purified cellular protein kinase that phosphorylates P protein of VSV *in vitro* show that the enzyme is highly similar, and most likely identical, to CKII. The biological relevance of this identity is evidenced by the demonstration that both enzymes can restore the transcription function of P protein through specific phosphorylation in the acidic domain I of P protein. The unusual role of CKII in activation of a RNA viral phosphoprotein may lead to unique considerations in viral gene regulation and host–virus interaction as discussed below.

CKII is a multipotential, ubiquitous, cyclic AMP-independent serine protein kinase with a number of properties that distinguish it from other protein kinases (see ref. 7 for a comprehensive review). Two such properties—namely, inhibition by heparin and the ability to use both ATP and GTP as phosphate donors—are widely used as diagnostic of CKII. Furthermore, peptide C has been shown to be a specific substrate for CKII and not phosphorylated by other protein kinases (6) such as CKI, protein kinase C, and cyclic AMP-dependent protein kinases. As shown here (Tables 1 and 2), our characterization of the purified P protein kinase as CKII has included all of these criteria.

CKII, purified from a variety of sources, exhibits the general subunit structure $\alpha_2\beta_2$ or $\alpha\alpha'\beta_2$, with the α and α' subunits having a broad size range of 36–44 kDa, and the β subunit being $\approx$25 kDa (7). The α polypeptide binds ATP and possesses some protein kinase activity in the absence of β, suggesting that it is the catalytic subunit. The β subunit, on the other hand, lacks protein kinase activity but undergoes phosphorylation when the holoenzyme is incubated with ATP (4, 7). It appears that CKII of BHK cells, which we have purified by monitoring its P protein phosphorylating activity, has an essentially similar structure (Fig. 3*A*). Moreover, upon autophosphorylation, the 25-kDa β polypeptide was phos-

phorylated (Fig. 3B). The evolutionary similarity of these enzymes is further underscored by the fact that the $\alpha\alpha'$ doublet in both preparations cross-reacted with anti-human α peptide. A minor polypeptide migrating just below the $\alpha\alpha'$ doublet in CKII but absent in PK could be a proteolytic fragment of α or α'; alternatively, it could represent an extra subunit or a contaminant unique to the bovine enzyme preparation (CKII).

CKII is known to require acidic amino acids glutamic or aspartic acid immediately C-terminal (+1 to +3) to the phosphoacceptor serine or threonine (7, 13). As shown in Fig. 4, mutation of five serine and threonine residues between residues 35 and 70 in domain I of P protein resulted in complete abrogation of phosphorylation. An examination of the amino acid sequence in this region reveals that among these five residues, Ser^{59} and Ser^{61} are followed (C-terminal side) by acidic residues (aspartic acid) at both the +1 and +3 positions and, therefore, could be optimal sites for CKII phosphorylation. Our recent analyses of the phosphorylation status of various mutant P proteins of the NJ serotype of VSV, expressed in COS cells, have shown that alteration of these two serines to alanines abolishes phosphorylation of P protein (A. Takacs, S.B., T. Das, and A.K.B., unpublished data). Using bacterially expressed mutant P proteins as substrates for purified PK or CKII *in vitro*, essentially identical results were obtained (unpublished observation). These results and the finding that a peptide containing only these serines as phosphate acceptors is phosphorylated efficiently by PK and CKII *in vitro* (Table 2) further suggest that CKII is the only cellular protein kinase phosphorylating the P protein. Thus, it appears that the preponderance of acidic amino acids in domain I of P protein serves at least two functions: (*i*) the acidity may directly contribute to the transactivation property of this domain (16) and (*ii*) it provides the proper environment for CKII-mediated phosphorylation, which is essential for RNA-dependent RNA transcription.

We have previously shown that phosphorylation of the P protein by cellular kinase is an essential prerequisite to its subsequent phosphorylation by the viral L protein-associated kinase and that only the final, fully phosphorylated form (P_2) appears to be transcriptionally active (3). By replacing the cell extract with purified PK or CKII, we have now obtained identical results (data not shown; Fig. 5). This is in conformity with a myriad of similar examples in which phosphorylation by CKII at one site potentiates a neighboring site for phosphorylation or dephosphorylation by other enzymes (17). Furthermore, phosphates introduced by CKII are relatively stable and undergo very slow turnover (7). This is in agreement with our earlier observation that phosphate groups of P_1, introduced by the cellular kinase, are highly resistant to phosphatases and do not undergo turnover during VSV transcription (3).

The unique role of CKII in RNA viral transcription offers the potential of screening for antiviral drugs through specific inhibition of CKII *in vitro*. In this connection, the antiviral xanthate compound D609 (tricyclo-decane-9-yl-xanthogenate), an inhibitor of VSV growth in cell cultures, is worth mentioning. The drug strongly inhibited phosphorylation of newly synthesized P protein in VSV-infected cells, with concomitant inhibition of secondary transcription and replication (18). However, primary transcription—mediated by the input viral ribonucleoprotein containing P protein that is already phosphorylated—remained unaffected. Although it remains to be shown that D609 inhibits CKII *in vitro*, these results clearly suggest an essential role of the cell kinase(s) in transcriptional activation of P protein *in vivo*. It would be important to study the effects of heparin and 2,3-diphosphoglycerate on VSV growth to further understand the role of P protein phosphorylation in VSV gene expression *in vivo*.

Our findings add the P protein of VSV to a large list of regulatory proteins that are phosphorylated by CKII; examples include the human heat shock protein 90, simian virus 40 large tumor antigen, translation initiation and elongation factors, RNA polymerases I and II, and DNA topoisomerases I and II (reviewed in ref. 7). By utilizing a cellular protein kinase that is widely distributed in eukaryotic cells to incorporate stable phosphate groups in an essential transcription factor, VSV probably ensures the ubiquity of its gene expression in the various host cells that it infects. It remains to be seen whether CKII is one of a variety of protein kinases reported to be packaged in mature vesicular stomatitis virions (1, 19) and whether phosphoproteins of other negative-strand RNA viruses are also phosphorylated by CKII.

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