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Comparative Studies on the Structural Phosphoproteins of Mammalian Type C Viruses

BIJAY K. PAL, ROBERT M. McALLISTER, MURRAY B. GARDNER, AND PRADIP ROY-BURMAN*

Departments of Pathology and Biochemistry, University of Southern California School of Medicine, Los Angeles, California 90033 and the Department of Pediatrics, University of Southern California School of Medicine, Childrens Hospital of Los Angeles, Los Angeles, California 90027*

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The major phosphoprotein common to woolly monkey sarcoma virus, gibbon ape lymphosarcoma virus, and type C viruses of the lower mammalian species (mouse, rat, cat), with the exception of the endogenous cat virus (RD-114), is the polypeptide of about 12,000 molecular weight. The protein-phosphate bond in this polypeptide of several viruses is of the phosphoserine variety excepting gibbon ape virus, which contains both phosphoserine and phosphothreonine. The primary phosphoprotein of RD-114 virus and the endogenous baboon type C virus, on the other hand, is the polypeptide of about 15,000 molecular weight which contains phosphothreonine as its phosphoamino acid. A second major phosphoprotein of molecular weight of 10,000 is detected only in viruses genetically related to rat species including those derived from the RPL cell line, from Sprague-Dawley rat embryo cells, and the Kirsten mouse sarcoma virus which was recovered from a mouse erythroblastosis virus after in vivo propagation through rat. These phosphorylated polypeptides of molecular weight 15,000, 12,000, or 10,000 are present in the virion structure in several different but nonrandom phosphorylated states.

We recently described specific phosphoproteins in mouse and feline type C viruses (18). Subsequently we identified structural phosphoproteins from an endogenous mouse type C virus (35), from two rat type C virus isolates (12; S. Rasheed, H. P. Charman, J. Bruszewski, P. Roy-Burman, and R. W. Rongey, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, V374, p. 263) and from three primate-derived type C virus isolates, namely woolly monkey sarcoma virus type 1, together with its associated virus (SSV-1/SSAV-1) (34, 39), gibbon ape lymphosarcoma virus (GaLV) (10), and baboon kidney cell-derived endogenous type C virus (BKD) (37). A comparison of the nature of the major phosphoprotein of the viruses suggested the presence of common characteristics between the phosphoproteins of the two primate-derived viruses (SSV-1/SSAV-1 and GaLV) and the lower mammalian (mouse, rat, cat) viruses with the exception of the endogenous cat virus (RD-114) (14), and between the phosphoproteins of the endogenous baboon virus (BKD) and the endogenous cat virus (RD-114). The major phorphoproteins were found to exist in several different but nonrandom phosphorylated states. Possible implications of these findings on the virus structure and function are discussed.

MATERIALS AND METHODS

Reagents, media, and sera. Reagent or analytical grade chemicals were used in all experiments. All radiochemicals were obtained from New England Nuclear. Ultrapure urea, sucrose, and protein molecular weight markers were purchased from Schwarz/Mann; agarose, acrylamide, and bisacrylamide from Bio-Rad Lab.; guanidine hydrochloride, Triton X-100, phosphothreonine, phosphoserine, and ninhydrin from Sigma Chemical Co.; phosphorylase *a* and pancreatic ribonuclease from Worthington Biochemicals Corp.; and Eagle minimum essential medium (MEM) and fetal bovine serum from Flow Labs. Sodium dodecyl sulfate (SDS) obtained from Matheson was recrystallized before use.

Cells and viruses. The viruses used in the present investigation were endogenous mouse type C virus (AT-124) (35), the baboon kidney-derived endogenous type C virus (BKD) (37), the woolly monkey sarcoma virus type 1, together with its associated virus (SSV-1/SSAV-1) (34, 39), the gibbon ape lymphosarcoma virus (GaLV) (10), Gardner-Arnstein and Snyder-Theilen strains of feline leukemia virus (FeLV-GA and FeLV-ST), the endogenous feline type C virus (RD-114) (14), the Sprague-Dawley rat embryo culture-derived type C virus (S. Rasheed et al. Abstr.

Annu. Meet. Am. Soc. Microbiol. 1974, V374, p. 263), and the RPL cell line (38)-associated rat type C virus (12). AT-124, SSV-1/SSAV-1, and RD-114 viruses were grown in human rhabdomyosarcoma (RD) cells (13). BKD and GaLV were propagated in human sarcoma cells, HT-1080 (21). The rat type C viruses were isolated from the Sprague-Dawley rat embryo fibroblast and RPL (38) cell cultures. All virus-infected cell lines were cultured in MEM supplemented with 10% fetal bovine serum, 2 mM glutamine, and 80 μg of gentamicin per ml.

Fractionation of virion phosphoproteins. Fractionation of virion phosphoproteins was as described (18). Briefly, viruses were labeled by growing the virus-producing cells in 50% phosphate-free MEM supplemented with 10% dialyzed fetal bovine serum in the presence of ^{3}H-labeled amino acid mixture (2 μCi/ml) and carrier-free [^{32}P]phosphate (80 μCi/ml). Two changes of the medium were made at 24-h intervals and the virus was purified from the pooled culture fluids by the published procedure (19, 22). The pelleted virus was then disrupted in 20 mM Tris-hydrochloride (pH 7.4) containing 0.1% Nonidet P-40 at 0 C for 1 h and then treated with RNase (0.5 mg/ml, final concentration) at 37 C for 2 h. The solution was then brought to 8 M Gu·HCl and 0.3% β-mercaptoethanol, heated at 56 C for 45 min and applied on a Bio Gel A 5-m column (1.5 by 87 cm) equilibrated with 6 M Gu·HCl for fractionation of virion polypeptides (15, 19).

Polyacrylamide gel electrophoresis. The protein peaks containing both ^{3}H and ^{32}P labels, isolated from the guanidine-agarose chromatography, were further analyzed by SDS-polyacrylamide gel electrophoresis after the procedure of Summers et al. (32). Dialyzed and lyophilized materials from the pooled peak fractions were dissolved in 10 mM sodium phosphate buffer (pH 7.2) containing 1% SDS and 0.1% β-mercaptoethanol by heating at 100 C for 2 min. Electrophoresis was carried out in 7.5% gels, containing 0.1% SDS, at 9 mA/gel for 4 h at room temperature. After the run, gels were taken out of the tube, frozen at −80 C, and cut in 1-mm slices as described (19). Two slices were taken as one fraction and counted in 6 ml of scintillation fluid (8.0 g of butyl-PBD, 0.5 g of PBBO in 750 ml of toluene, and 250 ml of Triton X-100) after shaking with 0.4 ml of 2% periodic acid for 30 min at 60 C (23). A Beckman LS-250 liquid scintillation counter with automatic quench correction was used for differential counting of ^{3}H and ^{32}P.

Viral phosphoproteins were also analyzed by urea-polyacrylamide gel electrophoresis following the method of Ornstein (17) and Davis (6) with modifications. Dialyzed and lyophilized samples of ^{3}H-labeled amino acids and [^{32}P]phosphate-labeled viral phosphoproteins, as obtained from guanidine-agarose chromatography, were solubilized in 62.5 mM Tris-hydrochloride (pH 6.85), 5.25 M urea, and 2% β-mercaptoethanol (sample buffer) by heating at 100 C for 2 min. The sample was mixed with 1 mg% of bromophenol blue and loaded on a preformed polyacrylamide gel. The gel (0.5 by 10.5 cm) contained a 1-cm long stacking part of 3% acrylamide, 5.25 M urea, 125 mM Tris-hydrochloride (pH 6.8), followed by a 9.5-cm long resolving part of 4 to 10% linear gradient of acrylamide in 375 mM Tris-hydrochloride containing 5.25 M urea, pH 8.8. Electrophoresis was carried out at 1 mA/gel for 1 h and then at 3 mA/gel for another period of 2.5 h using an electrophoresis buffer of 25 mM Tris and 192 mM glycine, pH 8.3.

Paper electrophoresis of viral phosphoprotein hydrolyzates. For the identification of the phosphoamino acids, the in vivo phosphorylated viral polypeptides were hydrolyzed in 6 N HCl at 110 C for 5 h (1). The hydrolyzate was dried on a boiling water bath and desiccated with NaOH beads in vacuum. The residue was dissolved in water and mixed with 50 μg each of phosphoserine and phosphothreonine, and subjected to high-voltage paper electrophoresis (1, 18) at 3,000 volts for 4 h at 0 to 4 C using Whatman 3MM paper and formic acid-acetic acid-water (45:200:755) buffer system, pH 1.6. After electrophoresis the paper was air dried in hood, stained with ninhydrin, and cut in 3-mm strips for radioactive counting.

Molecular weight determinations. The molecular weights of the smaller polypeptides (10,000 to 15,000) were estimated from the results of guanidine-agarose chromatography and those of the larger polypeptides from their relative electrophoretic mobility in SDS gels as described before (2, 18, 19). Proteins were designated by a lower case "p" and glycoprotein by a "gp" placed before the number indicating the molecular weight in thousands (2).

RESULTS

Separation and identification of the phosphoproteins. Purified AT-124 virus labeled with ^{3}H-labeled amino acids and [^{32}P]phosphate was treated with RNase in the presence of a detergent and then the polypeptides were separated by guanidine-agarose chromatography (18, 19). The proteins were resolved into five peaks of which two contained superimposed ^{3}H and ^{32}P labels (Fig. 1). One of these two peaks was eluted at the void volume of the column and was expected to contain ^{32}P label in the phospholipids of the virus (18). The other peak corresponded to the polypeptide, p12. Like SSV-1/SSAV-1 and GaLV (23), AT-124 was found to be deficient in the p15 component. Similar guanidine-agarose chromatography analysis for phosphoproteins in SSV-1/SSAV-1 (Fig. 2) and GaLV (data not shown) indicated that p12 was the major phosphorylated polypeptide in both cases, whereas p15 was found to be the major phosphoprotein of the BKD virus (Fig. 3). Interestingly, the BKD virus was deficient in the p12 polypeptide.

Also, there seemed to be an apparently minimal quantity of p30 in the pattern of Fig. 1 and 3, not consistently observed with other type C viruses (15, 18, 19, 23). Since our measurements were based on incorporation of ^{3}H-labeled amino acids only, the relatively lower incorpora-

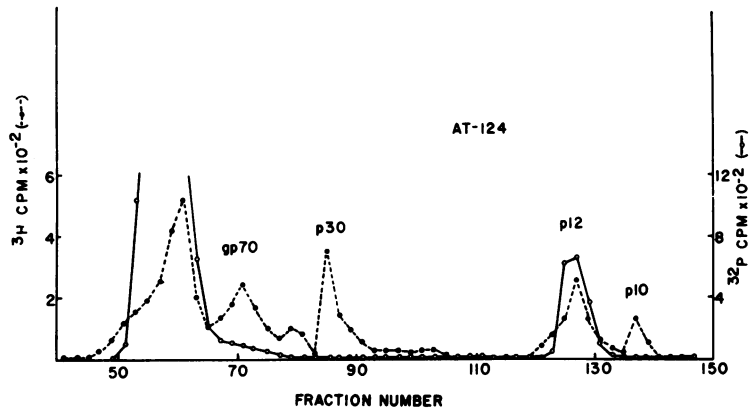

FIG. 1. *Separation of the polypeptides of AT-124 virions, labeled with ^{3}H-labeled amino acids and [^{32}P]phosphate, by guanidine-agarose chromatography.*

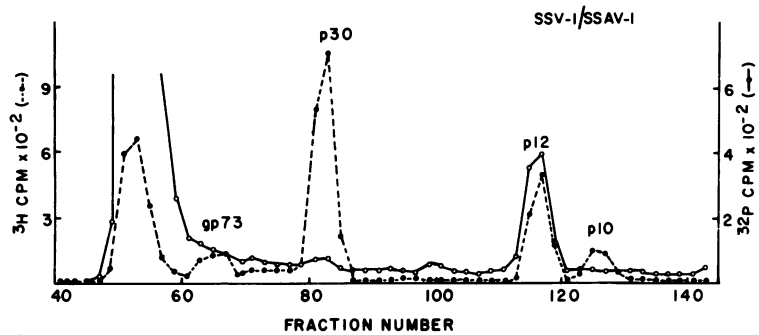

FIG. 2. *Separation of the polypeptides of SSV-1/SSAV-1 virions, labeled with ^{3}H-labeled amino acids and [^{32}P]phosphate, by guanidine-agarose chromatography.*

tion could also be a result of difference in amino acid composition of these p30 proteins.

Analysis of the rat type C viruses derived from the RPL cell line (Fig. 4) and Sprague-Dawley rat embryo cultures (data not shown) showed that both p12 and p10 contained superimposed ^{3}H and ^{32}P labels similar to the results obtained with the Kirsten mouse sarcoma virus (18). The data available on biologically active particles indicated that the Ki-MSV stocks we used contained transforming particles of at least 90% purity (P. Roy-Burman and V. Klement, J. Gen. Virol., in press).

Comigration of ^{3}H and ^{32}P radioactivities was also noted when the pooled fractions of the phosphoprotein peaks from the guanidine-agarose column were analyzed by SDS-polyacrylamide gel electrophoresis. This is illustrated in Fig. 5 for p15 of BKD, p12 of SSV-1/SSAV-1 and p12 of AT-124, and in Fig. 6 for p12 and p10 of the Sprague-Dawley rat type C virus. The p12 of SSV-1/SSAV-1 showed a dissociated minor component which did not contain ^{32}P label (Fig. 5).

Major phosphoamino acids identified in the viral phosphoproteins. For identification of the major phosphoamino acids of the in vivo phosphorylated viral polypeptides, p12 of GaLV, p12 of AT-124, and p15 of BKD were hydrolyzed in 6 N HCl at 110 C for 5 h and subjected to high-voltage paper electrophoresis with *o*-phosphoserine and *o*-phosphothreonine as markers (18). As shown in Fig. 7 (panel A), the major phosphoamino acid in p15 of BKD was found to be phosphothreonine which is also the phosphoamino acid of p15 of RD-114 virus (panel B). Consistent with the previous observation on mouse type C viruses (18), p12 of AT-124 virus contained primarily *o*-phosphoserine (Fig. 7, panel D). The major phosphoprotein (p12) of GaLV contained both phosphoserine and phosphothreonine as phosphoamino acids (Fig. 7, panel C).

Fractionation of the major phosphoproteins by urea-polyacrylamide gel electrophoresis. Both guanidine-agarose chromatography and SDS gel electrophoresis resulted in separation of viral polypeptides on the basis of molec-

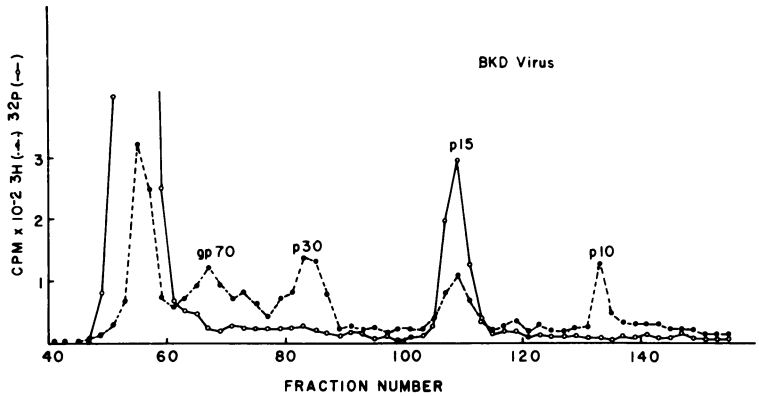

FIG. 3. *Separation of the polypeptides of BKD virions, labeled with ^{3}H-labeled amino acids and [^{32}P]phosphate, by guanidine-agarose chromatography.*

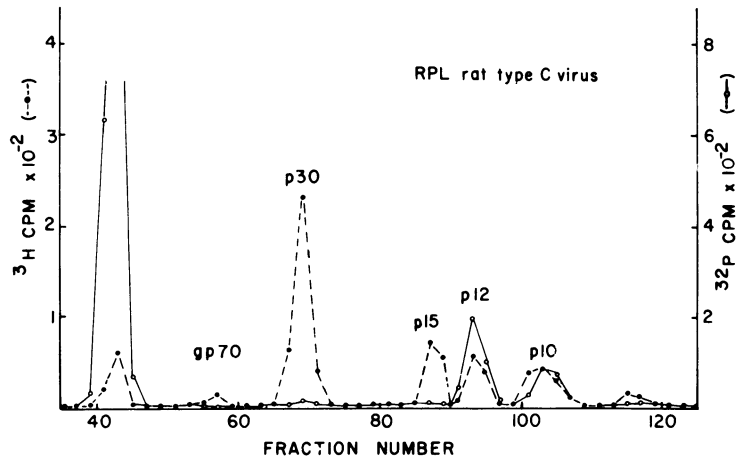

FIG. 4. *Separation of the polypeptides of the RPL rat type C virions, labeled with ^{3}H-labeled amino acids and [^{32}P]phosphate, by guanidine-agarose chromatography.*

ular size. It was necessary to study further separation among the molecules of similar size class utilizing possible differences in the total charge of the molecules. This was accomplished by electrophoresis in urea gels. The major phosphoproteins of GaLV (p12) and RD-114 (p15) labeled with ^{3}H-labeled amino acids and [^{32}P]phosphate were resolved into five to six major components (Fig. 8, panels A and B, respectively). Similarly, the phosphoprotein (p12) of feline leukemia viruses (18) were resolved into multiple components in urea gels. This is illustrated (Fig. 8, panel C) in the patterns of coelectrophoresis of ^{3}H-labeled amino acid-labeled p12 of FeLV-GA, and ^{14}C-labeled amino acid-labeled p12 of FeLV-ST. The superimposable nature of the multiple components of the differentially labeled p12 polypeptides of two isolates of FeLV suggested to us that the multiple peaks were not the result of breakdown of the polypeptides in urea gels. This distribution into multiple components was unique to p12 of FeLV because its other internal polypeptides, like p30 and p10, were resolved into single major components in urea gels (our unpublished data). An obvious explanation for multiple components could be the occurrence of the same polypeptide in various phosphorylated states. This postulation is supported by the data of Fig. 8 (panel A) and 9 (panel B) where it was shown that the greater the electrophoretic mobility of a component (left to right, cathode to anode) the higher was the ^{32}P (phosphate) to ^{3}H (amino acids) ratio. The possibility that the distribution might be due to degradation of the phosphoproteins was ruled out by the following experiments.

RD-114 virus was grown in the presence of

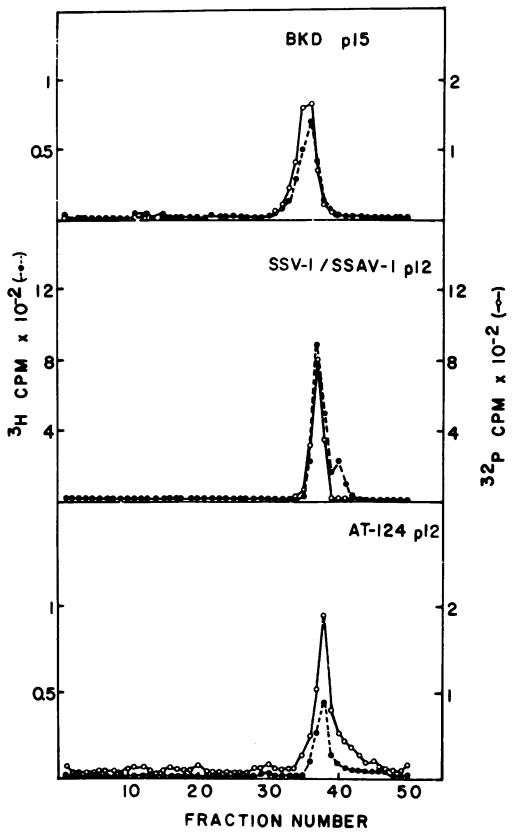

FIG. 5. *SDS-polyacrylamide gel electrophoresis of guanidine-agarose column-derived p15 of BKD, p12 of SSV-1/SSAV-1, and p12 of AT-124 all labeled with both ^{3}H-labeled amino acids and [^{32}P]phosphate.*

^{3}H-labeled amino acids and [^{32}P]phosphate, and the major phosphoprotein (p15) was isolated from the purified virion by guanidine-agarose chromatography (18). A portion of this p15 preparation was directly analyzed by SDS-polyacrylamide gel electrophoresis and a major single peak of superimposed ^{3}H and ^{32}P labels was obtained (Fig. 9, panel A). Other portions were subjected to urea gel electrophoresis in two identical gels. One of the gels was frozen, cut, and counted for radioactivities. It showed five major peaks with the increased mobility having an almost linear relationship with the increased ratio of ^{32}P to ^{3}H (Fig. 9, panel B). The total area comprising these five peaks was cut out from the second unfractionated gel and extracted with 20 mM Tris-hydrochloride (pH 7.4) containing 0.15 M NaCl at 0 C, and the solution was dialyzed overnight at 4 C against 0.1% β-mercaptoethanol in water and lyophilized. The dried materials were redissolved in 0.01 M sodium phosphate buffer (pH 7.2) containing 1% SDS and 0.1% β-mercaptoethanol, and subjected to SDS gel electrophoresis. Again a single major component of about 15,000 molecular weight was detected with superimposed ^{3}H and ^{32}P labels (Fig. 9, panel C) indicating that the polypeptides were not degraded under the conditions of urea gel electrophoresis.

Similar urea gel analysis of the p12 and p10 of the Sprague-Dawley rat virus showed resolution into variously phosphorylated multiple components (data not shown).

Comparison of the mammalian type C virus phosphoproteins. A list of the mammalian type C viruses studied and their major structural phosphoproteins is presented in Table 1. The polypeptide p12, was found to be a major phosphoprotein common to all type C viruses studied excepting RD-114 and BKD viruses where the major phosphoprotein was of molecular weight of about 15,000. The rat type C viruses and the Kirsten mouse sarcoma virus also contained, in addition to p12, another phosphoprotein of about 10,000 molecular weight. The mouse erythroblastosis virus also contained another major phosphoprotein which was either identical to, or comigrated with, the major glycoprotein (18).

Table 1 also shows that the endogenous mouse virus (AT-124) was deficient in the p15 polypeptide and the endogenous baboon virus

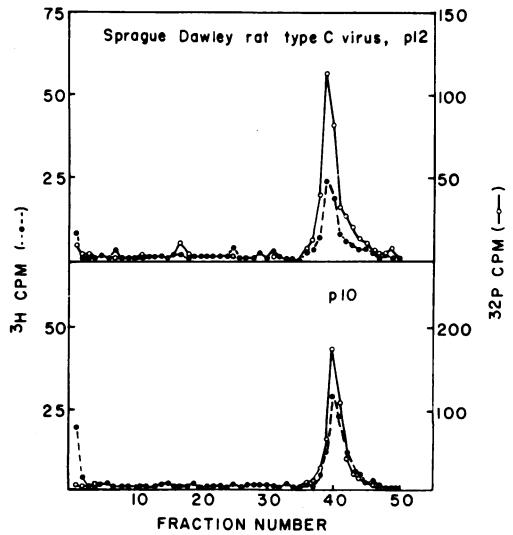

FIG. 6. *SDS-polyacrylamide gel electrophoresis of guanidine-agarose column-derived p12 and p10 of the Sprague-Dawley rat type C virus, labeled with both ^{3}H-labeled amino acids and [^{32}P]phosphate.*

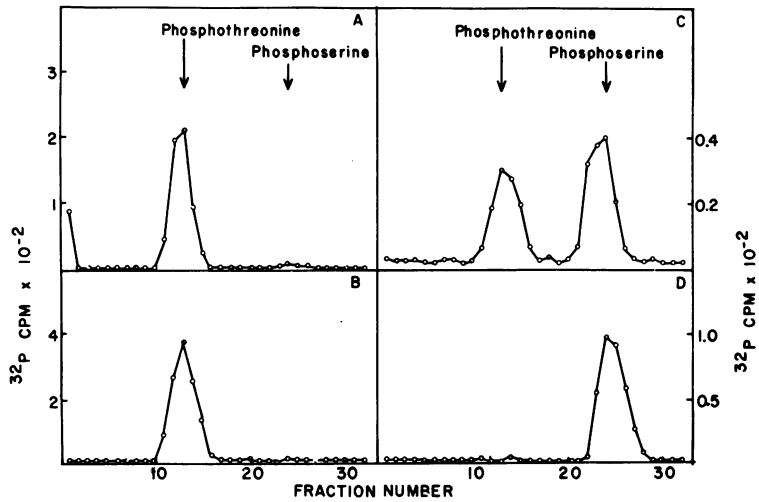

FIG. 7. *Identification of the phosphoamino acids in the in vivo phosphorylated virion polypeptides by high voltage paper electrophoresis. (A) BKD p15; (B) RD-114 p15 (previously reported in reference 18); (C) GaLV p12; (D) AT-124 p12. Arrows indicate the positions of the marker phosphoamino acids.*

(BKD) in the p12 polypeptide. In this respect, AT-124 virus was similar to the primate-derived viruses (SSV-1/SSAV-1 and GaLV) which were shown earlier to be deficient in p15 (23).

DISCUSSION

We present evidence that mammalian type C viruses contain phosphoproteins as their structural components. The proteins that are phosphorylated in vivo during virus synthesis belong to the major structural polypeptides. Phosphorylation is most probably carried out by protein kinases. We have not studied whether these phosphorylating enzymes are the same that were detected in a number of oncornaviruses (9, 31), or whether they represent cellular protein kinases (33). We have shown that the major phosphoprotein common to two primate-derived virus isolates (SSV-1/SSAV-1 and GaLV) and type C viruses of the lower mammalian species (mouse, rat, cat), with the exception of the endogenous cat virus (RD-114), is the polypeptide p12. Although the woolly monkey and the gibbon ape viruses were isolated from the respective primate tissues, their exact origin as yet remains unclear. These viruses have not been shown to be inducible from woolly monkey or gibbon ape cells in culture, nor has there been detectable evidence of virus-specific sequences in the cellular DNAs of these species (3, 26). On the other hand, a significant homology was detected between the sequences of these primate-derived viruses and the mouse type C viruses (5), and an immunological cross-reactivity was detected in the reverse transcriptases of the mouse and these primate viruses (36). In addition to showing a similarity in phosphoprotein of SSV-1/SSAV-1 or GaLV to mouse and other lower mammalian viruses, our data also shows that both of these primate-derived viruses resemble the endogenous mouse virus AT-124, in being deficient in the p15 structural polypeptide (23; and data presented in this report). All these results together may be interpreted to suggest an evolutionary relationship between woolly monkey or gibbon ape virus and mouse type C viruses, and that these primate-isolated viruses originated from endogenous xenotropic viruses of mice and are, thus, not endogenous viruses of primates (28)

Studies on the phosphoproteins of the endogenous cat virus RD-114, and the endogenous baboon type C virus BKD, reveal that the major phosphoprotein restricted to these two cases is the p15 polypeptide. The protein-phosphate bond in both cases is of the phosphothreonine variety which is less frequently found in biological polypeptides as compared to phosphoserine. The major phosphoamino acid in the p12 polypeptide of other viruses studied here is phosphoserine with the exception of GaLV which contains both phosphoserine and phosphothreonine. Thus there exists a similarity in the nature of the phosphoprotein of RD-114 and baboon viruses. Again, this similarity is consistent with the other relationships observed between endogenous primate virus and RD-114. RD-114 viral

RNA sequences are partially related to baboon virus RNA (4) and to baboon, and rhesus monkey cellular DNA (3) and the p30 protein of RD-114 is immunologically closely related to the baboon virus p30 (29). On the basis of these relationships it was proposed that RD-114 virus evolved from a progenitor of the Old World monkey viruses that was horizontally transmitted to ancestors of the cat at some time after these species diverged from one another (37).

A second major phosphoprotein detected in some type C viruses has a molecular weight of about 10,000. It is present in only rat type C viruses and in Kirsten mouse sarcoma virus which was derived from a mouse erythroblastosis virus after in vivo propagation through rat (11, 30). This Kirsten virus, as well as Harvey strain of mouse sarcoma virus, probably resulted from recombination events between

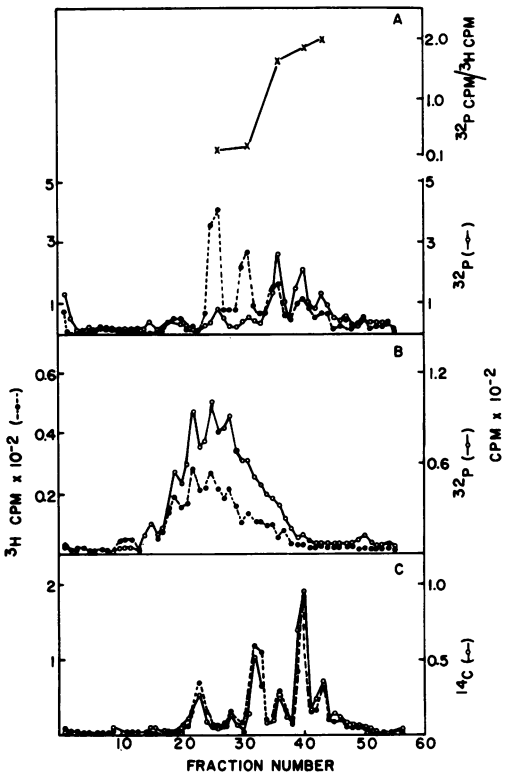

FIG. 8. *Further resolution of the guanidine-agarose column-derived phosphoproteins by urea-polyacrylamide gel electrophoresis. (A) p12 of GaLV labeled with ³H-labeled amino acids and [³²P]phosphate; ratio of the ³²P to ³H counts/min in the major peak fractions is shown by ×. (B) p15 of RD-114 labeled with ³H-labeled amino acids and [³²P]phosphate. (C) p12 of FeLV-GA (●) labeled with ³H-labeled amino acids and p12 of FeLV-ST (○) labeled with ¹⁴C-labeled amino acids.*

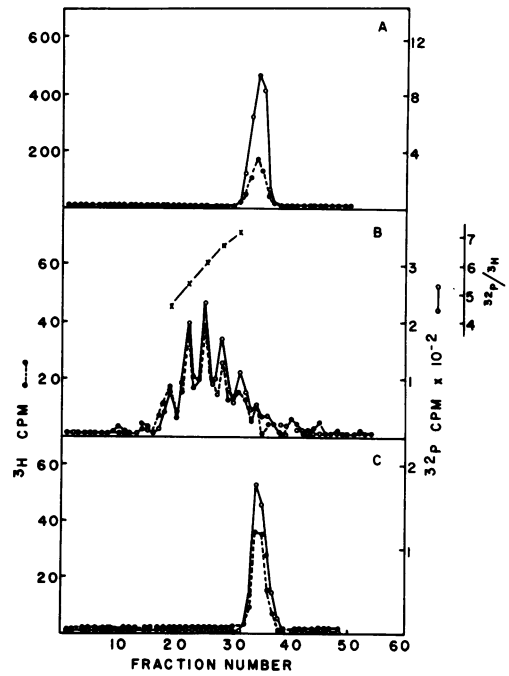

FIG. 9. *Polyacrylamide gel electrophoresis of the p15 of RD-114 labeled with ³H-labeled amino acids and [³²P]phosphate. The p15 was isolated by guanidine-agarose chromatography. (A) Electrophoresis in the presence of SDS. (B) Electrophoresis in the presence of urea; the ratio of the ³²P to ³H counts/min in the major peak fractions is shown by ×. (C) Electrophoresis in the presence of SDS of the eluted materials from fraction number 17 through 37 of a gel run identically as in B.*

mouse type C virus sequences and sequences in rat cells (25, 27; P. Roy-Burman and V. Klement, J. Gen. Virol., in press). Thus the p10 phosphoprotein may be a marker for viruses of rat origin. It is possible that p10 could be a breakdown product of p12 and such a precursor product relationship was indicated by the presence of immunological cross-reaction between p12 and p10 of the Kirsten strain of murine leukemia virus (20). In any case, the presence of p10 phosphoprotein is unique to rat-related type C viruses as it is not found in any other type C viruses studied.

The phosphoproteins of type C viruses probably have both structural and biological function. Maintenance of the integrity of complex intermolecular virion structure could be a major function. Since we find that the polypeptide p15, p12, or p10 is present in several different phosphorylated states, it is possible that each state is responsible for interaction with a specific component or a specific group of compo-

TABLE 1. *Major proteins and phosphoproteins of mammalian type C viruses*[a]

Species of isolation	Virus	Major glycoprotein	Major polypeptides			
		(gp70)	p30	p15	p12	p10
Mouse	Wild mouse type C virus isolates					
	WM-1540E (8)	+	+	+	⊕	+
	WM-292 (7)	+	+	+	⊕	+
	WM-275 (16)	+	+	+	⊕	+
	Mouse leukemia virus (Rauscher)	+	+	+	⊕	+
	AT-124, mouse endogenous virus	+	+	−	⊕	+
	Mouse erythroblastosis virus	⊕	+	+	⊕	+
	Kirsten mouse sarcoma virus	+	+	+	⊕	⊕
Rat	Sprague-Dawley rat embryo culture-derived type C virus	+	+	+	⊕	⊕
	RPL cell-associated rat type C virus	+	+	+	⊕	⊕
Cat	Feline leukemia virus (Gardner-Arnstein)	+	+	+	⊕	+
	RD-114, cat endogenous virus	+	+	⊕	+	+
Primate	BKD endogenous virus	+	+	⊕	−	+
	Woolly monkey sarcoma virus (SSV-1/SSAV-1)	+	+	−	⊕	+
	GaLV	+	+	−	⊕	+

[a] +, present; −, deficient; O, phosphoprotein.

nents of the viral structure. In other words, the various levels of phosphorylation may be necessary for appropriate assembly of virus structural components. It appears that the proportion of variously phosphorylated states is predetermined for a virus. This is illustrated in the urea gel electrophoresis experiments of the p12 phosphoprotein of FeLV-GA and FeLV-ST, both of which under conditions of propagation through the human rhabdomyosarcoma cell line should represent the subgroup B of feline leukemia virus (24). The proportions are identical for these two isolates as would be expected from nonrandom levels of phosphorylation. Other virus phosphoproteins, however, show different proportions. Thus, it is very likely that various phosphorylated states and their relative occurrence are characteristic of specific virus. Now the question arises what controls these levels. Protein kinase or kinases alone may not differentially direct the extent of phosphorylation of the same substrate polypeptide. But a combination of protein kinases and phosphoprotein phosphatases could result in variable distribution of phosphoryl groups in a polypeptide. It is conceivable that the polypeptides phosphorylated initially to a high level are subsequently stabilized in structural orientation through dephosphorylation by phosphatases. The extent of dephosphorylation may be determined by the state of association with other viral molecular components. Alternatively, we may be looking at an average profile of the low to high phosphorylations of the polypeptide in different virus particles. In such a case, phosphorylation will be a random nonspecific event and some kind of meaningless biological consequence. This is not very likely as our coelectrophoresis experiments certainly invoke nonrandom distribution. Although our attempts to explain the phenomena are highly speculative at this time, they at least provide a framework for further investigations on the structural role of phosphoproteins. The identification and characterization of the structural phosphoproteins of type C viruses should provide a basis for exploring the biological functions of these polypeptides and the related metabolic enzymes such as protein kinases and phosphoprotein phosphatases. A role for phosphoprotein in intracellular uncoating of the viral RNA, in regulating transcription of the viral genome, and in intracellular organization of viral molecular components are some of the avenues for further investigations.

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