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Purification and Characterization of Two Protein Kinases Associated with Rous Sarcoma Virus†

John H. Weis‡ and Anthony J. Faras*

ABSTRACT: The two major phosvitin-utilizing kinases have been purified from virions of the Prague C strain of Rous sarcoma virus by the use of ion-exchange and affinity chromatography. The two kinases isolated may be differentiated by their molecular weights as well as by their ability to utilize GTP as a phosphate donor. Protein kinase G, which will use either GTP or ATP as a phosphate donor, has a molecular weight of 120 000 as determined under nondenaturing conditions by glycerol gradient centrifugation and 28 000 when assayed under denaturation in sodium dodecyl sulfate (NaDodSO_4)–polyacrylamide gels. Protein kinase A, which will only efficiently use ATP as the phosphate donor, has an apparent molecular weight of 43 000 estimated by glycerol gradient sedimentation and 40 000 by NaDodSO_4–polyacrylamide electrophoresis. Both kinases possess the ability to autophosphorylate. Phosvitin is the major, and casein the minor, phosphate-accepting substrate for both kinases in vitro; however, kinase G will also phosphorylate histones to an extent similar to that observed with casein.

The presence of protein kinases associated with virions of retroviruses has been investigated by a number of groups (Houts et al., 1978; Tsiapolis, 1977a,b; Hizi et al., 1979; Hatanaka et al., 1972; Blaas et al., 1979; Rosok & Watson, 1979). These kinases have varied widely in their molecular weights, in their preference for phosphate donors and acceptors, and in their efficiency of phosphorylation. Three kinases have been identified from the virions of avian retroviruses: a 60 000 molecular weight protein preferring basic phosphate acceptor proteins (Rosok & Watson, 1979), a 45 000 molecular weight protein which utilizes the acidic phosphate acceptors (Fleissner & Tress, 1973), and a 25 000 molecular weight protein that can phosphorylate both acidic and basic phosphate acceptors (Hizi et al., 1979).

The role of the virion-associated kinases has also been investigated. Two reports have indicated that the phosphorylation of reverse transcriptase (Lee et al., 1975), or the phosphorylation of a reverse transcriptase associated protein (Tsiapolis, 1977a,b), can influence the rate of reverse transcription. A later report which investigated the phosphorylation of reverse transcriptase suggested that the β subunit of reverse transcriptase is a phosphoprotein (Hizi & Joklik, 1977). A number of the virion structural proteins have also been observed to be phosphorylated (Fleissner & Tress, 1973; Sen & Todaro, 1977 Leis et al., 1978). Included among these proteins are the two viral structural (gag-related) proteins pp19 and pp12 which are interesting due to their RNA binding capabilities.

The purpose of this investigation was to identify, purify, and characterize those protein kinases associated with Rous sarcoma virus. Futher studies will be initiated using these purified enzymes to specifically investigate their role(s) in the enhancement of reverse transcription by the phosphorylation of the viral polymerase.

Materials and Methods

Chemicals. The proteins used as phosphate acceptors and molecular weight markers as well as ATP and GTP were purchased from Sigma. DEAE-cellulose (DE-52), carboxymethylcellulose (CM-52), and phosphocellulose (PC-11) were purchased from Whatman. Adenosine triphosphate–agarose (ATP–agarose) was from P-L Biochemicals. [γ-^{32}P]ATP and [γ-^{32}P]GTP were prepared in this laboratory by the procedure of Walseth & Johnson (1979). The initial specific activities of the [^{32}P]ATP and [^{32}P]GTP preparations were 6500 Ci/mmol.

Virus. The Prague C strain of Rous sarcoma virus (RSV) was purified from crude virus pellets or ammonium sulfate precipitated virus from supernatants harvested from infected cell cultures. The virus was purified by banding once on a 40% potassium tartrate cushion in 15% sucrose followed by banding twice isopycnically in a 25–50% sucrose gradient (Faras & Dibble, 1975).

Protein and Enzyme Assays. Protein concentrations were determined by the method of either Lowry et al. (1951) or Sedmark & Grossberg (1977). Protein kinase activity was assayed in a buffer of 20 mM $MgCl_2$, 5 mM potassium phosphate, pH 7.5, and 1 mg/mL exogenous protein. For autophosphorylation studies, no exogenous protein was added. One unit of protein kinase activity was defined as that amount of enzyme required to transfer 1×10^{-12} mol of [γ-^{32}P]ATP

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to phosvitin in 60 min at 37 °C. The protamine-utilizing protein kinase was assayed by the method of Rosok & Watson (1979) by using a buffer of 25 mM tris(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl), pH 8.2, 10 mM $MgCl_2$, 5 mM dithiothreitol, and 1 mg/mL protamine. All protein kinase assays were for 60 min at 37 °C. Radioactivity was determined by perchloric acid precipitation of the labeled proteins and filtration of the precipitate onto glass fiber filters. Reverse transcriptase activity was determined by using sonicated calf thymus DNA buffer (Faras et al., 1972).

Chromatography. All chromatography columns were used with the following buffer: 10 mM potassium phosphate, pH 7.2, 4 mM β-mercaptoethanol, 0.2% NP-40, and 10% glycerol. The DEAE-52 and CM-52 columns were eluted with an increasing potassium phosphate buffer concentration while the PC-11 and ATP–agarose columns were eluted with increasing NaCl concentration. The NaCl concentration gradients were followed by refractive index. All purification steps were carried out at 4 °C. The final enzyme pools were concentrated by dialysis overnight in column buffer with 50% glycerol. The enzymes were stored at −70 °C.

Sodium Dodecyl Sulfate ($NaDodSO_4$)–Polyacrylamide Electrophoresis and Gradient Centrifugation. $NaDodSO_4$–polyacrylamide gels of a 5–15% linear gradient were prepared and run according to Laemmli (1970). Bovine serum albumin (M_r 65 000), ovalbumin (M_r 43 500), myoglobin (M_r 16 900), and cytochrome *c* (M_r 12 500) were used as molecular weight markers in all gels. After electrophoresis, gels were stained with Coomassie blue, destained in 10% acetic acid and 30% methanol, and dried on filter paper. Autoradiography of the dried gels was with Kodak No-Screen film.

Glycerol gradients (10–30%) were prepared containing 0.4 M NaCl, 0.01 M potassium phosphate, pH 6.8, 0.2% NP-40, and 0.2% β-mercaptoethanol.

Results

Virion Kinases. The impetus to purify the protein kinases associated with preparations of RSV was promopted by our initial observation that partially purified preparations of avian myeloblastosis virus (AMV) reverse transcriptase contained two phosvitin-utilizing kinases. These kinases were easily resolvable by glycerol gradient centrifugaiton by using either crude reverse transcriptase preparations or detergent-disrupted virions of Rous sarcoma virus (RSV) (Figure 1). The larger of the phosvitin-utilizing kinases migrated with an apparent molecular weight of 130 000 while the smaller kinase activity comigrated with ovalbumin at molecular weight of 43 500. The protamine-utilizing kinase that was previously reported by Rosok & Watson (1979) could also be detected.

Purified Prague C RSV was disrupted by adjusting the sample to 6.6% NP-40, 6.6% sodium deoxycholate (w/v), and 0.8 M KCl and incubating the preparation at 0 °C for 45 min. After disruption of the virus, the sample was centrifuged for 10 min at 10 000 rpm, and the pellet was discarded. The supernatant was diluted 10-fold in column buffer and loaded onto a DEAE-52 column (DEAE 1). After the supernatant was loaded, the column was washed with buffer and eluted with 0.3 M potassium phosphate (pH 7.2) (Figure 2A). Greater than 83% of the kinase activity, assayed with phosvitin as the phosphate acceptor, was bound to the column. This corroborated well with the amount of reverse transcriptase (95%) that bound to the column. The horizontal bar indicates those fractions pooled as the DEAE 1 pool. The pool contained 19% of the total protein before loading and 33% of the kinase activity. The DEAE 1 pool was diluted 10-fold with column buffer and passed over a CM-52 column. Greater than 94%

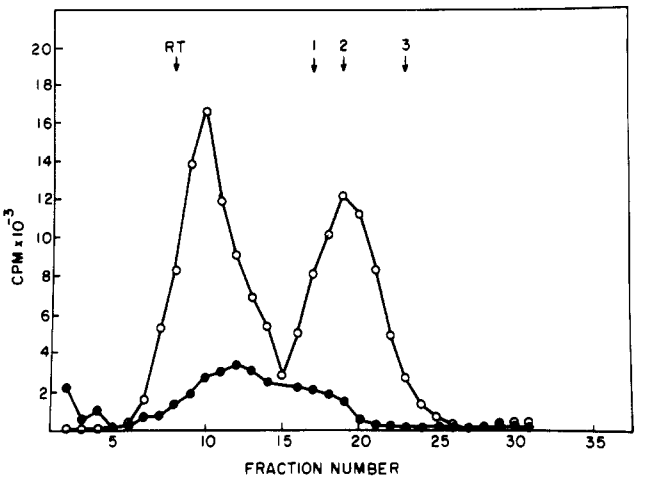

FIGURE 1: Analysis of kinase activity associated wth RSV. Purified RSV (0.3 mg) was disrupted with sodium deoxycholate as described and centrifuged through a 10–30% glycerol gradient (SW41 at 41 000 rpm and 4 °C for 46 h) with 100 μg each of bovine serum albumin, ovalbumin, and myoglobin, in separate tubes, as molecular weight markers. The kinase activity was detected by incubating 10 μL per fraction with 50 μL of buffer containing 1.1 μCi of [γ-^{32}P]ATP and either phosvitin (○) or protamine (●) as the phosphate acceptor. Reverse transcriptase, which was assayed as described, is indicated by RT. Molecular weight markers (1, bovine serum albumin; 2, ovalbumin; 3, myoglobin) were detected by the method of Sedmark & Grossberg (1977).

of the reverse transcriptase activity loaded onto the column was bound. However, only 38% of the kinase activity assayed with [γ-^{32}P]ATP and phosvitin was bound to the column. When the kinase activity was determined with [γ-^{32}P]GTP and phosvitin, only 11% of the enzymatic activity was bound to the column. The CM-52 column was washed with 2 volumes of buffer and eluted with a 200-mL linear gradient of 0.01–0.6 M potassium phosphate, pH 7.2, in the same buffer. From the elution profile of protein kinases from the CM-52 column (Figure 2B), it appears that there is only a minimal fraction of the GTP-utilizing kinase present in the elution profile while a broad peak of the ATP-utilizing protein kinase is apparent. The bar indicates those fractions collected as the carboxymethyl (CM) pool. This pool contained 6% of the protein and 46% of the protein kinase activity loaded onto the column. The CM pool was diluted 10-fold with column buffer containing 50 mM NaCl and applied to a phosphocellulose (PC)-11 column. The column was washed with buffer and eluted with a linear NaCl gradient of 0.1–1.0 M NaCl. Greater than 97% of the protein kinase activity was bound to the column (Figure 3A). The PC-11 pool contained 85% of the protein loaded on the column and 47% of the kinase activity. The PC-11 pool was diluted 10-fold in column buffer and loaded on a 5-mL ATP–agarose affinity column (Figure 3B). The column was washed with 20 mL of buffer and eluted with a 20-mL linear gradient of 0.05–0.7 M NaCl. Greater than 71% of the kinase activity was bound to the column. The protein kinase active fractions were dialyzed against 50% glycerol in column buffer overnight, collected, and frozen at −70 °C (designated ATP 1). The enzyme recovered was 2% of the initial protein kinase activity and 29% of the activity loaded on the ATP–agarose column. The final yield of protein kinase was 40 units, and it was designated protein kinase A.

The purification of the GTP-utilizing kinase found in the CM-52 flow-through volume was accomplished by reloading the protein kinase directly onto a second DEAE-52 (DEAE 2) column. The loaded column was washed with 2 volumes of buffer and eluted with a 70-mL linear gradient of 0.01–0.6

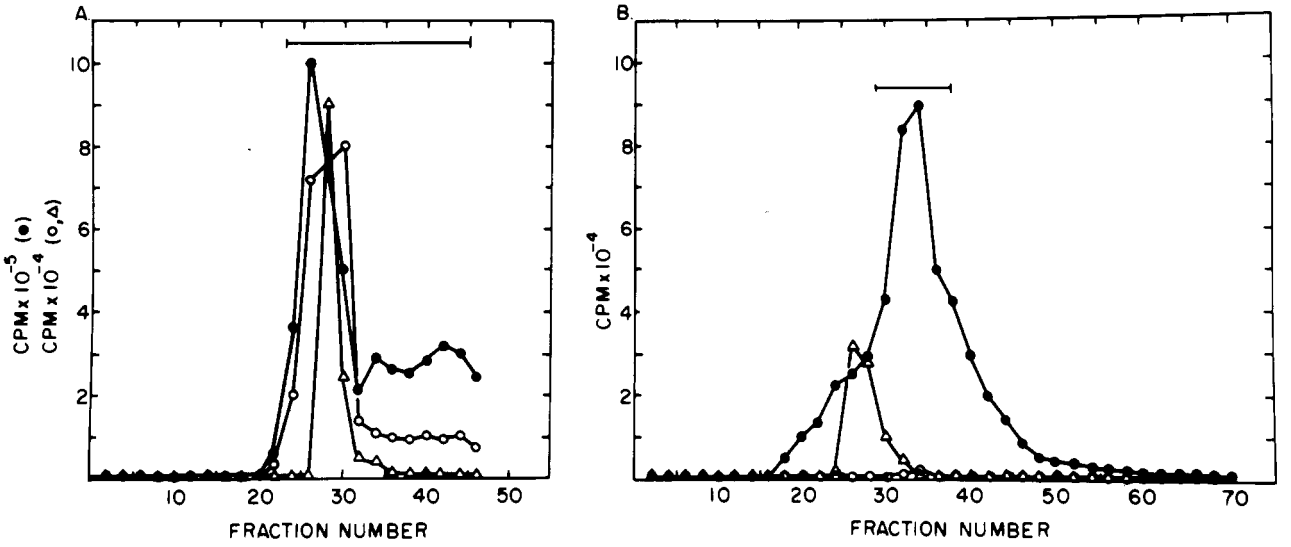

FIGURE 2: DEAE-cellulose (A) and carboxymethylcellulose (B) chromatography. (Panel A) The loaded virus was eluted with a 0.3 M potassium phosphate (pH 7.2) step. 46 fractions, each containing 4 mL, were collected. The bar indicates those fractions pooled and loaded onto the carboxymethylcellulose column (B). The kinase activity was assayed by using either [γ-^{32}P]ATP (●) or [γ-^{32}P]GTP (○). Each reaction contained 50 μL of phosvitin-containing buffer, 10 μL/column fraction, and 3 μCi of the apropriate triphosphate. Reverse transcriptase activity (△) was detected by using 10 μL/column fraction with 50 μL of calf thymus DNA buffer. All reactions were for 30 min at 37 °C. The samples were acid precipitated and counted. (Panel B) The loaded protein was eluted with a 200-mL 0.01–0.6 M potassium phosphate linear gradient. Both [γ-^{32}P]ATP (●) and [γ-^{32}P]GTP (○) were used to assay the column fractons. Kinase and reverse transcriptase (△) activities were determined as described in panel A. The bar indicates the pooled fractions collected for application to the phosphocellulose column.

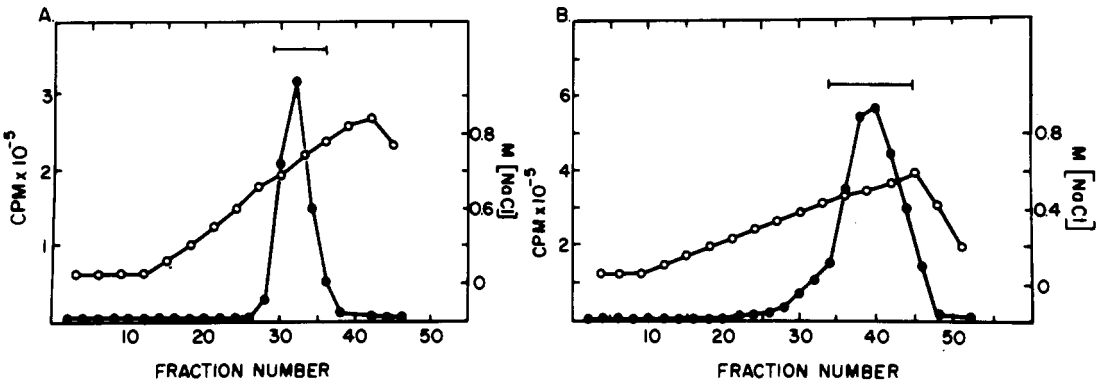

FIGURE 3: Phosphocellulose (A) and ATP–agarose (B) chromatography of kinase A. (Panel A) The loaded and washed PC-11 column was eluted with a 100-mL linear gradient of 0.1–1 M NaCl. Fractions containing 3.5 mL were collected and assayed. Each reaction contained 50 μL of phosvitin-containing kinase buffer, 5 μL/column fraction, and 3 μCi of [γ-^{32}P]ATP. The horizontal bar indicates the fractions pooled. This chromatography step eliminates virtually all the protamine-utilizing kinase (Rosok & Watson, 1979) present in the preparations thus far. (Panel B) The pooled active fractions from panel A were loaded onto a 5-mL ATP–agarose column. The column was eluted with a 20-mL linear gradient of 0.05–0.7 M NaCl. Each fraction contains 0.5 mL per fraction. Kinase activity was assayed by using 50 μL of kinase buffer with phosvitin and 3 μCi of [γ-^{32}P]ATP. The bar indicates those fractions pooled, concentrated by dialysis overnight against 50% glycerol in column buffer, and stored at −70 °C.

M potassium phosphate, pH 7.2 (Figure 4A). Greater than 92% of the kinase activity was bound to this column prior to elution. The fractions containing protein kinase activity were pooled, diluted 10-fold with column buffer supplemented with 50 mM NaCl, and loaded on a 5-mL ATP–agarose column (ATP 2). The column was washed and eluted with a 0.1–0.7 M NaCl gradient (Figure 4B). Greater than 78% of the kinase activity loaded on the column was bound. The pool of kinase activity was dialyzed overnight against 50% glycerol in the column buffer and stored at −70 °C. This fraction (ATP 2 pool) contained 2% of the original kinase activity of the disrupted virus pool. The final yield of this protein kinase was 43 units, and it was designated as protein kinase G.

Table I details the quantitation of the purification procedures. The final yield of both protein kinases was 4% of the original activity. This small yield was most likely due to the number of steps involved in purification, the preferential pooling of peak fractions, and the stability of the enzyme at 4 °C during purification. Nevertheless, as seen in Figures 1 and 6, the two purified enzymes are identical with the two major phosvitin-utilizing protein kinases present in the virion.

NaDodSO$_4$–Polyacrylamide Analysis of Protein Kinases A and G. In attempting to visualize the purified protein kinases by NaDodSO$_4$–polyacrylamide electrophoresis, we utilized the ability of the enzymes to autophosphorylate (Rosok & Watson, 1979; Erikson et al., 1979). Due to the low protein yield, we could not visualize any distinct bands by Coomassie blue staining. However, when the purified protein kinases were incubated in the presence of [γ-^{32}P]ATP but without any exogenous phosphate-acceptor proteins, distinct bands were readily discernible (Figure 5). (Autophosphorylation studies using [γ-^{32}P]GTP and either kinase G or virus did not differ in results from those studies with [γ-^{32}P]ATP.) Autophosphorylated detergent-disrupted virus does not contain any distinct bands which comigrate with either protein kinase A or G, indicating these kinases represent a minor subset of

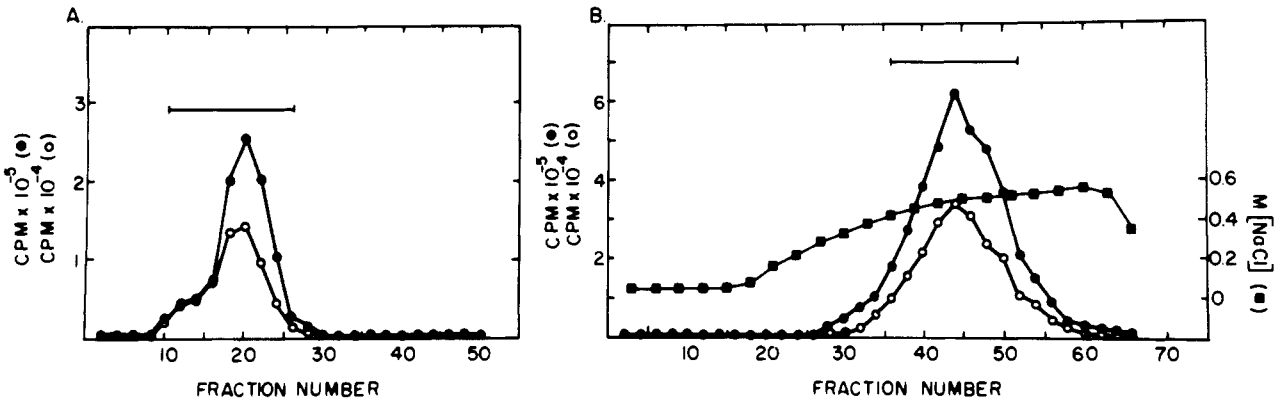

FIGURE 4: DEAE-cellulose (A) and ATP–agarose (B) chromatography of kinase G. (Panel A) The flow-through fraction from the carboxymethylcellulose column (Figure 2B) was loaded directly onto a DEAE-52 column. The kinase was eluted with a 70-mL linear gradient of 0.01–0.6 M potassium phosphate. Fractions containing 2.0 mL were collected and assayed. Each reaction contained 10 μL per fraction, 50 μL of phosvitin-containing buffer, and 3 μCi of either of [γ-^{32}P]ATP (●) or [γ-^{32}P]GTP (○). The active fractions were pooled as indicated by the horizontal bar. (Panel B) The active fractions from panel A were loaded on a 5-mL ATP–agarose affinity column. The enzyme was eluted with a 20-mL linear gradient of 0.1–0.7 M NaCl. Fractions containing 0.5 mL were collected and assayed as described in panel A [γ-^{32}P]ATP (●); [γ-^{32}P]GTP (○); NaCl concentration (■)]. The horizontal bar indicates the fractions pooled, concentrated by dialysis, and stored at −70 °C.

Table I: Purification of RSV Kinases A and G[a]

	fraction	vol (mL)	[protein] (mg)	kinase units	% yield	sp act. (units/mg)
kinases A and G	virus pool	600	234	2190	100	9.4
	DEAE-52 pool	80	44	724	33	17
kinase A	CM-52 pool	30	2.7	333	15	123
	PC-11 pool	25	2.3	141	7	61
	ATP-agarose pool	1	0.04	40	2	975
kinase G	CM-52 flow through	400	35.2	283	13	8.04
	DEAE-52 pool	37	22.9	208	10	9.08
	ATP-agarose pool	0.8	0.05	43	2	914

[a] Kinase units were defined as the ability to transfer 1×10^{-12} mol of [γ-^{32}P]ATP to phosvitin in 1 h at 37 °C as determined by acid-precipitable counts. The percentage yields of purification of both kinase A and kinase G were determined from the total ATP-utilizing kinase activity present in the disrupted virus pool. All samples were quantitated for kinase units at the end of the purification regime. Protein concentrations were determined by the method of Lowry et al. (1951) after removal of all buffer constituents by twice-repeated acetone precipitations.

virion-associated proteins capable of being phosphorylated. Protein kinase G appears to migrate as a slightly larger molecular weight protein than p27, a gag-related structural protein of the virus. Purified preparations of p27 do not possess any kinase activity (K. A. Staskus, unpublished experiments) nor does the protein appear to be phosphorylated in the virus even though it comprises a significant portion of the total protein of the virus. The bulk of protein kinase A migrates with an apparent molecular weight of 40 000; however, there appears to be some heterogeneity in either the size or the charge of the kinase preparation.

Glycerol Gradient Centrifugation of Protein Kinases A and G. The sedimentation of disrupted RSV, as illustrated in Figure 1, indicated there are two major phosvitin-utilizing kinases present within the virus particle. Since our final yield of purification was only 4% for both kinases, it was important to compare the sedimentation patterns of protein kinases A and G with those from the disrupted virion. Figure 6A illustrates the sedimentation of 4.3 units of protein kinase G and 3.2 units of protein kinase A. Both protein kinase gradients were analyzed for activity with either [γ^{32}P]ATP or [γ-^{32}P]GTP by using phosvitin as the primary phosphate acceptor. There is a slight decrease in the sedimentation velocity of the larger kinase with purification but no observable change in the migration of the smaller enzyme. This gradient also clearly identifies the 120 000 molecular weight protein kinase G, as the kinase able to efficiently utilize [γ-^{32}P]GTP.

To examine whether the proteins found in the peak gradient fractions were identical with those shown by autophosphorylation in $NaDodSO_4$–polyacrylamide gels, we autophosphorylated the peak fractions and electrophoresed the phosphorylated proteins in a $NaDodSO_4$–polyacrylamide gel. From the data in Figure 6, it is evident that the major phosphorylated gradient proteins are identical with the phosphorylated proteins identified as protein kinase A (p40) and protein kinase G (p28). Therefore, protein kinase G must exist in solution as a multimer, possibly a tetramer. Protein kinase A appears to be a monomer in solution but also to possess some molecular heterogeneity as suggested by lane E, Figure 5, and lane B, Figure 6B. This heterogeneity is not resolvable into distinct protein bands, which may indicate that there are many closely migrating molecular species of protein kinase A. Since the addition of a phosphate to a protein may alter its electrophoretic mobility (Erikson et al., 1977), the broad band may be due to the presence of subsets of the kinase, each being phosphorylated to a different degree.

Phosphate Acceptors: Analysis of Phosphorylation Substrates. The presence of protein kinases A and G was first detected by using phosvitin as the phosphate acceptor. These kinases, as seen in Figure 1, do not utilize protamine as an efficient substrate in contrast to a 60 000 molecular weight kinase isolated from AMV (Rosok & Watson, 1979). The efficiency of phosphorylation by kinases A and G was examined by employing a variety of phosphate-accepting proteins, including the basic protamine and histone molecules as well as the acidic casein and phosvitin proteins (Table II). Protein kinase A will only phosphorylate phosvitin, and, to a lesser degree, casein, when ATP is used as the phosphate donor.

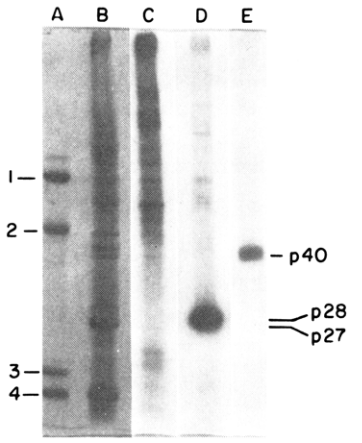

FIGURE 5: Autophosphorylaton of the purified kinase A and kinase G and the phosphorylation of disrupted RSV. A 5–15% $NaDodSO_4$–polyacrylamide gel was used to study the migration of the phosphorylated proteins. Each reaction contained 70 μL of kinase buffer without phosvitin and 3 μCi of [γ-^{32}P]ATP. After the incubation, each reaction was precipitated in 10% trichloroacetic acid (Cl_3CCOOH), reprecipitated in 100% acetone, resuspended in sample buffer, and layered into their respective wells in the gel. After electrophoresis, the gel was stained with Coomassie blue, destained, dried, and exposed to Kodak No-screen X-ray film. (Lane A) Molecular weight markers (1, bovine serum albumin; 2, ovalbumin; 3, myoglobin; 4, cytochrome *c*); (lane B) 50 μg of virus (Coomassie stain); (lane C) 25 μg of virus incubated with 0.05% NP-40 and [γ-^{32}P]ATP; (lane D) 0.3 unit of kinase G; (lane E) 0.4 unit of kinase A. Lanes A and B are the Coomassie-stained proteins while lanes C–E are the autoradiograms of the phosphorylated proteins. p40 indicates the migration of the autophosphorylated kinase A and p28 the position of the autophosphorylated kinase G.

Table II: Comparison of Phosphate Donors and Acceptors with Kinases A and G[a]

phosphate donor	phosphate acceptor	kinase A	kinase G
ATP	(autophosphorylation)	0.1	2.2
	phosvitin	76.5	205.6
	histones	<0.1	24.5
	protamine	<0.1	4.5
	casein	40.6	24.1
GTP	(autophosphorylation)	<0.1	1.9
	phosvitin	0.8	55.4
	histones	<0.1	9.2
	protamine	<0.1	3.1
	casein	0.2	7.6

[a] All reactions contained 50 μL of kinase buffer and either no exogenous protein (autophosphorylation) or 1 mg/mL each of phosvitin, histones, protamine, or casein. In addition, each reaction contained 2 μCi of [γ-^{32}P]ATP or 2.4 μCi of [γ-^{32}P]GTP and 0.3 unit of kinase G or 0.15 unit of kinase A. Each reaction was incubated at 37 °C for 30 min. The values expressed are the femtomoles of acid-precipitated ^{32}P-labeled proteins.

Protein kinase G will phosphorylate predominately phosvitin by using either ATP or GTP but will also phosphorylate casein and histones to a lesser extent.

Discussion

The purification and characterization of the two major phosvitin-utilizing protein kinases of RSV have been described in this report. The two protein kinases isolated appear to possess the same preference for phosphate-accepting substrates but do differ in their requirement for GTP as the phosphate donor.

Protein kinase A is only capable of efficiently utilizing ATP as the phosphate donor. It has an apparent molecular weight of 43 000 by glycerol gradient centrifugaiton either in the

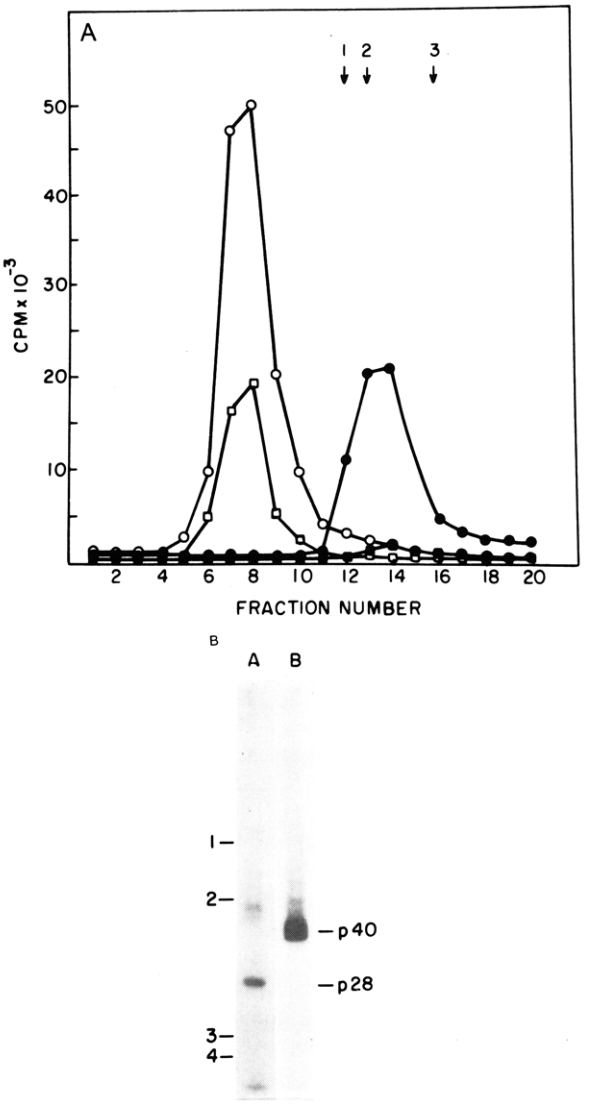

FIGURE 6: Analysis of kinase A and kinase G under nondenaturing and denaturing conditions. (Panel A) Kinase A (3.2 units) and kinase G (4.3 units) were analyzed by 10–30% glycerol gradient centrifugation. The sample was centrifuged in an SW41 rotor for 45 h at 40 000 rpm and 4 °C. Molecular weight standards (1, bovine serum albumin; 2, ovalbumin; 3, myoglobin) were also run in separate tubes. Kinase activity was detected by using [γ-^{32}P]ATP [(○) kinase G; (●) kinase A] and [γ-^{32}P]GTP [(□) kinase G; (■) kinase A] in kinase buffer with 1 mg/mL phosvitin. Each reaction contained 50 μL of buffer, 10 μL/gradient fraction, and 3 μCi of ^{32}P-labeled triphosphate. (Panel B) Autophosphorylaton of the peak kinase fractions from the glycerol gradients of kinase A and kinase G. Each reaction contained 60 μL of kinase buffer without phosvitin, 100 μCi of [γ-^{32}P]ATP, and 15 μL of the glycerol gradient fraction. The reactions were for 60 min at 37 °C. The phosphorylated samples were treated identically with those in (A). Molecular weight markers were run (1, bovine serum albumin; 2, ovalbumin; 3, myoglobin; 4, cytochrome *c*) and detected by Coomassie staining. p40 and p28 indicate the migration of the autophosphorylated kinases A and G, respectively.

highly purified form (Figure 5) or from detergent-disrupted virus (Figure 1). By analysis with $NaDodSO_4$–polyacrylamide gel electrophoresis, the autophosphorylated protein kinase A migrates with an approximate molecular weight of 40 000 (Figures 5 and 6). Interestingly, as alluded to previously, the kinase appears to migrate with some minor size heterogeneity. Whether this is due to an actual size or charge difference between molecules is not known.

Protein kinase A will preferentially phosphorylate phosvitin but will also phosphorylate casein to a lesser degree. Protamine will not be radioactively labeled by protein kinase A with either

[γ-^{32}P]ATP or [γ-^{32}P]GTP above the background levels.

At least two other kinases similar to protein kinase A have been reported, one from AMV (Houts et al., 1978) and the other from the PrC strain of RSV (Hizi et al., 1979). The 45 000 molecular weight protein kinase from AMV appears to be similar to protein kinase A in molecular weight, phosphate donors, and phosphate-acceptor proteins. However, the purification procedures of the kinases are quite different since only 5–10% of the AMV protein kinase will bind to DEAE-cellulose while greater than 83% of the PrC RSV kinase activity bound to DEAE-cellulose. This question is further clouded by our own observations of the 45 000 molecular weight kinase in preparations of reverse transcriptase which was eluted from DEAE-cellulose. The 45 000 molecular weight protein kinase previously isolated from the PrC strain of RSV (Hizi et al., 1979) also differed from our purified protein kinase A. Although both kinases had the same molecular weight from the disrupted virus, the molecular weight of the previously reported protein kinase shifted from 45 000 to 25 000 with purificaiton (as determined by glycerol gradient centrifugation). Furthermore, the protein kinase migrated in $NaDodSO_4$–polyacrylamide gels with an apparent molecular weight of 11 000. These data, contrasted with those present here, as well as the fact that the previously reported RSV protein kinase could utilize GTP efficiently as the phosphate donor (which protein kinase A cannot do), indicate the two enzymes are most likely not the same protein.

Protein kinase G is distinct from protein kinase A in its efficient use of GTP as the phosphate donor and is distinct from other protein kinases described with avian retroviruses by its nondenatured molecular weight. The enzyme will phosphorylate phosvitin and, to a much lesser degree than kinase A, casein. It will also phosphorylate the basic histone molecules. When glycerol gradient centrifugation analysis is employed, the enzyme has an apparent molecular weight of 130 000 from disrupted virions which shifts to 120 000 with extensive purification of the enzyme (Figures 1 and 6). $NaDodSO_4$–polyacrylamide gel electrophoresis of the autophosphorylated enzyme indicates the protein migrates very closely to, but slightly slower than, the viral protein p27. However, protein kinase G and p27 are definitely distinct since p27 is not a phosphoprotein and does not possess any kinase activity (data not shown).

Neither protein kinase A nor protein kinase G is present in the virion as a major phosphate-acceptor protein yet, with extensive purification, it is capable of being autophosphorylated. Whether the phosphorylaton of either enzyme has any effect upon its activity is not known. Similarly, Coomassie blue staining of the disrupted virus in $NaDodSO_4$–polyacrylamide gels does not specifically identify either kinase; thus, they must be considered minor components of the virion. However, the reproducible isolation of the kinase from RSV as well as AMV (J. H. Weis, unpublished experiments) indicates these enzymes are not random contaminants within the virion. Studies are currently under way to determine whether either of these virion-associated protein kinases can phosphorylate reverse transcriptase and their effect, if any, on reverse transcription.

Registry No. Protein kinase, 9055-08-7; phosvitin kinase, 9055-03-2.

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