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Enzymatic activities associated with a purified simian virus 40 T antigen-related protein

(adenovirus–simian virus 40 hybrid/D2 hybrid protein/ATPase/protein kinase/antibody inhibition)

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ABSTRACT **A protein antigenically related to the simian virus (SV40) *A* gene product has been purified to near homogeneity from cells infected with the adenovirus–SV40 hybrid virus Ad2$^+$D2 and shown to contain ATPase (ATP phosphohydrolase, EC 3.6.1.3) and protein kinase (ATP:phosphotransferase, EC 2.7.1.37) activity. Both enzymatic activities copurify with the protein through six stages including one gel filtration column, two ion exchange columns, and a heparin affinity column. Analogous fractions from extracts of cells uninfected or infected with adenovirus 2 alone do not contain these enzymatic activities. The D2 hybrid protein resolves into two forms (I and II) during ion exchange chromatography. Form I, the major species (85%) of the D2 hybrid protein, elutes from DEAE-Sephadex in 0.37 M NaCl and is able to catalyze the hydrolysis of ATP to ADP + P_i at a rate of 3 μmol/hr per mg. The remaining 10–15% of the D2 hybrid protein consists of form II which elutes from DEAE-Sephadex in 0.29 M NaCl and is able to hydrolyze ATP as well as to incorporate phosphorus from ATP into either the D2 hybrid protein itself or other protein acceptors such as phosvitin. Although both forms are able to bind DNA, the ATPase activity of form I cosediments with SV40 DNA more efficiently than does the protein kinase activity of form II during glycerol gradient centrifugation. The ATPase activity of form I is efficiently inhibited by addition of anti-T gamma globulin to the reaction mixture whereas control gamma globulin has no effect. Similarly, the phosphorylation of the D2 hybrid protein by form II is inhibited by anti-T gamma globulin. By contrast, phosphorylation of phosvitin is specifically inhibited by antibody only when the immune complex is removed from the reaction mixture. Thus, it appears likely that one and possibly two enzymatic activities are carried out by the D2 hybrid protein. These findings are discussed in terms of mechanisms of SV40 DNA replication and virally induced transformation.**

The tumor antigen, T antigen, encoded by the *A* gene of simian virus 40 (SV40) is a phosphorylated protein with an apparent molecular weight of 96,000 that is synthesized early after infection of host cells (1–3). Genetic evidence indicates that the *A* gene product functions as a regulatory component, involved both in viral replication and in transcription (2, 4–6). In addition, the tumor antigen is capable of stimulating cellular DNA replication and appears also to be required for the initiation and maintenance of transformation in various nonpermissive host cells (2, 7–13).

To understand how the (SV40) *A* gene product carries out these physiologically significant functions, we recently isolated in pure form a 107,000-dalton T antigen-related protein from cells infected with a defective adenovirus–SV40 hybrid, Ad2$^+$D2 (14, 15). The D2 hybrid protein consists of a polypeptide chain that is largely encoded by sequences of the SV40 *A* gene but contains a small (10,000–12,000 daltons) stretch of an unknown adenoviral coded protein at its amino terminus in place of the small T antigen portion of the SV40 tumor antigen (14, 16, 17). The purified D2 hybrid protein binds in a sequential manner to three specific sites contained within a sequence of 120 nucleotides located in a region of the SV40 genome that encompasses the origin of DNA replication and overlaps the start of early and late transcription (15). In addition, the highly purified D2 hybrid protein is capable of stimulating cellular DNA synthesis when injected into quiescent cells (8).

These experiments provide biochemical evidence for the involvement of the D2 hybrid protein in DNA replication although its mechanism of action remains obscure. One possibility is that, in addition to binding at origins of replication, the *A* gene protein catalyzes certain reactions required to initiate DNA synthesis. A search was therefore undertaken to test whether the purified D2 hybrid protein contained enzymatic activities such as site-specific nuclease, DNA unwinding, DNA polymerase, RNA polymerase, and ATPase that could be associated with initiation of replication. In addition, a recent report indicating that the transformation protein from avian sarcoma viruses ($p60^{src}$) contains a protein kinase activity prompted us to also test for a similar activity in the D2 hybrid protein (18, 19). In this communication we report that both an ATPase activity (ATP phosphohydrolase, EC 3.6.1.3) and a protein kinase activity (ATP:protein phosphotransferase, EC 2.7.1.37) are associated with purified D2 hybrid protein.

MATERIALS AND METHODS

Cells and Viruses. Monolayers of CV1 monkey cells were cultured in plastic dishes containing Dulbecco's modification of Eagle's medium supplemented with 5% (vol/vol) fetal bovine serum, streptomycin and penicillin (100 μg/ml). HeLa cells were grown in suspension cultures containing F-13 medium supplemented with 5% (vol/vol) horse serum. Plaque-purified hybrid virus Ad2$^+$D2 was propagated in monolayers of CV1 monkey cells as described (14).

ATPase Assay. The hydrolysis of ATP to ADP + P_i was carried out in 25–50 μl of PK buffer (0.01 M Tris, pH 8.0/0.15 M NaCl/5 mM $MgCl_2$/0.5% Nonidet P40 containing 1 μM [γ-^{32}P]ATP (300 Ci/mmol) and 0.1–1 μg of D2 hybrid protein. After incubation of the mixture at 20°C for 30–60 min, 1 μl was spotted on a polyethyleneimine plate (Polygram Cel 300, Macherey–Nagel Co., Westbury, NY) and subjected to ascending chromatography in pH 3.5 0.75 M KH_2PO_4. The products of the ATP hydrolysis reaction were visualized by autoradiography and, when necessary, the separated substrate and products were cut out from the plate and ^{32}P radioactivity was quantitated.

Protein Kinase Assay. The transfer of phosphorus from [γ-^{32}P]ATP to a protein acceptor was measured in 25–50 μl of PK buffer containing 1–5 mg of phosvitin per ml. After incu-

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Abbreviation: SV40, simian virus 40.

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bation of the reaction mixture at 20°C for 30–60 min, 1–5 μl was spotted on Whatman filter paper (20) and the reaction products were precipitated with cold 10% (wt/vol) trichloroacetic acid for 1 hr and washed with 5% trichloroacetic acid for 1.5 hr. The remainder of the reaction mixture was quenched with 5–10 μl of LS buffer [30% (wt/vol) sodium dodecyl sulfate/30% (wt/vol) 2-mercaptoethanol/0.15 M Tris, pH 6.8/bromophenol blue], boiled for 3 min, and subjected to sodium dodecyl sulfate gel electrophoresis. To visualize the phosphorylated products, slab gels either covered in SaranWrap or dried were exposed to x-ray film (SB-5 or R-1, Kodak). When necessary, bands corresponding to radiolabeled phosvitin and D2 hybrid protein were cut from the slab gel and the amount of radioactivity was measured.

RESULTS

Detection of ATPase and Protein Kinase Activities in Purified D2 Hybrid Protein. The 107,000-dalton D2 hybrid protein was purified from HeLa cells infected with Ad2 + D2 as described (15). After the final phosphocellulose chromatography step, the D2 hybrid protein was concentrated to approximately 1 mg/ml and assayed for ATPase and protein kinase activities by incubation with [γ-^{32}P]ATP in the presence of 5 mM $MgCl_2$. One microgram of the purified D2 hybrid protein was able to hydrolyze 1 nmol of ATP in 1 hr at 20°C (Fig. 1, lanes a and b). In addition, the D2 hybrid protein was able to transfer phosphorus from [γ-^{32}P]ATP to either the 107,000-dalton T antigen protein itself or to an exogenous phosphoprotein acceptor such as phosvitin (Fig. 1, lanes c and d). To demonstrate more conclusively that these enzymatic activities are, in fact, associated with the D2 hybrid protein, we determined whether ATPase and kinase activities would coelute with the complement-fixing activity of the D2 hybrid protein during purification.

Copurification of ATPase and Protein Kinase Activities with the D2 Hybrid Protein. A *de novo* purification of the D2 hybrid protein was performed in which ATPase and protein kinase as well as complement-fixing activities were assayed after each chromatographic step. The phosphotransferase activity was determined by measuring the transfer of ^{32}P from ATP to phosvitin as well as to the D2 hybrid protein itself. Proteins were extracted from the nuclei of infected cells by a series of ammonium sulfate fractionation steps and passed through a gel filtration column. At this relatively crude stage of purification, chromatography on Ultrogel A34 resolved at least two peaks of ATPase activity and two peaks of protein kinase activity (Fig. 2*A*). However, in each case, only one of the two peaks of enzymatic activity coincided with the fractions containing the complement-fixing activity. These fractions were pooled and applied to a DEAE-Sephadex column. Elution of the anion exchange resin with a linear gradient of NaCl (0.1–0.4 M) resolved two distinct peaks of complement-fixing activity (Fig. 2*B*). A major portion of the D2 hybrid protein (80–90%) eluted at approximately 0.37 M NaCl and was highly active in hydrolyzing ATP but contained little if any phosphotransferase activity. These fractions were arbitrarily designated form I D2 hybrid protein. By contrast, form II protein eluted at a lower ionic strength (0.29 M) and contained both an ATPase and a protein kinase activity.

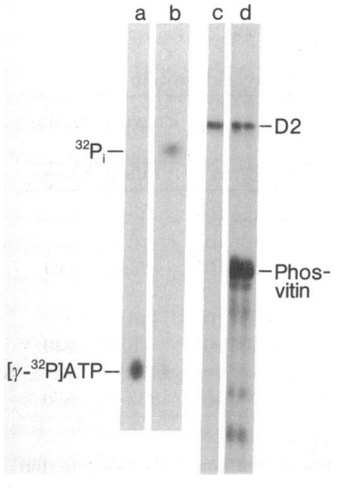

FIG. 1. ATPase and protein kinase activities associated with purified D2 hybrid protein. The 107,000-dalton D2 hybrid protein was purified as described (15) and assayed for ATPase and phosphotransferase activities. The hydrolysis of ATP was carried out in the absence (lane a) or presence (lane b) of 0.2 μg of purified D2 hybrid protein. The protein kinase reaction was performed with approximately 0.1 μg of the D2 hybrid protein and no added substrate (lane c) or in the presence of phosvitin at 1 mg/ml (lane d).

The fractions containing these two forms of the D2 hybrid protein were pooled separately and subjected to chromatography on phosphocellulose or heparin-Sepharose. Form I eluted from phosphocellulose as a single homogeneous peak with complete coincidence between fractions containing complement-fixing activity and ATPase activity (Fig. 2*C*). By contrast, form II protein eluted as two peaks (IIa and IIb) of complement-fixing activity when chromatographed on either phosphocellulose (data not shown) or heparin-Sepharose (Fig. 2*D*). In this case, only the more tightly bound form (IIb) contained protein kinase activity whereas both forms were able to hydrolyze ATP. One possible explanation for this finding is that the form II fractions pooled from the DEAE-Sephadex column were contaminated with form I and therefore fractions IIa and IIb correspond to forms I and II, respectively. Consistent with this idea is the observation that chromatography of the phosphocellulose form IIb on heparin-Sepharose resulted in the elution of a homogeneous peak of complement-fixing activity that coincided with the protein kinase activity (data not shown).

Analysis of the phosphocellulose-purified fraction of form I protein by sodium dodecyl sulfate/polyacrylamide gel electrophoresis revealed that, at this stage of the purification, the D2 hybrid protein was >99% pure, and virtually no protein other than the 107,000-dalton antigen could be seen after staining by Coomassie blue (Fig. 3, lane a). After purification by phosphocellulose or heparin-Sepharose chromatography, form II D2 hybrid protein also contained only one major species migrating at the position of 107,000 daltons (Fig. 3*B*). As a further criterion for copurification of the enzymatic activities with the D2 hybrid protein, we determined that the specific activities of the ATPase and protein kinase reactions increased with the complement-fixing ability of the antigen (Table 1). In addition, we have characterized ATPase and kinase activities from extracts of control cells either uninfected or infected with Ad2 alone and found that neither activity elutes from ion exchange columns in the same positions as the D2 hybrid protein. These data, taken together, strongly suggest that there are two forms of the D2 hybrid protein: a predominant form (I) that is associated with an ATPase activity and a minor form (II) that contains both an ATPase and a protein kinase activity.

Cosedimentation of Forms I and II with SV40 DNA during Glycerol Gradient Centrifugation. Because the D2 hybrid protein is known to bind to double-stranded DNA, specifically to the origin of replication in SV40 DNA, we determined whether forms I and II would interact with SV40 DNA during zonal centrifugation in the presence of a linear gradient of glycerol. It was expected that, if the D2 hybrid protein were responsible for catalyzing the enzymatic reactions, then their ATPase and protein kinase activities should be found associated with fractions cosedimenting with the viral DNA. When either purified form I or II D2 hybrid protein (20 μg) was mixed with SV40 DNA (50 μg), two peaks of complement-fixing activity

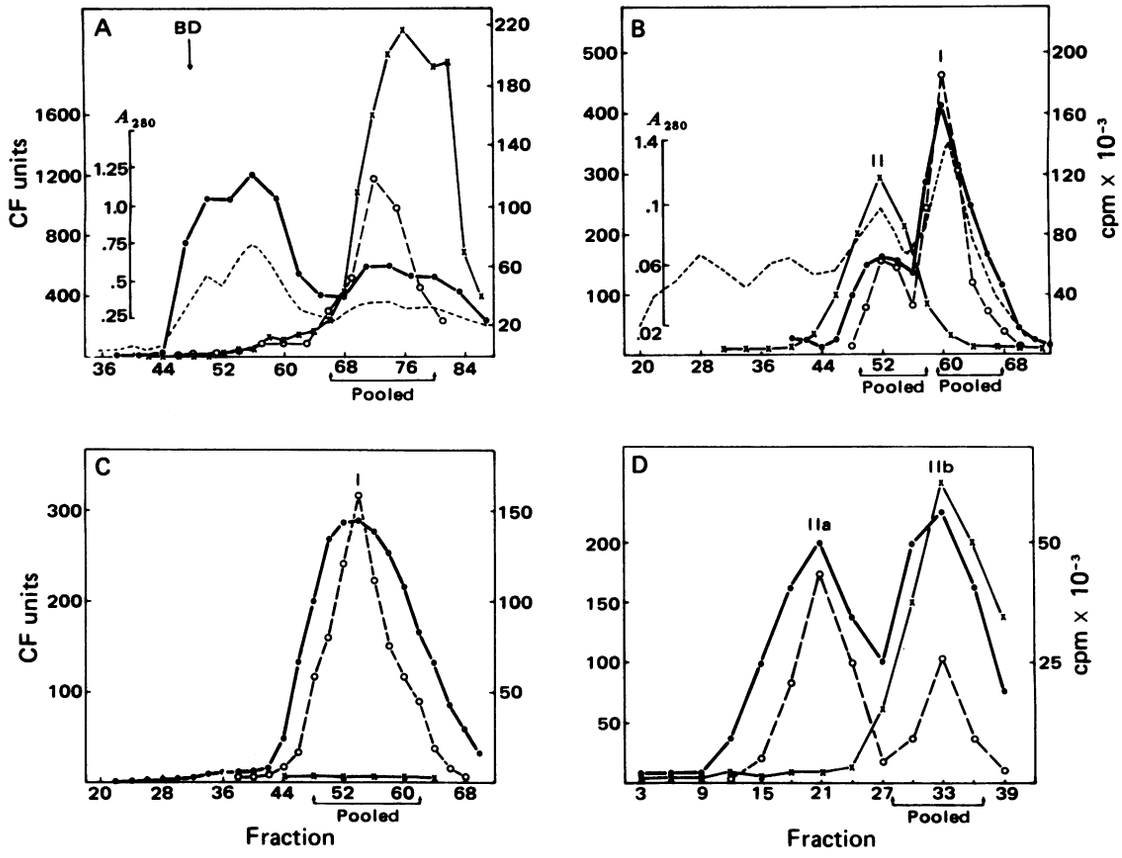

FIG. 2. Copurification of enzymatic activities with the D2 hybrid protein. HeLa cells (16 liters) infected with Ad2+D2 were harvested at 44 hr after infection, and nuclear proteins were extracted as described (15). After centrifugation at 100,000 × *g*, the extract was brought to 25% saturated ammonium sulfate and the precipitated proteins were removed. Nuclear proteins remaining in the supernatant were precipitated with 55% saturated ammonium sulfate and then subjected to gel filtration on Ultrogel (*A*) (BD, blue dextran). The fractions from this column that contained complement-fixing (CF) activity were pooled and subjected to chromatography on DEAE-Sephadex A-50 (*B*). Two peaks of complement-fixing activity (I and II) were resolved on the ion exchange column during elution with a 280-ml linear gradient (0.0–0.4 M) of NaCl. Peak I was pooled and passed through a phosphocellulose column (*C*); peak II was subjected to chromatography on a heparin-Sepharose (*D*). In each case, peaks I and II were eluted from these resins by a linear gradient of NaCl (0.1–0.6 M). After each chromatographic step, alternate fractions were assayed for complement fixation (○), ATPase (●), and protein kinase (×) activities. The absorbance at 280 nm was also monitored (- - -) after chromatography on Ultrogel A34 and DEAE-Sephadex A-50.

were resolved after glycerol gradient sedimentation (Fig. 4). The peak migrating near the middle of the gradient represents D2 hybrid protein that is not bound to DNA whereas the fractions near the bottom of the gradient represent protein bound to SV40 DNA with an average molar ratio of 6 molecules of protein per molecule of DNA. This interpretation was confirmed by identifying the fractions containing SV40 DNA by agarose gel electrophoresis; the position of unbound protein was independently established by sedimenting the D2 hybrid protein in the absence of SV40 DNA (data not shown). Although both forms of the protein bound DNA equally well, the ATPase activity of form I + II cosedimented more efficiently with DNA than did the kinase activity of form II. The reason for the loss in specific activity of form II during glycerol gradient sedi-

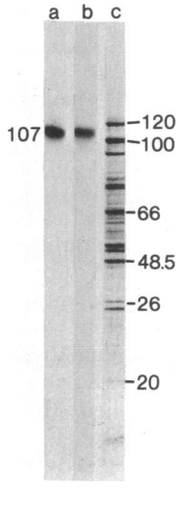

FIG. 3. Gel electrophoresis of purified form I and form II D2 hybrid protein. Approximately 3.0 μg of form I (lane a) and 2.0 μg of form II (lane b) D2 hybrid protein purified by phosphocellulose chromatography as described in Fig. 2 were subjected to electrophoresis on a 25-cm-long sodium dodecyl sulfate/polyacrylamide slab gel containing a 7–15% gradient of acrylamide (21). Molecular weight standards (lane c) were proteins from a total cellular extract of Ad2-infected cells; molecular weights are shown × 10^{-3}. In all cases, the protein bands in the gel were visualized by staining with Coomassie brilliant blue.

Table 1. Purification of D2 hybrid protein

Purification	Protein, mg	CFU/mg*	Enzyme,† units/mg ATPase	Enzyme,† units/mg Kinase
Nuclear extract	2000	3×10^3	—	—
$(NH_4)_2SO_4$ pellet (55%)	600	8×10^3	—	—
Ultrogel	50	7×10^4	26.6	29
DEAE-Sephadex				
I	6	1.25×10^5	146	—
II	2	7.5×10^4	66.6	240
Phosphocellulose I	0.8	5×10^5	1176	—
Heparin II	0.1	2×10^5	933	670

* Complement fixing units, as described (15, 22).

† One unit or ATPase or kinase activity is that amount of protein that hydrolyzes or incorporates 1 nmol of ATP in 60 min at 20°C.

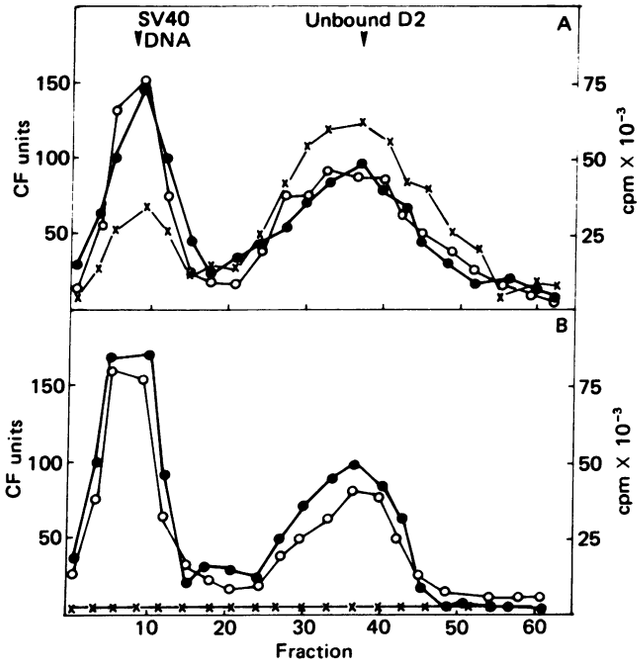

FIG. 4. Glycerol gradient sedimentation of the D2 hybrid protein in the presence of SV40 DNA. (*A*) Approximately 20 μg of form II D2 hybrid protein purified by heparin-Sepharose chromatography was mixed with 50 μg of SV40 DNA in the presence of buffer G [0.01 M Tris, pH 7.1/0.15 M NaCl/5 mM dithiothreitol/1 mM EDTA containing 10–30% (wt/vol) glycerol] and sedimented at 38,000 rpm for 24 hr at 2°C in a Beckman SW 41 rotor. (*B*) Similarly, 20 μg of form I D2 hybrid protein purified by phosphocellulose chromatography was mixed with 50 μg of SV40 DNA and sedimented in buffer G as described above. Fractions containing approximately 240 μl were collected from the gradients and aliquots were assayed for ATPase (●), protein kinase (×), and complement-fixation (○).

mentation is at present unknown. However, it is apparent that the major portion of the ATPase activity of form I cosediments with the SV40 DNA. Thus, at least the proteins responsible for catalyzing the ATPase activity has a high affinity for DNA, as would be expected of the D2 hybrid protein.

Inhibition of Purified ATPase and Kinase Activities by Anti-T Gamma Globulin. We determined the effect of specific anti-T gamma globulin on these enzymatic activities associated with the D2 hybrid protein. The anti-T gamma globulin was isolated from the sera of hamsters bearing tumors induced by SV40 (3). This antibody specifically and efficiently immunoprecipitates the 96,000-dalton SV40 T antigen, the 17,000-dalton little T protein, and the D2 hybrid protein (3, 14, 16, 17). Addition of increasing amounts of anti-T gamma globulin to the ATPase reaction efficiently inhibited the hydrolysis of ATP by purified form I D2 hybrid protein (Fig. 5*A*). By contrast, gamma globulin from preimmune sera had no effect. When increasing amounts of antibody were added to standard kinase reaction, the transfer of phosphorus to the D2 hybrid protein was also efficiently inhibited (Fig. 5*B*) but phosphorylation of phosvitin was not impeded (data not shown). However, when Sepharose beads containing covalently bound *Staphylococcus aureus* A protein were used to absorb the immune complex from the reaction mixture, the phosphorylation of phosvitin was greatly reduced (Fig. 5*C*). A similar absorption reaction performed with preimmune gamma globulin had no effect on the phosphorylation of either the 107,000-dalton protein or phosvitin. By contrast, neither anti-T nor control gamma globulin had any effect on the kinase activity purified from adenovirus-infected cells (data not included).

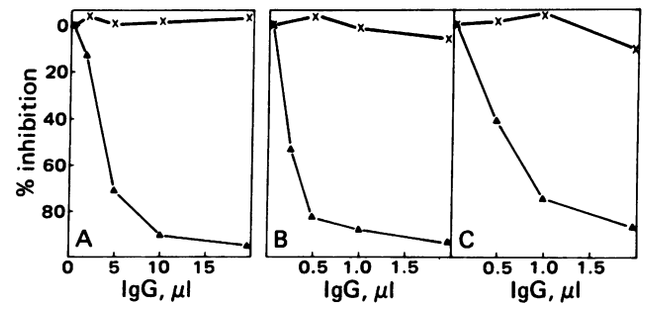

FIG. 5. Inhibition of ATPase and protein kinase activity by anti-T gamma globulin. (*A*) Increasing amounts of hamster anti-T gamma globulin (▲) or preimmune gamma globulin (×) were added to the ATPase reaction mixture containing approximately 1.0 μg of phosphocellulose-purified form I D2 hybrid protein. (*B*) The indicated amounts of either anti-T or preimmune gamma globulin were added to the protein kinase reaction mixture containing 0.1 μg of heparin-Sepharose-purified form II D2 hybrid protein and no exogenous substrate. (*C*) Protein kinase reactions were as in *B* except that phosvitin (1 mg/ml) was used as substrate for the phosphotransferase reaction and, prior to the addition of [γ-^{32}P]ATP, the antibody–antigen complexes were removed from the reaction mixture by absorption with 30 μl of a 50% solution of *Staphylococcus aureus* A protein covalently bound to Sepharose 4B (23).

DISCUSSION

We have taken advantage of the adenovirus–SV40 hybrid system to study potential enzymatic activities associated with SV40 T antigen. Highly purified fractions of the D2 hybrid protein were able to catalyze the hydrolysis of ATP to ADP + P_i and to transfer phosphorus from ATP either to the 107,000-dalton D2 hybrid protein itself or to an exogenous substrate such as phosvitin. Although the ATPase (form I) could be separated from the protein kinase (form II) during ion exchange chromatography, both enzymatic functions remained closely associated with the D2 hybrid protein throughout a series of rigorous purification steps. However, during glycerol gradient centrifugation the ATPase activity associated with the D2 hybrid protein cosediments more efficiently with SV40 DNA than does the protein kinase activity. In addition, the ATPase is specifically inhibited by anti-T gamma globulin but not by control antibody. These data provide direct biochemical evidence that at least one specific enzymatic activity may be associated with the SV40 *A* gene product.

It was somewhat surprising to find that there are two chromatographically distinct forms of the D2 hybrid protein that have different enzymatic properties. The purification procedure used previously (15) had not resolved multiple forms of the protein except that, on occasion, two peaks of complement-fixing activity were observed during phosphocellulose chromatography. In the current purification procedure we included an additional ammonium sulfate fractionation step and also greatly improved the resolution of the various ion exchange columns. These modifications, together with the enzymatic assays, facilitated the separation and subsequent detection of forms I and II D2 hybrid protein. Although we have reproducibly observed the two enzymatically distinct forms of the protein, we do not, at present, know what structural feature distinguishes them. One possibility is that form I protein is phosphorylated whereas form II is not. This hypothesis is not unreasonable in view of the fact that the D2 hybrid protein is known to be phosphorylated and that forms I and II are not distinguishable when analyzed by sodium dodecyl sulfate gel electrophoresis. Alternatively, it could be that one or both forms of the D2 hybrid protein contain tightly bound other viral or cellular components that alter its chromatographic and enzymatic properties.

This latter possibility raises a more central issue: Are the enzymatic activities associated with the D2 hybrid protein catalyzed by the SV40 coded *A* gene product? There are at least four formal possibilities: (*i*) enzymatic activities are due to a contaminating protein; (*ii*) the adenoviral coded portion of the D2 hybrid protein is responsible for its enzymatic activities; (*iii*) the D2 hybrid protein forms a tight complex with some other viral or cellular component to create an enzymatically reactive species; and (*iv*) the product of the SV40 *A* gene is an ATPase or a protein kinase or both. The data presented in this communication argue strongly against the possibility of a fortuitous contaminant because both enzymatic activities remain associated with the D2 hybrid protein through extensive purification and are specifically inhibited by anti-T gamma globulin. Moreover, extracts from uninfected HeLa cells and cells infected with adenovirus 2 alone do not appear to contain an ATPase and a protein kinase that copurify with the D2 hybrid protein. These data do not, however, exclude the possibility that the *A* gene product actually induces a specific host protein kinase that binds to the D2 hybrid protein.

The best available genome mapping data (24) indicate that the adenoviral sequences fused to the amino terminus of the SV40 *A* gene protein in Ad2$^+$D2 are coded either by the Ad2 100,000-dalton protein or by pVIII (14). The 26,000-dalton protein, pVIII, is a precursor of an adenoviral capsid protein (25) and we have no information concerning its potential enzymatic activity. We have separated the 100,000-dalton protein from the D2 hybrid protein and have shown that it does not contain either an ATPase or a protein kinase activity (data not shown). Moreover, the 96,000-dalton SV40 T antigen protein purified from adenovirus–SV40 hybrid Ad2$^+$D1 (unpublished results) is also associated with an ATPase and a protein kinase but, unlike the D2 hybrid protein, the D1 T antigen is entirely coded by SV40 (14). We therefore are left with the possibility that one or the other or both enzymatic activities are due either to the D2 hybrid protein alone or in conjunction with another, as yet unidentified, subunit(s). At present, we cannot rule out either of these possibilities and further investigation using different genetic and biochemical approaches will be required to settle the issue. In either case, a better understanding of these enzymatic functions may lead to the mechanisms involved in virally induced DNA replication and transformation.

Our preliminary characterization of the protein kinase activity reveals that form II D2 hybrid protein can transfer the γ phosphate of ATP to either the 107,000-dalton protein itself or to exogenous substrates such as phosvitin and casein but not histones, bovine serum albumin, or gamma globulin. The phosphorylation reaction was not stimulated by either cyclic AMP or cyclic GMP (data not shown). The use of phosvitin as substrate proved to be important for characterizing the kinase during purification but it is clear that phosphorylation of this egg protein is most likely a fortuitous event and provides no insight to the nature of the *in vivo* substrate. However, it is less clear why the D2 hybrid protein itself should be a substrate. It is possible, for instance, that phosphorylation of the D2 hybrid protein leads to a specific functional modification of the SV40 *A* gene protein. Thus, it is possible that the associated protein kinase activity is actually the cellular enzyme specifically responsible for phosphorylating T antigen. This idea is consistent with our finding that the D2 hybrid protein is phosphorylated much more efficiently than phosvitin. Alternatively, the phosphorylation of the D2 hybrid protein could be a chance occurrence and the main purpose of the protein kinase is to modify cellular components such as cytoskeletal proteins or replication enzymes that play a pivotal role in transformation.

By contrast, the ATPase activity of form I may be directly involved in the DNA replication function of the SV40 *A* gene protein. Studies of bacterial phage replication have shown that at least one ATPase, the product of *dnaB* is part of the initiation complex for DNA replication (26). In addition, we know that the SV40 *A* gene product is required to initiate viral replication and that it interacts specifically with the origin of DNA replication. Thus, it is not unreasonable to expect that an ATPase associated with the SV40 *A* gene product should be involved in DNA replication. One possibility is that the *A* gene protein binds at the origin of replication and, with the help of the ATP hydrolysis activity, locally unwinds portions of the duplex DNA, thus allowing polymerases to bind and initiate replication.

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