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Biochemistry

Specific *in vitro* adenylylation of the simian virus 40 large tumor antigen

(immunoprecipitation assay/protein–AMP complex/seryl phosphodiester linkage)

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ABSTRACT **Incubation of the simian virus 40 (SV40) large tumor antigen (T) from either transformed or lytically infected cells with adenosine [8-^{3}H]-, [α-^{32}P]-, or [α-[^{35}S]thio]-triphosphate in the presence of Mg^{2+} resulted in its labeling as defined by the appearance of an intact, appropriately immunoreactive band in NaDodSO$_4$/polyacrylamide gels. Radioactivity remained associated with the protein after boiling in buffer containing 3% NaDodSO$_4$, and 2-mercaptoethanol as well as after heating in 0.1 M HCl, 0.1 M NH$_4$OH, or hydroxylamine, but it was dissociated after incubation in 0.1 M NaOH at 37°C. After limited boiling of gel-purified [α-^{32}P]ATP + T complex in 5.6 M HCl, *o*-[^{32}P]phosphoserine was released, and snake venom phosphodiesterase or 0.5 M piperidine treatment of such a complex resulted in the liberation of [α-^{32}P]AMP. The reaction proceeded when either purified, soluble T or insoluble, specifically immunoprecipitated antigen was used as substrate. ATP and dATP were the preferred nucleotide substrates by comparison with the other six standard ribonucleoside or deoxynucleoside triphosphates. Partial tryptic digests of T + [α-^{32}P]ATP complexes revealed the presence of a single labeled peptide of $M_r \approx 12–14 \times 10^3$, and after exhaustive digestion, there was a single radioactive spot in the fingerprint. These data indicate that T can be adenylylated at a specific seryl residue(s) in a limited portion of the protein surface. Furthermore, adenylylation appears to be reversible and to proceed by a pyrophosphorylytic mechanism, since the nucleotide was released from the protein following incubation of adenylylated T with Mg^{2+}, sodium pyrophosphate, and poly(dT).**

Simian virus 40 (SV40) large tumor antigen (T) is a DNA binding phosphoprotein of M_r 81 × 10^3 with multiple biological functions and several *in vitro* biochemical activities (1). For example, it plays an essential role in the viral transforming mechanism, and T binding to the unique origin of SV40 DNA replication leads to the initiation of rounds of autonomous viral DNA synthesis in monkey cells and regulation of the early and late viral transcription units (2, 3, 50). In this regard, although certain salient features of the structures of the 5′ termini of newly initiated DNA daughter strands are known (4), the detailed chemistry linking origin binding of T to the replication initiation process is unknown.

In addition to its origin-specific DNA binding potential, T is an enzyme. Specifically, it displays intrinsic, DNA-independent adenosine 5′-triphosphatase (ATPase) activity (5–8). Moreover, highly purified T has both autokinase activity and an ability to promote the phosphorylation of certain exogenous protein substrates *in vitro* (5, 9–11). Recent evidence indicates the latter to be specifically associated with the antigen, although it has not, thus far, been possible to discriminate between the activity being intrinsic to T or a property of a small, tightly bound, cell-encoded subunit (10, 11). How these two activities relate to its *in vivo* function(s) is unclear. It is, perhaps, worth noting that the purified *Escherichia coli dnaK* protein, an element active in the initiation of λ DNA replication, also displays DNA-independent ATPase and autokinase activities (12). The similarities between the two circumstances stress the need to evaluate whether these activities contribute to the T-replication initiation function.

In this report, we describe a T-associated enzymatic activity that results in the reversible adenylylation of the antigen at a specific site. Since it requires ATP as substrate, it could, in theory, depend upon some of the same elements of protein structure as one or both of the T-associated enzymatic activities noted above.

MATERIALS AND METHODS

Cells and Viruses. SV80 cells, an SV40-transformed human cell line (13), were provided by the Massachusetts Institute of Technology Cell Culture Center. CV-1P cells were infected with SV40 and extracted as described (16). COS-1 (14) is a monkey cell line transformed by an origin-defective SV40 genome. AdSVR284, an adeno-SV40 hybrid virus (15) generously provided by T. Grodzicker, was grown in CV-1P cells at ≥10 plaque-forming units/cell.

Partial Purification of T from SV80 Cells. T was purified by a modification of a published method (10, 16) in which the final glycerol gradient centrifugation step was replaced by gel filtration in a column (1.7 × 28 cm) of Ultrogel AcA34 (LKB) equilibrated in buffer A (20 mM Hepes, pH 7.8/200 mM NaCl/1 mM dithiothreitol/0.02% Triton X-100/10% glycerol). Antigen was detected by ELISA (17) or by measuring its ATPase activity (5, 18) (or both).

Preparation of 2′,3′-Dialdehyde [α-^{32}P]ATP. It was prepared and purified as described (19, 20).

Reaction of T with Nucleotide. One to two micrograms of AcA34-purified T was incubated with 1–2 μM nucleoside [α-^{32}P]- or [α-[^{35}S]thio]triphosphate (410 or 650 Ci/mmol; 1 Ci = 37 GBq; Amersham) or 5–10 μM [8-^{3}H]ATP (30 Ci/mmol; Amersham) in buffer B (buffer A containing 100 mM NaCl) for 30 min at 37°C in the dark in a volume of 100–200 μl. Each reaction mixture was then gel filtered through Bio-Gel P10 (Bio-Rad; 3 ml packed bed volume) equilibrated in buffer B. Complexes were identified in the V_0 fraction and reincubated at 37°C for 30 min followed by immunoprecipitation and NaDodSO$_4$/polyacrylamide gel electrophoresis performed as described (10).

Approximately 5 μg of T, immunoprecipitated from a nuclear extract or a Nonidet P-40 cell lysate (10, 16), was exposed to 10 μg of monoclonal anti-T IgG (PAb419; ref. 21)

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Abbreviations: SV40, simian virus 40; T, large tumor antigen; TPCK, L-1-tosyl-amido-2-phenylethyl chloromethyl ketone; ATP[^{35}S], adenosine [α-[^{35}S]thio]triphosphate; SVE, snake venom exonuclease.

and 20 μl of a 50% (vol/vol) suspension of protein A-Sepharose beads (Sigma) for 1–1.5 hr at 23°C. The pelleted immunoprecipitate was washed three times with buffer B and incubated in the dark with 5 mM $MgCl_2$ and radioactive ATP for 3 hr at 23°C or overnight at 4°C. Immunoprecipitates were centrifuged, and the pellets were washed in buffer B three times before they were resuspended in 1 ml of buffer B for 30 min at 23°C. Samples were rewashed three times with 0.1 M Tris·HCl, pH 7.8/0.5 M LiCl and eluted by boiling in standard $NaDodSO_4$-containing sample buffer. $NaDodSO_4$/polyacrylamide gel electrophoresis was performed in 10% gels cast under 4% stacking gels. Gels were routinely stained with Coomassie brilliant blue, fluorographed when ^{35}S or ^{3}H was used, dried, and exposed to Kodak XR-5 film.

Analyses of the Nucleotide–Protein Linkage. Radioactive T bands were located and excised from the gel, crushed, and then eluted into 50 mM NH_4HCO_3/0.1% $NaDodSO_4$/1% 2-mercaptoethanol by gentle rocking for 24 hr at 37°C. Eluates were clarified by centrifugation and lyophilized. In preparation for snake venom exonuclease (SVE) cleavage, samples were precipitated with 1.5 vol of cold acetone, using 50 μg of bovine serum albumin as carrier. The pellet was treated twice with 1 unit of L-1-tosylamido-2-phenylethyl chloromethyl ketone treated-trypsin (TPCK-trypsin) (Worthington) for 5 hr each at 37°C (22). After inactivation with 5 units of aprotinin (Sigma), lyophilized samples were exposed to 2 units of SVE (Worthington) at 0, 4, and 18 hr and incubated continuously at 37°C for a total of 24 hr in 100 μl of 50 mM NH_4HCO_3/1 mM $MgCl_2$ (23). Treatment of the acetone-precipitated T + [α-^{32}P]ATP complex with 0.5 M piperidine was carried out at 37°C for 2 hr (24).

Phosphoamino Acid Analysis. Gel-purified [α-^{32}P]ATP-labeled T was trypsinized, lyophilized, and resuspended in 100 μl of constant boiling 5.6 M HCl and heated at 110°C for 2 hr in a sealed capillary tube (25). HCl was removed *in vacuo*, and the residue was dissolved in electrophoresis buffer (88% formic acid/glacial acetic acid/H_2O, 78:25:897, pH 1.9) containing 1 mg of phosphoamino acid markers per ml. Electrophoresis and chromatography were performed as described (26).

Tryptic Digestion of Immunoprecipitated T. Partial digestion of immunoprecipitated T with TPCK-trypsin was performed by the method of Schwyzer *et al.* (27). Tryptic digests for fingerprinting were prepared by performic acid treatment of acetone-precipitated complex followed by two additions of 10 units of TPCK-trypsin. Samples were fractionated by electrophoresis on cellulose TLC plates in 6% formic acid/1.2% acetic acid/0.25% pyridine, pH 1.9, for 75 min at 500 V followed by ascending chromatography for 5 hr in isobutyric acid/pyridine/acetic acid/butanol/H_2O, 65:5:3:2:29 (28).

RESULTS

Complex Formation Between T and ATP. Partially purified T from SV80 cells was incubated in the dark with adenosine [α-[^{35}S]thio]-triphosphate (ATP[^{35}S]) in the presence of increasing concentrations of Mg^{2+} and in its absence. It was then immunoprecipitated and electrophoresed after elution from an immune complex in boiling buffer containing 3% $NaDodSO_4$ and 2-mercaptoethanol. As shown in Fig. 1 *Left*, intact T was labeled in a Mg^{2+}-dependent reaction, with an optimum between 5 and 10 mM. Similarly, the purified antigen could be labeled with [α-^{32}P]ATP (Fig. 1 *Center*). Successful immunoprecipitation of radioactive T was performed with rabbit anti-$NaDodSO_4$ gel-purified T, hamster anti-SV40 tumor serum, and a monoclonal antibody to T (PAb416), but not with preimmune rabbit serum. If T was first immunoprecipitated with an anti-T monoclonal antibody, PAb419 (21), from crude extracts of SV80 cells, it could be labeled subsequently with [α-^{32}P]ATP or ATP[^{35}S]

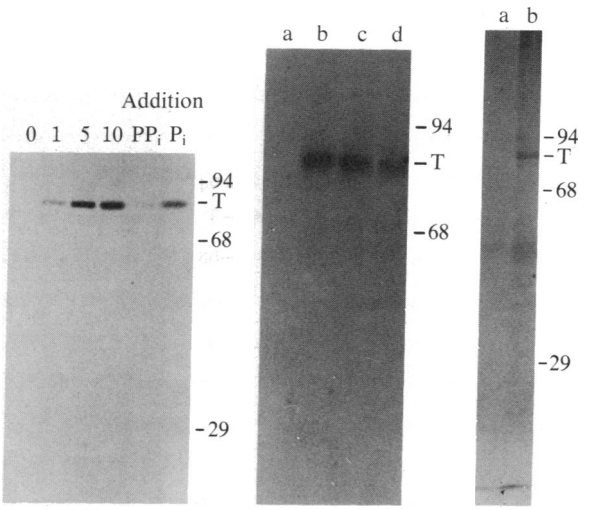

FIG. 1. Labeling of SV40 T after incubation with ATP. Assays in the *Left* and *Center* were performed with soluble T, purified from SV80 cells, and the assay in the *Right* was performed with a nuclear extract from SV80 cells initially immunoprecipitated with antibody. Molecular weight markers (shown as $M_r \times 10^{-3}$) are phosphorylase a, M_r 94,000; bovine serum albumin, M_r 68,000; and carbonic anhydrase, M_r 29,000. (*Left*) T was labeled with ATP[^{35}S] in the absence and presence of various concentrations (0, 1, 5, and 10 mM) of $MgCl_2$ (or 5 mM, where not indicated). During the reincubation following gel filtration, samples in the two right lanes were incubated in pH 7.8 buffer containing 1 mM $MgCl_2$, 1 μg of poly(dT), and 1 mM sodium pyrophosphate (PP_i) or sodium monophosphate (P_i) as indicated. T was immunoprecipitated prior to gel electrophoresis with monoclonal anti-T antibodies PAb416 and PAb419 (21). Coomassie blue-stained T bands (migration position labeled T on the right) of equal intensity were present in each gel slot. (*Center*) T was labeled with [α-^{32}P]ATP, and the final immunoprecipitation was carried out with preimmune rabbit serum (lane a), rabbit anti-$NaDodSO_4$ gel-purified T (lane b), hamster anti-SV40 tumor serum (lane c), and a monoclonal antibody to T (PAb416) (lane d). (*Right*) Equal quantities of T were labeled with [8-^{3}H]ATP after immunoprecipitation with nonimmune mouse immunoglobulin (lane a) and PAb419 (lane b).

(data not shown) as well as with [8-^{3}H]ATP (Fig. 1 *Right*). Purified soluble T was also labeled after incubation with [8-^{3}H]ATP (data not shown). These results are most consistent with the existence of a covalent linkage(s) between T and an adenine nucleotide(s).

T, immunoprecipitated from SV40-infected CV-1P cells, from COS-1 cells (Fig. 2 *Left*), and from CV-1P cells infected with the T-encoding AdSVR284 (data not shown), were also labeled after incubation with radioactive ATP. By contrast, when purified T from SV80 cells was incubated, in parallel, with α-^{32}P-labeled ATP, dATP, GTP, and dGTP, the protein was labeled more efficiently with ATP than its deoxy congener and failed to complex with the other two nucleotides (Fig. 2 *Center*). Barely detectable labeling was noted when T was incubated with similar concentrations of α-^{32}P-labeled UTP, CTP, dCTP, and TTP (data not shown). In keeping with these findings, ATP[^{35}S] labeling of T was markedly reduced when a 10^3-fold molar excess (relative to radioactive nucleotide) of ATP but not NAD and dGTP was present during the labeling reaction (Fig. 2 *Right*).

We also varied the input [α-^{32}P]ATP concentration from 1 μM to 10 mM in reactions with a fixed amount of immunoprecipitated T from SV80 cells. Labeling was directly proportional to nucleotide concentration, and ≈1% of the protein was labeled at 10 mM ATP (Fig. 3). In other experiments, labeling of purified, soluble T with [α-^{32}P]ATP was at least 5-fold more efficient than labeling of the immunoprecipitated protein. More efficient labeling of the purified pro-

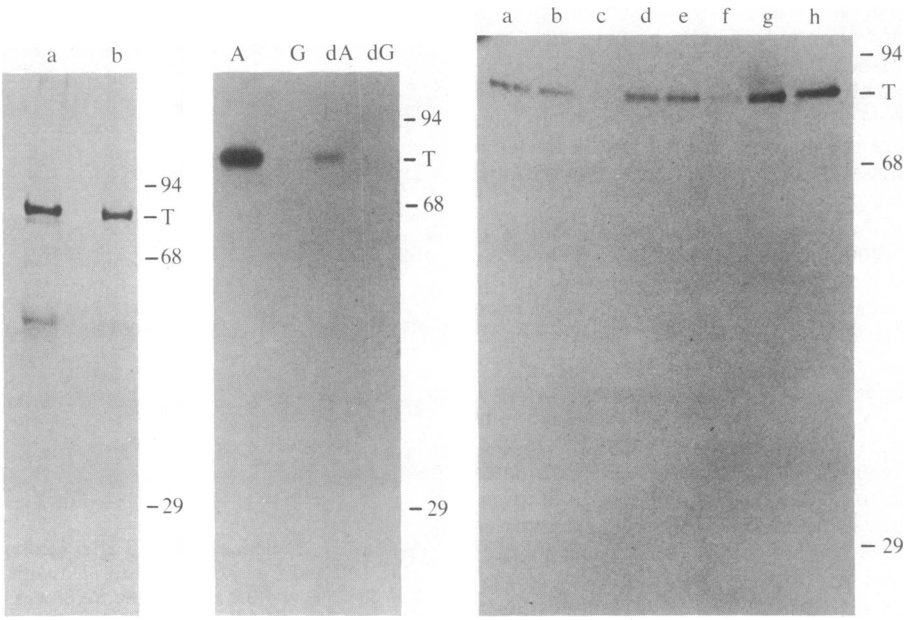

FIG. 2. Specificity of nucleotide labeling of SV40 T. The assay in the *Left* was performed with immunoprecipitated nuclear extracts, and assays in the *Center* and *Right* were performed with soluble purified T from SV80 cells. Molecular weight markers are as in Fig. 1. (*Left*) Extracts from COS-1 cells (lane a) and SV40-infected CV-1P cells (lane b) harvested 48 hr postinfection were labeled with ATP[^{35}S]. (*Center*) Equal aliquots of T were labeled with α-^{32}P-labeled nucleotides: ATP (lane A), GTP (lane G), dATP (lane dA), and dGTP (lane dG). (*Right*) Equal aliquots of T were labeled with 1 μM ATP[^{35}S] with the following additions: none (lane a), $\approx$3 μg of monoclonal anti-T PAb402 (an antibody known to inhibit T-associated ATPase) (lane b), 1 mM ATP (lane c), 1 mM NAD (lane d), 1 mM dGTP (lane e) and none (lanes f–h). After the gel filtration step, reincubation of samples in lanes a–e was as described in *Materials and Methods*, but 1 mM $MgCl_2$ was added to samples in lanes f–h. In addition, the sample in lane f contained 1 mM sodium pyrophosphate and 1 μg of poly(dT), and the sample in lane g contained only 1 mM sodium pyrophosphate. Samples were immunoprecipitated as in Fig. 1 *Left* and prepared for gel electrophoresis.

tein ($\geq$10-fold) was noted with the reactive ATP analogue 2′,3′-dialdehyde [α-^{32}P]ATP followed by sodium borohydride reduction (ref. 29; unpublished observations) than with ATP.

Identification of a Nucleotide-Linked Peptide. As noted above, T can form a stable complex with ATP more efficiently than with any other standard nucleoside triphosphate tested. To assess whether the nucleotide was linked to a specific site on the protein surface, an aliquot of purified T + [α-^{32}P]ATP complex was immunoprecipitated and exposed to limited tryptic cleavage. As shown in Fig. 4 *Left*, a single major radioactive band of $M_r \approx 12$–14×10^3 was detected. As noted by comparison with [^{35}S]methionine-labeled T, this band failed to comigrate with any of the observed methionine-containing, tryptic fragments including the M_r 17 $\times$ 10^3 band known to be composed of sequences from the amino terminus (27). We have confirmed the identity of that fragment by successfully immunoprecipitating it with the NH_2-terminal-specific antibody PAb416 (data not shown). Furthermore, a single ^{32}P-labeled peptide was observed after two-dimensional fingerprinting of an exhaustive tryptic digest of the labeled complex (Fig. 4 *Right*). Note that the well-described $M_r \approx 84 \times 10^3$, *in vitro* proteolyzed product of T (22, 30) was observed to be radioactive after incubation with α-^{32}P or ATP[^{35}S] (e.g., see Figs. 1 *Right* and 2 *Left*).

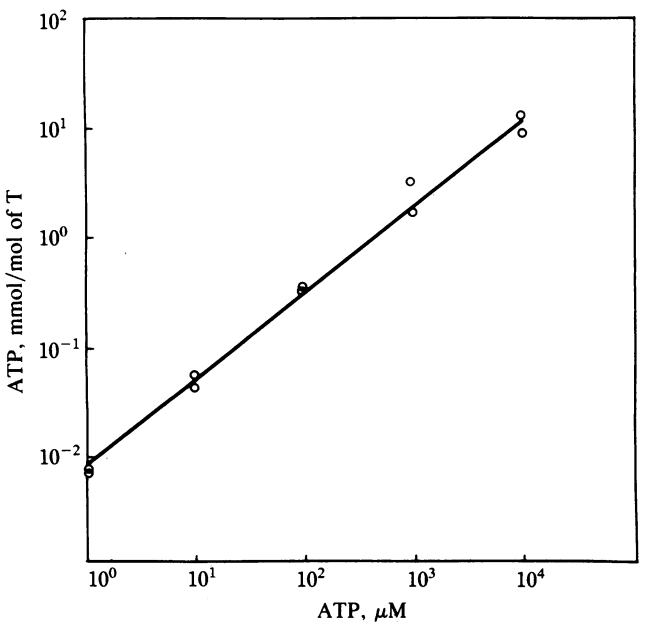

FIG. 3. Titration of ATP concentration in the T-labeling reaction. Aliquots of SV80 T ($\approx$5 μg) were immunoprecipitated with excess PAb419 and then labeled for 3 hr at 23°C with [α-^{32}P]ATP. Samples were eluted and electrophoresed. Coomassie blue-stained T bands were excised from the gel and the radioactivity (Cerenkov) was counted. Data were analyzed by using a least squares fit program, and r^2 = 0.9938.

Nature of the Nucleotide–Protein Linkage. The fact that NAD failed to inhibit T + ATP complex formation suggested that the product of this reaction was not ADP ribosylated T (31). To test this and other possible nucleotide–protein linkages, aliquots of immunoprecipitated [α-^{32}P]ATP-labeled T were incubated in 0.1 M HCl or 0.1 M Tris·HCl (pH 7) for 1 hr and 2 hr, respectively, at 37°C. $NaDodSO_4$ gel electrophoresis of each of these samples revealed that no significant amount of label was lost after these manipulations. By contrast, nearly all of the label was lost after incubating the complex in 0.1 M NaOH for 2 hr at 37°C. Furthermore, no label was lost after incubation of the complex in 0.2 M hydroxylamine at pH 7.5 or 3.86 M hydroxylamine at pH 4.75 for 30 min at 37°C. These data are consistent with the bound $^{32}PO_4$ being part of a phosphodiester bond to a serine or threonine (32). They are inconsistent with the presence of a phosphodiester linkage to a tyrosine, a phosphoamide, or a mixed anhydride linkage (33, 34). In keeping with these findings,

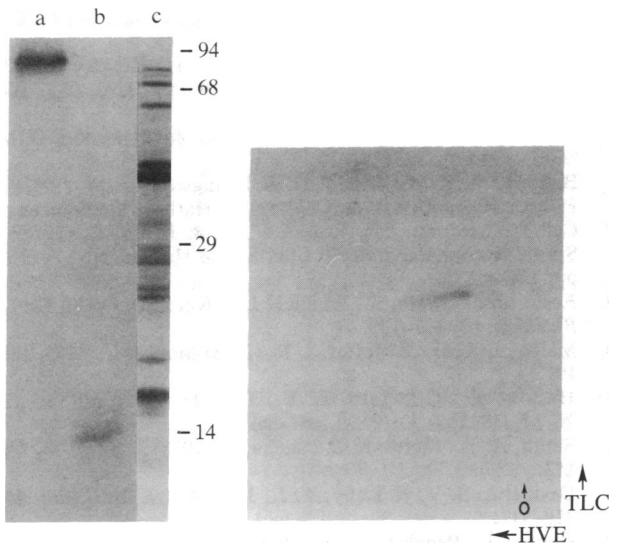

FIG. 4. Tryptic analyses of [α-^{32}P]ATP-labeled SV40 T. (*Left*) Partial tryptic digestion was performed on immunoprecipitated [α-^{32}P]-ATP-labeled T from SV80 cells. The purified soluble antigen was first reacted with nucleotide and then immunoprecipitated with hamster anti-SV40 tumor serum; undigested complex (lane a) and complex digested with 5 μg of TPCK-trypsin (1 unit) for 5 min at 4°C (lane b). Lane c contains a partial tryptic digest of [^{35}S]methionine-labeled T bound to hamster anti-SV40 tumor serum generated in parallel. The characteristic peptides of T described by Schwyzer *et al.* (27) are present, including the M_r 17 × 10^{-3} amino-terminal fragment. (*Right*) Two-dimensional tryptic peptide map of [α-^{32}P]ATP-labeled T. O, origin; HVE, high-voltage electrophoresis; TLC, ascending chromatography.

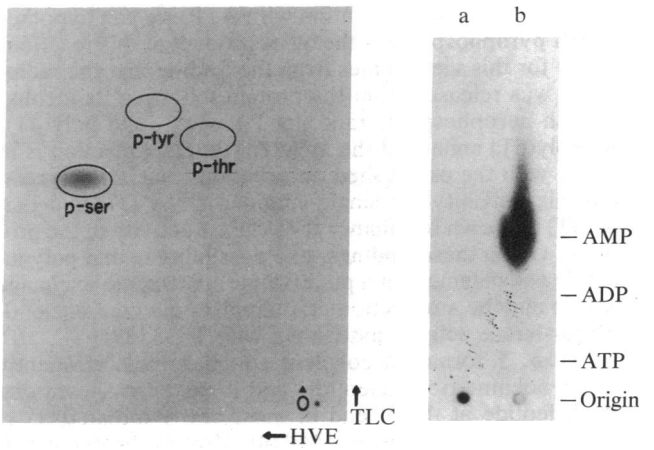

FIG. 5. Nature of the nucleotidyl–protein linkage. Immunoprecipitated T from SV80 cells was labeled with [α-^{32}P]ATP. (*Left*) Phosphoamino acid analysis. Amino acid markers were detected by ninhydrin staining. Free $^{32}P_i$, noted in other experiments, migrated off the plate. p-ser, Phosphoserine; p-tyr, phosphotyrosine; p-thr, phosphothreonine. O and HVE are as in Fig. 4. (*Right*) NaDodSO$_4$, gel-purified [α-^{32}P]ATP-labeled T was acetone precipitated (80% recovery) and prepared for SVE digestion. The sample in lane a was incubated without SVE and the sample in lane b was incubated with SVE. After lyophilization, the products were analyzed by TLC on PEI-cellulose plates in 1.2 M LiCl with the addition of 20 nmol each of AMP, ADP, and ATP as internal standards. Standards were visualized with short-wave UV light.

when purified T + [α-^{32}P]ATP complex was acid hydrolyzed with 5.6 M HCl for 2 hr, *o*-[^{32}P]phosphoserine was released (Fig. 5 *Left*). In addition, when the same complex was exposed to 0.5 M piperidine (data not shown) or SVE (Fig. 5 *Right*), only [α-^{32}P]AMP was detected by TLC. These data indicate that the radioactive label in the complex is present in the form of an AMP-*o*-serine linkage.

Apparent Reversibility of Complex Formation. After formation of the AMP complex with purified T, aliquots of the gel-filtered adduct were incubated with $MgCl_2$ and sodium pyrophosphate in the absence and presence of poly(dT) (Fig. 2 *Right*). Incubation with Mg^{2+} and either poly(dT) or sodium pyrophosphate resulted in no significant loss of label from the protein, whereas addition of all three reagents to this mixture resulted in its nearly complete disappearance. No significant loss of label was noted when P_i was substituted for pyrophosphate (Fig. 1 *Left*), and no reduction in stained immunoprecipitable T was detected after any of these incubations (data not shown). Hence, little or no protein was digested or otherwise lost during the incubation with pyrophosphate alone or with poly(dT). It must also be noted that this apparent releasing activity was less evident after incubation in 0.5 M LiCl than in 0.1 M NaCl and that there is no evidence that T itself is the adenylylating or "reversing" enzyme.

DISCUSSION

SV40 T, isolated from either transformed or lytically infected cells, can spontaneously form a covalent complex with ATP or dATP upon incubation in the presence of Mg^{2+}. The reaction proceeded as well in dark as in lighted conditions, indicating that it did not depend upon light-catalyzed nucleotide–protein linking reactions (35). From results obtained with the three forms of radioactive ATP employed here, it might be concluded that the product of the T + ATP reaction was a complex including T, adenosine, and a 5′-linked αPO_4 moiety. Furthermore, since NAD failed to compete with ATP for labeling of T and 5.6 M HCl hydrolysis of the [α-^{32}P]ATP-labeled complex resulted in the liberation of [^{32}P]phosphoserine, it seemed reasonable to conclude that ADP ribose was not the nucleotide bound to the protein. Further chemical and nuclease digestion analyses confirmed that [α-^{32}P]AMP was the bound moiety. The existing data do not eliminate the possibility that a fraction or all of the bound nucleotide exists as 3′,5′-oligo(A) moieties. Given the past finding of heteropolymeric RNA linked to gel band-purified T from ^{32}P-labeled cells (36), additional studies are necessary to assess this possibility.

The efficiency of *in vitro* T adenylylation was found to be low (≤11 mmol of AMP per mole of T) (Fig. 3). This may be a result of (*i*) functional heterogeneity of the T population, (*ii*) the presence of prelinked nucleotide at the same site(s), (*iii*) inhibition of labeling as a result of antibody or cellular protein binding to T, (*iv*) absence of an important cofactor, or (*v*) the possibility that the T–AMP complex is an unstable intermediate in a pyrophosphorylytic reaction.

Available data are most consistent with the presence of very few nucleotide molecules per molecule of adenylylated T—perhaps only one, given the tryptic fingerprinting results (Fig. 4 *Right*). The location of this peptide in the protein sequence is not known. Since the smallest major methionine-labeled partial tryptic peptide is the M_r 17,000 NH_2-terminal fragment of T (27), and the single, adenylylated, partial tryptic product migrated as a polypeptide of $M_r \approx 12\text{–}14 \times 10^3$, it seems unlikely that the latter is an adenylylated species of the former.

T is known to be a non-DNA-dependent ATPase (5–7) and has a closely associated autokinase activity (5, 7, 9–11). The nucleotide product of these two reactions is ADP (5, 6, 18). Given that the product of ATP modification is protein-linked AMP and not ADP, the adenylylation of T is not a direct measure of either of these two activities, although it remains possible that they depend upon common elements of T structure.

Since AMP [and/or oligo(A)] is the nucleotide product

linked to T following incubation with ATP, we can hypothesize that pyrophosphate is the other product of this reaction. Support for this view comes from the finding that the radioactivity was released from the protein following its incubation with pyrophosphate (and not P_i), Mg^{2+}, and poly(dT). That poly(dT) enhanced the apparent reversal reaction is in keeping with the established observations that T has a relatively high affinity for single-stranded DNA (37) and that poly(dT) is known to enhance the ATPase activity of the protein (6). Given these findings, one possibility is that polynucleotide enhancement of a putative pyrophosphate exchange reaction may be a reflection of a template-directed nucleotidyl transferase activity associated with T (38, 39).

In vitro, T formed a covalent complex most efficiently with an adenine ribonucleotide, and it has been shown that the nucleotide at the 5′ end of most newly initiated SV40 DNA daughter L strands is AMP (4). Most of the major 5′ L strand ends are encoded by thymidylate residues lying within a T antigen binding site, and the most prevalent ends are encoded by a sequence within the origin of replication (40–42). Thus, one possibility is that the reversible adenylylation of T is an enzymatic reaction necessary for the execution of an important step in the initiation of viral DNA synthesis—e.g., a priming activity. For example, a T–AMP complex might be tested as a primer of SV40 DNA replication (43). In this regard, the M_r 80,000 adenoviral terminal protein, once coupled to dCMP [via a 5′-*o*-seryl linkage (44, 45)] performs such a task in promoting the first step in adenoviral DNA replication (46–48). Similarly, the analogous protein of phage ϕ29, P3, initiates DNA replication as a protein–dAMP adduct, again with the nucleotide coupled to a serine (49). It is also conceivable that adenylylation is a manifestation of the role of T antigen in regulating early or late transcription (or both). Clearly, further experimental results will be needed before one can validate or invalidate these possibilities.

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