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Nonidentity of Some Simian Virus 40-induced Enzymes with Tumor Antigen

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The complement-fixing tumor (T) antigen induced by simian virus 40 (SV40) has been prepared from SV40-infected cell cultures, from infected cell cultures treated at the time of infection with 1-β-D-arabinofuranosylcytosine (ara-C), and from SV40-transformed cells. Upon partial purification, the T antigen exhibited the following properties: it was tightly adsorbed by calcium phosphate gel, it was precipitated by acetic acid at *p*H 5 or by ammonium sulfate at about 20 to 32% saturation, and it had a molecular weight greater than 250,000, as estimated by Sephadex G-200 gel chromatography. In contrast, deoxycytidylate (dCMP) deaminase, thymidylate (dTMP) kinase, and thymidine (dT) kinase were less strongly bound to calcium phosphate and were not precipitated at *p*H 5; these enzymes also had much lower molecular weights than the T antigen, as did dihydrofolic (FH_2) reductase. Furthermore, higher ammonium sulfate concentrations were required to precipitate dCMP deaminase, dTMP kinase, and FH_2 reductase activities than to precipitate the T antigen. Another difference was that the T antigen was not stabilized, but dCMP deaminase, dTMP kinase, and dT kinase, were stabilized, respectively, by dCTP, dTMP, and dT or dTTP. Deoxyribonucleic acid (DNA) polymerase activity resembled the T antigen in adsorption to calcium phosphate, in precipitation by ammonium sulfate or at *p*H 5, and in the rate of inactivation when incubated at 38 C. However, the polymerase activity could be partly separated from the T antigen by Sephadex G-200 gel chromatography. The cell fraction containing partially purified T antigen also contained a soluble complement-fixing antigen (presumably a subunit of the viral capsid) which reacted with hyperimmune monkey sera. The latter antigen was present in very low titers or absent from cell extracts prepared from SV40-infected monkey kidney cell cultures which had been treated with ara-C at the time of infection, or from SV40-transformed mouse kidney (mKS) or hamster tumor (H-50) cells. The T antigen, however, was present in usual amounts in SV40-transformed cells or ara-C treated, infected cells.

Acute simian virus 40 (SV40) infection of monkey kidney cultures results in the early induction of an intranuclear tumor antigen (T antigen), detectable by complement-fixation or immunofluorescence techniques (11, 12, 26, 32), and in the induction of four enzymes of deoxyribonucleic acid (DNA) metabolism [thymidine (dT) kinase, thymidylate (dTMP) synthetase, dihydrofolate (FH_2) reductase, and DNA polymerase (9, 20–23)]. Abortive SV40 infection of mouse kidney cell cultures also leads to the induction of T antigen and of dT kinase, DNA polymerase, deoxycytidylate (dCMP) deaminase, and dTMP kinase (16, 21). Moreover, the T antigen is found in SV40-transformed cell lines, and elevated enzyme levels occur in the transformed cell lines (11, 16, 22, 31, 33). The formation of the T antigen and of the enzymes is not inhibited by drugs which prevent DNA synthesis, but drugs that prevent protein synthesis do inhibit these inductions (4, 9–11, 16, 20, 21). Furthermore, it has been shown that the syntheses of both the T antigen and of dT kinase are reduced in cells infected with ultraviolet light-irradiated SV40, but that the loss of enzyme and T antigen inducing activity occurs at a slower rate than the loss of virus infectivity (5, 6).

The function of the T antigen is not known.

Because of the similarities mentioned in the preceding discussion, it has been suggested that the T antigen might be a virus-induced enzyme (11, 24). The present study demonstrates, however, that the bulk of the T antigen activity is not identical to dT kinase, dCMP deaminase, FH_2 reductase, or dTMP kinase. A partial separation of DNA polymerase activity from T-antigen has been achieved, suggesting that the polymerase and the T antigen also differ.

MATERIALS AND METHODS

Cell lines and virus preparations. The propagation of primary cultures of green monkey kidney (GMK) cells, the established CV-1 line of GMK cells, SV40-transformed mouse kidney (mKS) cells, as well as the preparation and assay of SV40 stocks, have been described (20, 21, 23). SV40-transformed mouse kidney (mKS) cells (21) and hamster tumor (H-50) cells (2) were grown in 8-oz (227-ml) prescription bottles in R5a medium containing 0.5% lactalbumin hydrolysate and 10% calf serum (8).

Buffer solutions. Tris(hydroxymethyl)aminomethane (Tris) buffered enzyme extraction solution (TEES) contains 0.15 M KCl, 0.003 M 2-mercaptoethanol, and 0.01 M Tris chloride buffer, *p*H 8.0 (25 C). DNA polymerase extraction solution (DPES) is TEES solution (*p*H 7.8) lacking 0.15 M KCl. Dialyzing solution (DS) contains 0.003 M 2-mercaptoethanol, 0.001 M ethylenediaminetetraacetate, and 0.01 M Tris chloride buffer, *p*H 7.8 (25 C).

Fractionation of cell extracts. Extracts were prepared from uninfected cells, transformed cells, and from GMK or CV-1 cultures infected for 40 to 44 hr with SV40. Harvested cells were collected by centrifugation, suspended in 9 volumes of buffer solution, frozen and thawed, treated for 1 min with a Raytheon sonic oscillator at 10 kc, and centrifuged for 1 hr at 100,000 × *g*. The supernatant fluid (fraction S3) was then incubated for 1 hr at 4 C with bovine pancreatic ribonuclease (five times crystallized, Sigma Chemical Co., St. Louis, Mo.) at a ribonuclease concentration of 20 μg/ml. The ribonuclease-treated material was then further fractionated, either by the calcium phosphate (CaP) method, or by the *p*H 5-acid precipitation (*p*H 5) method (20, 23). All steps were carried out at 4 C.

In both methods, protein fractions were precipitated by partially saturating the solutions with ammonium sulfate. The fraction precipitated by the addition of 0.23 g/ml of ammonium sulfate (32% saturation) was designated as As 0–32; that obtained by the further addition of 0.19 g of ammonium sulfate per ml was named As 32–60. The fraction obtained by adding 0.14 g of ammonium sulfate per ml to fraction S3, discarding the precipitate, and then adding an additional 0.14 g of ammonium sulfate per ml, was denoted As 20–40.

In the calcium phosphate (CaP) method, fraction S3 after ribonuclease treatment was mixed for 8 min with calcium phosphate gel [usually 1.5 mg (dry weight) of gel per mg of protein in fraction S3 (20)] and then centrifuged for 10 min at 17,000 × *g*. To the supernatant fluid (fraction CaP), solid ammonium sulfate was slowly added to precipitate the various protein fractions, the suspension was stirred for 30 min, and the precipitate was collected by centrifugation (fractions: CaP-As 0–32; CaP-As 32–60; etc.).

In some experiments, ammonium sulfate was added directly to fraction S3 to obtain fraction As 0–32. This latter fraction was then mixed with calcium phosphate gel. The supernatant fluid from the negative gel adsorption step was used for enzyme and antigen assays and for Sephadex gel chromatography.

In the *p*H 5-acid precipitation method, 0.2 M acetic acid was added dropwise to the ribonuclease-treated fraction S3. The precipitate formed at *p*H 5.0 was collected by centrifugation and dissolved in DPES buffer solution at one-third the original S3 volume (fraction *p*H5P). Solid ammonium sulfate (0.28 g/ml) was added and the suspension was stirred for 30 min; the precipitate was collected by centrifugation and redissolved in DPES solution at one-fourth the original fraction S3 volume. The solution was dialyzed for 3 to 4 hr against four changes (2 liters each) of DS buffer to give fraction *p*H5P-As 0–40.

To the supernatant solution from the *p*H 5-acid precipitation step (*p*H5S), 0.4 M sodium bicarbonate was added to adjust the *p*H of the solution to *p*H 7. Then, solid ammonium sulfate was added to obtain fractions *p*H5S-As 0–40, *p*H5S-As 35–60, and *p*H5S-As 32–60. Each fraction was assayed for enzyme activity and complement-fixation titer. The protein content of the various fractions was determined by the method of Lowry et al. (27).

Determination of SV40 T antigen and viral-capsid antigen. A microcomplement-fixation test employing sera from an SV40-immune monkey and from SV40-tumor bearing hamsters was used to detect specific viral and T antigens, respectively (28). Antigen titer was determined as the reciprocal of the highest dilution of antigen giving 3+ or 4+ fixation in the presence of 4 units of antibody (complement-fixation titer).

Electron microscopy. Selected samples were examined in a Hitachi HU-11B electron microscope at instrumental magnifications of 5,000 and 15,000 times. Samples were stained with phosphotungstic acid and uranyl acetate by methods previously described (35).

Enzyme assays. The assays for dT kinase, dTMP kinase, and dCMP deaminase have been described (18–20). In all experiments involving dCMP deaminase and dTMP kinase, 0.3 mM dCTP or 0.1 mM dTMP, respectively, was included in the TEES enzyme extraction solution as enzyme activators and stabilizers. The dCTP and dTMP were also included in the buffers used to redissolve fractions precipitated by ammonium sulfate.

DNA polymerase was assayed as described by Kit et al. (23), except that the buffer solution used in the assay consisted of Tris chloride buffer (6.5 mM) plus phosphate buffer, *p*H 7.5 (20 mM). Cell extracts were prepared with DPES buffer, and fraction *p*H5P or the fractions precipitated with ammonium sulfate were dissolved in DPES buffer.

TABLE 1. *Fractionation of enzymes[a] and T antigen from SV40-infected and SV40-transformed cells by the "calcium phosphate" method*

Expt	Source of fractions	Fraction[b]	Protein		T antigen (CF titer)	dT kinase	dTMP kinase	dCMP deaminase	DNA polymerase
			Concn (mg/ml)	Total amt (mg)					
a	mKS	S3	7.5	12	128		51		
		CaP	3.2	3.8	0		91		
		CaP-As 32–60	2.1	1.7	0		114		
b	mKS	S3	7.0	116				110	
		CaP	4.0	61				121	
		CaP-As 0–32	1.2	8				7	
		CaP-As 32–60	6.9	14				322	
c	H-50	S3	2.7	27.7	32	32			
		As 0–32	1.9	10.0	32	49			
		As 0–32 (CaP)	0.4	1.8	0	182			
		As 32–50	1.8	9.2	2	0.3			
d	SV40-infected CV-1	S3	4.7	40.3	256	17			
		As 0–32	2.3	14.9	256	47			
		As 0–32 (CaP)	0.5	2.9	0	172			
		As 32–55	2.6	11.3	32	0.5			
		As 55–83[c]	1.2	5.2	0	—			
e	SV40-infected GMK	S3	4.1	107		9			
		CaP	1.6	42		20			
		CaP-As 20–40	2.5	10		48			
f	SV40-infected CV-1	S3	4.4	38.3	512	10			
		CaP[d]	0.5	4.4	0	172			
		CaP-As 0–32	0.2	0.7	0	723			
g	SV40-infected CV-1	S3	5.3	35.0					2
		CaP	0.9	5.9					0

[a] Enzymes activities: dT kinase and dCMP deaminase, millimicromoles of dUMP formed per milligram of protein in 10 min at 38 C; dTMP kinase, millimicromoles of dTDP and dTTP formed per milligram of protein in 10 min at 38 C; DNA polymerase, millimicromoles of dTTP incorporated into DNA per milligram of protein in 30 min at 38 C.

[b] For abbreviations, see Materials and Methods.

[c] FH_2 reductase activity is found in this fraction.

[d] Calcium phosphate gel, 2 mg, per mg of enzyme protein.

Because of the marked inhibitory effects of 0.15 M potassium chloride, sodium chloride, or ammonium sulfate on DNA polymerase activity (23), partially purified fractions to be assayed for this enzyme were dialyzed against DS buffer.

Estimation of enzyme molecular weights by Sephadex gel chromatography. The method of Andrews was used (1). Columns (34 × 2.5 cm) were packed with a mixture of 2 parts (by weight) Sephadex G-200 (Pharmacia, Uppsala, Sweden), 1 part Sephadex G-100, and 0.2 parts Whatman cellulose powder CF-11, and were equilibrated with a solution consisting of 0.15 M KCl, 0.003 M 2-mercaptoethanol, and 0.05 M Tris chloride buffer, *p*H 8.0, 25 C (Sephadex G-167 columns). This solution was also used for elution of proteins. For calibration, the following proteins (2 to 5 mg/sample) were added to the column: crystalline glucagon (Eli Lilly & Co., Indianapolis, Ind.); horse heart cytochrome C and bovine liver L-glutamic dehydrogenase, type II (Sigma Chemical Co., St. Louis, Mo.); myoglobin (once crystallized), β-lactoglobulin (three times crystallized), ovalbumin (three times crystallized); mercaptalbumin (twice crystallized), aldolase (five times crystallized), human γ-globulin (fraction II), and thyroglobulin (all from Nutritional Biochemicals Corp., Cleveland, Ohio), and horse liver alcohol dehydrogenase, once crystallized (Worthington Biochemical Corp., Freehold, N.J.).

Either fraction S3 or purified enzyme fractions (2 to 4 ml) were applied to the columns. When dCMP deaminase or dTMP kinase was studied, 0.3 mM dCTP or 0.1 mM dTMP, respectively, was included in both the equilibrating and the eluting solutions. When DNA polymerase was studied, separate col-

TABLE 2. *Fractionation of enzymes[a] and antigens from SV40-infected and SV40-transformed cells by the "pH 5-acid precipitation" method*

Expt	Source of fractions	Fractions[b]	Proteins		Complement-fixation titer		DNA polymerase	dTMP kinase	dT kinase	dCMP deaminase
			Concn (mg/ml)	Total amt (mg)	T antigen	V antigen				
a	mKS	S3	4.7	63.5	16		0.18	41		
		pH5P	6.4	26.9	32		0.52	2		
		pH5P-As 0–40	4.8	14.4	32		0.45	0		
		pH5S	1.9	27.6	0		0	95		
		pH5S-As 32–60	3.7	13.0	0		0	236		
b	mKS	S3	6.5	49.0	32				5.1	
		pH5P	7.4	25.9	128				1.1	
		pH5P-As 0–40	7.4	14.8	128				1.4	
		pH5S	2.6	22.1	<4				12.5	
		pH5S-As 0–40	1.6	3.2	<4				35.3	
c	mKS	S3	5.5	82.5						62
		pH5S-As 35–60	6.8	23.8						178
d	SV40-infected GMK	S3	5.0	95.6	128	512	1.8			
		pH5P-As 0–40	7.6	38.0	256	1,024	2.9			
		pH5S			10	0	0			
e	ara-C treated and SV40-infected GMK[c]	S3	6.0	108	64	8	1.7			
		pH5P			256	16				
		pH5P-As 0–40	8.9	39.8	256	16	3.0			
		pH5S			0	0				
f	SV40-infected GMK	S3	3.8	30.7					4.0	550
		pH5P	3.8	9.9					—	32
		pH5S	2.1	14.8					5.8	910
		pH5S-As 0–35	1.3	2.6					15.1	76
		pH5S-As 35–60	3.0	6.0					0	1,550

[a] Units of enzyme activities as in Table 1.
[b] See Materials and Methods for abbreviations.
[c] Ara-C (20 μg/ml) added 2 hr postinfection.

umns were equilibrated with DPES buffer solution, and DPES buffer solution was used for eluting the enzyme.

A column was also packed with Sephadex G-200 for DNA polymerase studies. This column was equilibrated with DPES solution, and the latter solution was used for eluting the enzyme.

RESULTS

Fractionation of SV40-induced enzymes and antigens. The partial purification of dT kinase from virus-infected cells by the "*p*H 5-acid precipitation" method and by the "calcium phosphate negative adsorption" method has previously been described (17, 19, 20). Additional data on extracts from SV40-transformed cells and from SV40-infected monkey kidney cells are presented in Tables 1 and 2. In confirmation of previous results, it was found that dT kinase activity occurs in the *p*H 5 supernatant fluid and that some purification of the enzyme can be achieved by a calcium phosphate negative adsorption step. Most of the dT kinase activity is precipitated by the addition of ammonium sulfate to 20 to 32% saturation.

Tables 1 and 2 also show that the SV40-specific T antigen and DNA polymerase are precipitated by about the same concentration of ammonium sulfate as is dT kinase. However, both the T antigen and DNA polymerase are insoluble at *p*H 5 and they are more strongly adsorbed by calcium phosphate gel than is dT kinase. Thus, the bulk of the complement-fixing T antigen activity can be completely separated from dT kinase activity. These findings are summarized in Table 3.

It may also be seen from Tables 1 to 3 that dCMP deaminase and dTMP kinase activities are found in the *p*H 5 supernatant fraction and in

TABLE 3. *Properties of SV40-induced enzymes and tumor antigen*

Enzyme or antigen	Adsorbed by calcium phosphate gel	Precipitated at *p*H 5	Saturated $(NH_4)_2SO_4$ required for precipitation (%)
SV40 T-antigen	+	+	20–32
DNA polymerase	+	+	20–40
Thymidine kinase	−	−	20–32
dCMP deaminase	−	−	35–60
dTMP kinase	−	−	32–60
Dihydrofolic reductase			55–83

the supernatant fluid from the calcium phosphate gel negative adsorption step. Moreover, approximately 32 to 60% saturation of the solutions with ammonium sulfate is required to precipitate the dCMP deaminase and dTMP kinase activities.

Table 3 shows that even higher concentrations of ammonium sulfate (55 to 83% saturation) are required to precipitate dihydrofolate reductase.

Lack of effect of nucleotides on thermal stability of T antigen. It has previously been shown that complement-fixing T-antigen activity is destroyed by 5 min of heating at 55 to 60 C (11). SV40-induced DNA polymerase activity is also unstable to this treatment (23), but about half of the dT kinase activity induced by SV40 in monkey kidney cells survives this heating (15). Thymidine kinase activity is stabilized by dTTP or dT; the activities of dTMP kinase and dCMP deaminase are stabilized by 0.3 mM dCTP and 0.1 mM dTMP, respectively (17–19). To learn whether any of these metabolites enhanced the thermal stability of the SV40 complement-fixing T antigen, portions of fraction S3 from H-50 cells were incubated at 37 C with or without the nucleotides. Almost complete inactivation of the T antigen activity occurred after 1.5 hr at 37 C; addition of dT (1 mM), dTMP (0.1 mM), dTTP (0.1 mM), or dCTP (0.3 mM) did not afford protection. These results further distinguish the properties of T antigen from those of dT kinase, dTMP kinase, and dCMP deaminase.

SV40-viral capsid antigen. Fractions from SV40-infected GMK cells were assayed for viral capsid antigen, by use of specific hyperimmune monkey antisera in the complement-fixation test. Table 2, experiment d, shows that the titer of soluble viral-capsid antigen in fraction S3 was considerably greater than that of the SV40 T antigen; the soluble viral-capsid antigen was precipitated by acetic acid at *p*H 5. Approximately 40% saturation with ammonium sulfate was required to precipitate the viral capsid antigen from fraction *p*H5P, that is, the same concentration of ammonium sulfate that precipitated the T antigen activity.

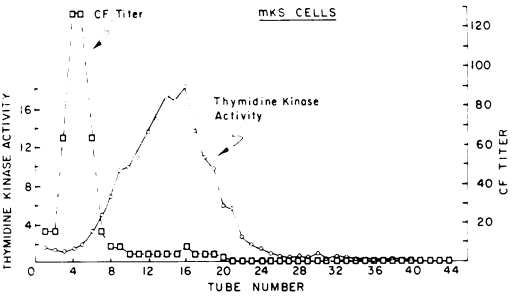

FIG. 1. *Sephadex G-167 gel chromatography of complement-fixing (CF) T antigen and thymidine kinase activities of fraction prepared from SV40-transformed mouse kidney (mKS) cells. A ribonuclease-treated S3 fraction (see Materials and Methods) was employed. After an initial 35 ml of buffer solution was filtered through the gel, 2.4-ml fractions (tube numbers 1 to 44) were collected. Thymidine kinase activity was assayed with ^{3}H-deoxyuridine as nucleoside substrate. Thymidine kinase activity: millimicromoles of dUMP formed per milligram of protein in 10 min at 38 C. SV40 T antigen titer: reciprocal of highest dilution of solution giving 3+ or 4+ fixation in the presence of 4 units of antibody. Approximately 33% of the thymidine kinase activity and 95% of the T antigen activity were recovered from the column.*

Formation of infectious SV40 and of viral-capsid antigen, but not T antigen, can be inhibited by 1-β-D-arabinofuranosylcytosine (ara-C) treatment of infected cells (4, 30). Table 2, experiment e, shows that the addition of 20 μg of ara-C per ml at 2 hr after infection of GMK cells with SV40 almost completely prevented formation of the soluble viral-capsid antigen but not of the T antigen or DNA polymerase.

To learn whether fraction *p*H5P-As 20–40 contained intact SV40 virions, portions of this fraction were examined by electron microscopy. SV40 virions were not detected; the fraction from SV40-infected GMK cells contained only amorphous protein material and was indistinguishable from the fractions from noninfected or ara-C treated, SV40-infected GMK cells.

Estimation of molecular weights of enzymes and antigens by Sephadex gel chromatography. Figures 1 and 2 illustrate the chromatography of enzymes and antigens on Sephadex G-167 and Sephadex G-200. Data on the estimated molecular weights of the virus-induced enzymes and antigens are summarized in Table 4.

dT kinase activity has been measured on fraction S3, the fraction precipitated from fraction S3 by the addition of ammonium sulfate to between 20 and 40% saturation, and on more highly

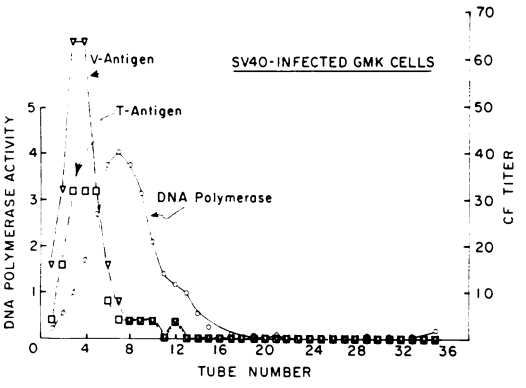

FIG. 2. *Sephadex G-200 gel chromatography of complement-fixing (CF) T antigen, viral-capsid (V) antigen, and DNA polymerase activities from SV40-infected GMK cells. Cells were infected with 66 plaque-forming units per cell of SV40 and harvested 42 hr after infection. Fraction pH5P-As 0–40 (see Materials and Methods) was used for chromatography. After an initial 35 ml of buffer solution was filtered through the gel, 2.1-ml fractions (tubes 1 to 35) were collected. DNA polymerase activity: millimicromoles of ^{3}H-dTTP incorporated into DNA per milligram of protein in 30 min at 38 C. Approximately 43% of the DNA polymerase, and 100% of the T-antigen and V-antigen, activities were recovered from the column.*

purified fractions (CaP-As 20–40). dT kinase exhibited a molecular weight of about 80,000 to 103,000, and most of the enzyme activity was eluted more than 10 fractions after the peak of the T antigen activity.

For the curve plotted in Fig. 1, ^{3}H-deoxyuridine (dU) was used as nucleoside substrate in the dT kinase assay. However, the assays on the mKs cell fractions were also performed with ^{3}H-dT as substrate. The dT kinase activity was eluted in the same position with either nucleoside substrate, supporting the conclusion that dU and dT are alternative nucleoside substrates of dT kinase (17).

The possibility was considered that SV40 progeny DNA might affect the chromatographic properties of dT kinase or the T antigen. Contrary to this hypothesis, Table 4 shows that the estimated molecular weights of dT kinase from SV40-infected cells, which had been treated with ara-C to prevent viral DNA synthesis, were the same as that from cells not treated with the drug. Moreover, the estimated molecular weight of the enzyme prepared from SV40-transformed cells, which are also blocked in viral DNA synthesis, was about the same as that prepared from productively infected cells or uninfected cells.

TABLE 4. *Molecular weight estimations by Sephadex gel chromatography of T antigens and enzymes from uninfected-, SV40-infected-, and SV40-transformed cell cultures*

Enzyme or antigen	Source of fraction	Fraction	Sephadex gel	Estimated molecular wt
T antigen	mKS	S3	G-167	>200,000
	H-50	As 20–40	G-167	>200,000
	GMK	pH5P-As 0–40	G-200	>250,000
	ara-C treated GMK	pH5P-As 0–40	G-200	>250,000
	SV40—infected GMK	pH5P-As 0–40	G-200	>250,000
	ara-C treated and SV40-infected GMK	pH5P-As 0–40	G-200	>250,000
DNA polymerase	mKS	S3	G-167	200,000
	GMK	pH5P-As 0–40	G-200	220,000
	ara-C treated GMK	pH5P-As 0–40	G-200	220,000
	SV40-infected GMK	pH5P-As 0–40	G-200	220,000
	ara-C treated and SV40-infected GMK	pH5P-As 0–40	G-200	220,000
dCMP deaminase	mKS	pH5S-As 32–60	G-167	120,000
dTMP kinase	mKS	pH5S-As 32–60	G-167	52,000
FH_2 reductase	SV40-infected CV-1	As 55–83	G-100	20,000
dT kinase	mKS	S3	G-167	90,000
	H-50	As 20–40	G-167	103,000
	GMK	As 20–40	G-167	80,000
	ara-C treated GMK	S3	G-167	100,000
	SV40-infected GMK	As 20–40	G-167	80,000
	SV40-infected GMK	CaP-As 20–40	G-167	80,000
	SV40-infected and ara-C treated GMK	S3	G-167	90,000

The approximate molecular weights of dTMP kinase, dCMP deaminase, and FH_2 reductase were much smaller than that of the complement-fixing T antigen (Table 4). However, the DNA polymerase activity had a molecular weight of about 220,000 and was eluted from the Sephadex slightly after glutamic dehydrogenase.

Figure 2 shows an experiment with partially purified fractions from SV40-infected GMK cells. The DNA polymerase activity was retarded by the Sephadex G-200 gel more than the complement-fixing T antigen, suggesting that they are different proteins. The SV40 viral-capsid antigen was eluted in the same tube fractions as the T antigen.

Little, if any, viral-capsid antigen was present when extracts were prepared from infected cells that had been treated with ara-C (Table 2). However, the T-antigen and DNA polymerase were found in normal amounts in the extracts from ara-C treated cultures, and their elution properties were not changed by the ara-C treatment of infected cells (Table 4).

DISCUSSION

The activities of six enzymes of DNA metabolism are enhanced in SV40-infected cell cultures (9, 16, 20–23). The present study shows that four of these enzymes have properties which are different from those of the SV40-induced complement-fixing T antigen.

As also shown by Gilden et al. (11), the T antigen is precipitated from cell extracts by the addition of acetic acid to *p*H 5. However, dT kinase, dTMP kinase, and dCMP deaminase are not. The T antigen is more strongly adsorbed by calcium phosphate gel than are these three enzymes. The T antigen is precipitated from solution by a different concentration of ammonium sulfate than are dTMP kinase, dCMP deaminase, and FH_2 reductase. Finally, the molecular weight of the T antigen, as estimated by Sephadex gel chromatography, is considerably greater than that of dT kinase, dTMP kinase, dCMP deaminase, or FH_2 reductase.

Two further observations contraindicating the identity of the bulk of the T antigen activity with that of the dT kinase can be mentioned. First, T antigen is more labile than dT kinase and it is not stabilized against thermal inactivation by metabolites which protect dT kinase activity. Second, recent studies from our laboratory show that normal levels of T antigen and DNA polymerase activity occur in SV40-transformed mouse kidney (mKS) cells that have been adapted to grow in media containing 100 μg of 5-bromodeoxyuridine (dBU) per ml. However, the dBU-resistant mKS cells display only about 1% of the dT kinase activity of parental mKS cells. These two observation could be explained by assuming that antigenic and active enzymatic sites occur at two different loci of the same protein, so that the enzymatic site could be protected by nucleotides or inactivated by mutation without disturbing the antigenic site. However, in view of the separation of T antigen and dT kinase by biochemical procedures, the most likely interpretation is that the T antigen and dT kinase are distinctive proteins.

The findings with respect to DNA polymerase and the T antigen are less definitive. DNA polymerase is similar to the T antigen in several properties. The polymerase is tightly adsorbed by calcium phosphate gel, it is precipitated at *p*H 5, and it is precipitated at about the same ammonium sulfate concentration as the T antigen. DNA polymerase and the T antigen activities are both rapidly inactivated by incubation at 37 C (22). However, Sephadex G-200 gel chromatoggraphy does effect a partial separation of the enzyme from the T antigen activity, suggesting that the polymerase and the T antigen are different.

The sixth enzyme that increases in SV40-infected cell cultures is dTMP synthetase. At this writing, the properties of the virus-induced enzyme have not been studied. It is noteworthy, however, that the molecular weight of dTMP synthetase purified from chick embryo extracts is about 60,000 (M. G. Lorenson, G. F. Maley, and F. M. Maley, Abstr. Meeting Am. Chem. Soc., New York, p. C127, 1966), much lower than that of the T antigen; thus, there is no *a priori* reason to believe that the T antigen and dTMP synthetase are the same.

The preparations of DNA polymerase and T antigen described in this study all had molecular weights in excess of 200,000. Various procedures were employed to prevent possible aggregation of these proteins. Ribonuclease treatment of the extracts (about 8 mg/ml of protein) reduced the contaminating ribonucleic acid content to less than 4 μg/ml (10, 23). Moreover, a study of the incorporation of ^{3}H-dT into fraction *p*H5P-As 20–40 and colorimetric determination of DNA revealed that only traces of DNA contaminated that fraction. In some experiments, the mercaptoethanol concentration of the solution used to prepare cell fractions and to elute the proteins from Sephadex gels was increased from 3 to 10 mM. Fractions were also dialyzed against sulfite, a procedure known to reduce protein aggregation (7, 34). However, these treatments did not alter the chromatographic properties of the polymerase and the T antigen on Sephadex gels.

A soluble complement-fixing antigen which reacted with hyperimmune monkey sera was present in the fractions prepared from SV40-infected monkey kidney cells, but not in those from H-50, mKS, or SV40-infected monkey kidney cells, which had been treated with ara-C. This antigen is thus distinct from the T antigen and is most probably a subunit of the virus capsid. An electron microscopic study of purified *p*H5P-As 0–40 fractions from infected cells failed to reveal the presence of intact virions in this fraction. These results recall the finding of subviral V antigen of SV40 in fluorouracil-treated cells (29). Experiments on the further characterization of this viral-antigen subunit are planned.

The data presented in this study are contrary to the hypothesis that the T antigen is an early virus-induced enzyme of DNA metabolism, but do not elucidate the biological role of the antigen. An appealing alternative possibility is that the T antigen participates nonenzymatically in the replication of either SV40 DNA or cellular DNA. Evidence for the existence of a class of nonenzymatic proteins, essential for the initiation of DNA synthesis in bacteria, has been advanced by Jacob et al. (13) and by Lark (25). Moreover, experiments by Kates and McAuslan (14) and by Ben-Porat and Kaplan (3), suggest that an early protein, distinct from dT kinase or DNA polymerase is needed in stoichiometric amounts for the synthesis of poxvirus DNA and herpesvirus DNA. The known properties of the T antigen are consistent with those of the initiator-replicator type protein. However, proof or disproof of this hypothesis must await the elucidation of the biochemical and biophysical properties of the nonenzymatic early proteins.

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