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Measurements of the Molecular Size of the Simian Virus 40 Large T Antigen

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A measure of the molecular weight of the large simian virus 40 T antigen was sought by SDS-polyacrylamide gel electrophoresis, random-coil chromatography, and sedimentation-velocity analysis in a density gradient. Large T antigen obtained from a simian virus 40-transformed human cell line either by immunoprecipitation or by standard preparatory methods migrated like a 94,000-molecular-weight (~94K)polypeptide in SDS-gels but was found to have an approximate molecular weight of 75,000 (±7%) by the other two methods. Comparable results were observed with T antigen obtained from lytically infected monkey cells. In view of the strong theoretical basis for the guanidine method and the agreement with the sedimentation data, these findings suggest that the molecular weight of this protein is ~75 to 80K as opposed to 94 to 100K and, therefore, that considerably less than the entire early region of simian virus 40 is required to encode it. This size estimate is in keeping with earlier results which revealed a normal-size T antigen in cells infected with viable deletion mutants lacking as much as 10% of the early region.

Classically, the simian virus 40 (SV40) T antigen has been viewed as a virus-specific protein detected in the nuclei of cells infected or transformed by the virus. More recent observations indicate that there are at least two T antigens and that the larger one is the product of SV40 gene *A* (3, 12, 21, 29, 40, 52, 53, 55; E. Paucha, R. Harvey, R. Smith, and A. E. Smith, INSERM Colloquium 49, in press), an early cistron defined by complementation studies with large numbers of temperature-sensitive (*ts*) mutants (8, 49). This does not appear to be the only early papova gene, for in the closely related virus, polyoma, others have identified a second complementation group—hr-t (16, 17, 19, 46). On the basis of experiments performed with SV40 *ts* mutants, the T antigen appears to play a role in initiation of rounds of viral DNA replication, suppression of early transcription, induction of host cell DNA replication, and virus-induced neoplastic transformation (6, 9, 27, 29, 34–36, 41, 50, 52).

Early estimates of the molecular weights of SV40 T antigen ranged from 70,000 (70K) (13, 39) to 600K (20). More recently, employing direct immunoprecipitation as a means of isolating the protein, a major polypeptide species has been reproducibly observed whose electrophoretic mobility in SDS-polyacrylamide gels has been that of a 94 to 100K polypeptide (52). This has been termed large T antigen as a means of contrasting it with a small cross-reacting molecule (approximately 17K in SDS-polyacrylamide gels), also present in infected and transformed cells (12, 21, 40, 45; Paucha et al., in press).

The amino acid composition of the large T protein is now known (44, 54), and, from it, one can calculate an average residue molecular weight of ~115. Assuming the molecular weight of T antigen to be 94K, an estimate of the predicted chain length of the protein would be 817 amino acids. The maximum size of the SV40 early region is approximately 2,500 base pairs (14, 26, 43, 47), and, therefore, the maximum coding capacity of such a DNA segment would be 833 amino acids. Thus, if the molecular weight of T antigen is truly 94K, nearly all of the early-region sequence should be committed to the large T antigen cistron.

Shenk et al. (45) have recently isolated and characterized a series of viable deletion mutants of SV40 which lack DNA sequences in the early region of the SV40 genome between 0.54 and 0.59 map units. The largest deletion among these was approximately 184 base pairs (dl 884). Surprisingly, cells infected by all of these 0.54 to 0.59 deletion mutants synthesize a T protein which, by comparative gel electrophoretic analysis, appears to be similar in size to the wild-type protein (12, 42, Paucha et al., in press). Thus, it appears that the majority of the sequences in the 0.54 to 0.59 region do not encode any segment of the 94K protein. This would be

problematic if the genuine size of the large T protein were 94K, since one would have expected deletions of this size to have resulted in a measurable reduction in the size of this molecule. Thus, unless T antigen is a hybrid SV40 host protein, one must consider the possibility that determinations of molecular weight by SDS-gel electrophoresis are misleading. In an effort to test this suggestion, we have attempted to measure the approximate molecular size of "94K" T antigen from both an SV40-transformed human cell line, SV80, and SV40-infected permissive cells by two empirical, non-electrophoretic transport methods: random-coil molecular-sieve chromatography and analytical sedimentation velocity in a density gradient. In keeping with prior results of random-coil chromatographic experiments (13), a molecular size estimate of 75 to 80K was obtained with both techniques for large T antigens from both SV80 and lytically infected cells. An analogous differential in molecular weight of the polyoma virus "100K" T antigen has recently been observed by Schaffhausen et al. (B. S. Schaffhausen, J. Silver, and T. L. Benjamin, Proc. Natl. Acad. Sci. U.S.A., in press), using random-coil chromatography and SDS-gel electrophoresis.

MATERIALS AND METHODS

Cells and viruses. SV80, an SV40-transformed human fibroblast cell line, (23) and CV1-P, an African green monkey kidney cell line, were grown in Dulbecco's modification of Eagle minimal essential medium containing 10% fetal calf serum at 37°C in a 10% CO_2-containing atmosphere in 90-mm plastic dishes (Falcon Plastics, Oxnard, Calif). CV1-P cells were infected at 90% confluence, with three-times plaque-purified SV40, strain 777, at a multiplicity of 1. Where indicated, SV80 cells were mass cultivated in roller bottles at the Massachusetts Institute of Technology Cell Culture Center. We are indebted to Donald Giard and Carrol McKinley for their assistance in this effort. A three-times plaque-purified clone of SV40, strain 777, was propagated in primary African green monkey kidney cells at a multiplicity of 10^{-4}.

Preparative immunoprecipitation of radioactive T antigen. To label SV80 T antigen, ≥90% confluent SV80 cells in 90-mm petri dishes were fed with standard medium lacking methionine and leucine. One hour later, [^{35}S]methionine (250 to 400 Ci/mmol; New England Nuclear Corp., Boston, Mass.) and [^{3}H]leucine (64 Ci/mmol; Schwarz-Mann, Orangeburg, N.Y.) were added at 50 μCi/ml each. To label T antigens from lytically infected cells, CV1-P cells were grown to ≥90% confluence in 90-mm petri dishes. They were then infected with three-times plaque-purified SV40, strain 777, at a multiplicity of 4 to 8. Twenty-four hours later, they were starved for methionine and leucine and then fed with the two radioactive amino acids as just described for SV80 cells. After a 3-h exposure to radioactive amino acids, both cultures were washed twice with 0.01 M sodium phosphate (pH 7.4)–0.14 M NaCl (phosphate-buffered saline), and the protein was extracted at 4°C with a solution of 20 mM Tris-hydrochloride, pH 9, 137 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10% glycerol, 1% Nonidet P-40, and 50 μg of phenyl methyl sulfonyl fluoride per ml (1.2 ml/plate), and immunoprecipitated as described by Schwyzer (44) and Schaffhausen et al. (Proc. Natl. Acad. Sci. U.S.A., in press). Briefly, the extracts were pooled and 10 μl of normal sheep serum per ml was added immediately. This was followed by 80 μl of a 50% suspension (vol/vol in water) of staphylococcal protein A-Sepharose (Pharmacia, Piscataway, N.J.). The suspension was gently agitated at 4°C for 1 h, and the beads were pelleted. Fractions of the supernatant were then incubated at 4°C with either hamster anti-T serum (4X-0416 from Roger Wilsnack and the Resources and Logistics Branch, National Cancer Institute Virus Cancer Program) or nonimmune hamster serum plus 80 μl of 50% protein A-Sepharose beads per ml. One hour later, the beads were centrifuged and washed three times with 100 mM Tris (pH 9)–0.5 M LiCl–140 mM β-mercaptoethanol (βME) (1). Immune complexes were then eluted from the beads with either 50 μl (per milliliter of original lysate) of buffer containing 20 mM Tris (pH 9.0)–5% SDS–700 mM βME–20% glycerol (85°C for 5 min) or 300 μl of solution A—6 M guanidine-hydrochloride (Schwarz-Mann), 0.1% Triton X-100, and 140 mM βME (37°C for 3 min). Eluates could be stored at −70°C for at least 2 weeks without loss of integrity as analyzed by SDS-polyacrylamide gel electrophoresis.

Purification of nonradioactive T antigen from SV80 cells. The 94K T protein was purified to ≥90% homogeneity from approximately 60-g lots of fresh SV80 cells by the method of Tenen et al. (54). The final product was characterized as a single-stained band, migrating in parallel with phosphorylase a (94K) by SDS-polyacrylamide gel electrophoresis.

T antigen radioiodination. Fractions of the protein (5 to 20 μg from SV80 cells) were labeled with ^{125}I by the chloramine T method of Greenwood et al. (22). The product migrated as a single 94K band in SDS-polyacrylamide slab gels, as defined by the presence of a single autoradiographic band (54).

Analytical density gradient sedimentation. These experiments were performed with 12 ml, 5 to 25% (vol/vol) linear glycerol gradients, prepared as described previously (54). Sedimentation was performed in a Spinco SW41 rotor for 15 to 18 h at 4°C. Fractions were collected after bottom needle puncture. Protein markers were added to each sample before commencing a run. β-Galactosidase (molecular weight, 515K) was assayed by the colorimetric method of Craven et al. (11); catalase (molecular weight, 247K) was assayed as described by Maehly (32); and alkaline phosphatase was assayed by a colorimetric assay with *p*-nitrophenylphosphate as substrate.

SDS-polyacrylamide gel electrophoresis. SDS-gelectrophoresis was performed in slab gels (25 cm by 10 cm by 1.0 mm) by the method of Laemmli (30). Polyacrylamide stacking gels (4%) were employed. Where indicated, gels were prepared for fluorographic analysis by the method of Bonner and Laskey (5) and dried under vacuum before autoradiography. All gels were stained with Coomassie brilliant blue. The fol-

lowing protein standards were routinely utilized. Phosphorylase a (94K), bovine serum albumin (68K), immunoglobulin G (IgG) heavy chain (53K), ovalbumin (44K), IgG light chain (25K), myoglobin (17K), and RNase A or cytochrome *c* (13K).

Random-coil chromatography. Chromatography was performed by the method of Fish et al. (18). Sepharose 4B-CL or Sepharose 4B (Pharmacia) was equilibrated in a solution of 6 M guanidine-hydrochloride (Schwarz-Mann), 140 mM βME, and 0.1% Triton X-100 (New England Nuclear), pH 4.0 (solution A). Comparable separations of proteins of 94K to 13K were obtained with both gel filtration media in this solution. All samples were incubated in this solution at room temperature for 48 h before column application. The various internal protein standards were detected by turbidometric analysis. These included: phosphorylase a (94K), bovine serum albumin (68K), IgG heavy chain (53K), ovalbumin (44K), yeast alcohol dehydrogenase (38K), and bovine heart cytochrome *c* (13K) or RNase A (12.6K), Moreover, in some instances, the column was calibrated between runs with the same standards, as ^{125}I-labeled proteins. The weight of each eluted fraction was ascertained, and the K_d values were calculated from such data by a published method (18, 38).

RESULTS

SDS-polyacrylamide gel electrophoresis of immunoprecipitated SV40 T antigen from SV80 and CV1-P cells. Immunoprecipitation was used to rapidly purify in vivo labeled T antigen from both SV80 and virus-infected permissive cells (44, 52; M. Schwyzer, in press). An SDS eluate was then analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 1). The prominent radioactive band, migrating in parallel with phosphorylase a, was observed in 12% polyacrylamide gels in the case of both SV80 cells and SV40-infected CV1-P cells. Immunoreactive bands between 70 and 90K were not observed in immunoprecipitation from either of these two cultures with this preparatory system. As noted in Fig. 1D and F, minor bands (relative to large T antigen) were occasionally observed in this region but were also seen in control serum slots. No ~94K T antigen band was detected when nonimmune hamster serum was substituted for anti-T serum.

In other experiments, the large T antigen from both SV80 cells and SV40-infected CV1-P cells comigrated with each other and with phosphorylase a (94K) in SDS-gels with acrylamide concentrations of 5, 7.5, 10, 12, 15, and 20%, and also in 7.5% polyacrylamide-SDS-6 M urea gels (data not shown). Prominent radioactive bands of lesser intensity at 57K, 55K, and 8K were seen in SV80 cells, whereas they were not observed in lytic T antigen or control serum-SV80 immunoprecipitates. The structural relationships of these other polypeptides to large T (if any) are presently under investigation. A discrete, small T antigen band (~17K) was present in both the SV80 and infected CV1-P immunoprecipitates.

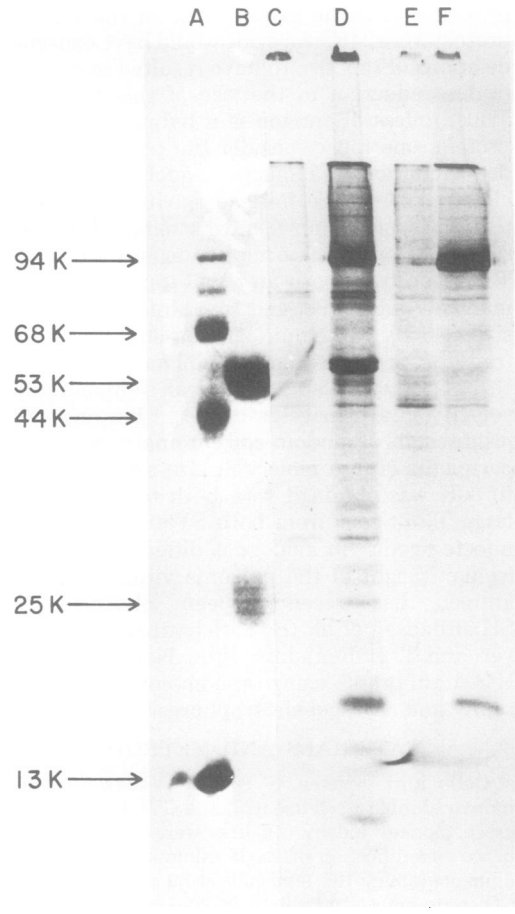

FIG. 1. *SDS-polyacrylamide gel electrophoresis of immunoprecipitated radioactive SV40 T protein(s): autoradiogram (48-h exposure) and photograph of a stained, 12% polyacrylamide-SDS slab gel. (A) Standard proteins (stained), phosphorylase a (94K), bovine serum albumin (68K), ovalbumin (44K), RNase (13K). The gel was stained with Coomassie brilliant blue. (B) Standard proteins, hamster IgG heavy chain (53K) and light chain (25K). (C) Precipitation of SV80 cells (~2 × 10^6 cells) with hamster preimmune serum. (D) Precipitation of SV80 cells (~2 × 10^6 cells) with anti-T serum. (E) Precipitation of SV40-infected CV1-P cells (~2 × 10^6 cells) with hamster preimmune serum. (F) Precipitation of CV1-P cells (~2 × 10^6 cells) with hamster anti-T serum. Virus infection, labeling, immunoprecipitation, and gel electrophoresis were performed as described in Materials and Methods. The sizing of the ~8K anti-T reactive band was performed in a 20% SDS-polyacrylamide gel with the above standards plus parathyroid hormone (9K), bovine pancreatic trypsin inhibitor (6K), and insulin (3.1K) (data not shown).*

Random-coil chromatography of standard proteins. A column of Sepharose 4B-CL was equilibrated with solution A. Individual

standard proteins and a mixture of bovine serum albumin and IgG heavy chain were incubated separately in solution A and then separately applied to the column. Their elution positions were marked by turbidometric analysis. As can be seen, phosphorylase a (94K), bovine serum albumin (68K), IgG heavy chain (53K), ovalbumin (44K), and cytochrome *c* (13K) all elute in separate positions and as discrete peaks (Fig. 2). A mixture of phosphorylase a and ^{125}I-labeled bovine serum albumin, ovalbumin, and RNase A (13K) also yielded a clear pattern of well-separated individual peaks with the same K_d values as were obtained in the above-noted experiment (data not shown). The plots of fractional elution values of these standards versus molecular weight developed by the method of Tanford et al. were linear (see Fig. 6 and 7) (18). Thus, at a minimum, this chromatographic system is capable of resolving polypeptide species between 94K and 12K. The error in individual measurements of molecular weight in this range was estimated at approximately 7%.

Random-coil chromatography of large T antigen from SV80 cells. A portion of SV80 radioactive material eluted from the immune complex-protein A beads into 6 M guanidine-hydrochloride-containing solution (solution A) was incubated at 23°C in the presence of several internal protein standards and then chromatographed in a column of Sepharose 4B equilibrated in solution A. A prominent radioactive peak of SV80 T antigen eluted at fractions corresponding to molecular weights of 74K (±7%) (Fig. 3), as determined from a plot of the elution fraction of individual standards versus molecular weight (see Fig. 6). When the major radioactive (74K) protein peak from SV80 cells was freed of guanidine by gel filtration and then analyzed by SDS-polyacrylamide gel electrophoresis, it again migrated in parallel with the unchromatographed "94K" polypeptide (see Fig. 6, inset). Thus, undegraded, large T antigen is the major component of the prominent protein peak in the guanidine chromatogram.

The 94T antigen was also extensively purified from SV80 cells by a series of conventional fractionation steps (54). When radioiodinated, it migrated in an SDS-gel as a single band with an apparent molecular weight of 94K (data not shown). When this material was analyzed by analytical gel filtration in 6 M guanidine, a single

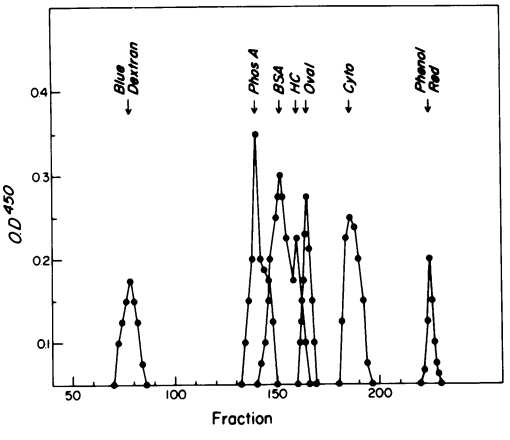

FIG. 2. *Random-coil chromatography of standard proteins. Standard proteins (~2 to 5 mg each) were incubated in 500 μl of solution A, as indicated in Materials and Methods, and applied individually to a column (1.2 by 108 cm) of Sepharose 4B-CL equilibrated in the same solution. Fractions of 0.5 ml were collected and 50- to 100-μl fractions of each were analyzed turbidometrically. The elution positions of several protein standards are shown by arrows: phosphorylase a (94K), bovine serum albumin (68K), hamster IgG heavy chain (53K), ovalbumin (44K), and cytochrome c (13K). The elution position of blue dextran and phenol red is also indicated by arrows. Where no solid dots are shown, no absorption of light was detected.*

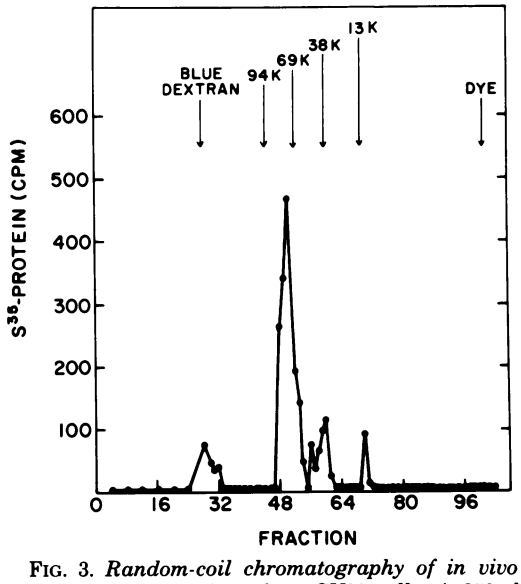

FIG. 3. *Random-coil chromatography of in vivo labeled large T antigen from SV80 cells. A 250-μl amount of $^{35}S/^{3}H$-labeled protein (2,200 cpm/μl) eluted from anti-T/protein Sepharose beads with solution A was incubated in that solution along with several internal protein standards (3 mg each) as described earlier. This sample was applied to a column (1.1 by 75 cm) of Sepharose 4B equilibrated in solution A. Portions (75 μl) of each 0.5-ml fraction were counted in Aquasol (New England Nuclear Corp.) in a liquid scintillation spectrometer. The elution positions (determined turbidometrically) of several internal standard proteins—phosphorylase a (94K), bovine serum albumin (68K), yeast alcohol dehydrogenase (38K), and cytochrome c (13K)—are designated by the arrows. The dye marker indicates the elution position of bromophenol blue.*

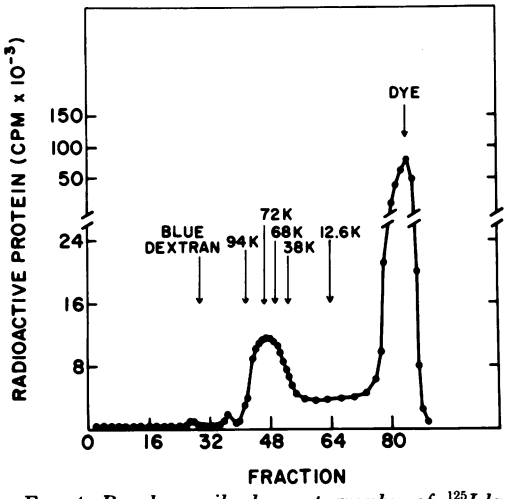

FIG. 4. *Random-coil chromatography of ^{125}I-labeled SV40 large T antigen. T antigen was purified from SV80 cell nuclei by the published method (54). It was iodinated as noted in Materials and Methods and was characterized as a single 94K radioactive band by SDS-gel electrophoresis (54). The specific activity of this preparation was estimated to be 4.5 × 10^6 cpm/μg; 6.4 × 10^6 cpm were preincubated in solution A and chromatographed on a column of Sepharose 4B as described in Materials and Methods. Fractions (0.6 to 0.7 ml) were collected. A 150-μl aliquot of each was counted. The elution positions of several protein standards (determined turbidometrically) are indicated by arrows: phosphorylase a (94K), bovine serum albumin (68K), yeast alcohol dehydrogenase (38K), and RNase A (13K). The dye marker indicates the elution position of bromophenol blue. The radioactivity eluting with the dye marker represents free iodine. The plateau of radioactivity trailing behind the 72K peak was a reproducible finding and was ≤10% precipitated by trichloroacetic acid. It may represent free iodine or iodopeptides which dissociate from the protein during the chromatographic procedure.*

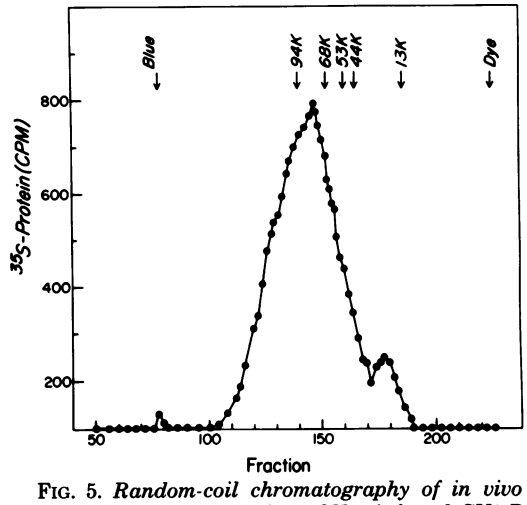

FIG. 5. *Random-coil chromatography of in vivo labeled large T antigen from SV40-infected CV1-P cells. A 400-μl amount of ^{35}S/^{3}H-labeled protein (2,000 cpm/μl), eluted from anti-T/protein A Sepharose beads in solution A, was incubated with standard proteins and chromatographed on a column of Sepharose 4B-CL (1.2 by 108 cm) as described earlier. A 0.1-ml portion of each 0.5-ml fraction was counted in a liquid scintillation spectrometer. The elution positions of several internal protein standards are shown by arrows: phosphorylase a (94K), bovine serum albumin (68K), hamster IgG heavy chain (53K), ovalbumin (44K), and cytochrome c (13K). The dye marker indicates the elution position of phenol red.*

radioactive peak was again identified (Fig. 4). The molecular weight of this material was found to be approximately 72K (Fig. 6).

Random-coil chromatography of large T antigen from permissive cells. An eluate of an anti-T immunoprecipitate from SV40-infected CV1-P cells was also incubated in solution A and then chromatographed by the guanidine-Sepharose method in the presence of internal standards. As shown in Fig. 5, a major peak of radioactive material eluted in fractions 130 to 150. The molecular weight of this peak was estimated as 79K (Fig. 7). A smaller peak of material was noted in fractions 170 to 185. Its molecular weight can be estimated as approximately 21K. One possibility is that this second peak corresponds to the small T antigen, the only prominent band of this general size in the SDS-gel electropherogram (Fig. 1F).

Sedimentation-velocity analysis of T antigen. T antigen, partially purified from nuclei of SV80 cells through the heparin-Sepharose step (54), was sedimented through a linear glycerol gradient (Fig. 8). Three internal standard proteins—*Escherichia coli* β-galactosidase (515K), bovine catalase (247K), and *E. coli* alkaline phosphatase (80K)—were included as markers. The antigen migrated slightly behind alkaline phosphatase (80K) in this and other such experiments. Using the method of Martin and Ames (33), we calculated a molecular weight of approximately 73K for this protein. In other experiments, values from 72 to 76K have been obtained.

DISCUSSION

Large T antigen isolated from an SV40-transformed human cell line, SV80, and an SV40-infected permissive monkey cell line, CV1-P, migrate in parallel as single ~94K bands in SDS-polyacrylamide gels. However, by random-coil chromatography in 6 M guanidine-hydrochloride and by sedimentation-velocity analysis, both of these proteins behave like ~75 to 80K molecules. Molecular-weight estimation by SDS-polyacrylamide gel electrophoresis has been shown to be subject to substantial error in selected instances (37, 56, 57). Molecular charge,

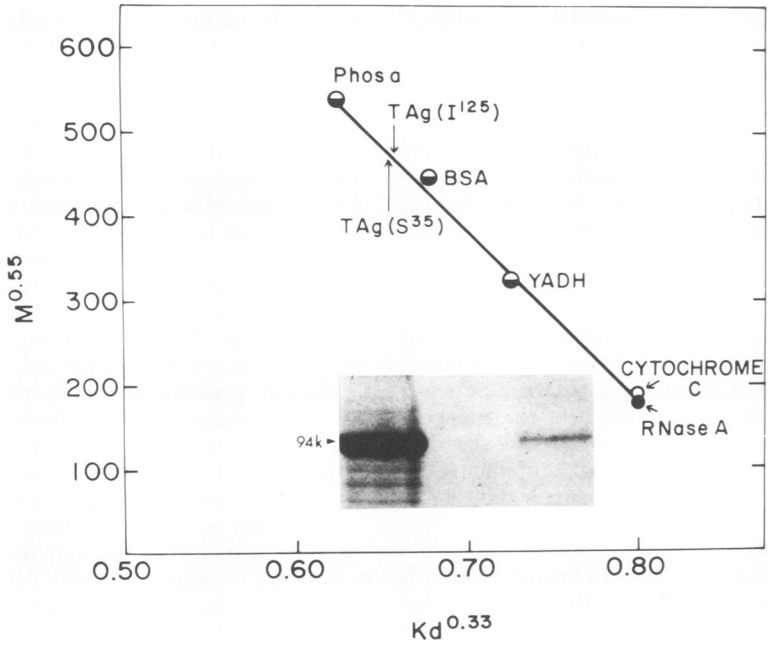

FIG. 6. *Estimation of SV80 large T antigen molecular weight by random-coil chromatography. The relationship of the elution fraction ($K_d^{0.33}$) for various internal standard proteins to their individual molecular weights ($M^{0.55}$) is shown (18, 38). (○) Results of the experiments with $^{35}S/^{3}H$-labeled SV80 large T antigen (Fig. 3). (●) Results of the experiments with ^{125}I-labeled T antigen (Fig. 4). The calculated molecular weights of $^{35}S/^{3}H$-labeled T antigen and ^{125}I-labeled T antigen were 74K and 72K, respectively. The variation in elution position of a known standard in these experiments was approximately 7%. In additional experiments, values as high as 75K have been obtained for the SV80 protein. (Inset) The major $^{35}S/^{3}H$-labeled T protein peak (74K) isolated after guanidine chromatography was freed of guanidine by gel filtration through Sephadex G-25 in 0.05% ammonium bicarbonate, lyophilized three times from water, redissolved in electrophoresis sample buffer, and then analyzed by SDS-polyacrylamide gel electrophoresis (cf., Materials and Methods). Shown is an autoradiograph (72-h exposure) of an SDS-polyacrylamide gel (10%). (Right) Radiolabeled protein from the 74K chromatographic peak; (left) nonchromatographed, immunoprecipitated $^{35}S/^{3}H$-labeled SV80 T antigen.*

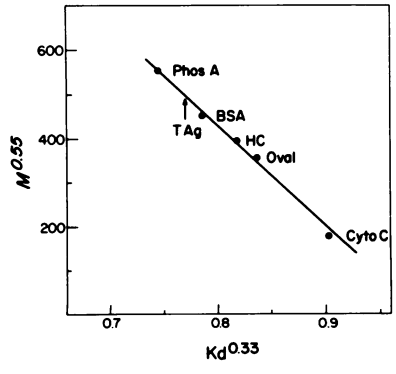

FIG. 7. *Estimation of T antigen molecular weight from SV40-infected CV1-P cells using random-coil chromatography. The relationships of elution position ($K_d^{0.33}$) for various internal standard proteins to their individual molecular weights ($M^{0.55}$) are shown. The calculated molecular weight for the large T antigen (arrow) is 79,300 (±7%). This data is from the experiment shown in Fig. 5.*

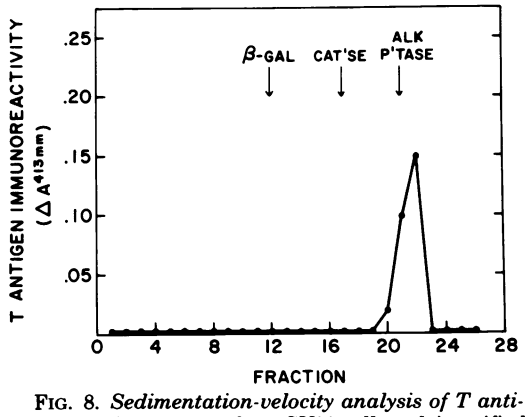

FIG. 8. *Sedimentation-velocity analysis of T antigen. T antigen (25 μg) from SV80 cell nuclei purified through the heparin-Sepharose step (54) was sedimented for 18 h at 4° C through a 12-ml linear glycerol gradient as described in Materials and Methods. Fractions (0.40 to 0.50 ml) were collected and assayed (5 μl each) for T antigen immunoreactivity by microcomplement fixation (54). Internal standard proteins included E. coli β-galactosidase (515K), bovine catalase (247K), and E. coli alkaline phosphatase (80K).*

protein aggregation, the presence of carbohydrate side chains, abnormal detergent-binding effects, and unusual conformational properties of certain proteins have all led to false molecular-weight estimates (frequently elevated) (37, 56, 57). Gel filtration in guanidine-hydrochloride is not accompanied by the high degree of resolution of bands possible with SDS-gel electrophoresis, but, as an analytical method, it is based on sound theory (18, 48). It is also less prone to yield erroneous molecular-size values than SDS-gel electrophoresis. This disparity, the known variation in relative electrophoretic migration of large T antigen in SDS-gels run with different buffer systems (42), and the agreement between the data obtained by two, independent transport methods, taken together, strongly suggest that the real molecular weight of this protein is more likely to be in the 75 to 80K range than 94 to 100K.

75K is nearly the same approximate value obtained by Del Villano and Defendi (13) with SDS-gel electrophoresis. Why they obtained this value by gel electrophoresis is unclear, but it should be noted that their antigen had been eluted from an immunoaffinity column with chaotropic ions and had also been reduced and alkylated before electrophoresis.

Whereas the present results do not permit an unequivocal determination of the magnitude of the T antigen cistron, they do allow one to suggest broad limits of the extent of this region. From the available amino acid composition of the protein, we can estimate an average residue molecular weight of 115 (54). With such a value, taking an estimated maximum size of the T protein as 80K, and assuming that external, covalent modifications do not significantly affect the molecular weight of the protein, we estimate the maximum size of the cistron to be approximately 2,100 nucleotides, which is considerably less than the entire early region of the SV40 genome. This estimate would be compatible with the recent observations indicating that deletion of as much as 9 to 10% of the viral early region have no measurable effects on the size of the large T protein (12). It would also fit with measurements of the size of the large T antigen messenger which appears to be approximately 2,200 nucleotides in length based on the results of the S_1/exonuclease VII method (A. Berk and P. A. Sharp, Proc. Natl. Acad. Sci. U.S.A., in press).

These results do not indicate any significant increase in size of SV80 large T antigen relative to the lytic protein as might be the case if the molecule in such a transformant were a host-viral fusion protein, with a major host component joined to all of the same sequence present in the lytic antigen. In fact, it is impossible from these results to assess whether SV80 large T antigen might contain some host sequences, a determination which must be made on the basis of more incisive protein chemical analyses. The comigration of these two large T antigens in SDS-gels, their presence as single, immunoreactive polypeptides, the apparent absence of immunoreactive large T antigen subspecies of 75- to 88K (51) in both types of immunoprecipitate, and the comigration (with phosphorylase a) of the guanidine-gel-filtered and unchromatographed large T antigens all argue against adventitious proteolysis significantly affecting sizes of the two proteins studied here. Others have previously observed similar electrophoretic comigration of lytic and transformed-cell large T antigens when precautions were taken to avoid adventitious proteolysis during extraction (51).

Of interest is the observation of three anti-T reactive bands (57K, 55K, and 8K) in SV80 cells in addition to large and small T antigen. Multiple "middle-size" anti-T reactive bands have been observed in polyoma virus-infected mouse cells (24; B. Schaffhausen et al., Proc. Natl. Acad. Sci. U.S.A., in press). Whether one or more of these molecules is structurally related to one or both of the already defined antigens and whether their apparent absence in infected CV1-P cells is physiologically significant are presently under investigation.

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