Electrophoretic Differences in the Capsid Proteins of Simian Virus 40 Plaque Mutants

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The structural proteins of three mutants of simian virus 40 (SV40) which differ in plaque size, temperature sensitivity, oncogenicity, host cell restriction, and immunological properties were studied. The polypeptide components of these SV40 strains could not be distinguished by their polyacrylamide gel electrophoretic patterns. When the dissociated virions of two of the mutants were analyzed by the isoelectric focusing technique in a urea gradient, the capsid protein peaks were found to differ significantly in their isoelectric points. The capsid protein of the small-plaque mutant had an isoelectric point of pH 6.51 as compared with pH 6.28 for the large-plaque strain. Isoelectric focusing of the isolated capsid protein revealed three components, a single major subunit and two minor forms. The coat proteins of two of the mutants, small-plaque and minute-plaque strains, were indistinguishable by this technique. The capsid protein peaks obtained by isoelectric focusing were further analyzed by polyacrylamide gel electrophoresis.

Stable mutant strains of simian virus 40 (SV40) differing in plaque size, oncogenicity, and temperature sensitivity have been described (12). The variants were originally isolated from a wild-virus stock on the basis of the plaque size produced in primary cultures of African green monkey kidney cells (AGMK) and have been designated as LP (large-plaque), SP (small-plaque), and MP (minute-plaque) strains. Significant differences in host range (10) and immunological properties have also been shown among these mutant strains (11).

Previous studies (2, 4, 5) have demonstrated that the structural proteins of the SP mutant of SV40 are composed of 4 to 6 polypeptides as determined by polyacrylamide gel electrophoresis. The major component, which comprises over 75% of the total virion, was shown to be capsid protein of an estimated molecular weight of 45,000. The antigenic and biological differences among the SV40 mutants suggested that they may be a manifestation of capsid protein heterogeneity, and comparative studies were undertaken. When the proteins of the SP and LP mutants were analyzed by electrophoresis on sodium dodecyl sulfate (SDS)-polyacrylamide gels, they could not be distinguished by their polypeptide patterns. Both variants shared the same number of polypeptides of

identical electrophoretic mobility and molecular weight. By employing the high resolving power of isoelectric focusing techniques, it has been possible to distinguish the capsid proteins of two of the SV40 plaque strains.

MATERIALS AND METHODS

Virus strains and cell cultures. The SV40 strains were obtained through the courtesy of K. K. Take-moto. The LP strain was grown in primary AGMK cell cultures whereas the SP and MP strains were cultivated in either primary AGMK cells or Vero cells, a continuous AGMK cell line. The purity of the stock virus strains was monitored frequently by plaque morphology on primary AGMK cell cultures. The methods for the propagation of virus, isotopic labeling, purification of virions, preparation of subviral components, and SDS-polyacrylamide gel electrophoresis have been described previously (2).

Isoelectric focusing of viral proteins. Prior to electrofocusing, viral proteins were dialyzed against 1% glycine and incubated at 37°C for 1 h in the presence of a nonionic detergent, Brij 35 (Pierce Chemical Co.), 2-mercaptoethanol, and urea at concentrations of 0.1%, 1.0%, and 4 M, respectively. Isoelectric focusing was performed in a 4 to 8 M urea gradient containing 1% ampholine (either pH 3 to 10 or pH 5 to 8 range) in a model 8101 column (LKB Instruments, Inc.) of 110-ml capacity. The dissociated viral samples were introduced in the middle of the column. Electrophoresis was carried out at 5°C with a

final voltage of 600 to 700 V for approximately 72 h. For the narrow pH range, pH 5 to 8, the anode solution, 1% sulfuric acid, was placed at the top of the column. The cathode solution containing ethanolamine in sucrose solution was placed at the bottom of the column. At the end of the electrophoretic analysis, the contents of the column were removed in 1.2-ml fractions at a constant flow rate maintained by pumping water from the top of the column with a peristaltic pump. The pH was assayed at 5 C in a radiometer pH meter with a combination electrode. Samples of fractions were also assayed for radioactivity.

RESULTS

SDS-polyacrylamide gel electrophoresis of SP and LP viral proteins. When purified SV40 SP virions labeled with ^{14}C -amino acids were mixed with ^3H -labeled LP virions, dissociated with 1% SDS, reduced with 1% mercaptoethanol, and analyzed by gel electrophoresis, the electrophoretic profiles were similar (Fig. 1). The major peak (I) was shown previously to be one of the capsid protein components having an estimated molecular weight of 45,000 (2). Peak II has also been found to be associated with the viral coat protein. The minor electrophoretic peaks IV, V, and VI were found to be associated with the DNA core of the virus.

Isoelectric separation of SP and LP virions. When purified virion preparations of SP and LP mutants labeled with different isotopes in their amino acids were combined, treated with nonionic detergent Brij 35, and

subjected to isoelectric focusing in the narrow pH 5 to 8 range, there was a clear difference in the isoelectric points of the major peaks (Fig. 2). The isoelectric point of the SP peak was 6.50, whereas the isoelectric point of the LP variant was 6.41. These results were reproducible with different preparations of virions and labeling with different isotopes.

Although the LP strain was routinely propagated on primary AGMK cell cultures and the SP strain on Vero cells, the results were similar with virus strains grown in primary AGMK cell cultures.

Isoelectric focusing of SP and MP virions. In a similar double-label experiment as described above, SP and MP viral proteins were mixed, dissociated, and analyzed by the isoelectric focusing technique (Fig. 3). As shown by the results, the profiles were indistinguishable.

SDS gel electrophoresis of the major isoelectric peak. To characterize further the major isoelectric peak, a sample of the pooled peak fractions was dissociated with 1% SDS and 0.1% mercaptoethanol, and was coelectrophoresed with SDS-dissociated SV40 virions in a 10% SDS polyacrylamide gel. The protein in this peak coelectrophoresed with the major capsid peak as previously described (2). The molecular weight was estimated to be 45,000 as determined by gel electrophoresis with standard protein markers.

Isoelectric focusing of the capsid proteins of SV40 SP and LP mutants. To confirm that

FIG. 1. Sodium dodecyl sulfate gel electrophoretic profiles of small-plaque and large-plaque polypeptides labeled with ^3H - and ^{14}C -amino acids. Electrophoresis was for 15 h at 3 mA per tube. Length of gel was 10 by 0.6 cm. Migration was from the origin at the left (cathode).

FIG. 2. Isoelectric focusing of the proteins of SV40 small-plaque mutant labeled with ^{14}C -amino acids and ^3H -amino acid-labeled SV40 large-plaque strain. The labeled virion proteins were mixed and treated as described in Materials and Methods, and isoelectric focusing was applied in a pH 5 to 8 ampholyte range at 5 C for 71 h at a final voltage of 700 V. Fractions (1.2 ml) were collected and pH and radioactivity in samples of each fraction were determined.

the capsid proteins of the SV40 mutants can be distinguished by their isoelectric points, similar analyses were performed with the isolated capsid proteins. Differentially labeled SV40 SP and LP mutant virions were mixed and degraded by dialysis against a pH 10.5 buffer as described by Anderer et al. (1). The capsid proteins were separated from the core protein-DNA complex by sedimentation in a 5 to 20% sucrose density gradient. The isolated capsid proteins then were subjected to isoelectric focusing after dissociation and reduction. As shown in Fig. 4, the isolated capsid proteins were heterogeneous, with a major peak and two minor peaks. The isoelectric point of the major SP capsid protein was 6.51, whereas the isoelectric point of the major LP peak was 6.28. To further characterize the three isoelectric peaks, samples of the isolated peaks were subjected to analytical SDS gel electrophoresis. The minor peaks each yielded single, sharp bands with estimated molecular weights of 45,000. The major isoelec-

tric peak of apparent 45,000 mol wt yielded a broad, single band with a suggestion of microheterogeneity (Fig. 5). To determine the homogeneity of the major peak from the isoelectric fractionation, the peak fractions were pooled and immediately refocused in 1% ampholine (isoelectric point range of 5 to 8). A single peak was observed with an isoelectric point of 6.50 for the SP mutant. The two minor peaks were not refocused because of limited level of radioactivity.

Isoelectric focusing of the capsid proteins of MP and LP. The isolated, capsid proteins and MP and LP strains were subjected to isoelectric focusing (Fig. 6). The isoelectric points of the major peaks of the MP and LP capsid proteins were 6.50 and 6.30, respectively, strikingly similar to the results obtained with the SP and LP capsid results of Fig. 4.

Isoelectric focusing of the capsid proteins of MP and SP strains. To answer the question whether SP could be distinguished from MP

FIG. 3. Isoelectric focusing profiles of small-plaque and minute-plaque viral proteins. The experimental conditions are similar to those given in Fig. 2.

FIG. 4. Isoelectric focusing of the capsid proteins of the small-plaque and large-plaque mutants. Capsid proteins were isolated by the method of Anderer et al. (8) in a 5 to 20% sucrose density gradient after dialysis against a pH 10.5 buffer. The conditions for electrofocusing are the same as for Fig. 2.

capsids by a subtle difference in any of the three isoelectric-focused peaks, the isolated capsids of both strains were analyzed by this technique. No differences in their isoelectric profiles could be observed (Fig. 7). These results also support the finding that the SP and MP virions were indistinguishable by their isoelectric focusing patterns (Fig. 3).

A summary of the isoelectric points of the capsid proteins of the SV40 plaque strains is presented in Table 1. The data presented are averages of at least two to three analyses, and the isoelectric points were found to be highly reproducible with different viral protein samples.

DISCUSSION

The mutants of SV40 in this study have strikingly different biological properties. It has been shown by Takemoto et al. (12) that the LP strain is temperature sensitive and unable to produce infectious virus when cells infected with the LP mutant are incubated at temperatures above 39 C. The SP and MP mutants, in contrast, were capable of multiplying at 41 C to titers almost equivalent to those obtained at 37 C. Furthermore, Takemoto and Martin (13) have shown that LP virions produced at 37 C show marked loss of infectivity after incubation at 50 C, whereas SP virions were completely stable and infectious under these conditions. The results were interpreted as structural protein defects in the LP mutant. The LP strain has also been reported to be inefficient at transforming mouse or human cells, requiring 50 times more infectious particles per transformation event than the MP mutants (14). Previous studies also have demonstrated that the LP strain is restricted in established cell lines, whereas the SP and MP strains grow well in many continuous cell lines (10). In addition, antigenic differences between the LP and SP strains, suggestive of capsid protein heterogeneity, have also been reported (11).

The results obtained in the present study show that it was possible to distinguish the capsid proteins of the LP strain from SP and MP strains by the high resolution obtained with the electrofocusing technique. Attempts thus far to distinguish between the SP and MP variants have been unsuccessful. One possible explanation for this difficulty is the instability of the SP mutant. Despite repeated plaque purification procedures, stock pools of this virus always contained a small proportion of progeny which produced MP. Alternatively, the SP and MP mutants may differ in early gene functions not demonstrable by coat protein heterogeneity.

FIG. 5. Polyacrylamide gel electrophoresis of the protein peaks after fractionation of dissociated large-plaque (LP) capsids by isoelectric focusing. A, Samples were taken from the minor peaks in an experiment similar to that of Fig. 5, except that only LP capsid protein was electrofocussed. The protein was dissociated with 1% sodium dodecyl sulfate (SDS) and analyzed by electrophoresis in SDS gels. B, Sample of the major isoelectrically focused capsid peak.

The significant differences in isoelectric points of the capsid proteins of the SP and LP SV40 mutants are probably due to an intrinsic charge difference caused by one or more amino acid substitutions. It is conceivable that differences in isoelectric point could be attributed to greater amidation of the SP coat protein and an isoelectric point of 6.51 as compared with a lower isoelectric point of 6.28 for the LP capsid. This variance in coat protein structure may account in part for the different host range, temperature sensitivity, and immunological properties of these strains. The capsid protein heterogeneity, significant enough to cause detectable differences in their isoelectric points, implies also genome differences which are presumably too subtle to be detected by the customary viral nucleic acid hybridization technique (13).

When intact virions were treated with non-ionic detergent Brij 35, only a single capsid peak was observed (Fig. 2). However, when the isolated coat protein was isoelectrically focused, after removal from the whole virion by alkaline dialysis and separation from the nucleoprotein complex by zone sedimentation, two minor components, in addition to the major protein subunit, were found with different isoelectric points. This heterogeneity is in accord with our earlier isoelectric and gel electrophoretic studies (2). The major isoelectric component (II) contained approximately 70% of the total radioactivity, whereas the two minor components, I and III, accounted for approximately 15% each of the total activity. When the major component was refocused, it yielded a single species

FIG. 6. Isoelectric focusing of the capsid proteins of the minute-plaque and large-plaque strains. The experimental details are similar to those given in Fig. 4.

FIG. 7. Isoelectric focusing profiles of small-plaque and minute-plaque capsid proteins. The experimental conditions are similar to those given in Fig. 4.

TABLE 1. *Summary of isoelectric points of the capsid protein of the SV40 mutants*

Virus strain	Isoelectric points of the capsid protein peaks		
	I	II	III
Small plaque	6.78 ($\pm$ 0.05)	6.51 ($\pm$ 0.02)	6.24 ($\pm$ 0.04)
Large plaque	6.57 ($\pm$ 0.03)	6.28 ($\pm$ 0.02)	6.10 ($\pm$ 0.02)
Minute plaque	6.78 ($\pm$ 0.03)	6.51 ($\pm$ 0.02)	6.23 ($\pm$ 0.03)

and had a similar isoelectric point, as found originally, with an estimated molecular weight of 45,000. The apparent molecular weight of 45,000 of the minor components as determined by gel electrophoresis is puzzling. In view of the limited coding capacity of the SV40 viral DNA, assuming a molecular weight of 3×10^6 , which allows for approximately 150,000 daltons of protein, the minor capsid components may represent either cleavage polypeptides or artifacts of the electrofocusing technique. Recently, multiple electrophoretic forms of yeast isocitrate dehydrogenase were found to be an artifact of the electrofocusing technique (6). It will be of interest to characterize the two minor components to determine their relationship to the major capsid component.

The finding of mutant strains of SV40 differing in physicochemical as well as biological properties is not without precedent. Murakami et al. (9) found in studies with three different strains of polyoma virus that two LP strains had similar antigenic properties and amino acid compositions, but differed significantly from a SP strain in these properties. Differences in electrophoretic mobility between LP and SP polyoma virions has also been reported (15). Electrophoretic mobility differences have also been found between strains of tobacco mosaic virus (7), pox virus variants (3), and variants of Southern bean mosaic virus (8).

Current studies are directed towards a comparative examination of the amino acid composition and peptide maps of the coat proteins of these SV40 mutants to further elucidate the structural differences.

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LITERATURE CITED

1. Anderer, F. A., M. A. Koch, and H. D. Schlumberger. 1968. Structure of simian virus 40. III. Alkaline degradation of the virus particle. *Virology* **34**:452-458.
2. Barban, S., and R. S. Goor. 1971. Structural proteins of simian virus 40. *J. Virol.* **17**:198-203.
3. Douglas, H. W., B. L. Williams, and C. J. M. Rondle. 1969. Micro-electrophoresis of pox viruses in molar sucrose. *J. Gen. Virol.* **5**:391-396.
4. Estes, M., E. S. Huang, and J. S. Pagano. 1971. Structural polypeptides of simian virus 40. *J. Virol.* **7**:635-641.
5. Girard, M., L. Marty, and F. Suarez. 1970. Capsid proteins of simian virus 40. *Biochem. Biophys. Res. Commun.* **40**:97-102.
6. Illingworth, J. A. 1972. Anomalous behavior of yeast iso-citrate dehydrogenase during isoelectric focusing. *Biochem. J.* **129**:1125-1130.
7. Knight, C. A., and M. A. Lauffer. 1942. A comparison of the alkaline cleavage products of two strains of tobacco mosaic virus. *J. Biol. Chem.* **144**:411-417.
8. Magdoff-Fairchild, B. S. 1967. Electrophoretic and buoyant density variants of Southern bean mosaic virus. *Virology* **31**:142-153.
9. Murakami, W. T., R. Fine, M. R. Harrington, and Z. Ben Sassan. 1968. Properties and amino acid composition of polyoma virus purified by zonal ultracentrifugation. *J. Mol. Biol.* **36**:153-166.
10. Ozer, H. L., and K. K. Takemoto. 1969. Site of host restriction of simian virus 40 mutants in an established African green monkey kidney cell line. *J. Virol.* **4**:408-415.
11. Ozer, H. L., K. K. Takemoto, R. L. Kirschstein, and D. Axelrod. 1969. Immunochemical characterization of plaque mutants of simian virus 40. *J. Virol.* **3**:17-24.
12. Takemoto, K. K., R. L. Kirschstein, and K. Habel. 1966. Mutants of simian virus 40 differing in plaque size, oncogenicity, and heat sensitivity. *J. Bacteriol.* **92**:990-994.
13. Takemoto, K. K., and M. A. Martin. 1970. SV40 thermosensitive mutant: synthesis of viral DNA and virus-induced proteins at nonpermissive temperature. *Virology* **42**:938-945.
14. Todaro, G. J., and K. K. Takemoto. 1969. Rescued SV40: increased transforming efficiency in mouse and human cells. *Proc. Nat. Acad. Sci. U.S.A.* **62**:1031-1037.
15. Thorne, H. V., W. House, and A. L. Kisch. 1965. Electrophoretic properties and purification of large and small plaque-forming strains of polyoma virus. *Virology* **27**:37-43.