CAPSID PROTEINS OF SIMIAN VIRUS 40

Marc Girard, Louise Marty and Françoise Suarez

Unité de Physiologie des Virus, Institut de Recherches Scientifiques sur le Cancer, B.P. 8, 94 - Villejuif (France)

Received May 25, 1970

Summary

Purified SV40 virions were disrupted by sodium dodecyl sulfate (SDS), β-mercaptoethanol and heat, and the proteins analyzed in polyacrylamide gels in the presence of SDS. Six different polypeptide chains were found. Agregation of proteins was ruled out by treatment with 8 M urea. The major protein had a molecular weight (M.W.) of approximately 45,000, two other proteins M.W.'s of 35,000 and 25,000 respectively. In addition, three minor components were found with M.W.'s of approximately 18,000, 14,000 and 10,000.

Introduction

Simian Virus 40 (SV40) is a small oncogenic virus, containing a DNA of approximately 2.5×10^6 daltons M.W. (1, 3), which can carry only a relatively limited amount of information.

As a prerequisite to the study of the proteins which are synthesized in SV40 infected cells, it was considered desirable to investigate the number of capsid proteins in the virion and their M.W.'s. As will be shown below, polyacrylamide gel electrophoresis at neutral pH, in the presence of SDS, revealed the existence of six polypeptide chains in the mature virion.

Methods

CV_I cells grown in 2 liter roller flasks were infected with the large plaque strain of SV40 at an input multiplicity of 0.1 pfu/cell. Virus and cells were a gift from P. Tournier. From the 5th to the 7th day after their infection, the cells were labeled with a mixture of ^{14}C labeled amino-acids (Commissariat à l'Energie Atomique, Saclay - France). Labeled virus was first

purified by centrifugation through 5 to 20 % sucrose gradients in NEB (0.01 M Tris-HCl pH 7.5, 0.01 M NaCl, 0.02 M EDTA) for 90 min. at 25,000 rev/min, 4°, in the SW 25.1 rotor of the Spinco. Fractions from the virus peak were layered over 15 ml of saturated KBr in 0.01 M Tris-HCl pH 7.5, 0.01 M EDTA (saturation was done at 4°), and centrifuged for 4.5 hours at 25,000 rev/min., 22°, in the SW 25.1 rotor. Purified virus was precipitated with 5 % trichloracetic acid in the presence of 0.6 mg/ml bovine serum albumine, washed twice with 5 % trichloracetic acid in the cold, twice with acetone, then resuspended in 0.01 M sodium phosphate buffer, pH 7.4. Virions were further disrupted by addition of 1 % β-mercaptoethanol and 1/3 volume of 10 % SDS. In one experiment, SV40 was concentrated by two precipitations with 5 % polyethylene glycol 6000 in the cold (2, 12), then purified as above. Specific radioactivity of the purified virus was usually 10^5 cpm/$O.D._{260}$.

The Mahoney strain of poliovirus was grown in HeLa cells in the presence of a mixture of tritium labeled amino acids (New England Nuclear, Boston, Mass.). Virions were purified by sucrose gradient centrifugation in the presence of 0.5 % SDS as previously described (6). Capsid proteins were then prepared as described above.

Immediately prior to electrophoresis, 1 % β-mercaptoethanol was added again to the samples of proteins to be analyzed. These were then heated at 100° for 1-2 min. They were layered over 8 cm long, 7.5 % polyacrylamide gels made according to Maizel (8). After 2.8 hours electrophoresis at 90 volts, the gels were frozen, and 1 mm thick slices were cut and counted in NH_4OH and Bray's solution as previously described (5).

Results and Discussion

Six polypeptide chains were repeatedly detected in purified SV40 virions (Fig. 1). The three major components were labeled A, B and C in order of decreasing M.W., the minor components D, E and F. In addition, material heavier than component A

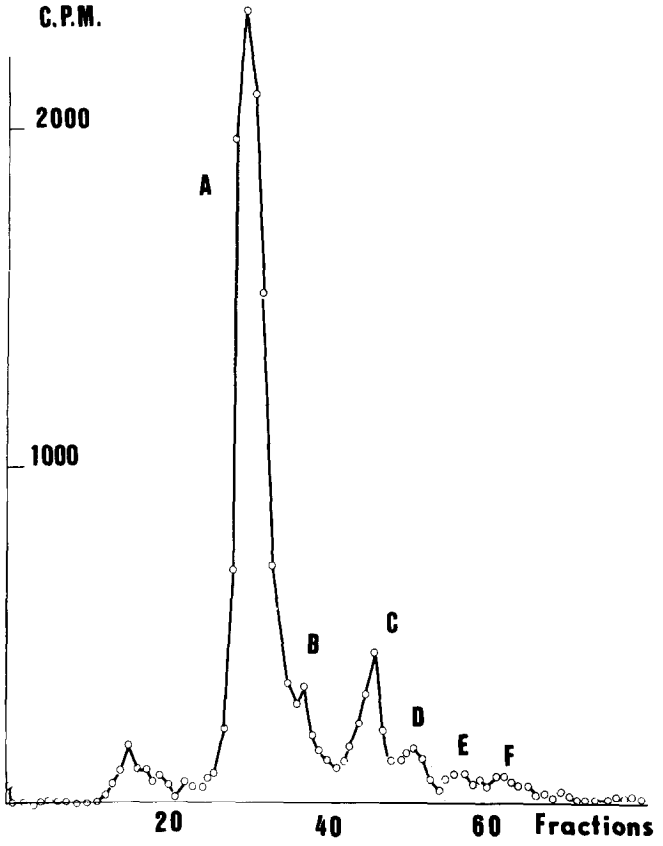

Fig. 1 : Gel electrophoresis pattern of SV40 capsid proteins. SV40 capsid proteins labeled with ^{14}C amino-acids were prepared as described under Methods and analyzed by electrophoresis in a 7.5 % polyacrylamide gel in 0.1 M sodium phosphate buffer, pH 7.4, 0.1 % SDS. Migration is from left to right.

was also observed (first peak to the left, Fig. 1) but its amount varied from experiment to experiment. Since, in addition, no such material was found in the presence of 8 M urea (see below), it very likely represented undissociated agregates. In the experiment shown in Fig. 1, peak B ran as a shoulder to the light side of peak A. It was however quite distinct from peak A in most experiments (see Fig. 2).

To exclude the possibility that some of the proteins observed were not actual part of SV40 virions, treatment of the virus with trypsin and sonication for various lengths of time were performed, as well as repeated cycles of purification through sucrose gradient

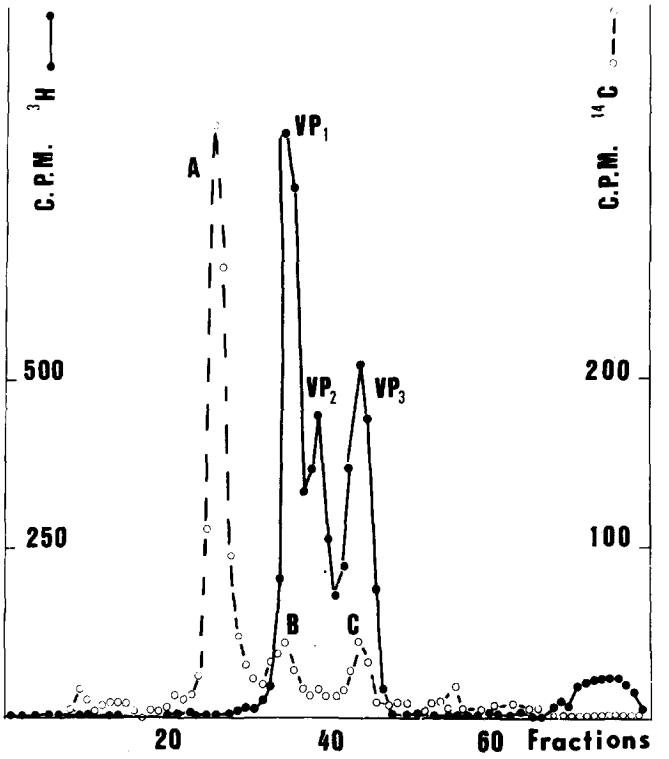

Fig. 2 : Determination of the molecular weights of SV40 major capsid proteins. SV40 capsid proteins labeled with ^{14}C amino-acids were mixed with tritium labeled poliovirus capsid proteins and analyzed by gel electrophoresis as in Fig. 1.

centrifugation and band sedimentation in saturated KBr. Also, samples of SV40 virions were further purified through isopycnic centrifugation in CsCl. None of these treatments had any effect on the gel electrophoresis pattern.

To rule out eventual agregation of proteins, a sample of SV40 capsid proteins in SDS and β-mercaptoethanol was heated at 100°, then made 8 M with respect to urea. Electrophoresis of this sample was performed in a 5 % polyacrylamide-8 M urea gel. Electrophoresis buffer also contained 8 M urea. The same six capsid proteins as in Fig. 1 were observed again, but the heavy agregate had disappeared.

The M.W.'s of the SV40 proteins were determined by comparing their electrophoretic mobility to that of poliovirus capsid

proteins, since it is known that in the SDS-neutral pH polyacrylamide gels, electrophoretic mobility is inversely related to log of M.W. (4,11), and since the M.W.'s of the three major poliovirus proteins have been accurately determined. Samples of ^{14}C labeled SV40 capsid proteins were co-electrophorized with tritium labeled poliovirus proteins (Fig. 2). The poliovirus proteins VP_1, VP_2 and VP_3 having M.W.'s of 35,000, 28,000 and 23,000 respectively (9), the M.W.'s of SV40 capsid components A, B and C were determined as 45-50,000, 32-35,000 and 23-25,000 respectively. There were too few counts in these experiments to allow for correct determination of the M.W.'s of the three minor SV40 capsid components D, E and F. However, on the basis of patterns obtained in other experiments (see Fig. 1), and of the M.W.'s determined above for components A, B and C, the M.W.'s of components D, E and F were evaluated to 18,000, 14,000 and 10,000 respectively.

In six repeated experiments, average radioactivity of protein A amounted to 72.5 % of the total radioactivity recovered in the gels. Approximately 11.5 % of the counts were recovered in each proteins B and C, and 3 %, 1.5 % and 1 % in proteins D, E and F respectively. Work is in progress to determine wether these three minor components are part of the capsid or internal proteins associated with the virus DNA. It is clear already, however, that the major sub-unit of the SV40 capsid is constituted of polypeptide A, the M.W. of which is approximately 45,000. A major protein of 45,000 M.W. has also been found for the capsid of polyoma virus (7). Anderer et al (1) and Schlumberger, Anderer and Koch (10), on another hand, described three proteins in SV40 virions, the M.W.'s of which were estimated at 16,900, 16,800 and 16,400 respectively. The reasons for the discrepancy between their results and ours are unknown. There is good agreement between our results and the pattern shown by Maizel for SV40 (see Fig. 2 in reference 8).

Acknowledgments

This investigation was supported in part by the Commissariat à l'Energie Atomique and in part by the Délégation Générale à la Recherche Scientifique et Technique.

References

1. Anderer, F.A., M.D. Schlumberger, M.A. Koch, H.H. Frank and H.J. Eggers, Virology 32, 511 (1967).
2. Calothy, G., M. Digeon and M. Raynaud, C.R.Acad.Sc., Paris 265, 1764 (1967).
3. Crawford, L.V. and P.H. Black, Virology 24, 388 (1964).
4. Dunker, A.K. and R.R. Rueckert, J. Biol. Chem. 244, 5074 (1969).
5. Girard, M. and L. Marty, Bull. Soc. Chim. Biol. 51, 31 (1969).
6. Granboulan, N. and M. Girard, J. Virol. 4, 475 (1969).
7. Kass, J. Virology 5, 381 (1970).
8. Maizel, J.V., In : Fundamental Techniques in Virology, K. Habel and NP. Salzman ed, Acad. Press (1969).
9. Maizel, J.V. and D.F. Summers, Virology 36, 48 (1968).
10. Schlumberger, H.D., F.A. Anderer and M.A. Koch, Virology 36, 42 (1968).
11. Shapiro, A.L., E. Vinuela and J.V. Maizel, Biochem. Biophys. Res. Commun. 28, 815 (1967).
12. Yamamoto, K.R., B.M. Alberts, R. Benziger, L. Lawthorne and G. Treiber, Virology 40, 734 (1970).