RESOLUTIONS AND IDENTIFICATION OF THE CORE DEOXYNUCLEOPROTEINS OF THE SIMIAN VIRUS 40

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SUMMARY: Low molecular weight basic core proteins of SV_{40} are resolved by Tris-Acetate-SDS polyacrylamide gel electrophoresis into a minimum of four polypeptides. These are the electrophoretic counterparts of the evolutionarily conserved histones F3, F2b, F2a2, and F2a1. Host African green monkey kidney cells contain an active protease activity which readily cleaves histone F3 during nuclear isolation in hypotonic buffers.

INTRODUCTION

Virion structural proteins of the papovavirus SV_{40} have been characterized as consisting of six major polypeptides (1, 2, 3, 4). In the present nomenclature these have the following designation and molecular weight range: VP1 (41-49,000); VP2 (30-34,000); VP3 (22-24,000) and VP4,5,6 (11-14,000). A collection of unresolved polypeptides in the VP4, 5, 6 size range have been shown to reside in subviral deoxynucleoprotein cores prepared by alkaline degradation of virions and to comprise about 10% of the protein mass (3). Current interest centers on these "core polypeptides" because they are suggested to be largely pre-formed and host-derived histones (5).

A complete catalogue of the component core polypeptides is required for an ultimate understanding of their function. We report here that the minimum number of core polypeptides in SV_{40} is four and that these correspond to the four evolutionarily conserved histones--F3, F2b, F2a2, and F2a1.

Abbreviations used are: (Tris), tris-(hydroxymethyl)-aminomethane; (SDS), sodium dodecyl sulfate.

Resolution of these low molecular weight polypeptides is achieved by electrophoresis in a modified Tris-Acetate-SDS polyacrylamide gel system.

METHODS

Vero (a continuous line of African green monkey kidney) cells were grown in Tricine buffered Eagles Medium and 10% fetal calf serum. Nuclei and unwashed nucleoproteins were prepared at 4°C from cells scraped from roller bottles by rolling 2 mm glass beads in cold phosphate buffered saline. Decanted cells were pelleted and resuspended in Reticulocyte Standard Buffer for 5 min; the suspension was then brought to 0.1% with Triton X-100 and homogenized in a glass Dounce homogenizer. Nuclei pelleted at 2,000 X g were further washed in reticulocyte buffer and 0.15 M NaCl-1mM EDTA, pH 7.6. Acid-soluble proteins, which included all histones, were extracted from cell fractions at 0.25 M HCl; precipitated at 25% trichloroacetic acid; washed in acidified (HCl)-acetone, acetone and air dried. Histones were further fractionated by Method 1 of Johns (6) with the inclusion of recycling steps employed by Oliver et al. (7).

Subconfluent Vero cells were infected by absorption of small plaque SV_{40} for 1 hr at 50 plaque-forming units per cell. After 5 to 7 days of infection in medium containing 5% serum, the entire culture was pelleted in a Spinco Type 42 rotor (2 hr, 35,000 rpm). The virus and cell pellet was resuspended in buffered saline supplemented to 1% with sodium deoxycholate and homogenized vigorously. After initial clarification at 15,000 X g, virus was cushioned onto 10 ml of 1.38 g/cc CsCl in a SW27 rotor (3 hr, 25,000 rpm). The density of the isolated virus band was adjusted to 1.34 g/cc with CsCl and centrifuged to equilibrium in a 50.1 rotor (16-18 hr, 40,000 rpm). The virus band at 1.340 g/cc was isolated and dialyzed vs. buffered saline. Subviral cores were prepared by anisotonic Tris-ethanolamine (pH 10.5) degradation method as described by Huang et al. (4).

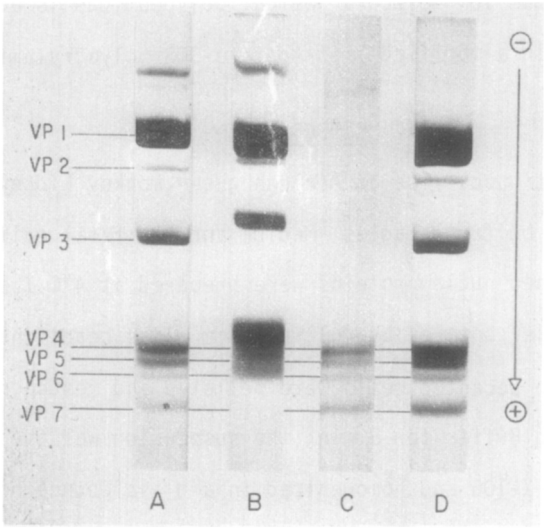

Figure 1. Coomassie blue stained 13% polyacrylamide gels on which various SV_{40} or subviral fractions have been electrophoresed. (A) Polyethylene glycol precipitated SV_{40} run on a Tris-Acetate-SDS gel; (B) same SV_{40} preparation as in (A) run on 0.1 M phosphate, 0.1% SDS gel (12); (C) proteins of SV_{40} cores prepared by alkaline degradation and isolated on a sucrose gradient; (D) SV_{40} virions purified as described in Methods and run on Tris-Acetate-SDS gel.

Virus or cell protein preparations (50 to 150 μg) were precipitated with 25% trichloroacetic acid, washed in acid-acetone (0.5% HCl v/v) and washed twice in acetone. The air-dried preparation was solubilized for electrophoresis by resuspension in 0.04 M Tris-0.02 M acetate, 2mM EDTA, 1% sodium dodecyl sulfate (SDS), 8 M urea, and 2% (v/v) 2-mercaptoethanol. This buffer is the 1X gel buffer of Fairbanks et al. (8) supplemented with urea and 2-mercaptoethanol. The mixture was heated a minimum of 2 hr at 40-50°C and then heated to 100°C for 2-3 minutes.

Electrophoresis was on 10 cm columns of 13% polyacrylamide gels prepared by a modification of the Tris-Acetate-1% SDS system of Fairbanks et al. (8). The modification was to raise the acrylamide concentration to 13% and the ratio of acrylamide:bis-acrylamide to 40:1. Gels were run at 6-10 v/cm until pyronin Y marker dye moved the desired distance. Gels were stained in 0.2% Coomassie blue by the procedure

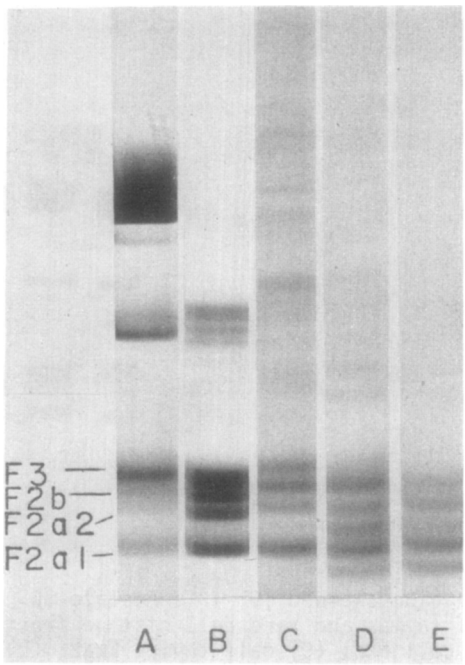

Figure 2. Coomassie blue stained 13% Tris-Acetate-SDS polyacrylamide gels of : (A) whole SV_{40} virion; (B) 50 µg of calf thymus histone (Sigma Chemical Co.); (C) 0.25 M HCl-soluble proteins of whole Vero monkey cells; (D) Vero 0.25 M HCl-soluble proteins from a crude 2,000 X g nuclear fraction after hypotonic swelling in reticulocyte buffer and homogenization; (E) Vero 0.25 M HCl-soluble proteins of a 0.15 M NaCl-1 mM EDTA washed nuclear fraction.

of Weber and Osborn (9) and were photographed using the spherical lens procedure of Oliver and Chalkley (10).

RESULTS AND DISCUSSION

The electropherograms A and B of Figure 1 show the protein patterns obtained from purified SV_{40} prepared using polyethylene glycol precipitation (11) and analyzed in two SDS polyacrylamide gel systems. On a Tris-Acetate-SDS gel (A), four core proteins designated VP 4, 5, 6, and 7 are resolved. In the conventional phosphate-SDS gel system (12), as used in earlier studies of SV_{40} proteins (1, 2, 3, 4), the relative mobility of viral proteins is different and the core proteins are not resolved.

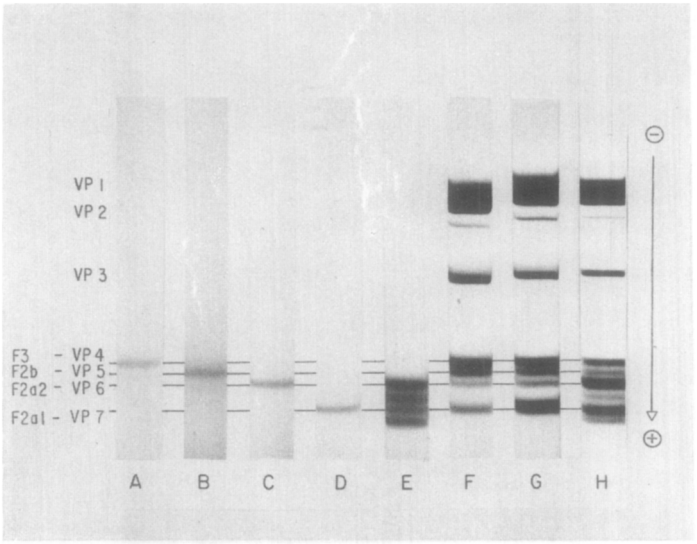

Figure 3. Coomassie blue stained 13% Tris-Acetate-SDS polyacrylamide gels of various calf thymus and Vero cell histone fractions. (A) calf thymus F3; (B) Vero cell F2b; (C) calf thymus F2a2; (D) calf thymus F2a1; (E) Vero cell histone fraction F2a contaminated with cleavage products of Vero F3; (F) SV_{40} complete; (G) SV_{40} plus calf thymus F2a1; (H) SV_{40} plus Vero cell fraction F2a as in (E).

As an indication of whether indeed these 4 polypeptides observed on Tris-Acetate-SDS gels reside in the core, the complement of polypeptides in cores obtained by isotonic alkaline degradation of SV_{40} (4) was determined. It is seen from Figure 1C that all four polypeptides are recovered from cores. Furthermore, the relative abundance of each of these 4 polypeptides (on the basis of dye binding) is the same in cores and whole virions purified by several alternative techniques (3). Comparing gels A and D of Figure 1, it is noted that a protein of molecular weight greater than VP1 is not detected when virions are purified as described in Methods.

We wished to compare these core proteins which are resolved on SDS gels with host cell (Vero) histones. All initial attempts at isolating Vero histones were complicated, however, by the presence in Vero cells of an apparent chromatin-associated protease activity which completely cleaves the host cell equivalent of VP4. It is found that the true in vivo

complement of Vero histones can be demonstrated only by 0.25 N HCl extraction of whole Vero cells; this is illustrated in Figure 2. When Vero cells are hypotonically treated in reticulocyte buffer for nuclear isolation, the histones recovered by acid-extraction (Figure 2D) lack a band which is present in acid-extracted whole Vero cells (Figure 2C). It is surmised that the two new bands on either side of F2a1 are cleavage products of what is shown below to be histone F3. This situation is unique to Vero cells insomuch as proteolysis has not been observed in Chinese hamster, Hela, or mouse cell lines similarly treated during chromatin isolation. Inclusion of the protease inhibitor sodium bisulfite (13) at 0.05 M does not prevent this loss of F3 and generation of extra bands.

As a consequence of this peculiarity of Vero chromatin, when histones are extracted and fractionated by the method of Johns (6), no F3 is recovered, and the cleavage products are contaminants of the F2a fraction. Histone F1 is of such low relative abundance in Vero cells, that it would not be expected to be seen in virions. Hence, to identify the SV_{40} core proteins, it was necessary to compare individual histone fractions of calf thymus histone (Sigma Chemical Co.) purified by recycling according to the methods of Oliver et al. (7).

Although direct comparison with all individual Vero histone fractions would be preferred, a comparison of core proteins with calf thymus histones is valid for identification purposes since, except for histone F1, mammalian histones are evolutionarily conserved (14). Also validating the identification is the fact that, in this gel system, bands in virions are the same as those in calf thymus and whole Vero cells as shown in Figure 2-A, B, C. Assignment of names to these electrophoretic bands is based on the comparisons of electrophoretic mobility illustrated in Figure 3. VP4, 5, 6, and 7 correspond to chemically fractionated histones F3, F2b, F2a2, and F2a1, respectively, as illustrated in

Figure 3 A-E. It is assumed that the same order of electrophoretic mobility holds for Vero histones such that the same designation can be given to those bands shown in Figure 2. To alleviate some of the uncertainties of band mobility differences inherent in parallel gels, some individual histone fractions have been mixed and coelectrophoresed with SV_{40} (Figure 3 G-H). Reinforcement of band intensity in these composite gels occurs in a fashion consistent with the band assignment which has been made. Unequivocal band identification will ultimately require comparative peptide mapping of each polypeptide.

From these analyses in the Tris-Acetate-SDS gel system, it is apparent that the minimum number of core proteins in SV_{40} should be revised upward to 4. It is also noteworthy that the relative proportion of each core protein is the same in whole virions and cores, but differs considerably from that in whole host cells. While histones are present at approximately equal proportions in Vero cells, histone F3 (=VP4) predominates in SV40 virions (Figure 2A vs. C). This suggests that histone incorporation into virions is not random but may be selective depending on the demands of a preferred structure of viral DNA in mature virions. It should be informative to use alternative gel systems such as used here to compare SV_{40} core proteins with those of other papovaviruses and SV_{40} mutants.

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