The results suggest that the mechanisms of action of cortisone on the lactose operon of E. coli and on enzyme production in rat liver cells incorporate identical stages, and that the most important of these stages is preliminary derepression of regions of the genome by substrate inducers.

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ISOLATION OF HIGH-MOLECULAR-WEIGHT INFECTIOUS DNA FROM TYPE 1 HERPES SIMPLEX VIRUS

F. P. Filatov, A. A. Manykin, and L. A. Monastyreva

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Isolation of DNA from type 1 herpes simplex virus (strain L2) is described; the DNA possessed the characteristics of an intact molecule: sedimentation rate, physical length, and infectivity. Data on infectivity of preparations of this DNA were obtained in cultures of chick embryonic fibroblasts.

KEY WORDS: herpes simplex virus; infectious DNA.

Current interest in the DNA of herpes viruses is due to the recently discovered possibility that the virus genome can be mapped by the use of restriction endonucleases, and in the case of the herpes viruses this could show the region of the molecule responsible for the oncogenic properties of some members of this group. Following such an investigation on the shorter DNAs of papova viruses and adenoviruses [8, 9, 11], this could shed some light on the question of virus carcinogenesis. This same approach to the study of virus DNA has a more general importance. It is evident that the preferred object for investigations of this sort must be the intact DNA molecule. In herpes viruses it has a molecular weight of about 10^8 daltons [5]. The isolation of such long molecules, incorporating as they do ribonucleoproteins [3, 7], is rendered more difficult by their high sensitivity to various external factors and, in particular, mechanical injury, leading to fragmentation during isolation.

The object of this investigation was to obtain preparations of intact DNA molecules from strain L2 of type 1 herpes simplex virus, which itself was isolated earlier in the writers' laboratory [1], and to assess their nativeness. A factor of considerable importance in this mater could be that as the system for reproduction and titration of the virus and also for assessment of the infectivity of its DNA a culture of chick embryonic fibroblasts (CEF) was used, although these cells are usually insensitive to type 1 herpes virus; it must, admittedly, be remembered that strain L2 is specially adapted to these cells. The criteria of nativeness of the DNA obtained were its sedimentation rate in a glycerol density gradient, electron-microscopic analysis of the preparation, and its infectivity.

D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow.

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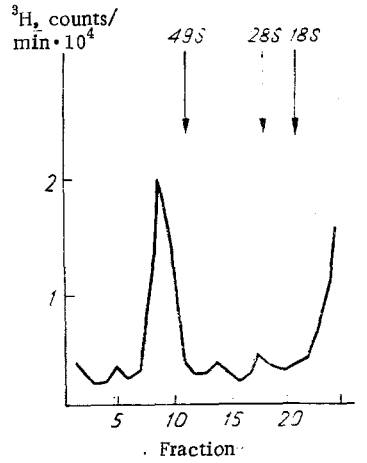

Fig. 1

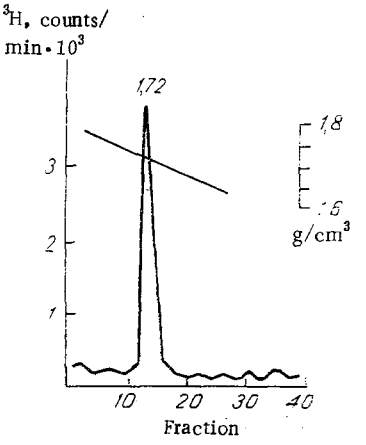

Fig. 2

Fig. 1. Sedimentation analysis of DNA from HSV-1 L23 labeled with thymidine-^{3}H in a 5-20% glycerol density gradient. MSE SS-65 ultracentrifuge, 3 × 6.5 rotor, 48,000 rpm, 4°C, 90 min. Arrows indicate positions of markers: DNA of phage Cd (49S) and ribosomal RNA (28 and 18S).

Fig. 2. Analysis of DNA from HSV-1 L23 in CsCl density gradient (for conditions of centrifugation see section "Experimental Method"). Distribution of ^{3}H-radioactivity.

EXPERIMENTAL METHOD

Strain L2 (clone L23) of human type 1 herpes simplex virus (HSV-1 L23), isolated in the writers' laboratory in 1956 from a patient with herpes labialis, and subsequently maintained through 40 passages (with diluted material) in a CEF culture until the beginning of these investigations, was used. A primary trypsinized CEF culture was obtained in the usual way and grown in medium No. 199 with 10% bovine serum. Once a monolayer had formed the cells were inoculated with the virus, and the maintenance medium did not contain serum. The infected cultures were incubated at 37°C. For working with a radioactive label (thymidine-^{3}H, specific activity 17.9 Ci/mmole) lactalbumin hydrolyzate was used as the nutrient medium. Thymidine-^{3}H was added to the medium 2 h after its infection with HSV-1 L23 in a final concentration of 100 μCi/ml. To obtain large quantities of the virus and of its DNA, HEp-2 cells were used, in which its titer reached $10^{7.2}$-$10^{7.5}$ PFU/ml when the corresponding values for the CEF culture were 10^6-$10^{6.5}$ PFU/ml. Plaque titration of HSV-1 L23 under agar was carried out on CEF by Porterfield's method [10].

The method of extraction of DNA from HSV-1 L23 was based on the procedure described by Wagner et al. [13], with certain modifications. CEF or HEp-2 cell cultures, on reaching the monolayer stage, were inoculated with HSV-1 L23 with a multiplicity of 1-5 PFU per cell. The cells were removed mechanically after 22 h, washed twice in cold TH buffer (0.2 M Tris-HCl, pH 7.5, 0.15 M NaCl) and disintegrated in a Downs' homogenizer (200 tractions). Next, 1 ml homogenate was mixed with 6 ml of 50% sucrose solution made up in TH buffer. A stepwise sucrose gradient was then formed by layering 35%, 30%, and 25% solutions successively on the material. Centrifugation was carried out in the MSE superspeed-65 ultracentrifuge at 27,000 rpm and 4°C for 16 h with the 3 × 25 rotor. After selection of the fractions containing the virus they were mixed, diluted four times with TH buffer solution, and centrifuged in the 8 × 25 rotor at 20,000 rpm for 20 min. The residue was then resuspended in standard RSB buffer containing 0.5% NP40, after which the material was centrifuged at 3000 rpm and the supernatant again centrifuged in the 3 × 6.5 rotor at 20,000 rpm for 30 min to sediment the virions. The residue was suspended in D buffer (0.01 M Tris-HCl, pH 8.0, 0.15 M NaCl, 0.01 M EDTA) containing 1% sarkosyl, and treated with pronase (final concentration 2 mg/ml), previously incubated for 3 h at 37°C. After incubation for 2.5 h at 37°C the DNA was extracted twice with phenol saturated with D buffer. All manipulations with DNA were carried out with plastic pipets with rounded edges and a bore of 2 mm, with a speed of movement of the material in the pipet of 0.025 ml/sec. The preparation of deproteinized DNA was dialyzed for 48 h against several changes of SSC buffer (0.05 M NaCl, 0.0015 sodium citrate). Sedimentation analysis of the preparation was carried out in a density gradient of 5-20% solutions of glycerol (Merck) on the MSE SS-65 ultracentrifuge in the 3 × 6.5 rotor at 4°C and 48,000 rpm for 90 min or in the 3 × 25 rotor at 4°C

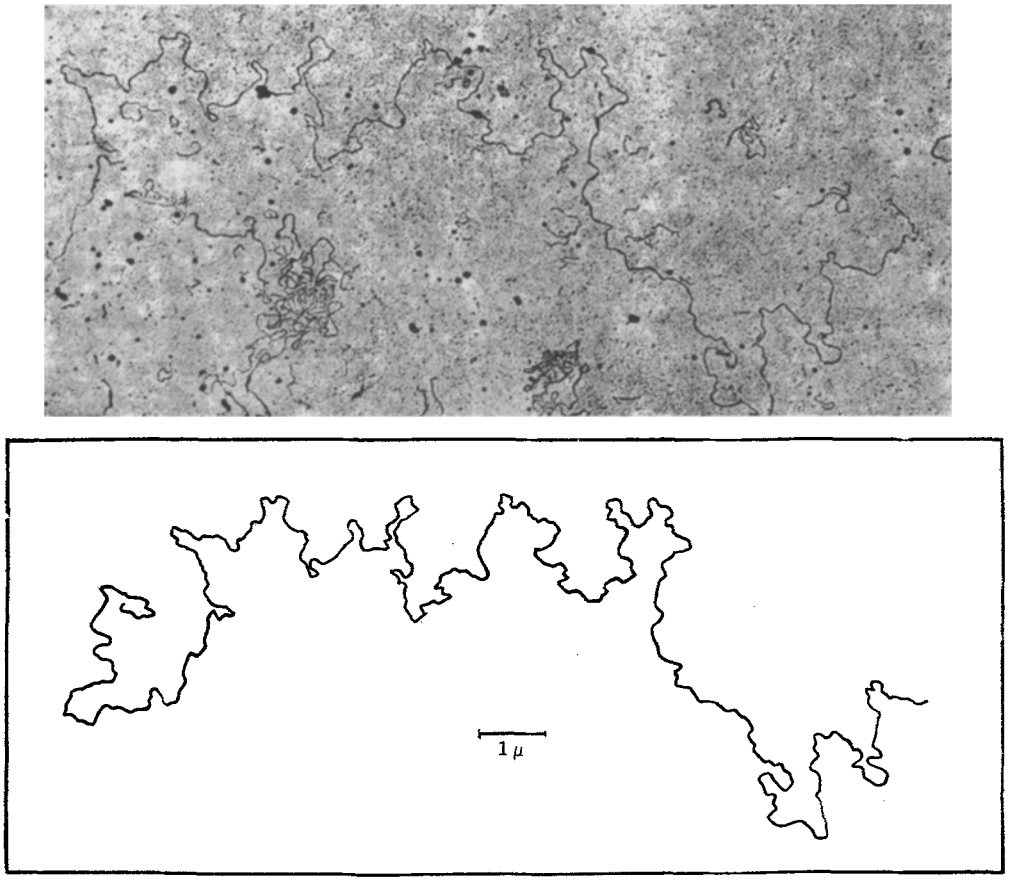

Fig. 3. Electron micrograph of DNA molecule from HSV-1 L23. Rotorary shadow-casting (protein film method). Length of molecule 46 μ.

and 15,000 rpm for 15 h. The buoyant density of the DNA was analyzed in a self-adjusting CsCl gradient (3 × 6.5 rotor, 4°C, 48,000 rpm, 66 h).

To estimate the infectivity of the DNA preparations, a monolayer of CEF ($5 \cdot 10^6$ cells) was inoculated with 0.2 ml of a mixture (1:1) of the DNA solutions with a solution of DEAE-dextran (Pharmacia, mol. wt. $2 \cdot 10^6$) 400 μg/ml, as described by Sheldrick et al. [12] and, after incubation for 1 h at room temperature, the top layer of agar was poured over it (the composition of the top layer was similar to that used for titration of the virus). Plaques were counted after 5 days.

Electron-microscopic analysis of the DNA was carried out by the protein film method [4]. The specimens were shadow-cast with a platinum and palladium alloy on a rotating stage at an angle of 7°. The magnification of the JEM-100B electron microscope was calibrated against a replica with 1152 lines/mm. The error of measurement of length was 2%.

EXPERIMENTAL RESULTS

Marked signs of the cytopathic action of the virus were found in the tissue culture 18-20 h after inoculation of the CEF or HEP-2 monolayer with HSV-1 L23. At this time the infected cells contained the largest amount of virus. Ultracentrifugation of a homogenate of these cells in a stepwise density gradient of sucrose showed that components containing infectious virus floated within a narrow concentration zone of the solution between 55 and 35%, in which the increase in infectivity also corresponded to the peak of radioactive label of the DNA and the increase in the level of absorption in UV light (wavelength 258 nm). Nevertheless the infectivity of the preparation in the above-mentioned zone was substantially less than expected, evidently because of aggregation of the virions in the sucrose, which was aggravated even more after concentration of the virus from the corresponding fractions of the gradient.

The virions were purified by treatment of the primary concentrates with the nonpolar detergents NP40 followed by sedimentation of the coarse cell fragments. This material was used in the present investigation for the extraction of herpes virus DNA as described above.

Sedimentation analysis of the DNA preparation thus isolated from HSV-1 L23 in a preparative ultracentrifuge (Fig. 1) showed that most of the DNA moved within a narrow zone as a homogeneous peak, the position of which corresponded approximately to a constant of 58S (mol. wt. about $90 \cdot 10^6$). The results of investigations of this DNA in a CsCl density gradient demonstrated the purity of the preparation (it contained no lighter cell DNA), the buoyant density of which was 1.72 g/ml (Fig. 2). Electron microscopic investigation showed that the DNA preparation isolated from HSV-1 L23 contained predominantly longer molecules up to 46 μ in length, corresponding to a molecular weight of about $90 \cdot 10^6$ (Fig. 3).

The infectivity of the DNA preparations was also tested. The results showed that DNA from HSV-1 L23 was infectious for CEF cultures and the specific activity was about 30-50 PFU/μg DNA. Plaque formation under agar took place a little later than in the case of inoculation with the virus; the result of the test was accordingly read on the 5th-6th day and not on the 4th day after infection. Plaques obtained as a result of infection of CEF cultures with DNA from HSV-1 L23 had the same distinguishing features as "virus" plaques.

The results of the experiments described above showed that the method can be used to obtain high-molecular-weight infectious DNA from herpes simplex virus and they thus confirmed the observations of Wagner et al. [13], who suggested this method. The resulting DNA was evidently native, if infectivity is regarded as a sign of nativeness. Infectivity is one of the most sensitive properties of the DNA of herpes viruses: Slight mechanical treatment, leading to fragmentation of the molecule, is all that is required to abolish it. Under the conditions used the specific infectivity of herpes simplex virus DNA was about two orders of magnitude lower than that described in the literature. This could be attributed to the special features of the HSV-1–CEF system noted above and with the fact that the CEF culture is, generally speaking, less adequate for work with herpes simplex viruses than those normally used, with the nonoptimal properties of the upper layer of agar, and also possibly with the fact that when inoculating the cells the molecules of HSV DNA were not deposited in the way that this is sometimes done [6]. The physicochemical properties of DNA from HSV-1 L23 (character of sedimentation, buoyant density in a CsCl gradient, length of the linear molecule) corresponded in general to the parameters given in the literature for intact DNA of herpes simplex virus [2, 5].

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