Identification and Genetic Mapping of a Herpes Simplex Virus Capsid Protein That Binds DNA

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Received 12 September 1983/Accepted 10 January 1984

Herpes simplex virus virion protein 19C (VP19C) is a constituent of both unenveloped (nuclear) and enveloped (cytoplasmic) capsids. In this paper we report that ^{32}P -labeled DNA, either supercoiled or linear double stranded, efficiently bound to VP19C electrically transferred from denaturing polyacrylamide gels containing electrophoretically separated proteins from purified capsids. Analyses of the polypeptides specified by herpes simplex virus type 1 $\times$ herpes simplex type 2 recombinants with respect to electrophoretic mobility and binding of ^{32}P -labeled DNA indicate that VP19C maps at the same location as infected cell polypeptide 32 and is derived from it.

Herpes simplex virus type 1 (HSV-1) and HSV-2 have been shown to specify the synthesis of at least 50 infected-cell polypeptides (ICPs) (8, 14, 17, 21, 25). Although the properties and functions of most of these polypeptides are unknown, many bind DNA. The most thoroughly studied of these is ICP8, the major DNA-binding protein specified by HSV, and it has been reported to bind more strongly to single-stranded DNA than to double-stranded DNA (1, 22).

The identification of DNA-binding activity of HSV-specified polypeptides has generally involved the use of DNA-cellulose affinity chromatography (1, 22, 24). Bowen et al. (3) have described the use of protein blotting on nitrocellulose sheets for the detection of DNA-binding proteins. Using the transfer method described by Towbin et al. (26) and others (2, 6) to increase the amount of protein transferred to nitrocellulose, Russell and Precious (23) detected DNA-binding activity with ^{32}P -labeled DNA to a number of adenovirus structural polypeptides which had been bound to nitrocellulose. In this paper, we report the application of this technique for the identification of a DNA-binding polypeptide contained in HSV capsids extracted from both the nuclei and cytoplasm of infected cells.

In these experiments, HSV-1(F) strain (10) capsids purified from either nuclei or cytoplasm of infected cells by centrifugation in sucrose density gradients as described elsewhere (5, 11) were solubilized in sodium dodecyl sulfate-containing disruption buffer and subjected to electrophoresis in 9.25% sodium dodecyl sulfate-polyacrylamide gels. The electrophoretically separated proteins were transferred electrically to nitrocellulose strips (4) and exposed to ^{32}P -labeled DNA as described in the legend to Fig. 1. The binding of nitrocellulose strips as described by Russell and Precious (23) was done by overnight immersion of the strips in a buffer (10 mM Tris, pH 7.2, 50 mM NaCl 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin) containing labeled probe (100,000 cpm/ml) at room temperature. After three washes in this buffer, the strips were dried and exposed to Kodak XS-1 film with the aid of a Dupont Lightning Plus intensifying screen. The results (Fig. 1) showed that HSV-1 capsids contained a polypeptide which bound DNA efficiently. This polypeptide comigrated with virion protein 19C (VP19C) (13) and bound supercoiled and

linear double-stranded pRB602 DNA (see legend to Fig. 1), which carries HSV-1 DNA sequences spanning the junction between the L and S components (20). Preliminary experiments indicated that the binding was not sequence specific under the conditions tested.

Studies on the VP19C specified by HSV-1(HFEM) and HSV-1(G) and by the HSV-1 $\times$ HSV-2 recombinants RB7G2 and RB7G3 suggest that VP19C is derived from ICP32. This conclusion is based on the following. Previous reports from this laboratory (15, 19) have shown that the electrophoretic mobility of HSV-1 ICP32 is slower than that of HSV-2 ICP32. The previously reported apparent molecular weights of HSV-1 and HSV-2 ICP32 were 51,500 and 50,500 (19), respectively, i.e., in approximate agreement with the reported apparent molecular weight of 55,000 for VP19C (13). Mapping studies (15) have identified a set of HSV-1(HFEM) $\times$ HSV-2(G) recombinants (RB7G2 and RB7G3) differing in the genetic origin of ICP32. Furthermore, the region defined by the crossovers shown in Fig. 2 does not encode other genes whose products correspond in size to that of VP19C. As shown in Fig. 3, the electrophoretic mobility of DNA-binding VP19C contained in capsids prepared from HSV-1(HFEM) and RB7G3 is slower than that of corresponding polypeptides specified by the parent, HSV-2(G), and recombinant RB7G2. These results indicate that the gene specifying VP19C is encoded within the genomic region specifying ICP32 and is probably derived from that polypeptide.

After this paper was submitted for publication, Costa et al. (7) published the map location of a gene specifying a 50,000-molecular-weight protein which the authors tentatively identified as VP19C on the basis of a report of Lemaster and Roizman (16), who mapped a structural protein of that size near the left terminus of the genome in the prototype orientation. However, Lemaster and Roizman (16) designated their protein as VP18.8. The latter protein is a phosphoprotein associated with capsid-tegment structures and is readily differentiable from VP19C, a prominent capsid protein.

Studies reported elsewhere (5) have suggested that VP19C is an internal component of the capsid. This conclusion is based on surface iodination of full and empty capsids. In these studies, surface iodination labeled VP5 and VP39 in empty capsids and predominantly VP22a and VP22 in full capsids. Only trace amounts of ^{125}I -labeled VP19C were obtained from preparations of empty capsids, and no demon-

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strable labeled VP19C was obtained from full capsids. VP19C was readily labeled by iodination of capsids disrupted by physical stress such as sonication of empty capsid pellets. By using two-dimensional gel electrophoresis techniques, these studies also suggested that VP19C may be composed of more than one species with different charges and which migrate in a single band on one-dimensional separations.

The VP19C is present in empty and full capsids (11, 13), and therefore this protein differs with respect to molecular weight and distribution from the DNA-binding protein described by Bayliss et al. (1) as being present in capsids. That protein had an apparent molecular weight of 43,000 and appeared to comigrate with VP21, which is a component of full but not empty capsids (11). The protein identified as VP21 did not bind DNA in our experiments. On the basis of

FIG. 1. Autoradiographic images of electrophoretically separated ICPs, capsid polypeptides, and ^{32}P -labeled DNA binding to separated capsid polypeptides. Lane 1, ICPs obtained from HSV-1(F)-infected Vero cells labeled with [^{35}S]methionine (New England Nuclear Corp., Boston, Mass.) for 2 h immediately before harvesting at 12 h postinfection; lane 2, polypeptides from full, DNA-containing nuclear capsids purified (5, 11) from nuclei of HSV-1(F)-infected Vero cells labeled with [^{35}S]methionine from 5 h until harvesting at 18 h postinfection. The labeled polypeptides served to identify the DNA-binding polypeptides. Lanes 3 to 5, ^{32}P -labeled DNA binding to transferred, electrophoretically separated polypeptides purified from unlabeled HSV-1(F)-infected Vero cells. Lane 3, Full nuclear capsids; lanes 4 and 5, cytoplasmic capsids banding at the same relative position as full nuclear capsids. Plasmid pRB602 carrying the HSV-1 *Hae*II fragment containing two *a* sequences (18, 21) was labeled with ^{32}P by one of two methods and used as a probe for DNA-binding activity. Lanes 3 and 4 were probed with supercoiled pRB602 (designated "S") labeled by growing the host bacteria in L broth containing 2 μCi of $^{32}\text{P}_i$ (New England Nuclear) per ml of broth depleted of phosphate by precipitation with magnesium chloride at pH 10 followed by neutralization to pH 7.4. Standard protocols were used to harvest plasmid DNA. Lane 5 was probed with pRB602 (designated "L") linearized by cleavage with *Bam*HI and end labeled with the Klenow fragment of DNA polymerase I (P-L Biochemicals, Milwaukee, Wis.) and [α - ^{32}P]dCTP (New England Nuclear) in the presence of 20 μM unlabeled deoxynucleoside triphosphates as described by Drouin (9).

FIG. 2. Summary of the map locations of HSV-1 and HSV-2 DNA sequences present in HSV-1 and HSV-2 recombinants used for mapping the gene specifying the major DNA-binding polypeptides present in HSV capsids. The construction and properties of the intertypic recombinants RB7G2 and RB7G3 have been described elsewhere (15). The top two lines represent the sequence arrangement and map coordinates of HSV DNA in the prototype arrangement. The top and bottom lines to the right of the designation of the recombinants represent HSV-1 and HSV-2 sequences, respectively. The heavy lines identify the provenance (HSV-1 or HSV-2) of the sequence present in each recombinant. The numbers between the double lines designate the approximate locations of the genes coding for various HSV-specified polypeptides.

FIG. 3. Autoradiographic images of electrophoretically separated ICPs and of DNA bound to capsid polypeptides. The two leftmost lanes show the autoradiograms of ICPs obtained from Vero cells labeled with [^{35}S]methionine for 2 h immediately before harvesting at 12 h postinfection with HSV-1(HFEM) or HSV-2(G) as indicated. The isolation and properties of HSV-1(HFEM) and HSV-2(G) were described in references 12 and 10, respectively. The four rightmost lanes show the autoradiographic images of pRB602 plasmid DNA bound to capsid polypeptides electrophoretically separated in polyacrylamide gels and then transferred electrically to nitrocellulose sheets as described elsewhere (4). The plasmid DNA was linearized with *Bam*HI and end-labeled with [^{32}P]cytidine. The polypeptides were obtained from the cytoplasmic capsids harvested from cells infected with HSV-1(HFEM), HSV-2(G), and with the HSV-1(HFEM) $\times$ HSV-2(G) intertypic recombinants RB7G2 and RB7G3 (15) as indicated. Purification of cytoplasmic capsids and end labeling and binding of DNA were done as described in the text.

studies described elsewhere (5), we suggest that VP21 may be an unprocessed member of the ICP35 family represented in capsids and virions as VP22a and VP22.

The function of ICP32 is not known. The observations that VP19C is an internal capsid protein and that it is a major component of the capsid capable of binding DNA suggest that it may function in packaging or anchoring the DNA in the capsid.

We thank Nancy Michael for excellent technical assistance.

These studies were aided by Public Health Service grants CA-08494 and CA-19264 from the National Cancer Institute and by American Cancer Society grant MV-2R. D.B. was a postdoctoral fellow receiving support from the Charles W. Webster and James E. Davis Fellowships administered by the University of Chicago Cancer Research Center. W.B. was a Public Health Service postdoctoral trainee (grant 5 T32 PHS AI-07182).

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