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DNA-Binding Proteins Present in Varicella-Zoster Virus-Infected Cells

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DNA-binding proteins present in varicella-zoster virus-infected cells were identified by DNA-cellulose chromatography of radioactively labeled cell extracts. Seven virus-specific proteins, ranging in molecular weight from ~175,000 to 21,000, showed affinity for single- or double-stranded DNA or both. These proteins include the varicella-zoster virus major capsid protein, a phosphorylated tegument protein, and a 125,000-molecular-weight species which may be analogous to the major DNA-binding protein of herpes simplex virus. We also identified a number of DNA-binding phosphoproteins by these procedures. Finally, protein blot studies were carried out to determine whether these proteins bind preferentially to virus rather than to host cell DNA.

Virus-coded proteins which bind to single- and double-stranded DNA have been identified in a number of herpesvirus systems (2–4, 19). The most extensively studied herpesvirus DNA-binding proteins are those found in cells infected with herpes simplex virus (HSV) type 1 (HSV-1) or HSV-2. HSV-1 and HSV-2 code for approximately 12 to 16 DNA-binding proteins which fall into a number of overlapping classes. Some bind exclusively to single-stranded DNA and others exclusively to double-stranded DNA, whereas the majority show some, although not equivalent, affinity for both single- and double-stranded DNA. These polypeptides vary from 175,000 in molecular weight (175K) to 12K and are present in all three temporal (immediate early, early, and late) classes of HSV-coded proteins (2, 11, 12, 17, 19, 20). A number of these proteins undergo phosphorylation-dephosphorylation events which alter their DNA affinity (28). The HSV DNA-binding protein which has been most extensively studied is an approximately 128K species designated ICP8 (10) or ICSP 11,12 (20). This protein binds cooperatively to single-stranded DNA, is involved in HSV DNA replication, and may represent a group-specific antigen for the herpesviruses (14–16, 18, 22, 27–29).

In this communication we report on our studies of DNA-binding proteins found in varicella-zoster virus (VZV)-infected cells. Using DNA-cellulose chromatography, we identified polypeptides present in [^{35}S]methionine-labeled, VZV-infected cells which bind to single- or double-stranded DNA or both. These proteins range from approximately 175K to 21K, and several appear to be analogous to those seen in the HSV system. We also identified DNA-binding phosphoproteins found in VZV-infected cells and carried out preliminary protein blot studies on the binding specificities of infected-cell and nucleocapsid DNA-binding proteins.

MATERIALS AND METHODS

Cells and viruses. VZV strains Oka and Ellen were obtained from the American Type Culture Collection, Rockville, Md. VZV strain Champ was obtained from A. Buchan, University of Birmingham, Birmingham, England. VZV strain Scott and the human foreskin fibroblast (HFF) cell line USUHS 184 were the generous gifts of G. Fischer, Department of Pediatrics, Uniformed Services University of the Health Sciences, Bethesda, Md. All VZV strains were propagated in HFF cell monolayers as described by Straus et al. (25). Briefly, infected cell monolayers were scraped and seeded onto uninfected monolayers at a ratio of 1:6. HSV-1 strain 17 and HSV-2 strain HG52 were propagated in Vero cell monolayers (7, 26). VZV nucleocapsids and HSV nucleocapsids were purified as described by Straus et al. (25).

Radioactive labeling of infected cells. Radioactively labeled extracts of HFF cell monolayers infected with strain Oka were used throughout the DNA-cellulose chromatography experiments. HFF cell monolayers were infected by addition of cells infected with VZV strain Oka at a ratio of 1:6. Infection was allowed to proceed until the cells showed 25 to 30% cytopathic effect (25 to 30 h). At this point, the medium was replaced with medium containing [^{35}S]methionine at 8 to 20 μCi/ml. Labeling continued until the cells showed 90 to 95% cytopathic effect (~60 h). The infected cell monolayers were washed twice with phosphate-buffered saline at 4°C and then scraped down and pelleted at 2,000 rpm for 5 min in an IEC PR6000 centrifuge. The cell pellets were stored at −60°C before being processed for DNA-cellulose chromatography. For specific labeling of phosphoproteins, the cells were infected as described above. At 25 to 30% cytopathic effect the cells were washed twice with phosphate-free medium for 1 h. The cells were incubated in the presence of medium containing 20 to 40 μCi of $^{32}P_i$ and harvested as described above.

Infected cell extracts. Harvested cell pellets were thawed, and all subsequent operations were performed at 4°C. Cells were resuspended in sonication buffer (0.05 M NaCl, 0.01 M $MgCl_2$, 0.001 M EDTA, 0.001 M 2-mercaptoethanol, 0.001 M dithiothreitol, 0.05 M Tris-hydrochloride [pH 7.6], 130 μg of lysozyme per ml, 23 μg of phenylmethylsulphonyl fluoride per ml, 20 μg of pancreatic DNase I per ml, and 0.4% sodium deoxycholate) at a concentration of 3×10^6 cells per ml in a

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final volume of 5 ml. The cells were then disrupted with three 20-s bursts from a Heat Systems-Ultrasonics sonicator (output setting, 5). The extract was incubated at 4°C for 2 h. After incubation, NaCl was added to a final concentration of 2.0 M. Incubation was continued for an additional 60 min. DNA and precipitated proteins were removed by centrifugation at 16,000 rpm with a Sorvall RC-5 centrifuge. The supernatant was dialyzed overnight against three changes of buffer A (0.05 M NaCl, 0.001 M EDTA, 0.001 M 2-mercaptoethanol, 0.05 Tris-hydrochloride [pH 7.6]).

DNA-cellulose chromatography. Native and denatured calf thymus DNA-cellulose matrices were prepared by the method of Alberts and Herrick (1). Columns (0.5 by 2 cm) of native and denatured DNA-cellulose were used throughout these studies. The columns were equilibrated in buffer A at 4°C. One half of the dialyzed extract was then applied to each column, which was washed extensively with buffer A and eluted by the stepwise addition of 0.2, 0.6, 1.0, and 2.0 M NaCl in buffer A. Thirty 1.0-ml fractions were collected. Aliquots (50 μl) of each fraction were added to 1.0 ml of ScintiVerse liquid scintillation cocktail (Fischer Scientific Co.) and counted with a Searle Mark III liquid scintillation counter. A 4.0 M guanidine hydrochloride wash was also performed on some columns in an attempt to strip off any residual, very tightly binding proteins.

Electrophoresis of DNA-cellulose column fractions. Fractions showing peak radioactivity from each of the salt steps were concentrated by overnight precipitation of 0°C after addition of a 0.1 volume of a protein extract from unlabeled, uninfected cells and 4 volumes of acetone. The protein extract was prepared as follows (R. T. Hay, Ph.D. thesis, University of Glasgow, Glasgow, Scotland, 1979). Uninfected HFF cells were swollen in distilled water for 15 min at 4°C and vortexed. An equal volume of a buffer containing 4 M NaCl, 0.04 M Tris-hydrochloride (pH 8.2), 0.002 M EDTA, and 0.002 M 2-mercaptoethanol was then added to lyse nuclei. The samples were incubated on ice for 30 min and then centrifuged at 100,000 × *g* for 1 h at 4°C. The supernatant was removed and dialyzed overnight against 0.05 M NaCl–0.02 M Tris-hydrochloride (pH 8.2)–0.001 M EDTA–0.001 M 2-mercaptoethanol–10% glycerol. A light precipitate was removed from the dialyzate by centrifugation at 10,000 × *g* for 30 min.

The acetone-precipitated proteins were collected by centrifugation at 3,000 × *g* for 15 min. The precipitate was solubilized in disruption buffer and heated at 100°C for 60 s. Samples were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10). The resulting gels were dried, and autoradiography was carried out with Kodak XAR-5 film and cassettes.

DNA probes for protein blots. VZV DNA was prepared by the method of Straus et al. (25). pBR322 plasmid DNA was the gift of John McGowan, Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, Md. VZV DNA and pBR322 DNA were labeled with ^{32}P by nick translation (21).

Protein blotting. Infected cells and nucleocapsids were suspended in disruption buffer, sonicated for 30 to 60 s, and heated at 100°C for 60 s. Samples were electrophoresed on 1.5-mm 12% sodium dodecyl sulfate-polyacrylamide slab gels by use of a vertical, water-cooled SE 620 slab gel electrophoresis unit (Hoefer Scientific Co., Inc.). The separated proteins were then electrophoretically transferred to nitrocellulose paper by use of a TE 52 Transphor apparatus (Hoefer Scientific). The transfer buffer used was 0.025 M Tris-hydrochloride–0.192 M glycine–20% methanol. Electrophoresis was carried out at 1.5 A, 60 V for 2 to 3 h. After the transfer was made, the nitrocellulose sheet was soaked in 200 ml of binding buffer (0.001 M EDTA, 0.01 Tris-hydrochloride [pH 7.0], 0.05 M NaCl, 0.02% Ficoll, 0.02% bovine serum albumin, and 0.02% polyvinylpyrollidine [5]). The nitrocellulose sheet was placed in a plastic Seal-A-Meal cooking pouch, and 10 ml of binding buffer containing the desired ^{32}P-labeled DNA (approximately 10^5 cpm/ml) was added. Air bubbles were removed, and the pouch was incubated for 2 h at room temperature with constant agitation on a rocking platform. Binding was terminated by removal of the radioactive mixture, followed by four 15-min washes with cold binding buffer. The washes were moni-

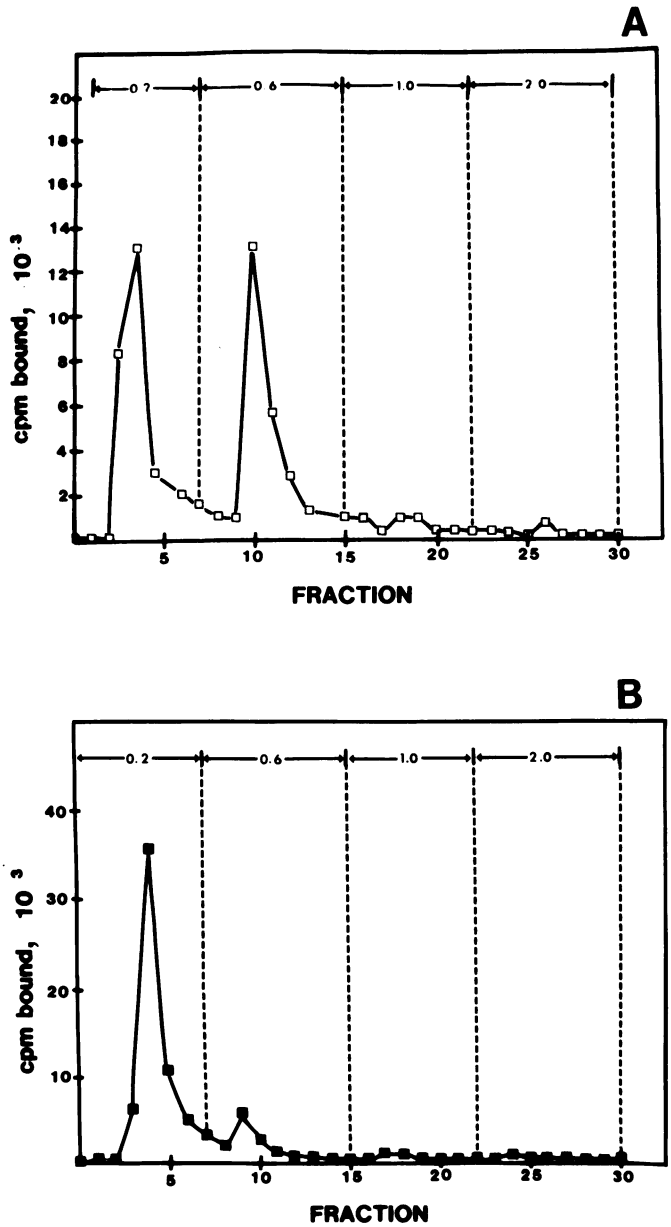

FIG. 1. Elution profiles of ^{35}S-labeled extracts of VZV-infected (A) and uninfected (B) HFF cells from single-stranded-DNA–cellulose columns. The NaCl concentrations used in the step gradient are indicated at the top of the profiles. Counts represent 50-μl aliquots from 1.0-ml fractions.

tored for radioactivity to ensure complete removal of unbound probe. The nitrocellulose sheet was then air dried and prepared for autoradiography as described above.

RESULTS

Elution from DNA-cellulose columns. Radioactively labeled proteins from uninfected and VZV-infected cells were eluted with steps of 0.2, 0.6, 1.0, and 2.0 M NaCl in buffer A as described above. The elution profiles of infected and uninfected extracts from both single- and double-stranded-DNA–cellulose columns showed significant differences. Specifically, the 0.6 M fractions from the infected cell columns were enriched in labeled material as compared with 0.6 M fractions from uninfected cell columns (Fig. 1 and 2). Thus, on the basis of the column elution profiles, labeled DNA-binding proteins in VZV-infected cells have, as a group, a higher affinity for DNA than does the majority of DNA-binding proteins in uninfected host cells labeled under the same conditions.

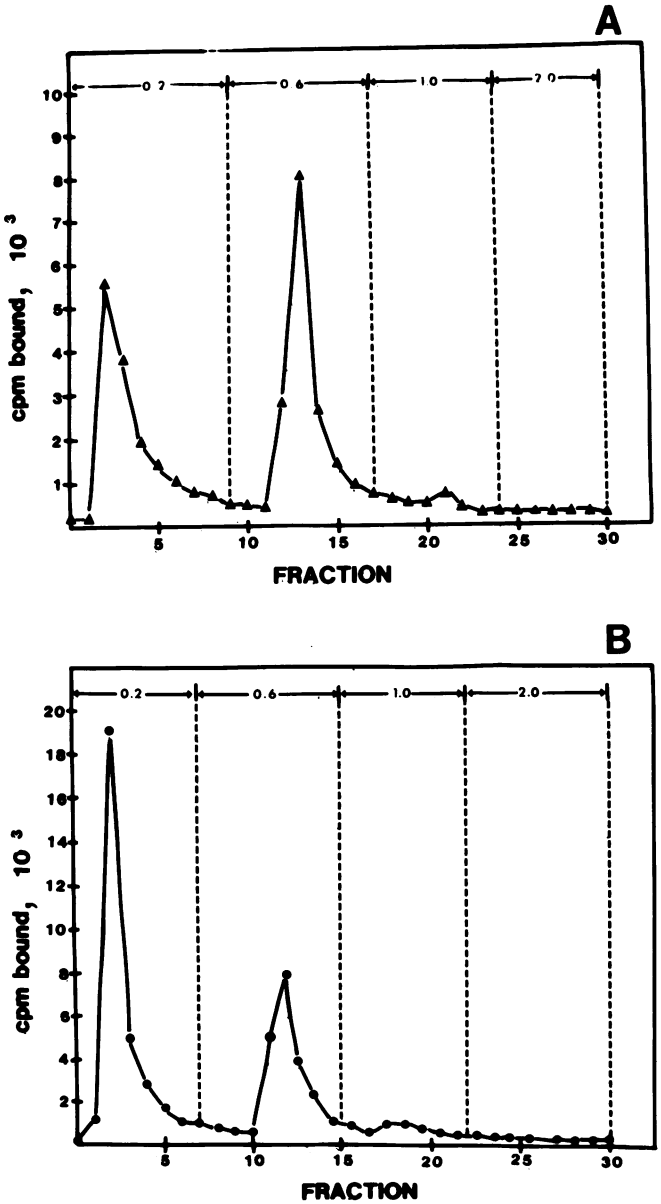

FIG. 2. Elution profiles of ^{35}S-labeled extracts of VZV-infected (A) and uninfected (B) HFF cells from double-stranded-DNA–cellulose columns. The NaCl concentrations used in the step gradient are indicated at the top of the profiles. Counts represent 50-μl aliquots from 1.0-ml fractions.

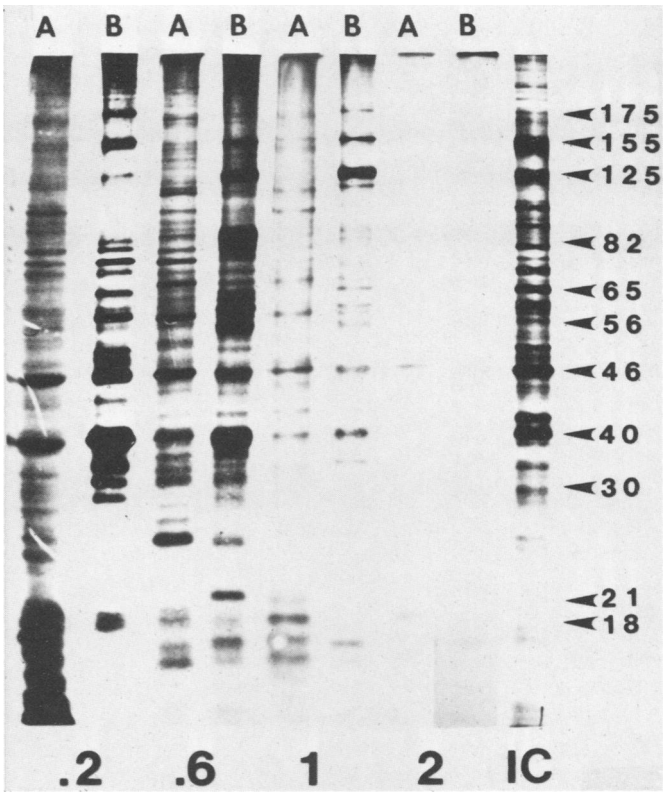

FIG. 3. Autoradiograph of single-stranded-DNA-binding proteins. Lanes labeled .2, .6, 1, and 2 represent peak fractions of uninfected (A) and infected (B) cell proteins eluted from single-stranded-DNA–cellulose columns at the indicated NaCl concentrations. Lane IC represents a VZV-infected cell extract prepared for DNA-cellulose chromatography.

Essentially all of the labeled proteins were eluted by this procedure. A wash with 4.0 M guanidine hydrochloride, designed to strip the columns of residual protein, did not result in elution of any significant amounts of labeled material. Comparison of electrophoretic profiles of sonicated infected cells with cell extract profiles indicated that the cell extracts were representative of the distribution of proteins present in infected cells (C. R. Roberts, Ph.D. thesis, Uniformed Services University of the Health Sciences, Bethesda, Md., 1984; C. R. Roberts et al., unpublished data).

Identification of VZV-specific DNA-binding proteins. Peak fractions from the DNA-cellulose columns were pooled, and the proteins were precipitated and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described above. After electrophoresis, the gels were dried and autoradiography was carried out at −70°C with Kronex intensifier screens (Kodak). Examination of the autoradiographs resulted in the identification of a minimum of seven DNA-binding proteins present in VZV-infected cells but not in uninfected host cells (Fig. 3 and 4). These seven major identifiable proteins were 175K, 155K, 125K, 82K, 56K, 40K, and 21K. All of these polypeptides bound to single-

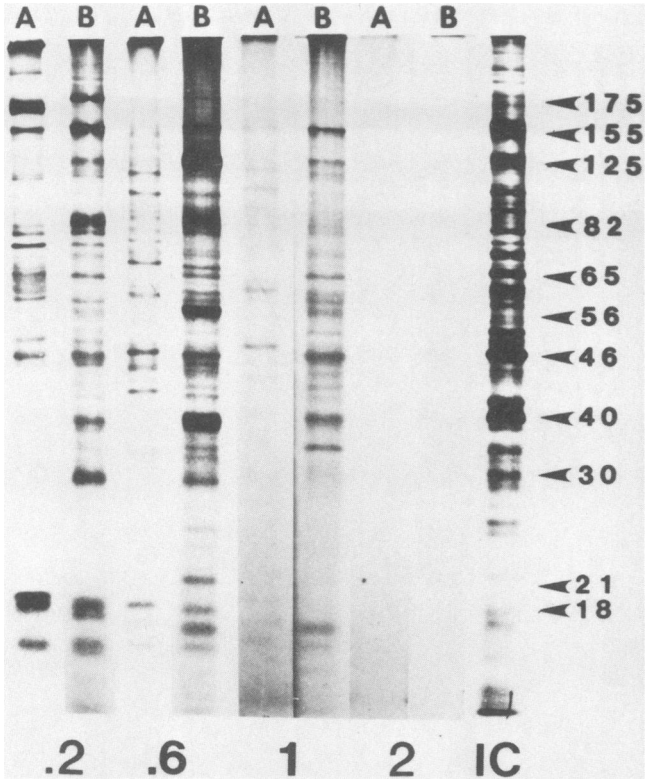

FIG. 4. Autoradiograph of double-stranded-DNA-binding proteins. Lanes labeled .2, .6, 1, and 2 represent peak fractions of uninfected (A) and infected (B) cell proteins eluted from double-stranded-DNA–cellulose columns at the indicated NaCl concentrations. Lane IC represents a VZV-infected cell extract prepared for DNA-cellulose chromatography.

stranded DNA with various affinities (Table 1). One of the two major single-stranded DNA-binding proteins was an 82K species. The interaction between this protein and single-stranded-DNA–cellulose withstood an 0.2 M NaCl wash. The protein was completely eluted with 0.6 M NaCl. No protein analogous to this species eluted from double-stranded-DNA–cellulose columns.

The other major species interacting with single-stranded DNA was a 125K protein. Approximately 50% of the 125K protein was eluted from single-stranded-DNA–cellulose columns by an 0.6 M NaCl wash, and the majority of the remainder eluted in the 1.0 M NaCl wash. A small quantity of the 125K protein was also seen in the 2.0 M NaCl wash. The 125K protein also bound to double-stranded-DNA–cellulose columns. In this case, the majority of the protein was eluted by an 0.6 M NaCl wash, with a small amount of residual protein eluting at 1.0 M. No labeled protein was detected in the 2.0 M NaCl wash. The remainder of the polypeptides listed above bound to both single- and double-stranded-DNA–cellulose with apparently equal affinity, based on these relatively crude criteria. Most of the polypeptides were eluted with 0.2 and 0.6 M NaCl, with some residual material eluting at 1.0 M NaCl.

TABLE 1. VZV DNA-binding proteins

Polypeptide	Mol wt ($\times 10^3$)	NaCl concn (M) for elution from[a]: SS DNA-celluose	DS DNA-cellulose	VZV polypeptide[b]	Mock-infected species of similar mol wt
1	175	0.2–1.0	0.2–0.6	S	+
2	155	0.2–1.0	0.2–1.0	S	+
3	125	0.6–1.0	0.6	NS	
4	82	0.6		NS	
5	56	0.2	0.6	S	
6	40	0.6	0.6	(S)	
7	21	0.6	0.6	(S)	+

[a] SS, Single-stranded; DS, double-stranded.
[b] S, Structural; (S), NS, nonstructural; presence of a VZV structural polypeptide of similar apparent molecular weight (C. R. Roberts, Ph.D. thesis, Uniformed Services University of the Health Sciences, Bethesda, Md., 1984).

Phosphorylated DNA-binding proteins. It has been reported in the HSV system that some DNA-binding proteins are phosphorylated (13, 28). To identify such proteins, we labeled VZV-infected and mock-infected cells with $^{35}P_i$ as described above. Extracts for DNA-cellulose chromatography were prepared, adsorbed to both single- and double-stranded-DNA–cellulose columns, and eluted with the same salt steps as those used for the ^{35}S-labeled proteins. The resulting autoradiograms indicated that 10 to 12 phosphorylated infected cell polypeptides bound relatively tightly to DNA. In contrast, very few uninfected cell DNA-binding phosphoproteins survived an 0.2 M NaCl wash from both single- and double-stranded-DNA–cellulose columns (Fig. 5 and 6). The ^{32}P-labeled DNA-binding proteins range from 175K to 27K. As was the case with ^{35}S-labeled proteins, most of the DNA-binding phosphoproteins bound to both single- and double-stranded-DNA with no apparent differences in binding affinity. A catalog of phosphorylated DNA-binding proteins from infected cells is shown in Table 2; a few of these have counterparts among the ^{35}S-labeled DNA-binding proteins, as detailed below.

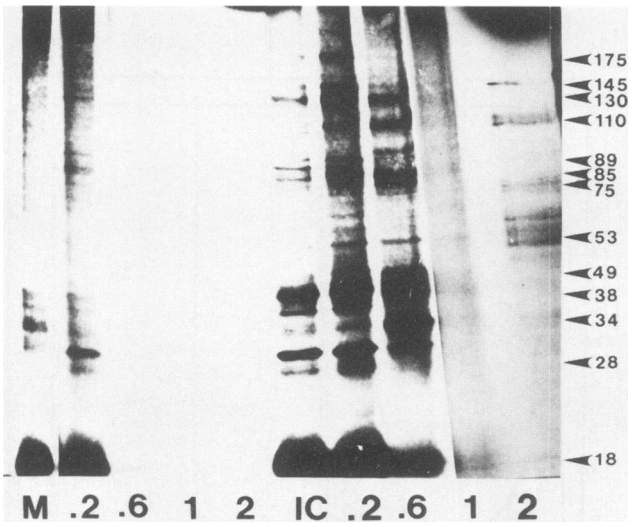

FIG. 5. Autoradiograph of ^{32}P-labeled single-stranded-DNA-binding proteins. The five lanes at the left represent DNA-binding phosphoproteins present in uninfected HFF cells, and the five lanes at the right represent DNA-binding phosphoproteins present in VZV-infected cells. Lane M, Proteins present in uninfected cell extracts; lane IC, proteins present in infected cell extracts. The remaining lanes correspond to peak fractions eluted from single-stranded-DNA–cellulose columns at the indicated NaCl concentrations (molar).

The majority of the 175K phosphoprotein eluted from double-stranded-DNA–cellulose at 0.2 M NaCl, with some residual protein eluting at 0.6 M NaCl. Essentially all of the protein was eluted from single-stranded-DNA–cellulose with an 0.2 M NaCl wash. This behavior is similar to that found with the ^{35}S-labeled 175K protein, although residual protein eluting at 1.0 M was not observed.

The 145K phosphoprotein was a new species which was not observed in ^{35}S-labeled extracts. This protein appeared to bind more strongly to double-stranded DNA than to single-stranded DNA, since roughly equivalent amounts of the protein eluted from double-stranded-DNA–cellulose columns with 0.2 and 0.6 M NaCl washes, whereas the majority of this protein eluted from single-stranded-DNA–cellulose columns with an 0.2 M NaCl wash. The 145K protein appears to be a minor polypeptide species which was detected only because of its phosphorylation.

The 130K phosphoprotein appeared to bind more tightly to double- than to single-stranded DNA, based on the fact that the majority of the protein was eluted from single-stranded-DNA–cellulose by 0.2 M NaCl, whereas approximately 50% of the protein was retained on double-stranded-DNA–cellulose columns at this salt concentration. The 130K protein was completely eluted from the single-stranded-DNA–cellulose columns with the 0.6 M wash. In contrast, a 2.0 M NaCl wash resulted in elution of a protein of this molecular weight from double-stranded-DNA–cellulose columns. This may be the same polypeptide as the ^{35}S-labeled 125K species.

Two other DNA-binding phosphoproteins are worthy of note. One is a 110K polypeptide and the other is a 35K polypeptide, but neither of these proteins is a major species in ^{35}S-labeled cell extracts. Both bind better to single- than to double-stranded DNA, with the 35K species appearing to bind almost exclusively to single-stranded DNA.

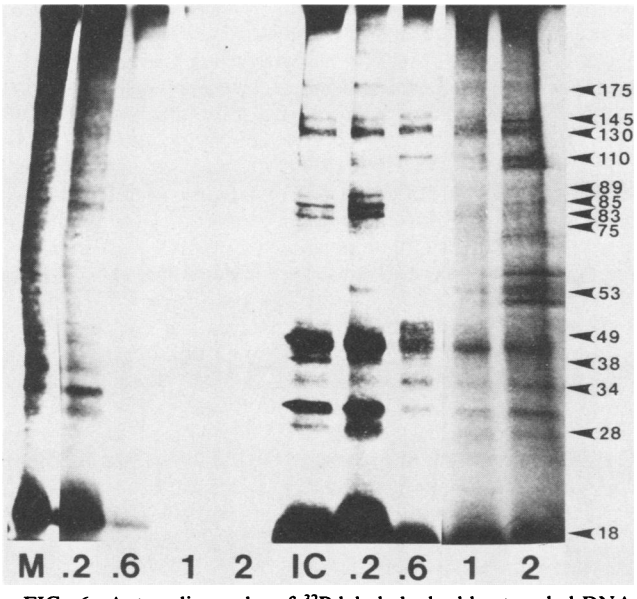

FIG. 6. Autoradiographs of ^{32}P-labeled, double-stranded-DNA-binding proteins. The five lanes at the left represent double-stranded-DNA-binding phosphoproteins present in uninfected cells, and the five lanes at the right represent double-stranded-DNA-binding phosphoproteins present in infected cells. Lanes M and IC, ^{32}P-labeled proteins present in uninfected and infected cell extracts, respectively. The remaining lanes correspond to peak fractions eluted at the indicated NaCl concentrations (molar).

TABLE 2. VZV DNA-binding phosphoproteins

Polypeptide	Mol wt ($\times 10^3$)	NaCl concn (M) for elution from[a]: SS DNA-celluose	NaCl concn (M) for elution from[a]: DS DNA-cellulose	VZV polypeptide[b]	Mock-infected species of similar mol wt
1	175	0.2	0.2–0.6	S	
2	145	0.2–0.6	0.2–0.6 (2.0)	NS	
3	130	0.2–0.6	0.2–0.6 (2.0)	NS	
4	110	0.2–0.6	0.6 (2.0)	NS	
5	75–89	0.2–0.6	0.2–0.6	(S)	+
6	53	0.2–0.6	0.2–0.6 (2.0)	NS	
7	38–49	0.2–0.6	0.2–0.6	(S)	
8	34	0.6	0.2–0.6	S	
9	29	0.2–0.6	0.2–0.6	?	+
10	27	0.2	0.2	?	+

[a] SS, Single-stranded; DS, double-stranded.
[b] S, Structural; NS, nonstructural; presence of a VZV structural polypeptide of similar apparent molecular weight (Roberts, Ph.D. thesis).

The lowest-molecular-weight DNA-binding phosphoprotein species in VZV-infected cells were a 29K-to-30K and a 27K-to-28K species. Polypeptides with similar molecular weights were also present in uninfected-cell extracts, but they were present either in smaller amounts or in less phosphorylated forms. These proteins bound to both single- and double-stranded-DNA–cellulose columns. The 27K-to-28K species was completely eluted by an 0.2 M NaCl wash, whereas the majority of the 29K-to-30K species eluted with an 0.2 M NaCl wash, with some residual protein eluting at 0.6 M NaCl. In addition to the proteins listed above, there were two faint, diffuse groups of bands corresponding to 89K to 79K and 45K to 38K. These proteins bound to both single- and double-stranded-DNA–cellulose columns. Roughly equivalent amounts were eluted by 0.2 and 0.6 M NaCl washes. Very little residual material eluted at 1.0 M.

A surprisingly large number of infected cell phosphoproteins were eluted from both single- and double-stranded-DNA–cellulose columns with a 2.0 M NaCl wash. These species were present in small amounts relative to the quantities eluting at lower salt concentrations and ranged from 145K to 27K. Small amounts of the 145K, 110K, 78K-to-89K, and 49-to-56K species were also present in 2.0 M NaCl washes from both single- and double-stranded-DNA–cellulose columns. Species of 130K, 38K, 35K, 29K, and 27K were detected only in the elutions from double-stranded-DNA–cellulose.

Finally, a major 18K-to-19K species was present in both infected and uninfected cell extracts. The infected cell 18K-to-19K protein appeared to bind more tightly to double-stranded DNA than did the 18K-to-19K species found in uninfected cells based on resistance to salt elution. Whether this difference in affinity represents a modification of a host cell protein or the presence of a virus-coded protein with a coincidental molecular weight is not known.

Protein blots of VZV polypeptides. To further identify VZV-specified DNA-binding proteins and to gain information regarding their binding specificity, we prepared a series of protein blots as described above. These blots involved uninfected host cell proteins, VZV-infected cell proteins, and proteins from purified VZV nucleocapsids. The blots were probed with radioactively labeled DNAs, including both homologous (VZV) and nonhomologous (pBR322) DNA. Figure 7 shows the results of probing a blot containing proteins from the above-mentioned sources with ^{32}P-labeled,

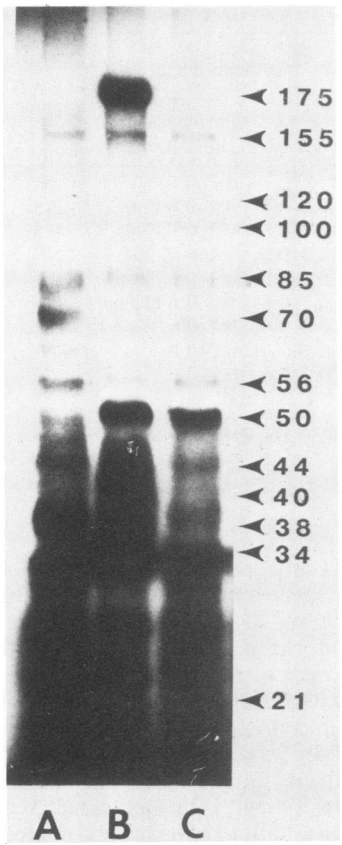

FIG. 7. Autoradiograph of a protein blot probed with ^{32}P-labeled, double-stranded VZV DNA. Lane A, Mock-infected cell proteins; lane B, VZV-infected cell proteins; lane C, VZV nucleocapsid proteins.

double-stranded VZV DNA. The uninfected-cell lane (lane A) shows binding of DNA due to the presence of DNA-binding proteins over the entire range of molecular weights. This is expected based on our experiments with DNA-cellulose column elution (Fig. 1). The 175K and 155K species identified previously, which interact with both single- and double-stranded-DNA, were present in the infected-cell lane (lane B). The 155K band in this lane most likely represents a mixture of the 155K double-stranded-DNA-binding protein present in uninfected cells (Fig. 4) and the VZV major capsid protein. The nucleocapsid lane (lane C) contained a 155K band, corresponding to the major capsid protein, but not the 175K band. A faint band corresponding to a 125K protein was also observed in some blotting experiments (see below).

There was a faint 68K band present in the nucleocapsid lane which does not correspond to any of the bands seen in the infected-cell protein lane. Two bands corresponding to approximately 56K and 50K were seen in both infected-cell and nucleocapsid lanes, as was a group of (at least) five bands ranging from approximately 44K to 30K, with a major band at about 34K. Some additional lower-molecular-weight material was seen in the infected-cell lane but not in the nucleocapsid lane. It is likely, however, that much if not all of this material is cellular, since a substantial number of bands in this molecular weight range were also seen in the uninfected lane.

We next attempted to ascertain whether any of the blotted VZV-specific proteins preferentially bound to VZV as opposed to heterologous pBR322 DNA. Both the VZV DNA and pBR322 DNA were nick translated and incubated against identical protein blots made up of three lanes: uninfected cell proteins, proteins from cells infected with VZV strain Scott, and proteins from cells infected with VZV strain Champ (Fig. 8). Within the limits of the assay, some differences were evident. In general there appeared to be a decreased affinity of VZV proteins for the plasmid sequences. The major exceptions were a 120K and a 34K species, which appeared to bind the heterologous DNA more tightly than VZV DNA. VZV-infected cell proteins which appeared to have a higher affinity for VZV DNA as compared with the plasmid sequences included a faint, diffuse 85K species, a 56K species, and a series of lower-molecular-weight bands (25K to 36K).

DISCUSSION

We have identified a minimum of seven major DNA-binding proteins present in VZV-infected cells based on DNA-cellulose chromatography of ^{35}S-labeled cell extracts. We have, in addition, identified 10 to 12 phosphorylated VZV DNA-binding proteins, the majority of which appear to be minor species. The properties of several of these proteins are similar to those found in other herpesvirus systems, particularly the HSV-1–HSV-2 system. The four most abundant DNA-binding proteins identified in VZV-infected cells were 175K, 155K, 125K, and 82K species.

Time course studies of protein synthesis in VZV-infected cells have identified two proteins ranging from 180K to 175K (Roberts, Ph.D. thesis; Roberts et al., unpublished data). One has some of the properties of an immediate early protein which appears soon (about 10 h) after infection and whose synthesis ceases 24 to 28 h postinfection. The other is an apparently late protein whose synthesis begins 28 to 30 h postinfection and continues through total cell cytopathic effect (about 72 h). This second polypeptide has an electrophoretic mobility which is slightly faster than that of the immediate early species and is a structural protein present in intact VZV virions but not nucleocapsids (Roberts, Ph.D. thesis). The 175K DNA-binding protein detected by single-stranded-DNA–cellulose chromatography in this study most likely corresponds to this VZV structural protein since the cells were harvested at ~72 h postinfection. Single-stranded-DNA–cellulose chromatography of labeled, uninfected extracts did not reveal a band at this position. However, it is possible that some of the material which eluted with an 0.2 M NaCl wash from double-stranded-DNA–cellulose to which infected cell extracts had been adsorbed may be from the host cell. The amount of host cell protein present, however, is probably small, since results of time course experiments show that the synthesis of the 180K-to-175K host cell protein ceases soon (5 to 10 h) after infection and synthesis of the immediate early 175K-to-180K infected cell protein ceases at ~21 h postinfection. At late times (45 to 50 h), the only band observed in this region of the gel corresponds to the 175K VZV structural protein (Roberts, Ph.D. thesis; Roberts et al., unpublished data). Further proof that the 175K structural protein binds DNA comes from the protein blot studies shown in Fig. 7. The infected-cell lane shows a heavy band at 175K. No band corresponding to a DNA-protein interaction is present at this location in the adjacent nucleocapsid lane or in the uninfected lane. Isolation of ^{32}P-labeled VZV virions and subsequent sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the virion proteins has shown that the 175K protein is highly phosphorylated (Roberts, Ph.D. thesis; Roberts et al., unpublished data).

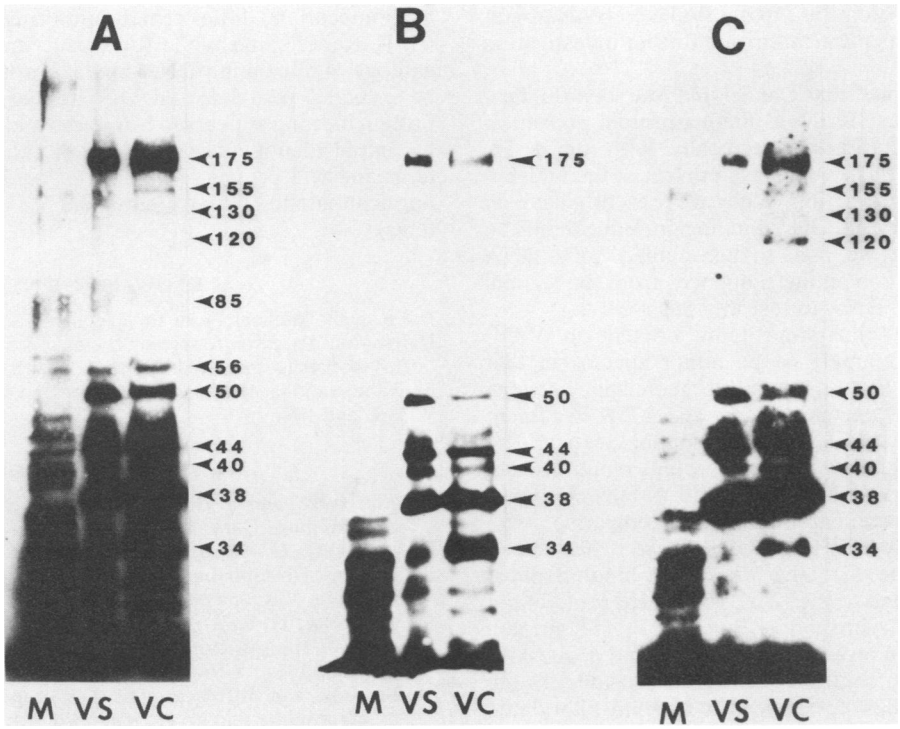

FIG. 8. Protein blot analysis of the binding of double-stranded VZV DNA compared with the binding of pBR322 sequences. (A) Results obtained by probing with VZV DNA; (B) lighter exposure of panel A allowing resolution of the bands less than 50K; (C) results obtained by probing an identical blot with pBR322 DNA.

Thus, the 175K DNA-binding phosphoprotein corresponds to the ^{35}S-labeled 175K protein described above.

The 175K phosphoprotein has been designated a tegument protein on the basis of its apparent location in the VZV virion (Roberts, Ph.D. thesis; Roberts et al., unpublished data). Six HSV-1 structural polypeptides (149K, 82K, 80K, 68K, 37.5K, and 12K) have been shown to be phosphoproteins (9, 13). All except the 37.5K and 12K species are believed to be contained in the tegument region of the HSV-1 virion (23, 24). The two lowest-molecular-weight proteins are present in nucleocapsids. Although none of these HSV-1 proteins has been specifically identified as a DNA-binding protein, they have, both as a class and individually, been described as basic (9, 13).

The VZV 175K protein may be similar to the viron basic phosphoproteins of human and simian cytomegaloviruses. These are 150K and 119K proteins, respectively. They are found in intact virions but not nucleocapsids, and both are highly phosphorylated. These proteins are also quite basic and as a result should be capable of binding negatively charged nucleic acids (W. Gibson, personal communication). Determination of the relationship of the 175K VZV protein to the cytomegalovirus basic virion proteins will await the development of appropriate immunological reagents.

The 155K DNA-binding protein is most likely the major VZV capsid protein. This protein bound to both single- and double-stranded-DNA–cellulose and was completely eluted only after washing with 1.0 M NaCl. In contrast, chromatography of mock-infected cell extracts indicated the presence of a 155K species which bound to double-stranded but not to single-stranded-DNA–cellulose columns and which was eluted by an 0.2 M NaCl wash. Confirmation of the fact that the 155K VZV major capsid protein was capable of binding DNA came from protein blot analysis. A 155K band was present in nucleocapsid lanes, indicating that it is an integral part of the VZV nucleocapsid structure. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of ^{35}S-labeled virions and nucleocapsids has shown that the major polypeptide present in nucleocapsids is a 155K species. The HSV-1 and HSV-2 major capsid proteins have also been reported to have some affinity for DNA (19).

The 125K DNA-binding protein appears to be analogous to the major 125K-to-130K DNA-binding proteins seen in other herpesvirus systems. This protein binds more strongly to single-stranded than to double-stranded DNA, and time course studies have shown that, like its proposed HSV-1–HSV-2 counterpart, the VZV 125K protein is synthesized relatively early after infection, with production significantly decreasing late in infection (Roberts, Ph.D. thesis; Roberts et al., unpublished data). Further comparison of the VZV 125K protein with HSV ICP8 will be pursued by (i) use of polyclonal and monoclonal antibodies and (ii) isolation of the VZV 125K protein and comparison of its DNA-binding properties with those of the HSV-1–HSV-2 ICP8. Such work is under way.

There does not appear to be a specific protein or proteins in the HSV-1–HSV-2 system similar to the 82K VZV major single-stranded-DNA-binding protein. This protein is nonstructural and is not readily identified in infected cell extracts. Major nonstructural single-stranded-DNA-binding proteins of HSV-1 and HSV-2 have been identified; these proteins, however, are present in detectable amounts in infected cell extracts, range from 40K to 42K, and are eluted at 0.3 M KCl or NaCl or both (19, 20). The VZV 82K protein, in contrast, was eluted at 0.6 M NaCl. The observed

enrichment of this protein by DNA-cellulose chromatography should facilitate its purification and further investigation of its properties.

It should also be noted that Dalziel and Marsden (8) have recently shown that a 21K DNA-binding protein present in HSV-infected cells interacts specifically with the a sequences of the viral DNA, which are present at the molecular termini and the L/S junction. While we presently have no evidence that the VZV 21K DNA-binding protein exhibits an analogous specificity, we plan to use cloned VZV DNA restriction fragments containing sequences from the termini and L/S joint of VZV DNA to test this possibility.

The DNA-binding phosphoproteins present in VZV-infected cells appear largely to be minor species in that ^{35}S-labeled proteins with comparable molecular weights were not seen. Two exceptions are the 175K tegument protein and, possibly, the 125K DNA-binding protein. The 125K-to-130K DNA-binding phosphoprotein binds to both single- and double-stranded-DNA–cellulose, but the apparent affinities of this protein are reversed in comparison with those of the ^{35}S-labeled 125K protein. These results could simply be due to the fact that the DNA-binding phosphoprotein is not related to the 125K ^{35}S-labeled protein, but it is also possible that phosphorylation of the 125K protein alters its DNA-binding properties. If this second proposal is correct, it would be in contrast to the data available for the HSV ICP8, which does not appear to be phosphorylated (28).

Similar difficulties with identification exist with the remainder of the phosphoproteins. The level of protein phosphorylation occurring in VZV-infected cells is much higher than that found in uninfected cells, and there is a finite possibility that some of the DNA-binding phosphoproteins found in infected cells represent phosphorylated host proteins. As a class, the VZV-specific phosphoproteins which interact with DNA do not appear to bind as strongly as do the proteins detected by ^{35}S labeling. A similar finding has been reported for phosphorylated and nonphosphorylated HSV-1 proteins (28).

Attempts at determining specificity of binding of some VZV DNA-binding phosphoproteins yielded interesting findings. The results obtained from probing protein blots with double-stranded VZV DNA show that major VZV DNA-binding proteins, such as the 175K and major capsid proteins, are readily detected by this technique. The protein blots also showed that a number of lower-molecular-weight VZV structural proteins also interact with DNA. One of these, the 50K species, has a molecular weight similar to that of HSV-1 VP19c, which has recently been identified as a DNA-binding protein (6). Results of the protein blot experiments in which VZV and nonhomologous DNA probes were compared indicate that there appear to be a number of infected-cell proteins (75K to 25K) which show a higher affinity for VZV DNA in this experimental system. Further work with filter binding or sedimentation techniques with individual proteins is required to fully document these apparent differences in affinity.

A consideration which must be borne in mind in regard to the protein blot experiments resides in the nature of the system itself. Successful DNA binding requires that the transferred proteins renature to a degree sufficient to reestablish their DNA-binding capacity. Some polypeptides do not appear to readily renature under the conditions used. An important example is the 125K major DNA-binding protein which showed little DNA binding in our experiments. The ICP8 protein of HSV-1 also shows little DNA binding and, by implication, little renaturation under our conditions (C. R. Roberts and W. T. Ruyechan, unpublished data). By analogy, multisubunit DNA-binding proteins would also not be expected to be detected. Despite these caveats, however, protein blotting appears to be a rapid and powerful technique for initial identification of infected cell DNA-binding proteins and will provide a number of guideposts for eventual purification and study of individual VZV DNA-binding proteins.

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