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SHORT COMMUNICATION

DNA BINDING PROTEINS INDUCED BY VARICELLA-ZOSTER VIRUS IN HUMAN CELLS

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DNA-binding proteins induced by varicella-zoster virus (VZV) were detected by native-and denatured-DNA affinity chromatography. Eleven DNA-binding proteins specific to the infected cells were separated by SDS polyacrylamide gel electrophoresis (PAGE); their molecular weights ranged from 180×10^3 to 22×10^3. When they were applied to an affinity column coupled with VZV-antibody, a polypeptide with a molecular weight of 34×10^3 was predominant on subsequent SDS-PAGE.

DNA-binding proteins induced by herpes simplex virus (HSV) have been isolated (Bayliss et al., 1975; Purifoy and Powell, 1976) and some of their biological properties with regard to DNA replication have been characterized (Powell et al., 1981). Furthermore, it has been reported that the major DNA-binding proteins of pseudorabies virus, equine abortion virus, bovine mammilitis virus and herpes simplex virus type 1 and 2 share common antigenic sites (Litter et al., 1981). Thus studies on the DNA-binding proteins of herpes group viruses seem important in elucidation of the replication and the immunological relationship of these viruses. This paper reports studies on DNA-binding proteins induced by VZV in human embryonic lung (HEL) cells.

The Kawaguchi strain of VZV (Takahashi et al., 1975) was used in the present study. HEL cells grown in plastic dishes (135 mm in diameter) were inoculated with VZV-infected cells at a ratio of 5:1 and labeled with ^{35}S-methionine (10 μCi/ml, 1220Ci/mmole, Radiochemical Centre, Amersham) in MEM medium depleted of methionine and containing 5% calf serum for 24 h after the appearance of 50% cytopathic change.

The labeled cells were washed three times with ice-cold phosphate buffered saline (PBS), collected with a rubber policeman and suspended in Tris buffer (20 m*M*, pH 7.5) containing 2 m*M* 2-mercaptoethanol and 500 μg/ml bovine serum albumin at a cell concentration of 10^7 cells/ml. The cell suspension was sonicated in an ultrasonic disruptor (Sharp) for 3 min in an ice bath and then mixed with an equal volume of 20 m*M* Tris

buffer (pH 7.5) containing 3.4 *M* NaCl and 10 m*M* EDTA and kept for 40 min in an ice bath. After centrifugation at 30,000 *g* for 20 min at 4 C, the supernatant was dialyzed overnight at 4 C against three changes of the column buffer (20 m*M* Tris pH 7.5, 50 m*M* NaCl, 1 m*M* EDTA, 2 m*M* 2-mercaptoethanol, 10% glycerol). The cell extract was then centrifuged at 100,000 *g* for 60 min at 4 C and the supernatant was subjected to DNA-cellulose chromatography. DNA-cellulose was prepared as described by Alberts and Herrick (1971) using salmon sperm DNA (Wako Pharmaceut. Co.). The column was washed extensively with the column buffer described above and then DNA-binding proteins were eluted with the same buffer containing increasing concentrations of NaCl of 0.15 *M*, 0.6 *M* and 2.0 *M*. The radioactivity of the fractions was determined and the peak fractions were collected and coprecipitated with calf serum with 5% trichloroacetic acid in an ice bath. The pellet was washed with ice-cold acetone and dried. The resultant pellet was resuspended in 1% SDS, 3% 2-mercaptoethanol, 10% glycerol, 0.01% phenol red and 50 m*M* Tris-HCl buffer (pH 8.2) and solubilized in boiling water for 3 min for analysis by SDS-PAGE. (Shiraki et al., 1982). Of the total radioactivity of the DNA binding proteins 77% was eluted at 0.15 *M* NaCl, 20% at 0.6 *M* NaCl and 2% at 2.0 *M* NaCl as sharp peaks of radioactivity (Fig. 1). The migration patterns of DNA-binding proteins of VZV-infected and mock-infected cells are shown in Fig. 2. Eleven DNA binding proteins specific to infected cells were identified and there was no significant difference in the elution patterns obtained by native-and denatured-DNA cellulose chromatography (data not shown).

On HSV infection, synthesis of host cell specific DNA binding proteins ceases immediately (" switch off "), and thus HSV-induced DNA binding proteins can readily be detected (Bayliss et al., 1975; Purifoy and

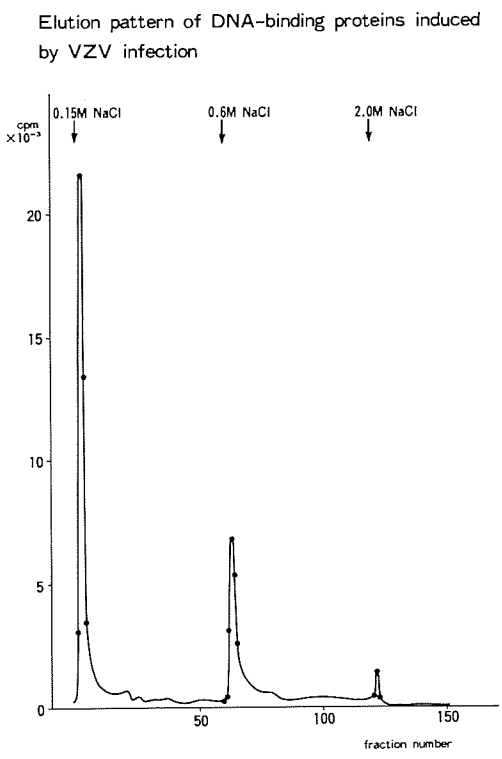

FIGURE 1. DNA-cellulose chromatography of proteins extracted from VZV-infected HEL cells.

Powell, 1976). However, since it was difficult to obtain VZ cell-free virus in high titer, a low input multiplicity of infected cells was used as inoculum, and this made it difficult to isolate VZV-induced DNA binding proteins. Subsequently, the DNA-binding proteins were applied to an affinity column coupled with VZV antiserum, which was prepared in green monkeys by repeated inoculation of VZV-infected green monkey kidney cells (Shiraki et al., 1982).

The affinity column was prepared as described previously (Shiraki et al., 1982). Briefly, activated Sepharose was suspended in antibody solution in BBS (30 m*M*-borate-buffered saline, pH 8.0) and the supension was gently mixed overnight in a cold room. Then the conjugated Sepharose was packed into small columns and washed with 0.05 *M*-

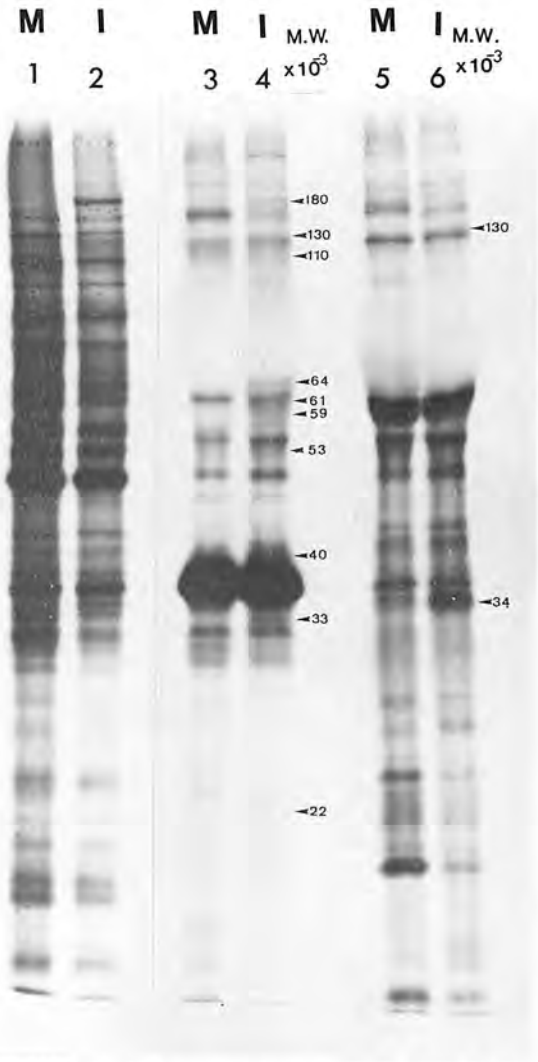

FIGURE 2. Autoradiography of DNA-binding proteins from extracts of mock-infected and infected cells eluted from a native DNA-cellulose column and subjected to electrophoresis in 10% polyacrylamide slab gel.

1. Extract of mock-infected cells before application to a DNA-cellulose column.
2. Extract of infected cells before application to a DNA-cellulose column.
3. Extract of mock-infected cells eluted from a DNA-cellulose column with column buffer containing 0.15 *M* NaCl.
4. Extract of infected cells eluted from a DNA-cellulose column with column buffer containing 0.15 *M* NaCl.
5. Extract of mock-infected cells eluted from a DNA-cellulose column with column buffer containing 0.6 *M* NaCl.
6. Extract of infected cells eluted from a DNA-cellulose column with column buffer containing 0.6 *M* NaCl.

I: Extract of infected cells

M: Extract of mock-infected cells

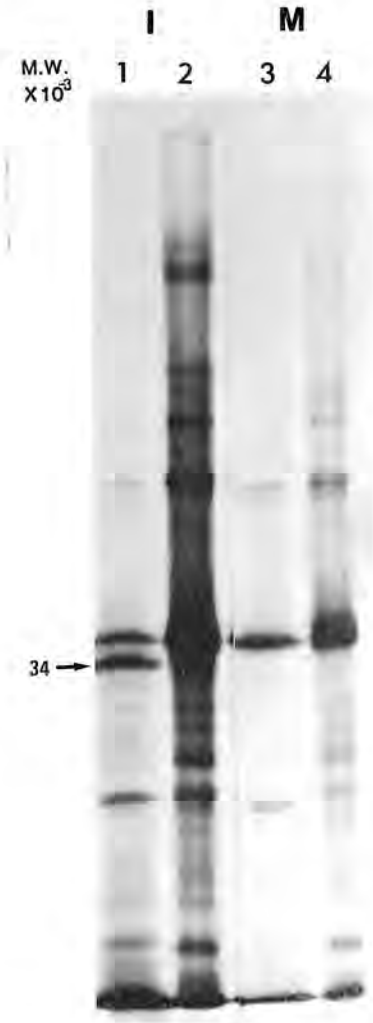

FIGURE 3. Autoradiography of DNA-binding proteins from extracts of infected and mock-infected cells eluted from a native DNA-cellulose column with 2 *M* NaCl and then applied to an affinity column coupled with VZV antibody and subjected to electrophoresis in 10% polyacrylamide gel.

1. Extract of infected cells eluted from a DNA-cellulose column and then applied to an affinity column coupled with VZV antibody.
2. Extract of infected cells eluted from a DNA-cellulose column before application to an affinity column coupled with VZV antibody.
3. Extract of mock-infected cells eluted from a DNA-cellulose column and then applied to an affinity column coupled with VZV antibody.
4. Extract of mock-infected cells eluted from a DNA-cellulose column before application to an affinity column coupled with VZV antibody.

I: Extract of infected cells
M: Extract of mock-infected cells

monoethanolamine in 10 m*M* borate buffer, pH 8.5, and finally washed with BBS. DNA-binding protein solution was applied to an affinity column, washed extensively with PBS and eluted with 3 *M* potassium thiocyanate in PBS. The eluted protein solution was concentrated by TCA precipitation and applied to SDS-PAGE.

As shown in Fig. 3, a polypeptide with a molecular weight of 34K was obtained predominantly by affinity column chromatography coupled with VZV antibody. Judging by comparison with the migration patterns of DNA binding proteins of mock-infected HEL treated similarly, other polypepitdes than the 34K polypeptide did not seem to be specifically bound and eluted.

It has been reported (Asano and Takahashi, 1980) that a virus specific polypeptide of 35K is synthesized in large amount soon after infection. The 34K DNA-binding protein detected in the present study seems to correspond to the 35K polypeptide detected in the previous study, but this requires confirmation. Other polypeptides than 34K polypeptide were not prominent, possibly because of their smaller amounts or lower antigenicities. Immunological studies on whether DNA binding proteins of VZV have common antigenic sites to those of other hereps group viruses will be pos sible when monoclonal antibodies to them are available.

Recently, an intranuclear DNA-binding protein (51K) was found in large amount in cells infected with cytomegalovirus, (Gibson et al., 1981). It is not certain whether 34K VZV DNA-binding protein is similar in biological

activity to the 51K cytomegalovirus DNA-binding protein. Studies on the properties of the 34K VZV DNA-binding protein are under way.

ACKNOWLEDGMENTS

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