DNA-Binding Properties of a Herpes Simplex Virus Immediate Early Protein

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The herpes simplex virus α , or immediate early, protein ICP4 has been shown to be central to the control of the early stages of virus replication. The detailed mechanism of this control is unknown. In this communication we show that purified ICP4 was unable to bind to DNA even though the protein was capable of such activity in a crude extract. Addition of either infected- or uninfected-cell extracts to the purified protein restored its DNA-binding activity. These results suggest that ICP4 binds to DNA only via a component of uninfected cells.

Herpes simplex virus (HSV) types 1 and 2 both induce the synthesis of more than 50 proteins in infected cells. The synthesis of these proteins occurs in a regulated fashion, the immediate early or α proteins being required before the synthesis of any other protein can occur (6, 7). One of the α proteins, ICP4 (ICSP 5-8, VP175, or Vmw175 [2, 6, 9, 11]), appears to have a central role in the switch from the early or α protein synthesis to the synthesis of β proteins. Evidence that ICP4 was involved in this switch came from studies of temperature-sensitive mutants such as tsB2, tsC75, tsLB2, TsT, TsD, and TsK. The lesions of all of these mutants lie in the sequences of repetitive DNA bounding the unique short region of the virus genome (3, 4, 12). All of these mutants are in various degrees defective in the switch from α to β protein synthesis. Thus, the properties of the ICP4 protein and the mechanism by which it brings about the α to β protein switch are of great interest.

We demonstrated previously (13) that the ICP4 protein, when present in crude extracts, binds to native or denatured DNA immobilized on cellulose. This observation has since been confirmed by others (1, 5, 14). Wilcox et al. (14) recently observed that the DNA-binding activity of ICP4 varied with its phosphorylation. The DNA-binding properties of ICP4 and the effects of mutations in the gene(s) encoding it suggested a role for this protein in the control of virus transcription. To investigate the DNA-binding activity of ICP4 in more detail, we decided to purify the protein and examine its properties. Since it is now clear that the large HSV-1 and HSV-2 immediate early proteins of >150,000 molecular weight are equivalent, we shall use the original nomenclature of Honess and Roizman (6) for the HSV-1 protein for the HSV-2

equivalent. Thus, in this paper, both the HSV-1 and HSV-2 proteins will be designated ICP4, and our earlier ICSP 5-8 nomenclature for the HSV-2 equivalent will be dropped.

The virus strains used (HSV-2 186 and HSV-1 HFEM) and the methods for cell culture, virus infection, and infected-cell harvest have all been described previously (10). Infected cells (5×10^9) were labeled with a mixture of ^{14}C -labeled leucine, isoleucine, and valine (0.5 $\mu\text{Ci/ml}$; Amersham International Ltd.) in the Glasgow modification of Eagle medium supplemented with 1% newborn calf serum and one tenth the normal concentration of the labeling amino acids. The infected cells were harvested at 5 h after infection, by which time the synthesis of ICP4 was ceasing (9). The infected cells were extracted with a high concentration of salt exactly as described by Powell and Purifoy (10). After dialysis against 20 mM Tris-hydrochloride (pH 7.5)–50 mM KCl–1 mM EDTA–0.5 mM dithiothreitol–0.2% Nonidet P-40–20% glycerol (DE buffer), the cell extracts were subjected to chromatography on denatured DNA-cellulose. The proteins which bound to DNA on the column were eluted by stepwise addition of 0.1, 0.3, and 1.0 M KCl in DE buffer. All fractions (1.0 ml) from the column were assayed for acid-insoluble radioactivity as previously described (10). The elution of ICP4 was detected by polyacrylamide gel electrophoresis of column fractions by a method described previously (9). ICP4 bound to the column and was eluted with 0.3 M KCl in DE buffer (Fig. 1). The fractions from the DNA-cellulose column containing the ICP4 protein were pooled and dialyzed against DE buffer. The partially purified ICP4 preparation was applied to a column of phosphocellulose prepared as described previously (10). Proteins bound to the column were then eluted by stepwise addition of

FIG. 1. Purification of ICP4 from HSV-2 186. ICP4 was purified by a combination of high salt extraction and chromatography on (A) a 0.6-cm-diameter by 9-cm denatured DNA-cellulose column, (B) a 1.5-cm-diameter by 9-cm column of phosphocellulose, and (C) a 0.6-cm-diameter by 6-cm denatured DNA-cellulose column. In each case the samples were applied to the column in DE buffer. The salt (KCl) concentration in the buffer used for elution are indicated with arrows at the top of each graph. The line indicates the position of isotope in fractions (●). The position of ICP4 in the eluates was as follows: (A) in the peak eluting with 0.3 M KCl, which contained about 7% of the protein applied to the column; (B) in the peak eluting with 1.0 M KCl, which contained about 3.4% of the protein applied to the column; (C) in the peak of material not bound to the column. In each case the recovery of label from the column was more than 90% of that applied.

DE buffer containing 0.1, 0.2, 0.3, 0.4, 1.0, and 1.5 M KCl. This mode of chromatography was chosen to avoid unnecessary dilution of the ICP4. Fractions (1.8 ml) eluting from the column were collected and assayed for acid-precipitable radioactivity and for the presence of ICP4. ICP4 bound strongly to the column and only eluted with high salt concentrations (1.0 M; Fig. 1 and 2).

ICP4 as recovered from the phosphocellulose column was almost free from contamination with other virus proteins (Fig. 2). The salt from the eluate was removed by dialysis against DE

buffer, and the dialyzed eluate was applied to a fresh column of denatured DNA-cellulose. It was found that the ICP4 no longer bound to the column and appeared in the break-through material from the column (Fig. 1 and 2). This result was unexpected, but was found whenever ICP4 was purified by phosphocellulose chromatography before DNA-cellulose chromatography. As the recovery of protein from this column was high (90%), the result could not be explained by ICP4 binding irreversibly to the column. If ICP4 had been denatured by the purification procedure such a result might have been expected. To rule out this possibility and to explore the mechanism of DNA binding by ICP4, we investigated the ability of ICP4 eluted in the unbound fraction from the DNA-cellulose column to bind to fresh DNA-cellulose columns in the presence of other infected-cell proteins. Extracts of infected or uninfected HEP-2 cells were prepared as described above with the omission of radiolabels. The cells were extracted with high salt concentrations, and the extracts were dialyzed against DE buffer. Purified ICP4 which had failed to bind to DNA-cellulose was then mixed with an equal volume of the extracts of either uninfected or infected HEP-2 cells. The artificial mixture of ICP4 and cell extract was then applied to a fresh column of denatured DNA-cellulose equilibrated in DE buffer. The column was washed with 0.05 and 0.1 M KCl in DE buffer, and the bound

FIG. 2. Polyacrylamide gel electrophoresis of samples containing ICP4. Samples: (A) whole-cell extract of HSV-2-infected cells; (B) fractions from the phosphocellulose column (Fig. 1, panel B) containing ICP4; (C) fractions not bound to the DNA-cellulose column (Fig. 3); (D) fractions bound to this column in the presence of uninfected-cell extracts; (E) fractions bound to an identical column in the presence of infected-cell extracts. In both D and E the samples were eluted with 1.0 M KCl. Closed arrows, ICP4; open arrows, a contaminating protein.

FIG. 3. Binding of ICP4 to DNA-cellulose in the presence and absence of cell extracts. ICP4 preparations from phosphocellulose columns were mixed with either infected- or uninfected-cell extracts as described in the text. After chromatography on denatured DNA-cellulose, the ICP4 protein did not bind to the column in the absence of extracts (A) but did in the presence of either uninfected- (B) or infected-cell extracts (not shown). The line indicates the position of radioisotope (●).

proteins were eluted with 1.0 M KCl in the same buffer.

The results (Fig. 3) showed that 52% of the purified ICP4 bound to the column in the presence of uninfected-cell extracts. Exactly the same results were obtained when the purified ICP4 was cochromatographed with infected-cell extracts. We estimate that the crude extracts used in these experiments approximated the concentrations found in the cells from which the pure ICP4 was derived. We have not been able to improve on the DNA binding by ICP4 by varying the amount of cell extract.

The methods described in this communication allow considerable purification of one of the herpesvirus immediate early proteins, ICP4. The method is rapid and relatively simple. The product, although not completely homogenous, represents a considerable improvement over unfractionated extracts. The purified protein should enable further studies to be made on the role of the protein in transcription in *in vitro* systems and should allow the preparation of monoclonal antibodies to the protein.

The DNA-binding properties of ICP4 have been the subject of several studies (1, 5, 13, 14). Although there are some minor discrepancies in these reports, all show the ability of ICP4 to bind to DNA. All of these previous studies involved crude preparations of ICP4. The purified protein in this study was found not to have DNA-binding properties. This result could imply that the ICP4 protein had been denatured during the purification process. Two lines of evidence suggest that this is not correct. The herpes simplex virus DNA polymerase enzyme, itself very thermolabile, survives a similar purification procedure without harm and without damage to its DNA-binding properties (K. L. Powell, unpublished observations). Furthermore, the addition of infected- or uninfected-cell extracts to the purified proteins resulted in restoration of their DNA-binding activities. Alternatively, we could have selected one of the forms of ICP4 (2) which has reduced DNA-binding properties (14). However, were this to be the case, we would not expect to be able to restore its DNA-binding ability simply by chromatography with cell extracts at 4°C.

The behavior of ICP4 in the DNA-binding experiments suggests that ICP4 may possess no inherent ability to bind DNA but may rather bind to other proteins in crude extracts. Such ability, although capable of overinterpretation, may, in the light of previous observations, reflect a role for the protein in the control of transcription through binding to RNA polymerase as has been suggested before (8, 11).

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