DOUBLE-STRANDED DNA BINDING OF ADENOVIRUS TYPE 12 TUMOR ANTIGEN

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1. Introduction

Hamsters carrying adenovirus type 12 (Ad12) induced tumors develop complement-fixing antibodies to virus-specific tumor (T) antigen that can also be detected in cells transformed in vitro or productively or abortively infected with Ad12 [1]. This protein that is synthesized in absence of viral DNA synthesis remains poorly biochemically characterized and has not yet been associated with any biological activity [2].

In adenovirus-infected cells two species of proteins binding to single-stranded DNA appear shortly before viral DNA synthesis [3]. Their association with a DNA replication complex may imply a role in DNA replication [4]. It was recently reported that the larger of these two proteins from Ad2-infected KB cells reacted with antibodies to Ad1 and Ad2 T antigen [5].

In a search for biological significance of Ad12 T antigen, we have attempted to establish whether or not T antigen corresponds to one of the Ad12-induced proteins with affinity for single-stranded DNA. We have found that Ad12-specific T antigen from tumor and transformed hamster cells does not bind to single-stranded DNA but has a strong affinity for double-stranded DNA.

2. Materials and methods

2.1. *Isolation of T antigen*

Two Ad12 T antigen-positive established hamster cell lines grown in roller bottles were used: the HT2 line of Ad12-induced tumor cells [6] and the T637 line of Ad12-transformed BHK21 cells [7]. For purification purposes a portion of the cells was labeled with a ^3H mixture of amino acids. The cells were broken by freezing and thawing and sonication in 10 mM potassium phosphate buffer, pH 7.4, containing 1 mM dithiothreitol. Following centrifugation at 15 000 *g*, the pellets were re-extracted. The combined supernatants were centrifuged for 60 min at 105 000 *g*. The T antigen was precipitated from the supernatant by ammonium sulphate at 35% saturation. This material is referred to as crude T antigen. It was further purified by hydroxylapatite chromatography [8] and then by a gel filtration on Sephadex G-100 in 10 mM potassium phosphate buffer, pH 7.4, and 1 mM dithiothreitol. Where needed, material was concentrated in an Amicon ultrafiltration chamber using membrane PM10. The entire procedure, which resulted in 150–200-fold purification, was carried out at 4°C. T antigen was measured by a micromodification of the complement fixation (CF) reaction in a final vol of 75 μl with 2 units of complement. After a 60 min incubation 25 μl of a 2% suspension of sensitized sheep erythrocytes was added. Hemolysis was estimated in a spectrophotometer at 413 nm. The antibody (ascitic fluid of tumor-bearing hamsters) to Ad12 T antigen was obtained from Flow Laboratories, Rockville, Maryland.

2.2. *DNA-cellulose chromatography*

DNA cellulose was prepared using purified native or denatured calf thymus DNA as described by Alberts and Herrick [9]. The columns with double-stranded and single-stranded DNA were equilibrated with 5 mM potassium phosphate buffer, pH 6.2, containing 1 mM dithio-

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threitol, 0.1 M NaCl and 10% glycerol [10]. The crude and purified T antigen preparations were applied to columns in the same buffer; DNA-binding material was eluted with 10 mM Tris–HCl buffer, pH 8.0, containing 0.6 M NaCl, 1 mM dithiothreitol and 10% glycerol.

3. Results

When a crude T antigen preparation was applied to a Sephadex G-100 column, all CF positive material eluted as a single sharp peak (fig.1A). No CF activity was detected in material eluting from the column after the T antigen peak, even after it was concentrated 20 times in an Amicon ultrafiltration chamber. Purified (200-fold) T antigen preparation eluted from the column in the same position (fig.1B). Complete recovery of CF activity from the column was observed.

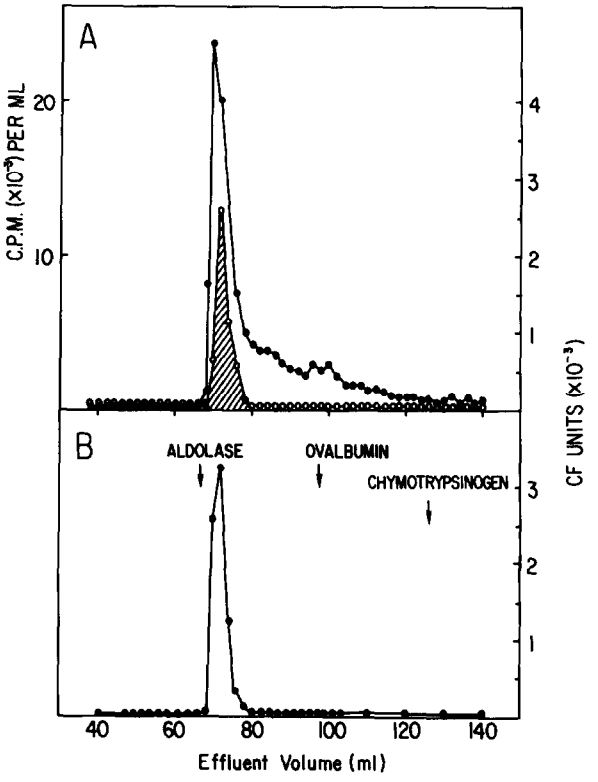

Fig.1. Gel filtration of Ad12 T antigen on Sephadex G-100. (A) Crude T antigen isolated from three roller bottles of HT2 cells was applied to Sephadex G-100 column (3 × 40 cm) and eluted at a flow rate of 5 ml/h as described in Materials and methods. (•) Radioactivity; (○) CF activity. (B) Purified T antigen (8000 CF units) from HT2 cells was applied to the column and eluted as described in A. One CF unit is arbitrarily designed here as 25 μl of the highest dilution of antigen that completely fixed complement. The arrows indicate the elution positions of the markers. (•) CF activity.

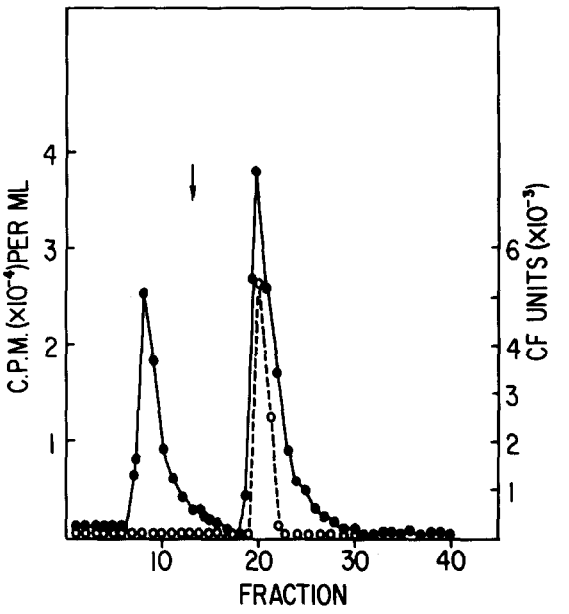

Fig.2. T antigen chromatography on double-stranded DNA cellulose column. Crude T antigen from T637 cells (8500 CF units) was applied to the column containing 4 g of double-stranded DNA cellulose and eluted as described in Materials and methods. Two ml fractions were collected. The arrow marks the switch of buffer. (•) Radioactivity; (○) CF activity.

The mol. wt of T antigen from both cell lines was estimated at approx. 80 000 from the elution position relative to those of six known markers.

When the crude T antigen from either HT2 or T637 cells was applied to the double-stranded DNA cellulose column at pH 6.2 in low salt concentration buffer, all T antigen CF activity was retained on the column (fig.2). It was eluted at pH 8.0 in 0.6 M NaCl. With purified T antigen, virtually all added material was found to be bound to double-stranded DNA (fig.3). Recovery of T antigen CF activity from the column under these conditions was almost quantitative, though loss of activity was observed when dithiothreitol was omitted from column buffers.

Application of the same material to a single-stranded DNA column under identical conditions showed that

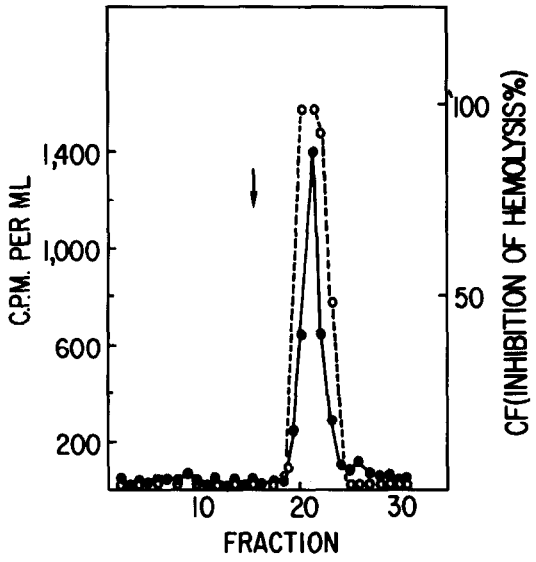

Fig.3. Double-stranded DNA cellulose column chromatography of purified Ad12 T antigen. 10 000 CF units of purified T antigen from HT2 cells were applied to the column. The arrow indicates the switch of buffer. (•) Radioactivity; (○) CF activity.

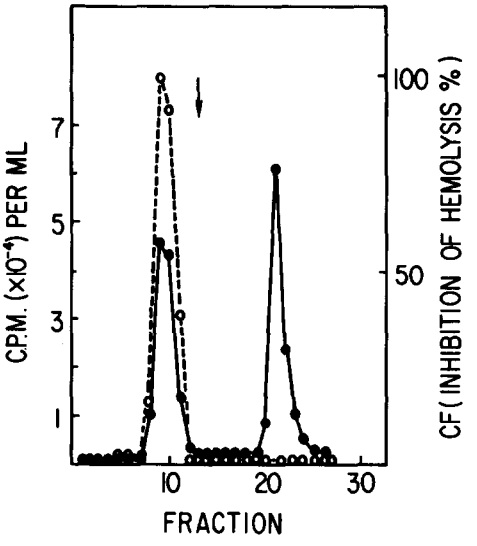

Fig.4. T antigen chromatography on single-stranded DNA cellulose column. Crude T antigen from T637 cells was applied to the column containing 4 g of single-stranded DNA cellulose and processed as described in Materials and methods. The arrow marks the switch of buffer. (•) Radioactivity; (○) CF activity.

all material with T antigen CF activity eluted in the pH 6.2, 0.1 M NaCl buffer (fig.4), indicating that no binding to single-stranded DNA had taken place. Similarly, no binding of T antigen to single-stranded DNA was observed under classical conditions at pH 8.1 [9].

4. Discussion

These results indicate that Ad12 T antigen from HT2 and T637 cell lines binds strongly to double-stranded DNA. This binding may be useful for future purification purposes.

Our estimate of the molecular weight of T antigen is in excellent agreement with that reported for the thermolabile component of T antigen in Ad12-infected KB cells [11]. The larger of the two Ad12-induced single-stranded DNA-binding proteins is smaller, with a mol. wt. of 60 000 [12]. The difference in size and the lack of affinity of T antigen for single-stranded DNA argues that these two proteins are not identical. The discrepancy between our results and the report that the larger Ad2-induced single-stranded DNA-binding protein has T antigen reactivity [5] may reflect the difference between the Ad2 and the Ad12 systems or different specificities of the antisera used.

T antigen is clearly distinct from the virus inhibitory factor (VIF) present in both cell lines used in this study [13]. VIF, a protein also of presumably viral origin, behaves differently during purification and does not bind to DNA (V. Rubio and W. A. Strohl, in preparation).

Simian virus 40 (SV40) T antigen has DNA-binding properties similar to those reported here [10] and is also of similar size [14]. Its binding to SV40 DNA at the origin of replication suggests direct involvement in the initiation of DNA synthesis [15].

Analogy to SV40 system which might suggest a similar biological role for Ad12 T antigen is consistent with the observation that inhibition of T antigen synthesis in Ad12-infected cells by cyclic AMP is associated with inhibition of induction of DNA synthesis [16].

Acknowledgements

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