IDENTIFICATION OF EARLY PROTEINS INDUCED BY HIGHLY ONCOGENIC HUMAN ADENOVIRUS 12 DURING LYTIC INFECTION AND IN HAMSTER TUMORS

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Summary: "Early" proteins (EP) induced by infection of cultured human KB cells with human adenovirus 12 (Ad12) were resolved by electrophoresis on sodium dodecyl sulfate (SDS)-polyacrylamide slab gels. Ad12 infected cells were incubated either in the presence of cycloheximide (CH) (to enhance the synthesis of early mRNA relative to cell mRNA) or cytosine arabinoside (ara C) (to prevent the expression of late viral genes) for 8 hr and the proteins were labeled with [^{35}S] methionine (met) in an isotonic (110 mM NaCl) or hypertonic medium (210 mM NaCl) in the presence of ara C. Seven Ad12 induced EPs with apparent molecular weights of 60,000 (60K), 16.5K, 15K, 13K, 12.5K, 11K, and 10K (EP1 to EP7, respectively) have been identified when infected cells were labeled in an isotonic medium with CH pretreatment or in hypertonic medium with or without CH pretreatment. Sera from hamsters bearing Ad12 induced tumors (Ad12 T antisera) immunoprecipitated at least four of these polypeptides.

INTRODUCTION

Adenovirus 12 (Ad12) is a well studied member of the "highly oncogenic" group (group A) of human adenoviruses. The factors responsible for the increased oncogenicity of Ad12 compared to members of group B and C have not been elucidated. Since the viral functions expressed in Ad12 induced tumor and in transformed cells are also expressed during "early" stages of lytic infection of permissive cells (2,9), isolation and characterization of early proteins may assist in understanding the increased oncogenic potential of Ad12. Detection of early proteins by in vivo labeling is hindered by the large background of host cell protein synthesis. In order to enhance the synthesis of early viral proteins relative to that of the host cell, we labeled infected cells both in hypertonic medium and after pretreatment with CH; these procedures were shown to reduce host protein synthesis in other virus-cell systems (1,6,7,15). In this report, we describe the identification of at least seven early polypeptides (EP) induced by Ad12 upon infection of KB cells. Ad12 tumor antiserum was found to contain antibodies to four of these EPs.

Abbreviations: CH, cycloheximide; DOC, sodium deoxycholate; PBS, phosphate buffered saline.

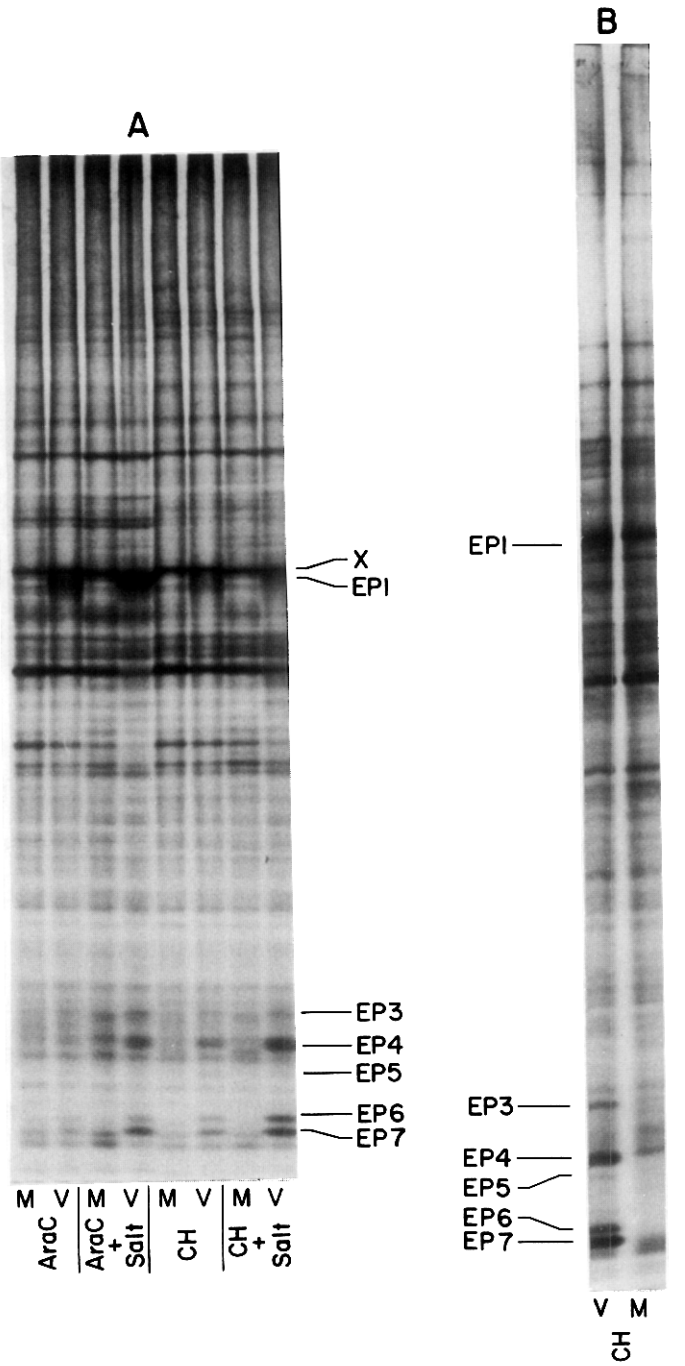

Fig. 1. Gel electrophoretic analysis of [^{35}S] met labeled polypeptides synthesized by Ad12 infected and mock infected KB cells. M and V indicate mock infected and virus (Ad12) infected cells, respectively. Infected and mock infected cells were incubated at 37° in the presence of 20 μg/ml of ara C or 25 μg/ml of CH for 8 hours. Cells were

Table 1. Molecular weights of Ad12 induced early polypeptides.

Polypeptide	MW
EP1	60,000
EP2	16,500
EP3	15,000
EP4	13,000
EP5	12,500
EP6	11,000
EP7	10,500

The molecular weights were determined (16) in a 15% SDS-polyacrylamide gel using the following standards: Ad2 hexon (120K), phosphorylase a (94K), bovine serum albumin (68K), Ad2 fiber (62K), Ad2 core (48,5K), ovalbumin (43K), Ad2 hexon associated polypetpides (24K), γ-globulin l-chain (23.5K), Ad2 major core (18.5K), Ad2 polypeptide 14.5K, lysozyme (14.3K), Ad2 polypeptide VIII (13K), and cytochrome C (11.7K). The relative mobility was calculated with reference to Ad2 virion polypeptide X.

MATERIALS AND METHODS

Virus infection and labeling. Suspension cultures of KB cells were infected with Ad12 (Huie, plaque 9, free of adenovirus associated viruses) at a multiplicity of 100 PFU/cell as described for Ad2 (5). Ad12 infected and mock infected cells were incubated in the presence of 20 μg/ml of ara C or 25 μg/ml of CH for 8 hours, were washed with met-free growth medium containing 20 μg/ml of ara C, and labeled with [^{35}S] met (200-250 μCi/mmol) for 3 hours in isotonic (110 mM NaCl) or hypertonic (210 mM NaCl) medium as described in the legend of Fig. 1. The labeled polypeptides were analyzed by electrophoresis on polyacrylamide gradient slab gels containing sodium dodecyl sulfate (6).

Radioimmune precipitation. Indirect radioimmune precipitation of Ad12 induced early proteins was performed as described previously (4) with modifications. In brief, infected or mock-infected cells were resuspended in a solubilizing buffer (20 mM Tris-HCl, pH 7, 10% glycerol, 50 mM NaCl, 5 mM EDTA, 1 mM β-mercaptoethanol, 1% DOC, 1% NP-40, and 0.8 M urea) and sonicated for 5 minutes at full power in a Raytheon DF-101 sonic oscillator. The sonicates were clarified at 12,000 X g for 15 minutes. Equal number of counts from infected and mock infected cell extracts were mixed with 100 μg of normal hamster IgG or IgG from the sera of hamsters bearing Ad12 induced tumors (a generous gift of Dr. R. Gilden). The mixtures were incubated at 4° for 17 hours, 45 μg of goat antihamster IgG (obtained from Huntington Research Center) was added, and further incubated for 2 hours. The precipitates formed were sedimented at 900 X g for 10 minutes, washed 3 X with PBS containing 1% DOC and 0.5% NP-40, and analyzed by gel electrophoresis as described (6).

then washed 3 times with met-free growth medium containing 20 μg/ml of ara C and labeled with [^{35}S] met (25-30 μCi/ml) for 3 hours in the presence of ara C. For labeling in hypertonic medium, cells were preincubated for 15 minutes in hypertonic medium (referred to as "+ salt") before the addition of ^{35}S-met. Labeled cell extracts were prepared for electrophoresis essentially as described (6). Fig. 1A and B are results of different experiments.

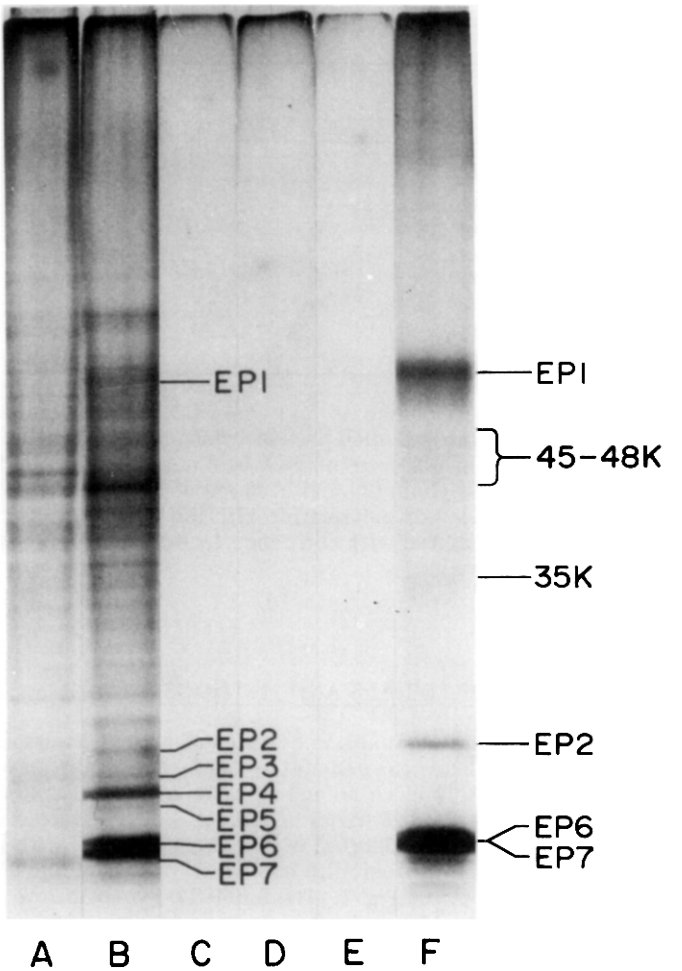

Fig. 2. Identification of Ad12 induced EPs immunoprecipitated by Ad12 T antiserum. Autoradiogram of SDS-polyacrylamide of cell extracts from Ad12 infected and mock infected KB cells before and after immunoprecipitation with normal hamster serum or Ad12 T antiserum. The various designations indicate the following: A, mock infected cells (no immunoprecipitation); B, Ad12-infected cells (no immunoprecipitation); C and D, mock infected and Ad12-infected cells, respectively immunoprecipitated with normal hamster serum; E and F, mock infected and infected cells immunoprecipitated with serum from hamsters bearing Ad12 induced tumors (Ad12 T antiserum).

RESULTS AND DISCUSSION

As shown in Fig. 1A when infected cells were labeled in a hypertonic medium with CH pretreatment (ara C + salt, Fig. 1A), or pretreated with CH and labeled in either isotonic (CH, Fig. 1A, B) or hypertonic medium (CH + salt, Fig. 1A), six polypeptides were observed in Ad12 infected cells that were not detected in mock infected cells.

The apparent molecular weights of these polypeptides are 60,000 (60K), 15K, 13K, 12.5K, 11K, and 10K (EP1, EP3 to EP7, Table 1). When the infected cells were labeled in an isotonic medium without CH pretreatment (ara C, Fig. 1A), only EP1 and reduced amounts of EP7 could be seen.

To identify EPs that are expressed in Ad12 induced tumor extracts of Ad12 infected cells pretreated with CH and labeled in hypertonic medium were immunoprecipitated with IgG prepared from sera of hamsters bearing Ad12 induced tumors (Ad12 T antisera). Ad12 T antiserum specifically precipitated EP1, EP6, and EP7 from [^{35}S] met labeled Ad12 infected cell extracts (Fig. 2, lane F), but not from mock infected cells (Fig. 2, lane E) (lanes A and B show labeled polypeptides from mock infected and uninfected cells prior to immunoprecipitation. An additional polypeptide, EP2 with apparent molecular weight of 16.5K, which could not be distinguished from a polypeptide of the same size seen in the extracts from mock infected cells in Fig. 1 was readily detected (Fig. 2). Normal rat IgG did not precipitate labeled proteins from mock infected or Ad12 infected cells (Fig. 2, lanes C and D).

EP1 may correspond to the early Ad12 induced polypeptide, ISCP-3, reported by Russel and Skehel (13), and to the Ad12 coded single stranded DNA binding protein (58-60K) identified by Rosenwirth et al (12). Polypeptides comparable to our EP2 to EP7 have not been previously reported. EP3 and EP5 are faint in Fig. 1A but could be seen clearly in Fig. 1B. EP6 and EP7 were always readily observed in cells labeled in hypertonic medium with or without CH pretreatment. In addition to the seven Ad12 induced early polypeptides that we have identified we also have observed faint bands of 45-47K and 35K region specific for Ad12 infected cells after immunoprecipitation with Ad12 T antiserum (Fig. 2). Because of the low amount of radioactivity present in these polypeptides more work is needed to establish their identity with certainty.

A combination of hypertonic medium and CH pretreatment appears to enhance the synthesis of low molecular weight polypeptides (EP3 to EP7) in Ad12 infected cells. The synthesis of EP1, however, is reduced by CH pretreated cells. The synthesis of some host polypeptides (e.g. X in Fig. 1A, ara C + salt, V) is selectively blocked in a hypertonic medium, but is not shut off in CH pretreated cells. Hypertonic medium

has been reported to inhibit the synthesis of cellular polypeptides relative to viral polypeptides in cells infected with RNA and DNA viruses (1, 7, 15). The mechanism by which hypertonic conditions selectively supress host protein synthesis is not known. However, it has been suggested that under hypertonic conditions, polypeptide chain initiation is more efficient in viral polyribosomes and in certain host polyribosomes than in the majority of host polyribosomes. Recently it has been shown that CH pretreatment enhances the synthesis of Ad2 induced EPs in vivo (6) and in vitro (14). It has been suggested that cycloheximide pretreatment may result in more efficient initiation of protein synthesis (8,11).

The total molecular weights of Adl2 EPl and EP3-7 is 138,500, about 12% of the coding capacity of the Adl2 genome (2). Saturation-hybridization analysis has revealed that 22% of the single strand viral genome equivalent is expressed in polyribosomes of Adl2 transformed hamster cells (3). Since viral genes expressed in Adl2 transformed cells are a subset of early genes, it seems likely that not all Adl2 induced early polypeptides are identified in the present study. We are analyzing Adl2 induced early proteins by two dimensional gels in order to detect additional early polypeptides.

The polypeptides precipitated by Adl2 antiserum are candidate transforming proteins based on the assumption that the transformed phenotype is maintained by the functioning of early viral coded protein(s). Although there is no proof for this assumption, the Adl2 EcoRI-C fragment which contains an Adl2 early gene block (10) can transform cells by transfection (F. Graham and S. Mak, personal communication). It is of further interest that candidate transforming proteins of 53K and 15K have been identified in Ad2 infected and transformed cells by radioimmune precipitation with antiserum to cells expressing mainly transforming genes (5).

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