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Comparison of the interactions of the adenovirus type 2 major core protein and its precursor with DNA

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ABSTRACT

The interactions of the major core protein of adenovirus type 2 (Ad2) protein VII, and its precursor, protein pre-VII, with viral DNA, were studied using UV light induced crosslinking of ^{32}P-labelled oligonucleotides to the proteins. Proteolytic fragments of these two proteins that contain DNA-binding domains were identified by virtue of their covalently attached, alkali-resistant ^{32}P-radioactivity. The overall efficiency of crosslinking of protein pre-VII to DNA, in H2ts1 virions assembled at 39°C, was comparable to that of the crosslinking of protein VII to DNA in Ad2 virions. However, a protease V8 fragment comprising the N-terminal half of protein pre-VII crosslinked to DNA at least ten times more efficiently than the corresponding N-terminal fragment of protein VII, which is truncated by the removal of 23 amino acids from the N-terminus of protein pre-VII during virion maturation.

INTRODUCTION

Adenoviruses encode a set of basic core proteins, V, VII and μ that package viral DNA within the virion (1 - 10). It is well established that protein VII is the major DNA-binding protein of the virion core (9-12, see reference 13 for a review) and that protein V forms an outer shell around the protein VII-DNA complex (7-9, 14, 15). By contrast, little consensus has been reached on the molecular structure of the core nucleoprotein (9, 10, 16 - 20, see reference 13 for a review). Nor has the nature of the nucleoproteins within which adenoviral DNA resides in infected cells, the templates for viral gene expression, been well defined, although several authors have suggested that cellular histones replace viral core proteins during the infectious cycle (17, 18, 21-23). Be that as it may, it is clear that viral DNA synthesised during the course of a productive infection must associate with the precursor (24) to the major core protein, pre-VII. Whether such association occurs before, or as a result of, virion assembly is not entirely clear (25-27, see reference 28 for discussion), although the final assembly intermediates, immature or young virions, contain both viral DNA and protein pre-VII (26, 29, 30). During the final stage of virion maturation, pre-VII and several other precursor proteins (24, 30, 31) are cleaved by the virion endopeptidase defined by the H2ts1 mutation (30,

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33). Such maturation cleavages are essential, for H2ts1 virions made at a non-permissive temperature are not infectious (30, 34, cited in 35). The failure of H2ts1 virion assembled at 39°C to support productive infection might stem, at least in part, from different interactions of protein pre-VII and protein VII with viral DNA. Indeed, it has been reported that such non-infectious H2ts1 virions do not complete a final uncoating step, loss of the core proteins (35).

We have recently developed a method to examine the interactions of the major adenovirus core protein with viral DNA. This approach relies upon covalent attachment of ^{32}P-labelled nucleotides (or oligonucleotides) to proteins by UV-light induced crosslinking and subsequent identification of those proteins carrying covalently attached nucleotides (36). In an attempt to learn more about the core protein-DNA interactions involved in the construction of adenovirions, and the significance of the protein pre-VII to protein VII cleavage, we have now extended this approach to protein pre-VII.

MATERIALS AND METHODS

Preparation of labelled viruses. HeLa cells in suspension culture were infected with 3pfu/cell adenovirus type 2 (Ad2) as described previously (37). Ten hours after infection at 37°C the cells were gently pelleted, washed with PBS and resuspended in 1/10th the initial culture volume of phosphate-free SMEM (GIBCO) containing 7mCi ^{32}P-orthophosphate (New England Nuclear, carrier-free). Cells were then incubated at 37°C and diluted two-fold every two hours with phosphate-free medium until the initial cell density was achieved, when 1/10th. the volume of normal medium was added. Cells were harvested 46 hours after infection and virus released and purified as described previously (15, 37). Labelled virions were prepared from H2ts1-infected cells maintained at 39°C in the same manner. When H2ts1-infected cells were maintained at 33°C, a permissive temperature, ^{32}P-labelled orthophosphate was added 18 hours after infection and cells were harvested after 4 days of incubation. Typical specific activites obtained under these conditions with all three viruses were on the order of 5×10^6 Cerenkov cpm/O.D. 260 unit, after purification by two cycles of sedimentation to equilibrium in CsCl gradients.

Irradiation Conditions. A 200 watt mercury arc lamp (Oriel Corporation) served as the irradiation source. As described in detail previously (36), this produced an intensity of radiation between 240 and 320 nm of approximately 150 milliwatts/sq.cm. at the position where samples were irradiated. Virions were irradiated directly upon purification.

Degradation of DNA with Formic acid/Diphenylamine. Crosslinked and uncrosslinked samples in cesium chloride were mixed with twice their volume of a solution of 2% diphenylamine in 90% formic acid (38). Samples were then incubated at 37°C for 20 hours, rather than 8 hours as

used in our previous experiments (36), a modification that improved the recovery of crosslinked protein VII displaying only small alterations in mobility in 12% polyacrylamide gels.

Analysis of crosslinked proteins. SDS-polyacrylamide gel electrophoresis (39), partial proteolysis (40), alkali treatment of gels and Coomassie blue staining have been described in detail previously (36).

RESULTS

Crosslinking of protein pre-VII to adenoviral DNA.

To examine the interactions of the precursor to protein VII with viral DNA we have taken advantage of the inactivation of the virion protease by the ts1 mutation of Ad2: virions assembled at 39°C, in H2ts1-infected cells, hereafter referred to as H2ts1-39°C virions, by contrast to wild-type virions or those produced at 33°C in H2ts1-infected cells, contain precursors to several virion proteins, including VII, VI, VIII, IIIa and the 55kd. terminal protein (30, 33). Virions labelled with ^{32}P-orthophosphate were prepared from Ad2-infected HeLa cells and from H2ts1-infected cells maintained at 33 or 39°C and purified as

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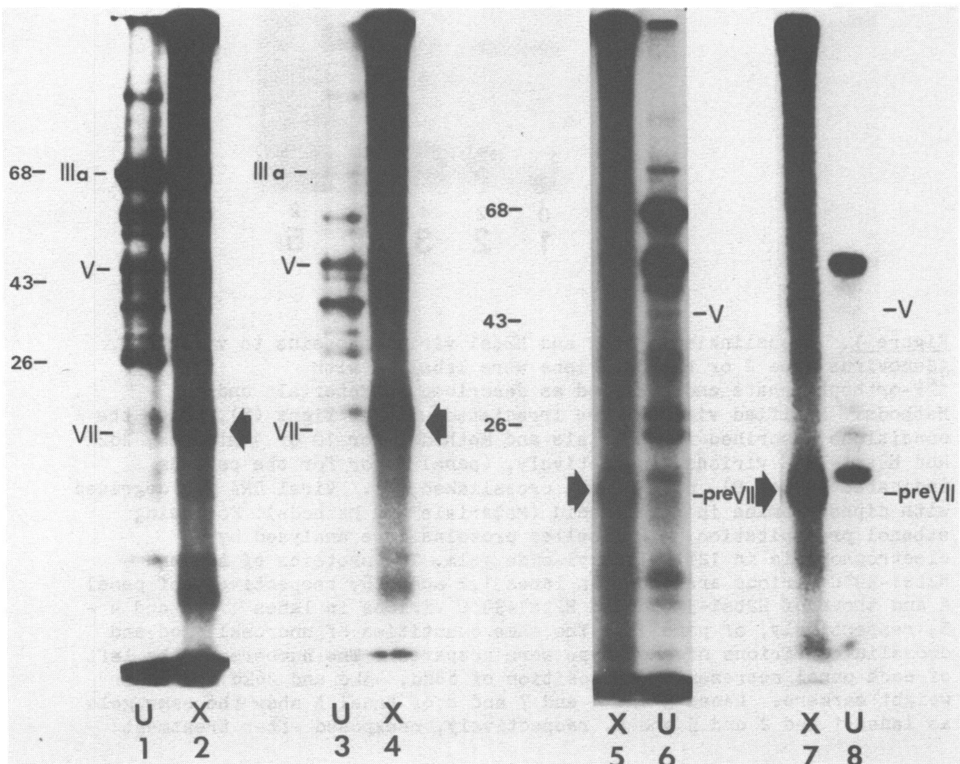


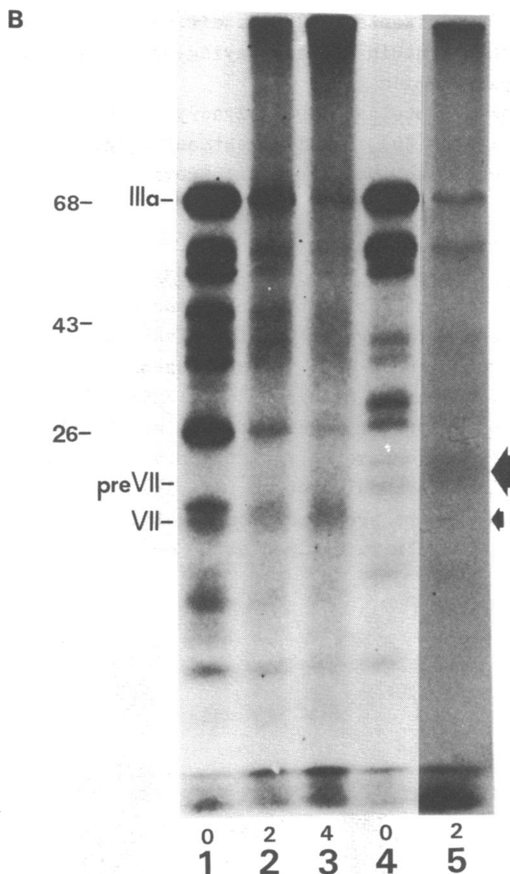


Figure 1. Crosslinking of Ad2 and H2ts1 virion proteins to viral DNA. Adenovirus type 2 or H2ts1 virions were labelled with ^{32}P-orthophosphate and purified as described in Materials and Methods. Purified virions were irradiated with UV light (X), under the conditions described in Materials and Methods, for 10 or 4 minutes, Ad2 and H2ts1-39°C virions, respectively, (panel A) or for the periods indicated (panel B), or were not crosslinked (U). Viral DNA was degraded with diphenylamine in formic acid (Materials and Methods). Following ethanol precipitation, ^{32}P-labelled proteins were analysed by electrophoresis in 12% polyacrylamide gels. The proteins of Ad2 and H2ts1-39°C virions are shown in lanes 1,2 and 5,6, respectively of panel A and those of H2ts1-33°C and H2ts1-39°C virions in lanes 1 - 3 and 4 - 5, respectively, of panel B. The same quantities of uncrosslinked and crosslinked virions of each type were compared. The numbers at the left of each panel represents the position of 68kd, 43kd and 26kd molecular weight markers. Lanes 3 and 4 and 7 and 8 of Panel A show the same gels as lanes 1 and 2 and 5 and 6, respectively, reexposed after treatment

with 1M NaOH. The major virion phosphoprotein, IIIa, the major core protein or its precursor, VII or pre-VII and the minor core protein, V, are indicated. The major, alkali-resistant, ^{32}P-labelled species induced upon crosslinking are marked by the arrowheads in each part of the figure.

described in Materials and Methods. Wild-type and mutant virions were irradiated with UV-light for various periods under the conditions described in Materials and Methods. Equal quantities of each unirradiated and irradiated sample were then diluted and treated with diphenylamine in the presence of formic acid at 37°C to degrade viral DNA (36). Labelled proteins were then examined by electrophoresis in 12% polyacrylamide-SDS gels followed by autoradiography. The results of a qualitative comparison of ^{32}P-labelled proteins recovered from uncrosslinked and crosslinked Ad2 and H2ts1-39°C virions are shown in Figure 1A. Many of the Ad2 virion proteins are phosphoproteins (41, 42) and can be seen in uncrosslinked samples (lane 1, Figure 1A). The pattern of phosphoproteins seen in H2ts1-39°C virions (lane 6, Figure 1A) did not correspond exactly to that of wild-type virions, for several proteins are replaced by their precursors in these mutant virions, as discussed previously. Novel, ^{32}P-labelled species, marked by the arrowheads in Figure 1A, appeared upon crosslinking of either Ad2 or H2ts1-39° C virions (lanes 2 and 5, respectively, Figure 1A). To confirm that these novel species indeed represented crosslinked proteins, rather than, for example, degradation products of higher molecular weight phosphoproteins, the gels were treated with 1M NaOH (36) and autoradiograms again prepared. The phosphoproteins of mature virions were largely alkali-labile (compare lanes 1 and 3, Figure 1A), as we have reported previously (36), as were those of H2ts1-39° C virions (compare lanes 6 and 8, Figure 1A). The samll quantities of ^{32}P-radioactivity associated with uncrosslinked protein VII or pre-VII were completely hydrolysed under these conditions (lanes 1, 3, 6 and 8, Figure 1A). By contrast, the novel ^{32}P-labelled proteins characteristic of crosslinked samples were largely alkali-resistant (compare lanes 2 and 4, and 5 and 7, Figure 1A).

The major crosslinked species recovered from Ad2 virions (lane 4, Figure 1A) has been shown to be generated as a result of crosslinking of protein VII to ^{32}P-labelled viral DNA (36). The corresponding, major crosslinked protein of H2ts1-39°C virions displayed a slightly lower mobility than protein pre-VII present in uncrosslinked virions (seen most clearly in lanes 4 and 5 and 6, Figure 1B). This shift in mobility was similar to that displayed by crosslinked protein VII (compare lanes 1 and 5, Figure 1B), suggesting that pre-VII was the protein most efficiently crosslinked to DNA when H2ts1-39°C virions were irradiated. The results obtained when crosslinked species of H2ts1-33°C and H2ts1-39°C virions

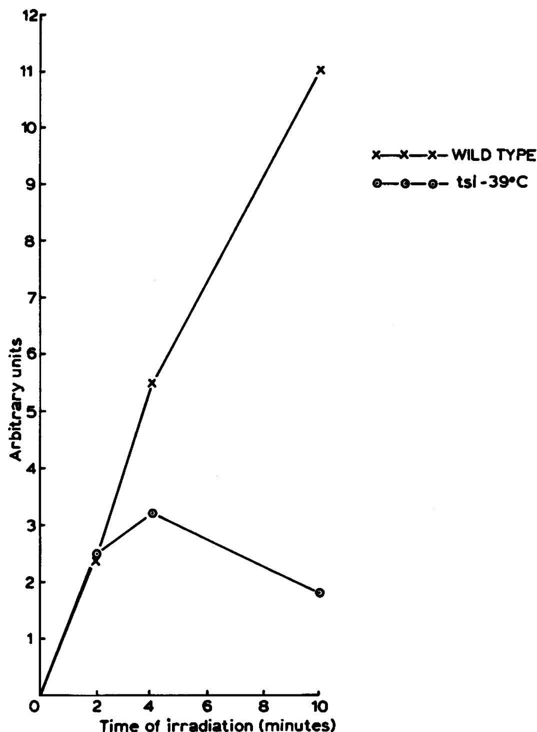


Figure 2. Efficiency of crosslinking of the major DNA-binding proteins of Ad2 and H2ts1-39°C virions. Purified Ad2 or H2ts1-39°C virions were exposed to UV light for the periods indicated in the figure. Crosslinked proteins from equal quantities of virions were analysed as described in the legend to Figure 1. The quantities of the major crosslinked species, marked by the arrowheads in Figure 1, were determined by scanning autoradiograms like those shown in Figure 1A, representing a range of exposures, using a BioRad model 620 video densitometer. The integrated areas under the relevant peaks are expressed as arbitrary units on the abcissa.

were compared are consistent with conclusion: the major crosslinked species obtained when H2ts1-33°C were irradiated was identical to that recovered from Ad2 virions (Figure 1B, lanes 1 - 3). In H2ts1-39°C virions, protein VII was replaced by pre-VII, also poorly labelled with ^{32}P-orthophosphate (lanes 1 and 4, Figure 1B) and upon UV-irradiation a new species, migrating slightly more slowly than pre-VII appeared (compare lanes 4 and 5, Figure 1B). Additional evidence confirming the identity of the crosslinked H2ts1-39°C protein marked with the arrowhead in Figure 1A, lane 7, is described in the next section.

A comparison of the yield of crosslinked protein VII and protein pre-VII species as a function of the dose of irradiation is shown in Figure 2. Purified, ^{32}P-labelled Ad2 or H2ts1-39°C virions were irradiated for increasing intervals and comparable quantities of each crosslinked sample analysed as in the experiment shown in Figure 1A. Autoradiograms of alkali- treated gels were scanned using a BioRad model 620 video densitometer and the integrated peak areas used as a measure of the quantity of ^{32}P-radioactivity associated with protein VII or protein pre-VII. The data obtained in this way (Figure 2) indicate that, at low doses, formation of protein VII to DNA crosslinks was directly proportional to the dose of irradiation. At the lowest doses tested, the efficiencies at which protein VII and its precursor were crosslinked to viral DNA were identical (Figue 2). However, as the dose of irradiation was increased, less crosslinked protein pre-VII than protein VII was recovered and by 10 minutes of crosslinking the quantity of ^{32}P-labelled protein pre-VII had decreased to a value below that observed after 2 minutes of irradiation (Figure 2). We do not believe this difference to be a result of a lower efficiency of crosslinking of protein pre-VII to DNA (see Figure 2), but rather the result of increased trapping of the protein pre-VII-DNA complex at the top of the gel or of the substantial change in the mode of DNA binding (see next section).

DNA-binding domains of protein VII and its precursor.

We have previously used partial proteolysis of crosslinked protein VII from Ad2 virions to confirm the identity of the major crosslinked species and to identify a domain of that protein that contained covalently attached nucleotides (or oligonucleotides) (36). We therefore wished to perform a similar analysis of the putative, crosslinked protein pre-VII obtained from H2ts1-39°C virions. Before examining core proteins ^{32}P-labelled as a result of crosslinking to viral DNA, however, we compared the products of partial protease V8 digestion of protein VII and its precursor.

A schematic representation of the sequences of these two proteins, indicating the locations of methionine and arginine residues and potential sites of protease V8 cleavage, is shown in Figure 3A. The products generated when ^{35}S-methionine labelled protein pre-VII from extracts of Ad2-infected cells harvested during the late phase of infection or ^{3}H-arginine labelled protein VII from purified Ad2 virions were digested _in situ_ with protease V8 are illustrated in Figure 3B. We had previously identified the protein pre-VII cleavage products exhibiting apparent molecular weights of approximately 9kd and 12kd. (lanes 1 and 6, Figure 3B) as fragments C and P (or H), respectively, (Figure 3A), on the basis of their apparent sizes and intensities of ^{35}S-methionine labelling (36). Protease V8 digestion of ^{3}H-arginine labelled protein VII liberated an approximately 12kd. fragment comigrating with the larger product of digestion of protein

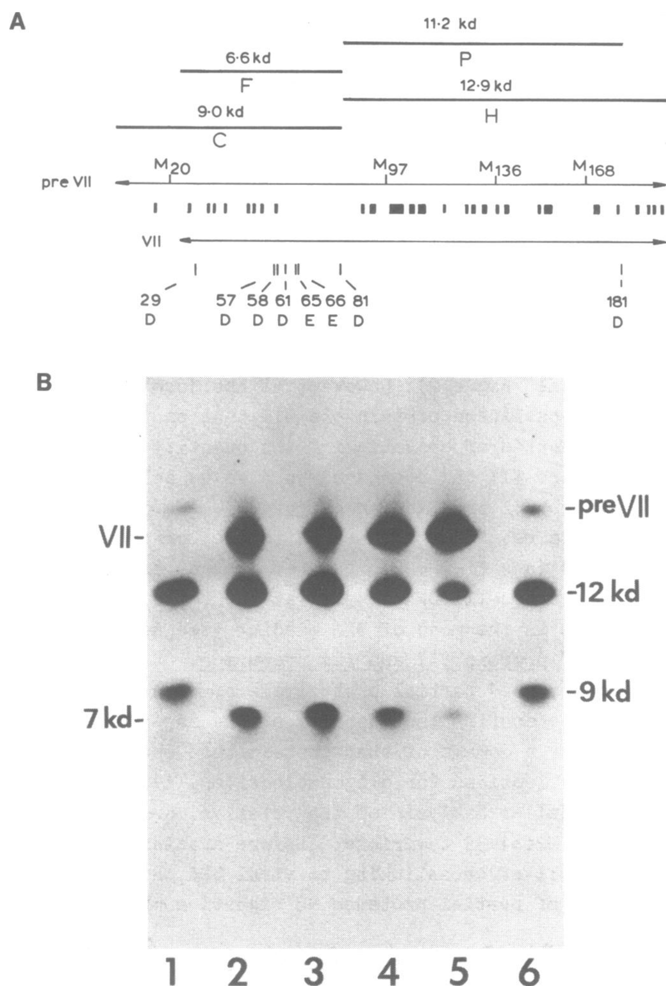


Figure 3. Protease V8 cleavage of protein VII and its precursor. Panel A shows linear representations of the sequences of proteins VII and pre-VII (44-46), in which the locations of methionine (M) and arginine (I ▮ ▮ I) residues are indicated. The vertical lines below the proteins indicate the positions of aspartic and glutamic acid residues, potential sites of protease V8 cleavage (48). The horizontal lines above the proteins indicate the protease V8 fragments observed and their calculated molecular weights.

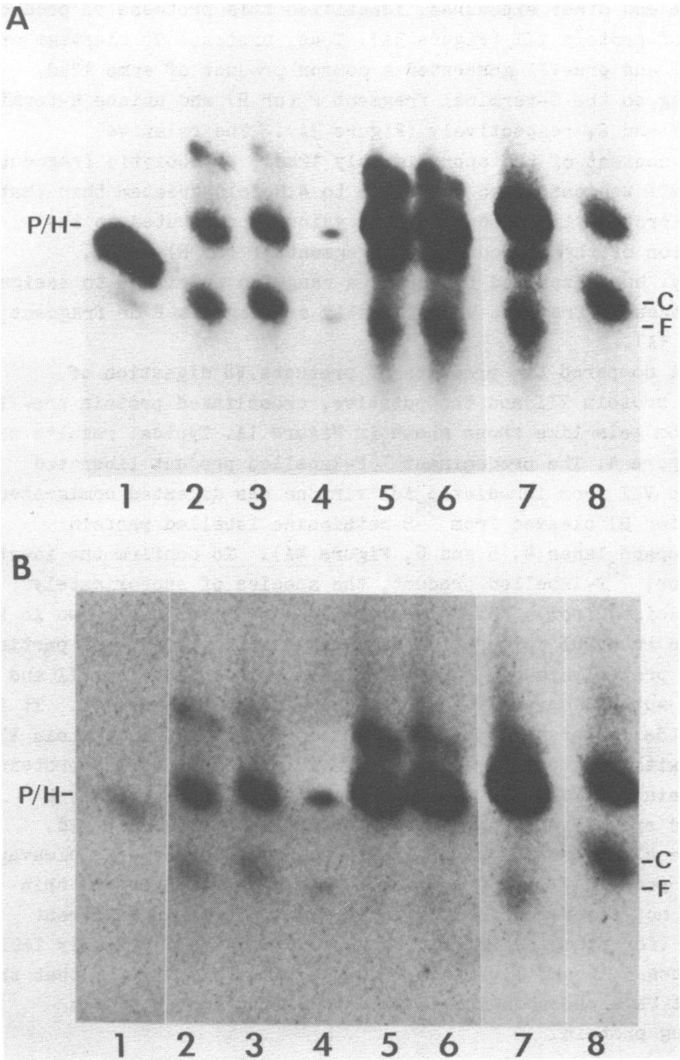
Panel B shows typical results of partial protease V8 proteolysis of ^{35}S-methionine labelled protein pre-VII and ^{3}H-arginine labelled protein VII. These proteins were cut from 12% polyacrylamide-SDS gels to which had been applied extracts of Ad2-infected cells labelled with ^{35}S-methionine at 17 to 19 hours after infection (lanes 1 and 6) or

^{3}H-arginine labelled Ad2 virions (lanes 2 to 5). The proteins were digested with 1(lane 5), 2 (lane 4) 3 (lane 3) or 4(lanes 1,2 and 6) µg/protease V8 during migration through a 3.5% stacking gel (41) and resolved by electrophoresis in a 15% polyacrylamide-SDS gel.

pre-VII, and a second fragment of some 7kd.(lanes 2-5, Figure 3B). The latter contains no methionine (lane 4, Figure 4C, 36). This property, the size of the smaller protease V8 cleavage product of protein VII and its arginine content relative to that of the approximately 12kd. fragment, which was estimated by densitometry of the autoradiogram shown in Figure 3B and other exposures, identified this protease V8 product as fragment F of protein VII (Figure 3A). Thus, protease V8 cleavage of proteins VII and pre-VII generated a common product of some 12kd. corresponding to the C-terminal fragment P (or H) and unique N-terminal fragments, F and C, respectively (Figure 3A). The relative ^{3}H-arginine content of the approximately 12kd. proteolytic fragment of protein VII was estimated to be 3.5 to 4.3-fold greater than that of the smaller proteolytic product. These values contributed to the identification of these products as fragments P (or H) and F, respectively, but displayed too broad a range to permit us to assign the larger protease V8 fragment unequivocally as fragment P or fragment H (see Figure 3A).

We next compared the products of protease V8 digestion of crosslinked protein VII and the putative, crosslinked protein pre-VII obtained from gels like those shown in Figure 1A. Typical results are shown in Figure 4. The predominant ^{32}P-labelled product liberated when protein VII from irradiated Ad2 virions was digested comigrated with fragment P (or H) cleaved from ^{35}S-methionine labelled protein pre-VII (compare lanes 4, 5 and 6, Figure 4A). To confirm the identity of this major, ^{32}P-labelled product, the species of approximately 12kd. was excised from a 15% polyacrylamide gel like that shown in Figure 4A and rerun in a 20% polyacrylamide gel with the products of partial protease V8 proteolysis of ^{35}S-methionine labelled proteins VII and pre-VII. An autoradiogram of such a gel is shown in Figure 4C. It is clear that the larger product of digestion of crosslinked protein VII comigrated with fragment P (or H) of ^{35}S-methionine labelled protein VII or protein pre-VII. In the experiment shown in Figure 4A, a ^{32}P-labelled species of slightly greater mobility than the 12kd. fragment was also observed among the products of protease V8 cleavage of crosslinked protein VII (lanes 5 and 6, Figure 4A). However, this species was not recovered in reproducible quantities in different experiments (for example, lane 7, Figure 4A) and was completely labile to 1M NaOH (lanes 5, 6 and 7, Figure 4B). We therefore believe that this species must be a phosphoamino acid-containing fragment from a contaminating protein.

The smallest product of protease V8 digestion of crosslinked protein VII exhibited the same size as fragment F (see Figure 3), that is, it migrated slightly ahead of fragment C of ^{35}S-methionine labelled protein pre-VII (lane 4, Figure 4A). The ^{32}P-radioactivity of this fragment was not eliminated upon alkali treatment, although the recovery of the labelled fragment was somewhat variable (compare lanes 5, 6 and 7 Figure 4B), probably the result of variable loss of the protein fragment itself during alkali treatment. Despite this complication, it is clear that fragment F of crosslinked protein VII retained a significantly greater proportion of its ^{32}P-radioactiviy than a marker



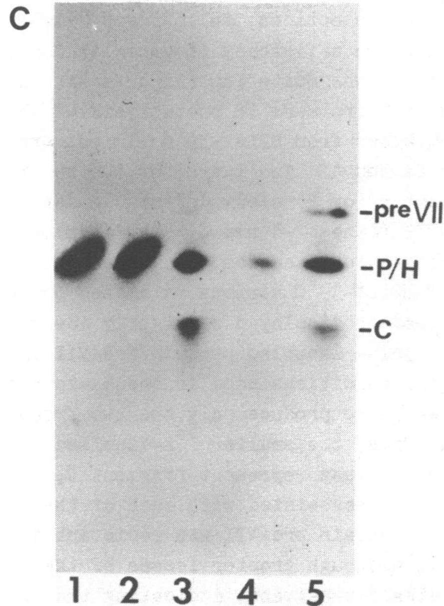


Figure 4. Protease V8 cleavage of the major crosslinked proteins of Ad2 and H2ts1-39°C virions.
Panel A. The major crosslinked, ^{32}P-labelled proteins of Ad2 (lanes 5 and 6) or H2ts1-39°C (lanes 2 and 3) virions were excised from gels like those shown in Figure 1A and digested in a second gel with 2 (lanes 2 and 5) or 4 (lanes 3 and 6) μg. protease V8, as described in the legend to Figure 3B. A ^{35}S-methionine labelled protein pre-VII marker was similarly digested in lane 4. Lanes 7 and 8 show a second example of protease V8 digestion of ^{32}P-labelled, crosslinked protein VII and pre-VII, respectively, from an independent experiment. The gels were not loaded with equal quantities of ^{32}P-labelled, crosslinked proteins VII and pre-VII. A 15kd phosphoprotein marker was applied to lane 1. Lanes 1 to 6 are from one gel and 7 and 8 are from a second. The polypeptides produced upon protease V8 digestion are designated according to the nomenclature given in Figure 3A.
Panel B shows an autoradiogram of the same gel as shown in panel A, made after treatment of the gel with 1M NaOH.
Panel C. The major product of protease V8 cleavage of ^{32}P-labelled protein VII, some 12kd., (lanes 1 and 2) was excised from a gel like that shown in panel A, and re-electrphoresed in a 20% polyacrylamide-SDS gel. Lanes 3 and 5 and lane 4 show protease V8 digested ^{35}S-methionine labelled protein pre-VII and protein VII, respectively.

phosphoprotein (compare lanes 1, 5, 6 and 7, Figures 4A and 4B). As the small quantities of ^{32}P-radioactivity present in uncrosslinked protein VII, and indeed uncrosslinked protein pre-VII, are completely alkali labile (Figure 1A, 36), this result indicated that protease V8 fragment F of protein VII (Figure 3A) also carried covalently bound,

^{32}P-nucleotides, or oligonucleotides, following UV light irradiation of Ad2 virions, although the efficiency of crosslinking of viral DNA to this region of the protein was quite low (Figures 4A and 4B). The results of similar partial protease V8 proteolysis of the major crosslinked protein obtained from H2ts1-39°C virions are shown in lanes 2, 3 and 8 of Figures 4A and 4B. The larger of the two products liberated comigrated with fragment P (or H) cleaved from ^{35}S-methionine labelled protein pre-VII (lanes 2-4 and 8, Figure 4A). This result, and those discussed in the previous section, indicate that the major crosslinked protein of H2ts1-39°C virions is indeed protein pre-VII. The smaller ^{32}P-labelled product displayed a slightly lower mobility than fragment C of ^{35}S-methionine labelled protein pre-VII (lanes 2-4 and 8, Figure 4A). Under the conditions used in these experiments, protease V8 cleaves protein pre-VII to produce only the two fragments C and P (or H) shown in Figure 3A. Thus, the smaller ^{32}P-labelled product seen in lanes 2 and 3 of Figure 4A must represent fragment C, modified in some way. The ^{32}P-radioactivity associated with each of the two protease V8 cleavage products of protein pre-VII was resistant to 1M NaOH (lanes 2, 3 and 8, Figure 4B), although greater losses of the smaller fragment were incurred during alkali treatment, indicating that each carried covalently bound nucleotides, or oligonucleotides, transferred from labelled viral DNA. A striking difference in the relative efficiencies of crosslinking of the N-terminal fragments of protein VII and pre-VII can be seen when the products of proteolysis of the crosslinked proteins are compared (lanes 5, 6, 7 and 2, 3 and 8, Figure 4A). Scanning of autoradiograms like that shown in Figure 4A established that the crosslinking of the N-terminal domain of protein pre-VII, fragment C, was almost as efficient as the crosslinking of fragment P (or H). By contrast, the corresponding N-terminal domain of protein VII was crosslinked at least ten-fold less efficiently than fragment P (or H).

DISCUSSION

We have recently described a new approach to the identification of proteins, and of protein domains, that contact DNA (36). This method takes advantage of the transfer of labelled nucleotides (or oligonucleotides) from DNA to proteins upon UV irradiation of nucleoproteins and the sensitivity to alkali of the common phosphoamino acids (43). In the experiments reported here, the recovery of crosslinked proteins displaying minimal alterations in mobilty, when compared to their uncrosslinked counterparts, has been improved by more extensive chemical degradation of labelled DNA. Such improved recovery has enabled us to identify a second DNA-binding domain of the major adenovirus core protein, protein VII, that crosslinks to viral DNA with one-tenth the efficiency, or less, (Figure 4) displayed by the major DNA-binding domain described previously (36). The minor DNA-binding domain identified here lies within the N-terminal fragment, F, produced upon protease V8

digestion of protein VII (Figure 3). Although this segment is quite arginine-rich, it contains no runs of arginine residues, like those present in protease V8 fragment P (or H) (Figure 3A). The most obvious structural feature of fragment F of protein VII is a strong α-helix, predicted to comprise residues 55 to 67 (44). It is unlikely that charge-charge interactions between arginine side chains and the DNA phosphate backbone would be detected by UV light induced crosslinking (see reference 50). We therefore believe that the crosslinking of N-terminal fragment F of protein VII to viral DNA is likely to be the result of an interaction of residues within the α-helix formed by amino acids 55 to 67 with the DNA.

The overall efficiencies of crosslinking of protein VII and its precursor to viral DNA are comparable (Figures 1 and 2). Nevertheless, a striking difference was observed when the crosslinking of protease V8 fragments of the two proteins to DNA was examined. The N- and C-terminal fragments of protein pre-VII were crosslinked to viral DNA at comparable efficiencies, in marked contrast to the approximately ten-fold greater efficiency of crosslinking of the C-terminal fragment P (or H) of protein VII (Figure 4). Thus, the relative efficiency of crosslinking of the N-terminal segment of protein pre-VII was at least ten-fold greater than that of the corresponding segment of protein VII. It is possible that the structure of H2ts1-39°C virions, containing several uncleaved precursor proteins (30,33), is sufficiently different from that of wild-type virions to induce the altered mode of DNA-binding displayed by protein VII. However, the N- and C-terminal fragments of protein pre-VII are also crosslinked to DNA with equal efficiencies when HeLa cells infected with H2ts1-39°C virions are irradiated (Pradeep K. Chatterjee, unpublished observations). As removal of the outer capsid proteins occurs in such mutant infected cells (35, Pradeep K. Chatterjee, unpublished observations), it seems likely that the altered DNA binding properties we have observed are intrinsic to protein pre-VII.

Protein pre-VII contains 23 amino acids at its N-terminus that are removed by the virion endopeptidase during virion maturation (see Figure 3A 30, 33, 44, 46). Secondary structure analysis (47) of the unique segment of protein pre-VII predicts no α-helical or strong β-sheet structures, nor does this region contain a segment rich in positively-charged residues (Figure 3A). It does, however, appear to contain a strong turn, (P_t 1.26-1.52) comprising residues 4 to 9. The N-terminal segment of protein pre-VII removed during virion maturation therefore contains no obvious DNA-binding domain. We cannot rule out the possibility that the unique N-terminal segment of protein pre-VII can be directly crosslinked to adenoviral DNA at high efficiency. Nevertheless, it seems more likely, in view of its structural features, that the N-terminus of protein pre-VII participates indirectly in DNA-binding, altering the conformation of the protein such that the helical region

comprising residues 55 to 67 adopts a position more favourable to its crosslinking to viral DNA upon UV irradiation. Such an altered conformation would also be consistent with the reduced stability of nucleoprotein cores released from H2ts1-39°C virions (11).

Although the molecular details of the interactions whereby proteins VII and pre-VII bind to adenoviral DNA have not yet been established, it is clear that the removal of the N-terminal 23 amino acids of pre-VII dramatically alters the interaction of the protein with DNA. Such an alteration has obvious biological consequences: virions that contain protein pre-VII, rather than protein VII, are not infectious (30, 34) and the viral DNA entering cells from pre-VII containing virions becomes degraded at about the time it would normally be replicated (P.Chatterjee, M.E.Vayda and S.J.Flint, submitted for publication). Thus, the change in the binding to viral DNA induced upon protein pre-VII maturation appears to be essential to the ability of the virus to initiate the productive cycle.

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REFERENCES

1. Laver, W.G., Pereira, H.G. Russell, W.C. and Valentine, R.C. (1968). J. Mol. Biol. 37, 379-386.
2. Maizel, J.V., Jr., White, D.O. and Scharff, M.D. (1968). Virology 36, 126-136.
3. Russell, W.C., Laver, W.G. and Sanderson, P.J. (1968). Nature 219, 1127-1130.
4. Russell, W.C., McIntosh, K. and Skehel, J.J. (1971). J. Gen. Virol. 11, 35-46.
5. Laver, W.G. (1970). Virology 41, 488-500.
6. Prage, L. Pettersson, U., Höglund, S., Lonberg-Holm, K. and Philipson, L. (1970). Virology 42, 341-348.
7. Everitt, E., Sundquist, B., Pettersson, U. and Philipson, L. (1973). Virology 62, 130-147.
8. Everitt, E., Lutter, L. and Philipson, L. (1975). Virology 67, 197-208.
9. Brown, D.T., Westphal, M., Burlingham, B.T., Winterhoff, U. and Doerfler, W. (1975). J. Virology 16, 366-387.
10. Vayda, M.E., Rogers, A.E. and Flint, S.J. (1983). Nuc. Acid. Res. 11, 441-460.
11. Mirza, M.A. and Weber, J. (1977). Virology 80, 83-97.
12. Sato, K. and Hosokawa, K. (1981). Biochem. Biophys. Res. Comm. 101, 1318-1323.
13. Nermut, M.V. (1984). In 'The Adenoviruses', Ginsberg, H.S., ed. pp5-34. Plenum Press, New York.
14. Nermut, M.V. (1979). Archiv. Virol. 62, 101-116
15. Chatterjee, P.K., Vayda, M.E. and Flint, S.J. (1985). J. Virol. 55, 379-386.

16. Corden, J., Engelking, H.M. and Pearson, G.D. (1976). Proc. Natl. Acad. Sci. USA 73, 401-404.
17. Tate, V. and Philipson, L. (1979). Nuc. Acid. Res. 6, 2769-2785.
18. Sergeant, M., Tigges, M.A. and Raskas, H.J. (1979). J. Virology 29, 888-898.
19. Nermut, M.V. (1980). Archiv. Virol. 64, 175-196.
20. Mirza, A. and Weber, J. (1982). Biochim. Biophys. Acta. 696, 76-86.
21. Corden, J.L. and Pearson, G.D. (1979). Abstr. ICRF Tumor Virus Meeting on SV40, Polyoma and Adenoviruses, Cambridge, p70.
22. Brown, M. and Weber, J. (1980). Virology 107, 306-310.
23. Daniell, E., Groff, D.E. and Fedor, M.J. (1981). Mol. Cell. Biol. 1, 1094-1105.
24. Anderson, C.W., Baum, P.R. and Gesteland, R.F. (1973). J. Virol. 12, 241-252.
25. D'Halluin, J-C, Martin, G.R., Torpier, G., and Boulanger, P.A. (1978). J. Virol. 26, 357-363.
26. Edvardsson, B., Everitt, E., Jornvall, H., Prage, L., and Philipson, L. (1976). J. Virol. 19, 533-547.
27. Edvardsson, B., Ustacelebi, S., Williams, J. and Philipson, L. (1978). J. Virol. 25, 641-651.
28. Philipson, L. (1984). In 'The Adenoviruses', Ginsberg, H.S., ed. pp309-337. Plenum Press, New York.
29. Ishibashi, M. and Maizel, J.V. (1974). Virology 57, 409-424.
30. Weber, J. (1976). J. Virol. 17, 462-471.
31. Challberg, M.D., Desiderio, S.V. and Kelly, T.J., Jr. (1980). Proc. Natl. Acad. Sci. USA 77, 5105-5109.
32. Boudin, M.L., D'Halluin, J-C., Cousin, C. and Boulanger, P. (1980). Virology 101, 144-156.
33. Tremblay, M.L., D'ery, C.V., Talbot, B.G. and Weber, J. (1983). Biochim. Biophys. Acta. 743, 239-245.
34. Begin, M. and Weber, J. (1975). J. Virol. 15, 1-7.
35. Mirza, M.A.A. and Weber, J. (1979). 30, 462-471.
36. Chatterjee, P.K., Vayda, M.E. and Flint, S.J. (1986). J. Mol. Biol. (in press).
37. Flint, S.J., Gallimore, P.H. and Sharp, P.A. (1975). J. Mol. Biol. 96, 47-68.
38. Kunkel, G.R. and Martinson, H.G. (1978). Nuc. Acid. Res. 5, 4263-4271.
39. Laemmli, U.K. (1970). Nature 227, 680-685.
40. Cleveland, D.W., Fischer, S.G., Kirschner, M.W. and Laemmli, U.K. (1977). J. Biol. Chem. 252, 1102-1106.
41. Russell, W.C. and Blair, G.E. (1977). J. Gen. Virol. 34, 19-35.
42. Axelrod, N. (1978). Virology 87, 366-383.
43. Cooper, J.A. and Hunter, T. (1981). Mol. Cell. Biol. 1, 165-178.
44. Sung, M.T., Cao, T.M., Coleman, R.T. and Budelier, K.A. (1983). Proc. Natl. Acad. Sci. USA 80, 2902-2906.
45. Sung, M.T., Lischwe, M.A., Richards, J.C. and Hosokawa, K. (1977). J. Biol. Chem. 252, 4981-4987.
46. Alestrom, P., Akusjarvi, G., Lager, M., Yeh-Kai, L. and Pettersson, U., (1984). J. Biol. Chem. 259, 13980-13985.
47. Chou, P.Y. and Fasman, G.D. (1978). Annual Rev. of Biochem. 47, 251-276.
48. Drapeau, G.R., Boily, Y. and Houmard, J. (1972). J. Biol. Chem. 247, 6720-6726.
49. Kyte, J. and Doolittle, R.F. (1982). J. Mol. Biol. 157, 105-132.
50. Wang, S.Y. (1976). Photochemistry and Photobiology of nucleic acids Volume I and II. Academic Press, Inc., New York.