JOURNAL OF VIROLOGY, Aug. 1985, p. 379–386
0022-538X/85/080379-08$02.00/0

Vol. 55, No. 2

Interactions Among the Three Adenovirus Core Proteins

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Received 21 August 1984/Accepted 19 April 1985

Interactions among the three adenovirus core polypeptides V, VII, and μ were examined, using the reversible chemical cross-linker dithiobis(succinimidyl propionate) and two-dimensional polyacrylamide gel electrophoresis. Cross-linked species obtained from gradient-purified adenovirus type 2 cores were well represented among the cross-linked products of pentonless virions and crude core preparations. The more efficiently formed cross-linked core species were also identified with the arginine-specific cross-linker, *p*-azidophenyl glyoxal. In addition to dimers of polypeptides V and VII, efficient cross-linking of V to VII, V to μ, and VII to V to μ was detected in adenovirus cores. Notably absent were cross-linked species corresponding to higher multimers of polypeptide VII. A major core-capsid interaction appeared to be via the association of polypeptide V with a dimer of polypeptide VI.

The nucleoprotein core of human subgroup C adenoviruses contains the linear, double-stranded DNA genome (36.6 kilobases) bearing the covalently linked 55,000-dalton (55 kDa) terminal protein at each 5′ end (10, 25, 26). Within the core, the viral DNA molecule is condensed some 350-fold, presumably the result of the presence of about 1,200 copies of the major core protein, VII, some 200 copies of protein V, and an unknown number of copies of polypeptide μ (8, 14, 23, 24, 27, 31). These core proteins are virus specific, rich in arginine and alanine, and with the exception of V, deficient in lysine (14, 23, 28, 29).

Nucleoprotein cores can be isolated in good yield from purified adenovirions by sucrose gradient centrifugation once the outer shell of capsomers has been stripped by exposure to acetone or pyridine (14, 24). Such purified nucleoprotein cores are inherently unstable and susceptible to rearrangement or aggregation or both when exposed to chemical or enzymatic manipulations (3, 31; Michael E. Vayda and S. J. Flint, manuscript in preparation). Thus, the absence of a full biochemical description of the core structure is not surprising. It has been established that polypeptide VII is most tightly bound to viral DNA (3, 31), but the details and consequences of protein-DNA and protein-protein interactions within the core remain poorly understood (see reference 31 for a discussion). Nevertheless, several models of core structure, based for the most part upon stoichiometric considerations, electron microscopic examination of cores, and analogy with nucleosome structure, have been proposed (3, 5, 18, 20), in some cases with strikingly different conclusions (3, 5, 18).

As one of several approaches to elucidation of the organization and molecular structure of the nucleoprotein core of adenovirions, we carried out nearest-neighbor analyses with a cleavable protein cross-linker. The results of such studies are presented here.

MATERIALS AND METHODS

Cells and virus. HeLa cells were maintained in suspension culture at 2×10^5 to 4×10^5 cells per ml in minimum essential medium for suspension cultures (GIBCO Laboratories) supplemented with 5% calf serum (Flow Laboratories, Inc.). Adenovirus type 2 or type 5 were propagated by low multiplicity of infection (1 to 5 PFU per cell) of such cells. Stocks were prepared as described previously (2, 9) and titrated on HeLa cells by the method of Williams (32). To prepare labeled virions, HeLa cells in suspension were concentrated to 1/10 the initial culture volume and infected with 40 PFU of adenovirus type 2 or type 5. After adsorption for 1 h, the culture was diluted to its original volume with fresh medium and incubated at 37°C for 18 to 20 h. The cells were then collected by centrifugation, suspended in 1/10 the original culture volume of RPMI 1640 without arginine (RPMI-1640 Select-Amine, GIBCO) containing 20 μCi of [^{3}H]arginine (15 to 30 Ci/mmol; New England Nuclear Corp.) per ml, and incubated at 37°C. One hour later, cultures were diluted with an equal volume of RPMI 1640 without arginine, and after incubation at 37°C for a further 3 h, they were diluted fivefold with the same medium. Six hours later, cultures were diluted to their original volume with normal medium. Cells were harvested 12 to 14 h later. Virus was released from the infected cells by repeated cycles of freezing and thawing and purified by centrifugation to equilibrium at least twice in CsCl gradients (17).

Preparation of cores. Cores were released from purified, pentonless virions by exposure to 10% pyridine and purified in 15 to 25% sucrose gradients by the method of Prage et al. (24) as described previously (31) except that the buffer used in gradients was 10 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] (pH 6.8). Crude core preparations were made by dialysis of pyridine-disrupted virions against four changes of 10 mM PIPES buffer (pH 6.8).

Cross-linking. Dithiobis(succinimidyl propionate), obtained from Pierce Chemical Co., was used in a majority of the cross-linking experiments. This was dissolved to 100 mM in dimethyl sulfoxide (DMSO) at room temperature. The solution was then diluted 100-fold at room temperature with continuous stirring into 5 mM PIPES buffer (pH 6.8) containing 10 mM NaCl and 1% Triton X-100. Freshly prepared cross-linker solution was added directly in a fivefold volume excess to the pooled gradient fractions, pentonless virions, or crude core preparations in 5 mM PIPES buffer (pH 6.8). The time of incubation at 21°C in the presence of the cross-linker ranged from 40 s for adenovirus cores to 2 min for pentonless virions, depending upon the degree of cross-linking desired. At the end of this period, excess cross-linker was quenched by addition of ammonium acetate to 2 mM,

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and free sulfhydryl groups in the proteins were blocked by addition of *N*-ethylmaleimide (Pierce Chemical Co.), dissolved in DMSO, to a final concentration of 2 mM. Phenylmethylsulfonyl fluoride was added to a final concentration of 1 mM, and sodium acetate was added to a final concentration of 0.2 M. Cross-linked and uncross-linked but similarly treated samples were then ethanol precipitated. The precipitates were treated with *N*-ethylmaleimide in DMSO and dissolved in 50 mM Tris-hydrochloride (pH 6.8) containing 2% sodium dodecyl sulfate and 5 mM *N*-ethylmaleimide. The samples were incubated in boiling water for 1 min and immediately loaded onto polyacrylamide gels.

Protein-protein cross-linking with *p*-azidophenyl glyoxal was performed by incubation of the sample in 10 mM sodium borate (pH 8.3) containing 1% Triton X-100 and 5 mM cross-linker (dissolved in DMSO) in the dark for 30 min at room temperature, a method modified from that of Politz et al. (22). Half of the sample was then irradiated for 5 min with light from a 200-W mercury arc lamp with a 5-mm-thick borosilicate glass plate to remove UV light of wavelength less than 320 nm. The other half of the unirradiated sample kept in the dark served as the uncross-linked control. Samples were precipitated with ethanol and prepared for electrophoresis as described above.

One- and two-dimensional polyacrylamide gel electrophoresis. All gels were cast and run by the method of Laemmli (13) except for the omission of β-mercaptoethanol or other reducing agents in first-dimension gels. First-dimension tube gels varied in composition from 8 to 12% acrylamide and contained bis-acrylamide to acrylamide at a ratio of 1:50. After electrophoresis, these gels were equilibrated for 1 h (8% gels) or 2 h (12% gels) in 150 mM Tris-hydrochloride (pH 6.8) containing 0.2% sodium dodecyl sulfate, 2% β-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride at room temperature with gentle shaking. They were then positioned on the second-dimension gel with 0.8% agarose containing the same buffer. Two-dimensional gels were prepared for autofluorography by soaking in PPO (2,5-diphenyloxazole)-DMSO and one-dimensional gels were soaked in En^{3}Hance as described previously (2, 4).

To determine the relative amounts of V, VII, and μ in core peaks, autoradiograms made by exposure of dried gels were traced with a Joyce-Loebel double-beam recording microdensitometer. The signals given by polypeptides II and VI in different exposures of the same lane were used as internal standards to relate the relative amount of μ measured in long exposures to those of V and VII measured in short exposures.

RESULTS

Nucleoprotein cores were released from purified virions by exposure to pyridine and purified in 15 to 25% sucrose gradients as described in Materials and Methods. Gradient fractions from core peaks like that shown in Fig. 1A were pooled into three classes (a, b, c), and equal portions of each were subjected to electrophoresis in 17% sodium dodecyl sulfate-polyacrylamide gels to identify low-molecular-weight components. It is clear from lanes a, b, and c of the insert in Fig. 1A that polypeptides VII, V, and μ were the major protein constituents of gradient-purified nucleoprotein cores, and capsid proteins were concentrated towards and at the top of gradients, as expected on the basis of earlier work (24, 31). The leading edge of the core peak, fraction a in Fig. 1A, where lane a is shown overexposed for the major core proteins, contained considerably less contaminating hexon than the trailing edge, fraction c.

Autoradiograms like that shown in the insert of Fig. 1A were used to estimate the molar ratio of polypeptide μ to polypeptide V, and lighter exposures were used to measure the VII-to-V ratio, using the amounts of II and VI as internal standards. The complete sequences of the adenovirus type 2 genes encoding the two larger core polypeptides have been determined (1, 28; M. Sung, personal communication) and that of polypeptide VII has been confirmed by partial protein sequencing (15, 28, 29). These two proteins contain 41 and 48 arginine residues, respectively. Although the gene encoding polypeptide μ has not been identified experimentally, the protein has been shown to comprise 42% arginine and 14% histidine and estimated to contain 12 arginine residues (12; M. Sung, personal communication). With the values of 48, 41, and 12 arginine residues per molecule of V, VII, and μ, respectively, and the relative concentrations of these polypeptides in cores measured by densitometry, molar ratios of VII-to-V and μ-to-V were estimated to be 6:1 and 1:1, repectively. This calculation assumes that the individual core proteins are synthesized by using the same pool of free arginine and that there is no discrimination among new and old populations of core proteins during virion assembly. The VII-to-V ratio in cores we have estimated, 6:1, is identical to that made previously with whole virions (8, 23).

Portions from each of the fractions a, b, and c of the core peak were treated with the cross-linker dithiobis(succinimidyl propionate) (specific for lysine residues) immediately after their fractionation. After cross-linking for 40 s at 21°C, samples were prepared for electrophoresis as described in Materials and Methods. An analysis of cross-linked and uncross-linked samples by one-dimensional electrophoresis is shown in Fig. 1B. Even at this low level of cross-linking, the cross-linked core samples, for example those in lane 2, displayed extra bands, such as P and R, which were not present in the uncross-linked sample, aU (lane 1), and were less abundant in fractions from nearer the top of the gradient (lane 4). On the basis of their mobilities, species P and R were assigned apparent molecular sizes of 37 and 75 kDa, respectively.

To assess how accurately cross-linked species obtained from gradient-purified cores represented the position of nearest neighbors within the virion, we turned to pentonless virions which were cross-linked in a similar fashion. The major novel bands P and R were observed in cross-linked pentonless virions (Fig. 1C, lane 2) as in cores (Fig. 1C, lane 1), although it was necessary to extend the time of cross-linking of pentonless virions to 100 s. At this level of cross-linking, the hexons were too heavily cross-linked to enter the gel (Fig. 1C, lane 2), indicating that the reaction of dithiobis(succinimidyl propionate) with the hexon polypeptides was almost complete before the cross-linker had equilibrated with the internal proteins. This result attests to the rapidity of the coupling reactions, thereby eliminating fears that extensive modification, resulting in denaturation of quaternary structure, might occur before productive cross-linking.

The results of cross-linking pentonless virions with a novel cross-linker are also shown in Fig. 1C. This reagent, *p*-azidophenyl glyoxal, was designed for protein-nucleic acid cross-linking and has been used specifically for the cross-linking of 16S rRNA to 30S ribosomal proteins of *Escherichia coli* (22). Such 1,2-dicarbonyl reagents as cyclohexanedione, glyoxal, and substituted glyoxals have been used in previous studies for the modification of arginine residues

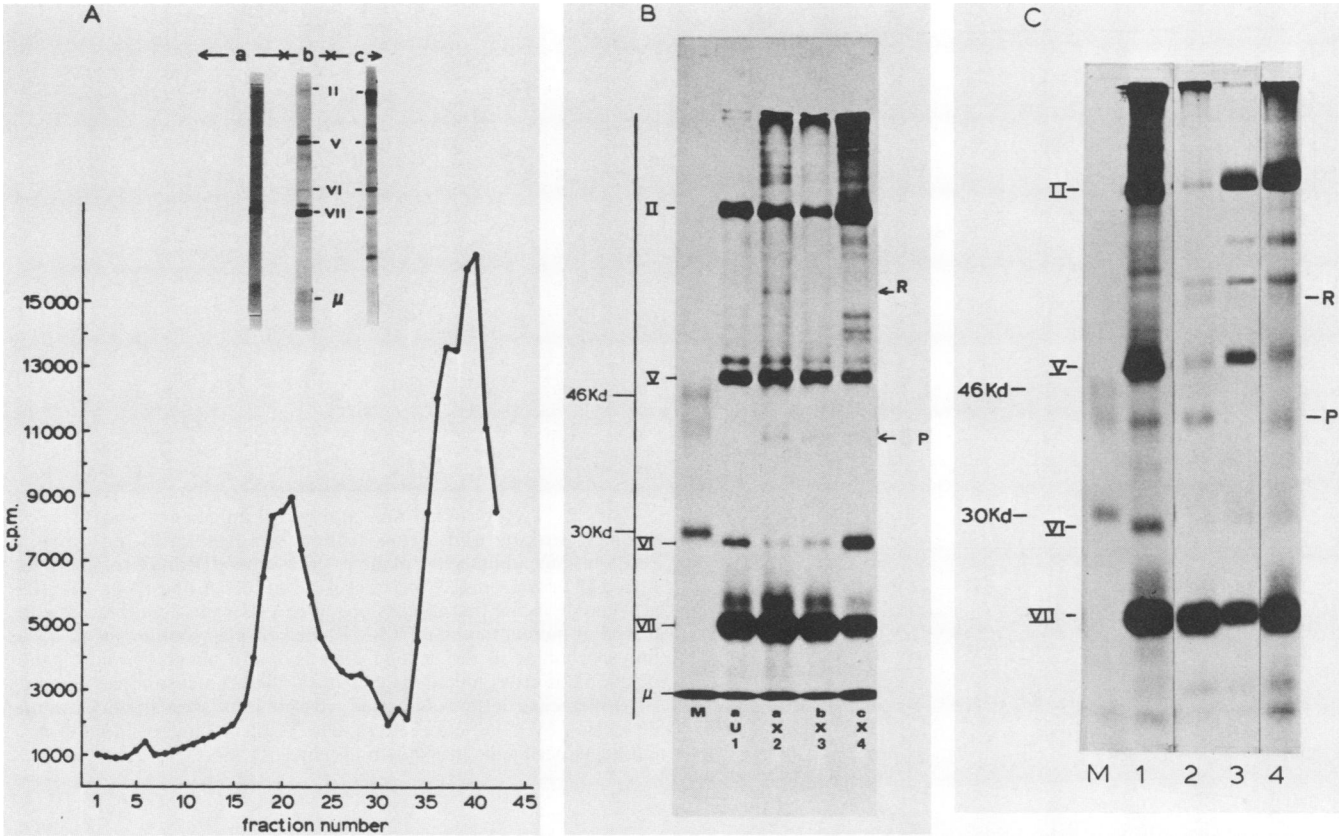
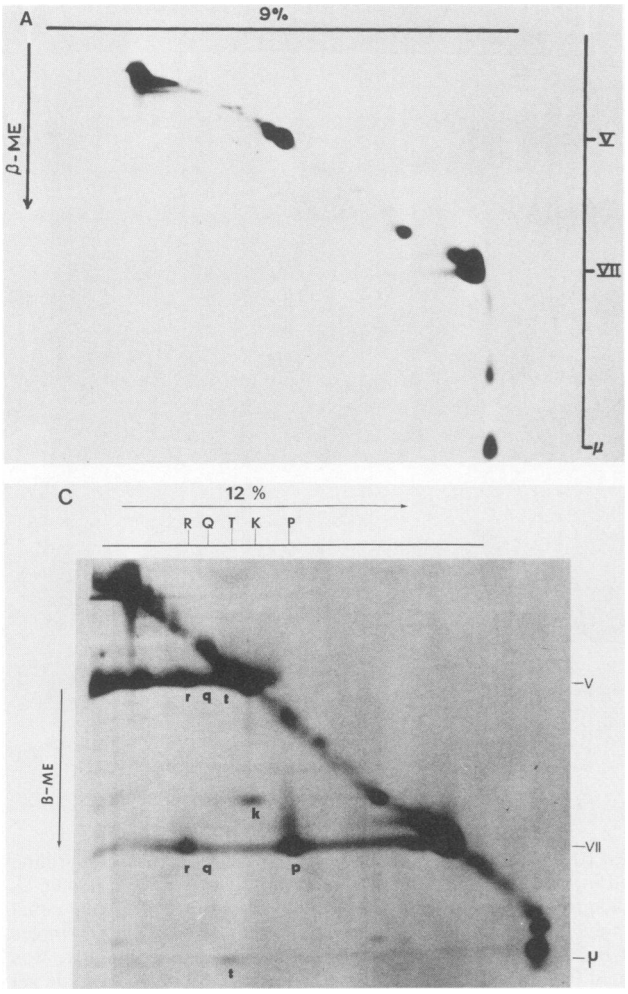


FIG. 1. One-dimensional analysis of cross-linked, gradient-purified cores. [^{3}H]arginine-labeled adenovirus type 2 virions were pyridine disrupted and fractionated in a 15 to 30% sucrose gradient (A) as described in Materials and Methods. Gradient fractions were pooled as indicated to form fractions a, b, and c. The inserts show autoradiograms made from a 17% polyacrylamide gel to which were applied equal portions of the fractions indicated. After cross-linking with dithiobis(succinimidyl propionate) as described in Materials and Methods, samples were analyzed in a 10% polyacrylamide gel (B, lanes 2 to 4). A portion of fraction a, uncross-linked but otherwise treated similarly, was applied to lane 1aU. Novel species seen in cross-linked samples are designated P and R. (C) An autoradiogram of a 10% polyacrylamide gel to which had been applied cross-linked, gradient-purified cores, equivalent to fraction b (lane 1), pentonless virions cross-linked for 100 s with dithiobis(succinimidyl propionate) (lane 2), pentonless virions cross-linked with *p*-azidophenyl glyoxal (lane 4), and pentonless virions treated with *p*-azidophenyl glyoxal but not exposed to UV light, i.e., not cross-linked (lane 3). In panels B and C, marker proteins, whose molecular weights are listed in kilodaltons at the left, were loaded in the lanes marked M.

in proteins (11, 21, 22). We considered it worthwhile to use *p*-azidophenyl glyoxal in our cross-linking studies, because major constituents of the nucleoprotein core, polypetides VII and μ, are rich in arginine and poor in lysine residues (1, 12, 14, 23, 27, 28; M. Sung, personal communication). The results presented in Fig. 1C, lane 4, show that the major cross-linked species P and R were indeed also formed when pentonless virions were exposed to *p*-azidophenyl glyoxal. However, the cross-linked species P, which is a dimer of VII (see below), contributed a larger portion of the cross-linked species. (Compare lanes 2 and 4, Fig. 1C.) No cross-linked species that were not also present after cross-linking with dithiobis(succinimidyl propionate) could be detected after exposure of pentonless virions to *p*-azidophenyl glyoxal (compare lanes 2 and 4, Fig. 1C). Thus, the use of the lysine-specific cross-linker did not appear to limit the number of interactions among core proteins that could be investigated. The hexons were not as heavily cross-linked by *p*-azidophenyl glyoxal as by dithiobis(succinimidyl propionate), a result that was not unexpected because the photoactive azide moiety of the cross-linker was activated only after prior equlibration of the cross-linker with pentonless virions. Protein cross-links to unpaired guanosine residues that might be present in single-stranded regions of the DNA inside virions were not analyzed in this study. In summary, the results presented in Fig. 1 indicate that the major cross-linked species generated from gradient-purified cores were not limited by use of a lysine-specific cross-linker and were qualitatively identical to a subset of those obtained when pentonless virions were cross-linked. Thus, we conclude that the major interactions among the polypeptides associated with the core are not disrupted during core purification.

To identify the individual components in each cross-linked species, the polypeptides were separated in a second dimension after one-dimensional separation and cleavage of the cross-linker (6, 16, 30). The results of such an analysis for portions from the samples of aU and aX shown in Fig. 1A are presented in Fig. 2A and B. The results of cross-linking an independent core preparation are shown in Fig. 2C. No off-diagonal components were observed in the uncross-linked sample (aU) (Fig. 2A), confirming that species P and R discussed previously indeed represented cross-linked products. In the two-dimensional gels shown in Fig. 2B and 3, cross-linked species are designated by capital letters, P, R, and so on, and identified by their mobility in the first dimension. Their components, identified in the second di-



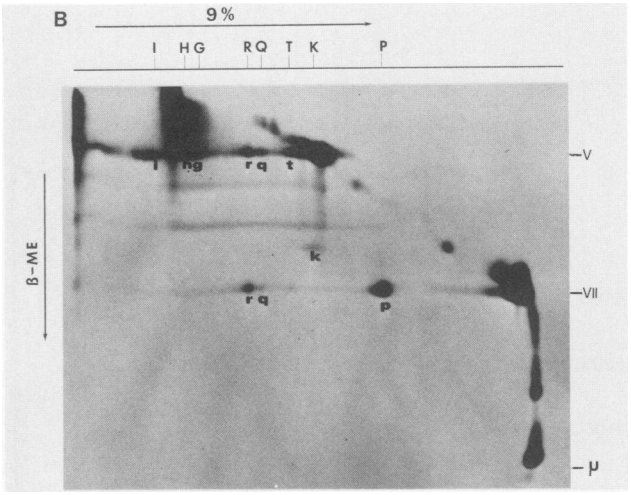


FIG. 2. Two-dimensional polyacrylamide gel analysis of uncross-linked and cross-linked gradient-purified cores. Dithiobis(succinimidyl propionate)-cross-linked (B and C) or uncross-linked (A) core samples were resolved in 9% (A and B) or 12% (C) acrylamide in the first dimension. Such gels were equilibrated with 0.3 M β-mercaptoethanol (β-ME) before electrophoresis in 11% polyacrylamide in the second dimension. In panels B and C, the positions of cross-linked species in the first dimension are marked by upper case letters and their components seen in the second dimension by lower case letters. The results of independent cross-linking experiments are shown in panels B and C.

mension, are designated p, r, and so on. The properties of cross-linked species discussed in the subsequent paragraphs are summarized in Table 1.

The only component of core cross-linked species P was polypeptide VII (Fig. 2B). The molecular weight of 37 kDa exhibited by species P is in excellent agreement with that predicted for a dimer of VII (8, 23, 28). Previous cross-linking studies of whole virions with tartryl diazide have also identified a major cross-linked band corresponding to a dimer of VII (7). It is striking that higher multimers of protein VII, trimers, tetramers, and so on, could not be detected under any of the conditions of cross-linking or electrophoresis used in these experiments (Fig. 2B, 2C, and 3). Upon two-dimensional electrophoresis, it became clear that the cross-linked species designated R in Fig. 1B and C in fact comprised two products, denoted R and Q in Fig. 2B and C. Cross-linked species Q yielded polypeptides V and VII as its off-diagonal components in the vertical direction (Fig. 2B and C), and its estimated molecular weight, about 70 kDa, is consistent with that predicted for a cross-linked product comprising one molecule of protein VII (some 19 kDa) and one molecule of protein V (approximately 48 kDa). Cross-linked species R, estimated to be about 75 kDa, also yielded polypeptides comigrating with polypeptides V and VII (Fig. 2). The difference between species R and Q appeared to be the result of the presence of polypeptide μ in the former: this protein could be detected as a component of species R when the gels shown in Fig. 2B and C were exposed for very long periods and is shown for disrupted virions in Fig. 3B. In the experiment shown in Fig. 3B, polypeptides IX and μ were well resolved in the second dimension, and a concentration of μ can be seen aligned with the VII and V components of species R. In view of the less than ideal behavior of polypeptide μ during electrophoresis in polyacrylamide gels (see also Fig. 1A), we cannot be sure that species Q does not also contain μ. Nevertheless, the apparent molecular weights exhibited by species Q and R and the data shown in Fig. 3A indicate that these species differed by one copy of the μ protein. We are unable to decide which of the cross-linked species Q or R corresponds to the V-VII cross-linked species reported in the previous study of whole virions which did not detect μ (7). The major μ-containing species which could be detected in cores (and other preparations) was T (Fig. 2C). The composition of this and additional cross-linked products detected in core samples, such as G, H, and I (Fig. 2B and C), is discussed in subsequent paragraphs.

The cross-linked species containing core polypeptides, such as P, Q, and R, were also detected when the products of dithiobis(succinimidyl propionate)-cross-linked, pentonless virions were examined in two dimensions (Fig. 3A). However, the high overall level of cross-linking necessary to

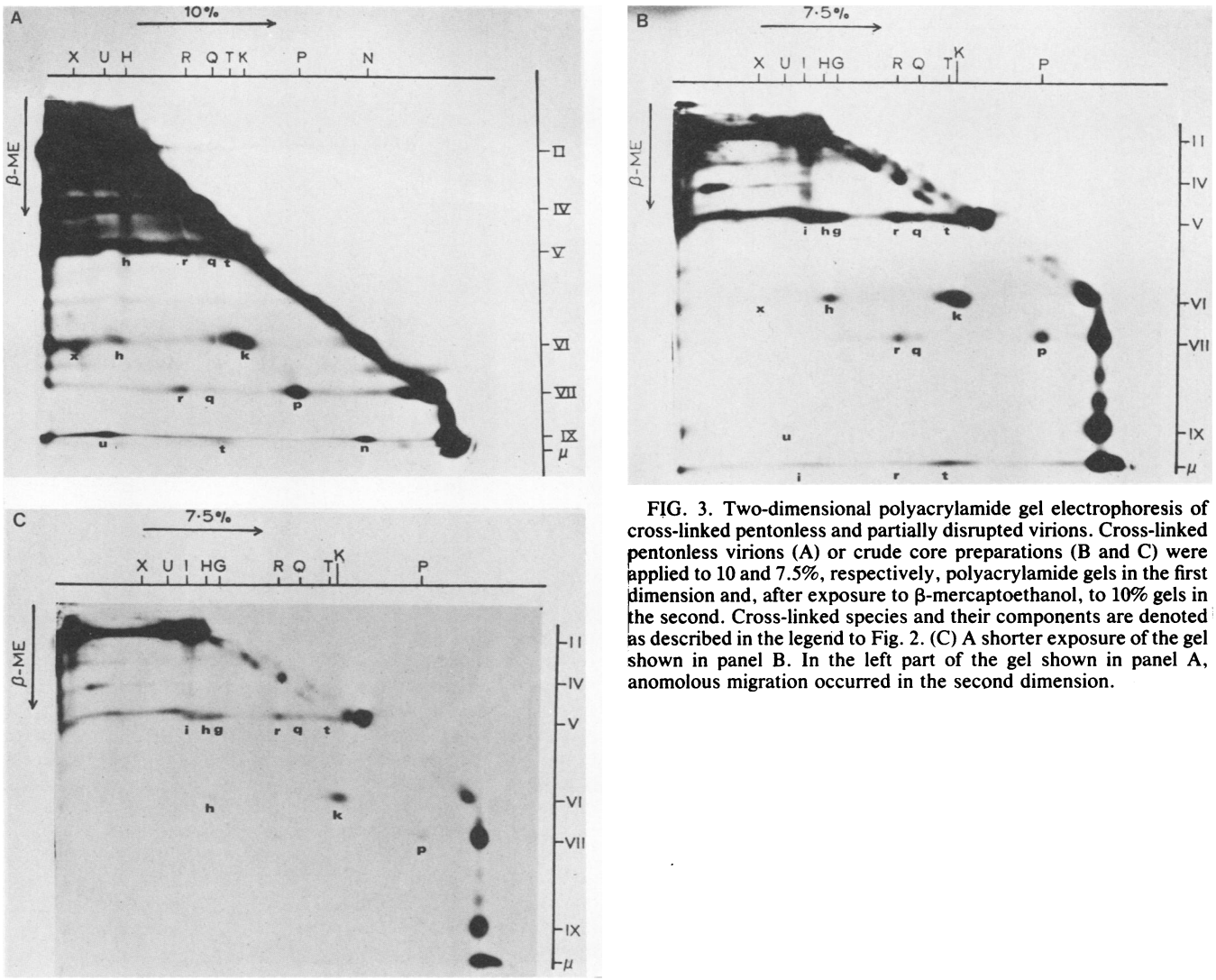


FIG. 3. Two-dimensional polyacrylamide gel electrophoresis of cross-linked pentonless and partially disrupted virions. Cross-linked pentonless virions (A) or crude core preparations (B and C) were applied to 10 and 7.5%, respectively, polyacrylamide gels in the first dimension and, after exposure to β-mercaptoethanol, to 10% gels in the second. Cross-linked species and their components are denoted as described in the legend to Fig. 2. (C) A shorter exposure of the gel shown in panel B. In the left part of the gel shown in panel A, anomolous migration occurred in the second dimension.

generate core cross-links in these structures induced such heavy cross-linking of hexon polypeptide II and other capsid proteins (Fig. 3A) that the resolution and analysis of individual cross-linked species was difficult. Although *p*-azidophenyl glyoxal cross-linked core polypeptides of pentonless virions readily, with reduced reaction with capsid components (Fig. 1C), such cross-linking was not readily reversible. To circumvent such difficulties, crude cores were prepared by brief exposure of pentonless virions to pyridine, quickly dialyzed against 10 mM PIPES buffer (pH 6.8), and treated with dithiobis(succinimidyl propionate) for 60 s at room temperature. A two-dimensional analysis of the resulting cross-linked species is shown in Fig. 3B and C. The corresponding uncross-linked sample exhibited no off-diagonal spots other than one corresponding to a trimer of polypeptide II (data not shown), whose formation is presumably mediated by intermolecular disulfide bonds. In addition to cross-linked species P, Q, and R, the predominant products when purified cores were cross-linked (Fig. 2B and C), numerous other species were detected. Among them were those designated G, H, I, and T, also observed among the products of core cross-linking, which, like P, Q, and R, contained core polypeptides (Fig. 2B, 3B, and C). Thus, species G contained only polypeptide V and exhibited the molecular size, 97 kDa, expected of a protein V dimer (8, 14, 23). Careful alignment of the off-diagonal spots by using, for example, the V and VII components of species R as reference points established that species H comprised polypeptides V and IV (Fig. 3B and C). Its estimated molecular size, 101 kDa, suggested that it was composed of one molecule of V and two of VI. Such a species has been previously identified after cross-linking of virions (7) and, indeed, was produced at high efficiency when pentonless virions were cross-linked (Fig. 3A).

The other core protein-containing species, I and T, apparently contained polypeptides V and μ but no polypeptide VII (Fig. 2C and 3B). The behavior of polypeptide μ was less than ideal, even during one-dimensional electrophoresis (see, for example, Fig. 1A), and even when arginine-labeled virions were employed, it represented a minor labeled virion protein. The assignments of the μ protein discussed here are, therefore, based on the presence of concentrations of the μ protein at relevant positions in all cross-linked samples analyzed. Thus, variable locations of μ such as that at the P position in Fig. 3B but not in Fig. 3A were ignored.

The series of products designated I in Fig. 2 and 3 were

TABLE 1. Summary of cross-linked species

Cross-linked species	Mol wt[a]	Components cross-linked	Comments
Core protein cross-links			
P	37	VII-VII	High efficiency in all samples
T	58	μ-V-μ	Moderate efficiency; best identified in crude cores
Q	70	V-VII	Moderate efficiency; easily identified in gradient-purified cores, and crude cores less easily identified in pentonless virions.
R	75	μ-V-VII	High efficiency in all samples
G	97	V-V	High efficiency in all samples
I	>110	Multimers of V with μ	High efficiency in all samples
Core protein-capsid protein cross-link			
H	101	VI-VI-V	Moderate efficiency in all samples
Capsid protein cross-links			
K	53	VI-VI	Most efficient cross-link of all
N	25	IX-IX	High efficiency, identified only in pentonless virions
U	126	II-IX	Low efficiency, not detected in cores
X	>130	II-VI	Low efficiency, not detected in cores

[a] Best estimate based on mobility in different percentages of polyacrylamide first-dimension gels.

poorly resolved from one another and therefore difficult to analyze in detail. The estimated molecular weight of I, ≥110 kDa, suggests that it contained a dimer of protein V cross-linked to more than one molecule of μ. In this context, it should be noted that species T was also composed only of V and μ polypeptides and appeared, on the basis of its estimated molecular size, 58 kDa, to represent one molecule of polypeptide V cross-linked to one or two molecules of μ. Although the molecular weight estimate of species T was not sufficiently accurate to discriminate between these alternatives, the intensity of the μ spot released from species T tended to favor cross-linking of two molecules of μ with one of V to form species T. As the V spot released from species I was, if anything, more intense than that of the V spot released from species T (Fig. 3C) and the I μ spot is less intense than the μ released from in T (Figure 3B), it would appear that species I comprised a dimer of polypeptide V cross-linked to one molecule of protein μ. Species T was less efficiently formed when purified cores were cross-linked (compare Fig. 2B and 3B). Similarly, a VII-μ cross-link was detected only when pentonless virions were treated with dithiobis(succinimidyl propionate) (compare Fig. 3A and B). Partial unfolding of the core from a quasi-spherical structure within the capsid to a 30-nm-thick fiber upon its release from virions (18, 30) may be responsible for separating the polypeptides beyond a cross-linkable distance.

The remaining cross-linked species were composed of capsid proteins and, with the exception of K, a dimer of polypeptide VI (Fig. 3A and B), were more efficiently generated from pentonless virions than crude or purified core preparations, as would be expected. The most clearly observed species were polypeptide II cross-linked to IX(U) or to VI(X) and a dimer of polypeptide IX(N). The latter was observed with high efficiency only when pentonless virions were cross-linked (compare Fig. 3A and B).

DISCUSSION

Nearest-neighbor analysis in a cluster of macromolecules by means of chemical cross-linking is a well-established procedure (6, 16, 30). The validity of conclusions drawn from such studies depends to a large degree on the extent to which the system is modified before the actual formation of intermolecular cross-links. In these studies, exchanges between disulfide bonds already existing or introduced and free sulfhydryl groups in proteins were eliminated by blocking all free sulfhydryl groups with *N*-ethylmaleimide before and after the disruption of virions or core assemblies and at the end of the cross-linking reaction. Such treatment also eliminates artifacts arising from air oxidation of free sulfhydryl groups. That the dithiobis(succinimidyl propionate) cross-linker employed in most experiments was indeed well behaved under the conditions employed is illustrated by the generation of identical cross-linked species from different structures and by the induction of the same major cross-linked core species by a cross-linker with completely different specificity, *p*-azidophenyl glyoxal (Fig. 1). Moreover, the results shown in Fig. 3A illustrate a unique example where a chemical cross-linker provides topographical information in a cluster of macromolecules and distinguishes proteins on the outside of the virion, cross-linked heavily in this case, from the core proteins which are inside and cross-linked to a lesser degree.

Purified adenovirions have been subjected to cross-linking studies previously (7). However the procedures employed, conditions such as pH 8.5 and a 30-min exposure to 20 mM cross-linker (tartryl diazide), undoubtedly the best then available, limited such investigations to sturdy structures like intact virions. We have used the much more efficient cross-linker dithiobis(succinimidyl propionate) under substantially milder conditions, namely pH 6.8 for 40 s at a cross-linker concentration of 1 mM, procedures that enabled us to study such more fragile structures as gradient-purified nucleoprotein cores. We also wished to establish how polypeptide μ, not identified at the time of the studies of Everitt et al. (7), might interact with the other core proteins.

The finding that the major cross-linked species produced from gradient-purified cores were also well represented when crude cores or pentonless virions were cross-linked (Fig. 2 and 3) strongly suggests that the major interactions among the core proteins that are characteristic of virions were not disrupted during core preparation, at least when the purified cores were cross-linked immediately upon collection

from the gradients. In view of the instability of cores and their tendency to aggregate, this finding will be of importance to the interpretation of other approaches to the elucidation of core structure (M. E. Vayda and S. J. Flint, manuscript in preparation; P. K. Chatterjee, M. E. Vayda, and S. J. Flint, submitted for publication). Conversely, the subset of core protein-protein interactions that could be most readily identified in virions (Table 1) would appear to represent those that participate in maintaining the core in its compact, intravirion conformation but are disrupted upon release of the core, when it unfolds to a thick fiber conformation (19, 31). Quite a large number of interactions among the core polypeptides have been identified. These include the formation of V-V and VII-VII dimers, both at high efficiency in agreement with previous reports (7). The absence of higher multimers comprising polypeptide VII is interesting in light of models suggesting that the basic DNA packaging unit of the core comprises six molecules of this protein (5). The cross-linking data presented here are not necessarily inconsistent with this notion, but they do restrict the type of symmetry that could be displayed by the polypeptide VII chains within the structural unit (31) that they form. The lysine residues of polypeptide VII with which dithiobis(succinimidyl propionate) can react are restricted to the N-terminal one-third of the protein (28). Thus, for example, a head-to-tail arrangement of six molecules of VII present in a repeating unit or any other quaternary structure possessing a sixfold axis of rotation are ruled out by these cross-linking data.

The μ protein, identified by its labeling with [^{3}H]arginine, molecular weight, and presence as the only small virion polypeptide present in gradient-purified cores (31; Fig. 1A), was detected primarily in association with polypeptide V (species T and I, Fig. 2 and 3, Table 1). The only cross-linked species observed comprising VII and μ(R), observed in cores or disrupted virions, also contained polypeptide V. The absence of VII-μ but presence of V-μ species among the cross-linked products of cores might suggest that the association of VII and μ polypeptides in species R is mediated by a bridge of polypeptide V. On the other hand, the absence of VII-μ cross-links might reflect the fact that polypeptides VII and μ are not within sufficiently close contact within the core (approximately 0.6 to 0.8 nm) to be cross-linked by dithiobis(succinimidyl propionate) or the limited location of potentially reactive residues within the μ protein: polypeptide μ contains no lysine residues (12; M. Sung, personal communication) so it seems likely that the only primary or secondary amine available for reaction with dithiobis(succinimidyl propionate) is the N terminus of this protein.

Polypeptide μ is extremely basic (12; M. Sung, personal communication) and might be expected to interact with DNA and thus lie in close proximity to the polypeptide VII units. Nevertheless, the high efficiency with which μ becomes cross-linked to polypeptide V (Table 1), which appears to form an outer shell around the polypeptide VII-DNA complex (3, 7), indicates that at least the region of μ containing groups reactive with dithiobis(succinimidyl propionate) is located towards the outer perimeter of the core in close association with polypeptide V.

The only interactions between the core and the outer capsid of the virion that we could detect were mediated by polypeptide V (Table 1), consistent with its previously reported location (3, 7): the prominent cross-linked species comprised polypeptide V cross-linked to a dimer of polypeptide VI (Table 1). The failure to observe a VI-V cross-linked species suggests that dithiobis(succinimidyl propionate) forms VI-VI dimers extremely efficiently: indeed almost half of polypeptide VI was found in species K, a dimer of VI, in all experiments performed, despite the low levels of cross-linking employed (Fig. 2 and 3). It is clear that polypeptide V can also be cross-linked to the larger capsid polypeptides, probably including II and IV (Fig. 2 and 3).

The II-IX and II-VI associations seen when pentonless or partially disrupted virions were cross-linked (Fig. 3) confirm previous conclusions, based on other methods, about the locations of these capsid constituents. Thus, immunoprecipitation of partially disrupted virions located polypeptide IX with groups of nine hexons and polypeptide VI with individual hexon trimers (7). The IX-IX dimer, cross-linked at an efficiency approaching that of the VI-VI dimer in pentonless virions, has not been reported previously.

In summary, we have confirmed or identified six interactions among the three core proteins of subgroup C adenoviruses (Table 1). When this information is combined with the results of DNA-protein cross-linking studies (P. K. Chatterjee, M. E. Vayda, and S. J. Flint, submitted for publication) and of analysis of the nucleoprotein units that comprise the adenovirus core (M. E. Vayda and S. J. Flint, manuscript in preparation), it should be possible to construct a reasonably detailed picture of the adenovirus core.

ACKNOWLEDGMENTS

We thank R. Lunt for excellent technical assistance and M. Sung and R. Roberts for communication of data before publication.

This work was supported by a grant from the American Cancer Society (MV189E). S.J.F. is the recipient of a Public Health Service Research Cancer Development Award (K04AI00441).

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