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Comparative Studies on the Soluble Components of Adenovirus Types 9 and 15 and the Intermediate Strain 9-15

ERLING NORBY

Department of Virology, Karolinska Institutet, Faculty of Medicine, Stockholm University, Stockholm, Sweden

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Five different soluble components of adenovirus types 9, 9-15, and 15 have been identified. These are: (i) a slowly sedimenting, trypsin-resistant, incomplete hemagglutinin (HA). (This component was demonstrable by hemagglutination-enhancement (HE) tests in the presence of heterotypic antisera against members of Rosen's subgroups II and III, but *not* of subgroup I); (ii) a slowly sedimenting, trypsin-resistant, complete HA, causing only a partial agglutination of cells; (iii) a rapidly sedimenting, incomplete HA, demonstrable by HE tests in the presence of heterotypic antisera against members of all Rosen's subgroups. (Trypsin treatment of this component caused a conversion into slowly sedimenting incomplete HA); (iv) a group-specific complement-fixing (CF) antigen devoid of HA activity; and (v) a rapidly sedimenting, trypsin-sensitive, complete HA, which in the electron microscope was found to represent a dodecahedral aggregate of 12 pentons (a dodecon). On the basis of their biological and physicochemical characteristics, the first four components were interpreted to represent (i) fibers, (ii) a polymer of a few, probably two, fibers, (iii) pentons, and (iv) hexons, respectively. The length of fibers extending from dodecons and virions was estimated to be 11 to 14 nm. A similar value was suggested from exclusion chromatography experiments. Adenovirus types 9 and 15 fibers were recovered in a position intermediate to that of fibers of types 3 and 4, the lengths of which are 10 and 17 nm, respectively. The sequence of elution of different components of types 9 and 9-15 from an anion exchanger was fibers, fiber-aggregate, pentons, hexons, and dodecons. Type 15 components appeared in the same order except for the fact that dodecons eluted before hexons. The molarities of NaCl required to elute the different types 9 and 9-15 components, excluding hexons, were identical. They were distinctly different from those of the corresponding type 15 components. However, hexons of all three serotypes eluted in proximity to each other and there was a slight tendency for type 9-15 hexons to take a position intermediate to those of types 9 and 15.

The separation of human adenoviruses into 31 different serotypes is based on results of neutralization and hemagglutination-inhibition (HI) tests. However, neither one of these two tests is strictly type-specific for all members of the adenovirus group. Some cross-reactions between different serotypes in one or both of the tests were observed (21). In addition, some strains have been identified which behave like one serotype in the neutralization test but like a completely different one in the HI test. One example of such an intermediate strain is type 9-15. This serotype was demonstrated to be closely related to type 15 in the neutralization test, but to type 9 in the HI tests (1, 28). In addition, it was found that the biological characteristics of the type 9-15 hemagglutinin (HA) were similar to those of type 9, but partly different from those of type 15 (28). Further comparative serological studies of types 9, 15, and 9-15 have revealed that, although there was no cross-reaction between types 15 and 9-15 in HI tests when soluble complete hemagglutinins were used as antigens, a clear-cut relationship was found in HI tests when soluble hemagglutinins were exchanged for virion-associated hemagglutinins (29). In order to explain these facts, it was suggested (9) that the capsid of serotype 9-15 contains a mosaic of structural components derived from both serotypes 9 and 15. As an introduction to a study of these relationships on the level of structural antigens, experiments were designed for a separation and characterization of

soluble components of types 9, 15, and 9-15. Some different soluble components of type 9 already have been studied (16).

The terminology for structural components adopted for use in the following paper was proposed by Ginsberg et al. (4). Thus nonvertex capsomers, vertex capsomers plus projections, and isolated vertex projections will be denoted hexons, pentons, and fibers, respectively. In addition, the term dodecon proposed by Gelderblom et al. (2) will be used to describe a complete HA composed of a symmetrical aggregate of 12 pentons (7). Furthermore, the term complete HA will be applied to a component giving direct hemagglutination, whereas the term incomplete HA refers to a component requiring the presence of heterotypic antibody in order to display its agglutinating activity. A complete HA, by definition, can cause the establishment of bottom patterns of either maximal or partial agglutination of red cells.

Previous studies of members of Rosen's subgroup II (20), which includes serotypes 9 and 15, have demonstrated the occurrence of three different soluble components (2, 3). Listed in order of their elution from an anion exchanger by NaCl solutions of increasing molarity, these were (i) a type-specific antigen postulated to represent fibers, (ii) a group-specific antigen identified as hexons, and (iii) a dodecahedral aggregate of 12 pentons (2) carrying complete HA activity. Similar results were obtained in this laboratory in parallel studies of adenovirus type 9 (16). However, the latter study described in addition the occurrence of an incomplete HA postulated to represent isolated pentons. In this study, these four main types of components that occur in preparations of adenovirus types 9, 9-15, and 15 have been further characterized and compared. In addition, one more kind of soluble component from Rosen's subgroup II is described.

MATERIALS AND METHODS

Virus and cell cultures. The prototype strains of adenovirus type 9 ("Hicks") previously studied, type 15 (ch. 38), and the intermediate type 9-15 (strain 5399, isolated by J. van der Veen, Nijmegen, Holland) were used. The two latter strains were kindly provided by R. Wigand, Homburg (Saar), Germany. Virus materials were prepared in a human bone marrow cell line, denoted Masa cells. The techniques for handling these cells were described previously (8, 10). The monolayer cultures were maintained on Parker's medium 199 that contained 2% inactivated calf serum. The materials were harvested at an advanced state of virus-induced cell degeneration. The medium and the cells then were concentrated by forced dialysis against polyethylene glycol (Carbowax 6000, Union Carbide Corp., New York) and were frozen and thawed three times; thereafter, cell debris was removed by low-speed centrifugation. Soluble components were separated from nonsoluble products, i.e., virions and empty capsids, by three consecutive centrifugations at Pi 5.3 (26,400 × *g*; rotor 40, Beckman Instruments Inc., Fullerton, Calif.). Remaining infectivity amounted to 10^{-5}, or less, of that of the starting material.

Preparation of hyperimmune sera. Tenfold concentrates (4ml) of prototype strains of types 2, 3, 5, 6, 9, 11, and 15 and of virions of adenovirus type 3, purified by isopycnic banding (13) and mixed with Freund's complete adjuvant, were injected intramuscularly into rabbits. Five weeks later, the animals were given an intravenous booster of 1 to 2 ml; they were then exsanguinated after another week.

Tests for biological activities. Determination of complete and incomplete hemagglutinins and of hemagglutination-inhibition antibody-consuming (HIC) components was performed in tubes by use of the techniques previously described (8, 14, 16, 17, 27). Four hemagglutination-enhancing (HE) units of serum, determined in a chess-board titration, were applied per antigen dilution in tests for incomplete HA. When one and the same serum indicated two different incomplete hemagglutinins, the adjustment of serum concentration to be used was made with reference to the component requiring the lowest serum dilution for its identification. Complement-fixing antigen was determined by the drop technique as modified by Svedmyr et al. (22). Two units of complement and two units of an antiserum against purified virions of adenovirus type 3, which did not react with nonviral components, were applied per antigen dilution.

Separation techniques. The previously described techniques for zonal centrifugation in linear 5 to 20% sucrose gradients (7), equilibrium centrifugation in CsCl gradients (13), exclusion chromatography on Sephadex G200 (Pharmacia Fine Chemicals, Uppsala, Sweden) (14, 15), and anion exchange chromatography on DEAE-Sephadex A25 (Pharmacia Fine Chemicals; 8, 10, 16) were used.

Electron microscopy. Purified virus components to be studied were dialyzed against a 1% ammonium acetate solution. One drop of the dialyzed material and one drop of a 2% solution of sodium tungstosilicate (STS) were mixed on dental wax. When needed, purified bovine albumin in a final concentration of 0.01% was added to the mixture in order to improve its spreading on the grid. Examinations were made by use of a Philips EM200 electron microscope at primary magnifications of 40,000 to 50,000.

Treatment with trypsin. Samples to be treated were dialyzed against a 0.15 M NaCl solution containing 0.001 M $CaCl_2$. It was then buffered to *p*H 7.2 with 0.05 M tris(hydroxymethyl)aminomethane (Tris-chloride). Trypsin (twice crystallized; Fluka AG, Buchs SG, Switzerland) dissolved in the same solution was added to give a final concentration of 0.4%. After incubation for 3 hr in a 37 C water bath, the activity of the enzyme was stopped by addition of equal amounts of soybean trypsin inhibitor (five times crystallized, Nutritional Biochemicals Corp., Cleveland, Ohio).

RESULTS

Characteristics of the hemagglutinating activity of adenovirus types 9, 15, and 9–15. Adenovirus

type 9-15 has been demonstrated to agglutinate both rat and human type O erythrocytes (28). Thus, with respect to this biological activity, it appears to be more closely related to type 9 than to type 15 because the latter type does not agglutinate human type O erythrocytes (20). In order to further characterize the properties of the hemagglutinins of types 9-15 and 15, the effect of repeated erythrocyte absorptions on the content of hemagglutinins and of treatment of HA-red cell aggregates with receptor-destroying enzyme (RDE) was studied. RDE has been demonstrated to effectively elute the complete HA of type 9 (5, 16). Tenfold concentrates of types 9, 9-15, and 15 materials in amounts of 4.5 ml were adsorbed five consecutive times with 0.5 ml of packed human type O erythrocytes. In the case of type 15, they were adsorbed with recently washed rat erythrocytes. Each adsorption was allowed to last for 1 hr at room temperature, after which the red cells were sedimented by low-speed centrifugation. After each adsorption, a sample was collected from the supernate. The pellet of cells from the first adsorption was resuspended in a one-eighth dilution of RDE (standard reagent cholera filtrate product; N. V. Philips-Roxane, the Netherlands) in phosphate-buffered, 0.07 M (*p*H 7.2) physiological saline (PBS). After another incubation for 1 hr at room temperature, the cells were removed by low-speed centrifugation and the supernate was collected. The results of tests for the presence of complete and incomplete HA in the different samples from one experiment are summarized in Table 1.

The effect of the repeated adsorptions on remaining HA activity of all three kinds of materials was identical. As was already described for type 9 (16), all complete HA activity was eliminated rapidly, whereas the titer of incomplete HA, identifiable after the first adsorption, was not significantly changed by four additional adsorptions. The titer of incomplete HA, demonstrable in the presence of an antiadenovirus type 15 serum in the case of types 9 and 9-15, and an anti-adenovirus type 9 serum in the case of type 15, was 10 to 5% that of complete HA in all three types of materials. RDE treatment of aggregates of HA and human type O erythrocytes of types 9 and 9-15 caused an elution of 50 to 100% of all HA from the cells. The effect of a corresponding treatment of rat cells agglutinated by type 15 HA was different. No, or only trace amounts of, HA was eluted. However, the latter were not considered significant because similar small quantities of HA occasionally also could be demonstrated in controls, including agglutinated cells resuspended in PBS. It should be mentioned that rat erythrocytes were relatively fragile. Repeated washings and RDE treatment caused considerable hemolysis.

Separation of soluble components of types 15 and 9-15 by zonal centrifugation. Gelderblom et al. (2) demonstrated that the complete HA of types 13, 15, and 19 sedimented at a rate of about 60*S*, a value corresponding to that described also for the complete HA of types 3 (7) and 9 (16). A similar kind of relatively heavy complete HA was identifiable in type 9-15 materials (Fig. 1). After zonal centrifugation in a linear 5 to 20% sucrose gradient at Pi 7.0 (63,600 × *g*; rotor SW25, Spinco) for 5.5 hr, the complete HA of this

TABLE 1. *Effect of five consecutive erythrocyte adsorptions on the concentration of complete and incomplete HA in preparations of soluble components of adenovirus types 9, 9-15, and 15*

No. of adsorptions[b]	Activities (HAU/0.4 ml) of materials of different serotypes[a]					
	Type 9		Type 9-15		Type 15	
	Complete HA	Incomplete HA[c]	Complete HA	Incomplete HA[c]	Complete HA	Incomplete HA[c]
0	6,400		1,600		12,280	
1	80	320	40	80	80	1,280
2	<10	320	<10	80	<10	1,280
5	<10	320	<10	80	<10	640
RDE eluate from cells of adsorption 1	6,400		800		<10	<10

[a] The absence of an activity value indicates that the quantity of incomplete HA could not be determined because of the presence of excess amounts of complete HA.

[b] Each adsorption included an incubation with packed red cells (human O-types 9 and 9-15, rat-type 15) in a final concentration of 10% for 1 hr at room temperature.

[c] Determined in the presence of antiadenovirus type 15 sera in the case of type 9, and of type 9 sera for types 9-15 and 15 materials.

serotype was recovered in the high density region of the gradient. In contrast, incomplete HA activity demonstrated in the presence of an anti-adenovirus type 15 serum as well as all group-specific CF antigen were associated with markedly more slowly sedimenting components recovered close to the meniscus of the gradient.

In order to further separate the slowly sedimenting components, virus materials from which all heavy complete HA had been removed by erythrocyte adsorptions, as described above, were subjected to zonal centrifugations at Pi 5.9 (54,000 × *g*; SW25, Spinco) for 42 hr. Four different components that exhibited corresponding biological activities were demonstrated in preparations of all three types. The distribution of adenovirus type 9 and 9-15 components was identical. However, there was a variation in the relative concentrations of the different components in type 15 materials as compared to the corresponding types 9 or 9-15 components. The general pattern of distribution of different activities is exemplified in Fig. 2, which shows the results of a fractionation by prolonged zonal centrifugation of type 15 components.

Group-specific CF antigen was recovered in the high-density region of the gradient. Similar or slightly slower sedimentation characteristics were exhibited by an incomplete HA, which was demonstrable in the presence of antisera against members of subgroups I (types 3 and 11), II (type 9 or 15, depending upon the type of material fractionated), and III (types 2, 5, and 6).

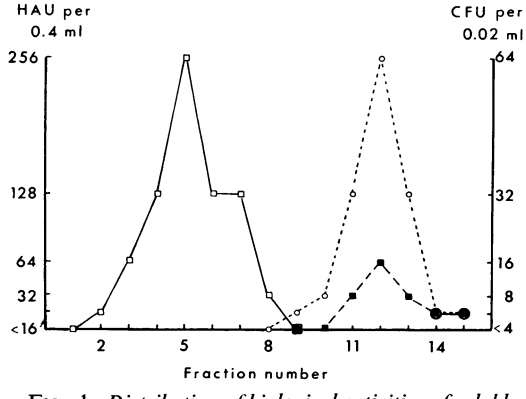

FIG. 1. *Distribution of biological activities of soluble components of adenovirus type 9-15 after zonal centrifugation in a linear 5 to 20% sucrose gradient at Pi 7.0 (63,600 × g; SW25, Spinco) for 5.5 hr. Fractions were collected from the bottom of the tube. The following activities were recorded:* (□) *complete HA;* (■) *incomplete HA determined in the presence of an antiadenovirus type 15 serum;* (○) *group-specific CF antigen (hexons) reacting with an antiserum against virions of adenovirus type 3.*

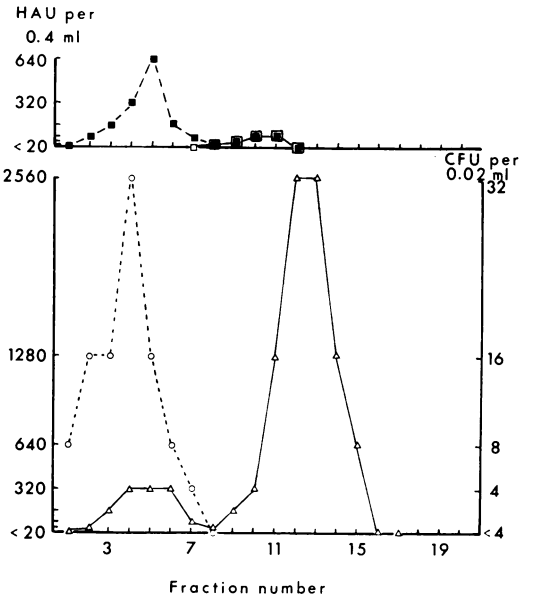

FIG. 2. *Distribution of biological activities after zonal centrifugation of a preparation of soluble adenovirus type 15 components from which the rapidly sedimenting complete HA had been removed by erythrocyte absorptions. Centrifugation was performed at Pi 5.9 (54,000 × g; SW25, Spinco) for 42 hr in a linear 5 to 20% sucrose gradient. The bottom of the tube is to the left. The following activities were recorded:* (□) *complete HA (bottom patterns of partial agglutination);* (■) *and* (△) *incomplete HA determined in the presence of antiadenovirus types 3 and 6 sera, respectively;* (○) *group-specific CF antigen (hexons) determined with an antiserum against adenovirus type 3 virions.*

In addition to this type of incomplete HA, HE tests with the antisera against members of subgroups II and III, but not of subgroup I, revealed the presence of one more incomplete HA. This component sedimented relatively slowly and was recovered in the low-density half of the gradient. The absence of any capacity of antisera against members of subgroup I to react with this type of incomplete HA was carefully controlled in chessboard titrations. The patterns of red cell agglutination caused by the slowly sedimenting incomplete HA varied somewhat depending upon the type of antiserum used and its relative concentration of HE antibody. Most often the patterns of agglutination of this component were only partial, contrasting with patterns of the rapidly sedimenting type of incomplete HA, which always were maximal. As can be seen from Fig. 2, adenovirus type 15 materials contained relatively more slowly sedimenting than rapidly sedimenting incomplete HA. In contrast, most preparations of adenovirus type 9 or 9-15 materials con-

tained somewhat larger quantities of rapidly sedimenting than of slowly sedimenting incomplete HA.

The fourth type of component identified after zonal centrifugation was a complete HA. This component sedimented slightly more rapidly than the incomplete HA recovered in the low-density half of the gradient. It was clearly identified in type 15 materials, but was present only in trace amounts in types 9 and 9-15 materials. The distribution of the activity of this slowly sedimenting complete HA was not easily recognized. This was due to the facts that it caused only a partial agglutination and that marked prozones of negative agglutination patterns occurred in fractions of lower densities. The latter phenomenon most likely was due to the presence in the low-density part of the peak of this complete HA activity of increasing amounts of slowly sedimenting incomplete HA, which might be capable of causing a competitive blocking of receptors on red cells. Presumably, the same situation of interference is the explanation for the absence of any detectable complete HA activity in unfractionated erythrocyte-adsorbed material.

Separation of soluble components, excluding complete HA, of serotypes 9, 15, and 9-15 by exclusion chromatography. The distributions of biological activities remaining in preparations of soluble adenovirus types 9, 15, and 9-15 materials, from which all rapidly sedimenting complete HA had been removed by erythrocyte adsorptions, were similar after exclusion chromatography on Sephadex G200. However, only in type 15 materials was the above-mentioned slowly sedimenting complete HA, exhibiting partial agglutinating activity, present in sufficient quantities to be recovered in demonstrable amounts in the fractions. The results of an exclusion chromatography experiment of this kind of material have been chosen, therefore, to exemplify the distribution of different activities (Fig. 3). Normal calf serum was included as a reference in this fractionation. One incomplete HA, demonstrable with sera against members of all three subgroups, eluted somewhat after the void volume, the 19*S* gamma globulin peak. A similar distribution was exhibited by a complete HA, identifiable by a partial agglutination of cells. This complete HA was identified with the slowly sedimenting complete HA in zonal centrifugation experiments. Thus, although this component sedimented markedly more slowly than group-specific CF antigen (hexons), it eluted distinctly before this type of component in exclusion chromatography experiments. The group-specific CF antigen eluted as a separate peak of activity in a position intermediate to that of the group-specific in-

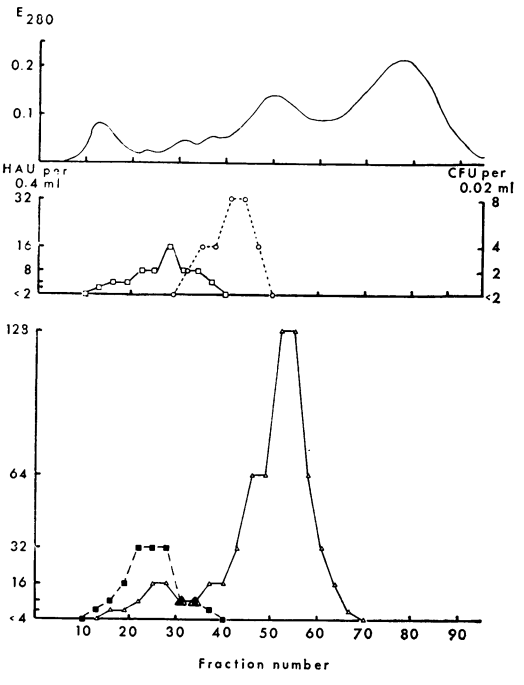

FIG. 3. *Distribution of biological activities after exclusion chromatography on Sephadex G200 of a preparation of soluble components of adenovirus type 15 from which the rapidly sedimenting complete HA had been removed. The topmost curve gives extinction values at 280 nm mainly representing components of normal calf serum added to the material. The meanings of different symbols is identical to those presented in Fig. 2.*

complete HA and the peak of 7*S* gamma globulin. Immediately after the latter, a peak of a second incomplete HA, demonstrable only with antisera against members of Rosen's subgroups II and III, was eluted.

In previous studies (14), it was found that exclusion chromatography was an excellent means of separating fibers of varying lengths derived from different serotypes. Since the slowly sedimenting incomplete HA described above most likely represents fibers, comparative exclusion chromatography experiments were performed with mixtures of this component and the corresponding kind of component derived from other serotypes. Adenovirus types 3 and 4 fibers, which are 10 nm (7) and 17 to 18 nm (26) long, respectively, were included as references. A mixture of fibers of these two serotypes as well as of types 9 and 15, all prepared by prolonged rate zonal centrifugation, as described above and in previous publications (14, 17), and normal calf serum as an additional reference were fractionated on

Sephadex G200. As can be seen from Fig. 4, the incomplete HA of types 9 and 15 eluted together at a position intermediate between the fibers of types 4 and 3.

Electron microscopy of complete HA and virions of adenovirus types 15 and 9-15.

The rapidly sedimenting complete HA of adenovirus types 15 and 9-15 was isolated by zonal centrifugation under the conditions presented in Fig. 1. Electron microscopic examination revealed the presence of star-shaped structures (Fig. 5a, b), confirming, in the case of type 15, the results published by Gelderblom et al. (2). The symmetrical arrangement of the different components was similar to that previously described for complete HA of types 3 (7), 4 (26), 9 (17), and 11 (11). By analogy, also complete HA of types 15 and 9-15 most likely represent dodecahedral aggregates of 12 pentons plus, probably, some internal structure. The dimensions of the individual parts of these complete hemagglutinins corresponded to that of the complete HA of type 9 (16), another member of Rosen's subgroup II. The capsomer-like structures appeared slightly funnel-shaped and had an outer diameter of 7 to 8 nm. The length of the projections extending from these structures was estimated at 11 to 14 nm. The diameter of the core of complete HA was 24 to 28 nm, whereas the overall diameter,

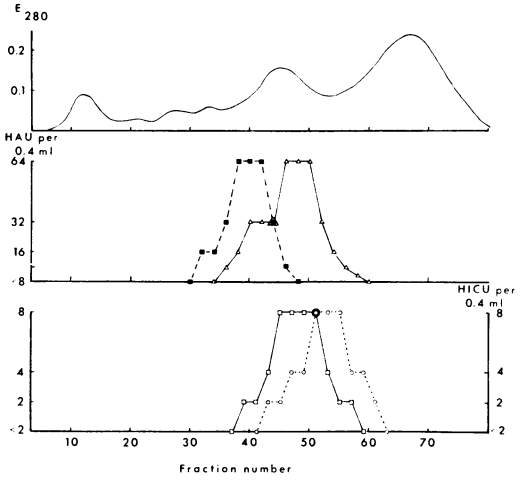

FIG. 4. *Distribution of activities after exclusion chromatography of a mixture of purified preparations of slowly sedimenting incomplete HA of types 4, 9, and 15 and HIC test-positive material of adenovirus type 3* (○). *Incomplete HA of types 4, 9, and 15 were determined in the presence of mixtures of antiadenovirus types 5, 9, and 15* (■), *types 4 and 15* (□), *and types 4 and 9 sera* (△), *respectively. The top line represents extinction values at 280 nm of components of normal calf serum added to the mixture of virus components.*

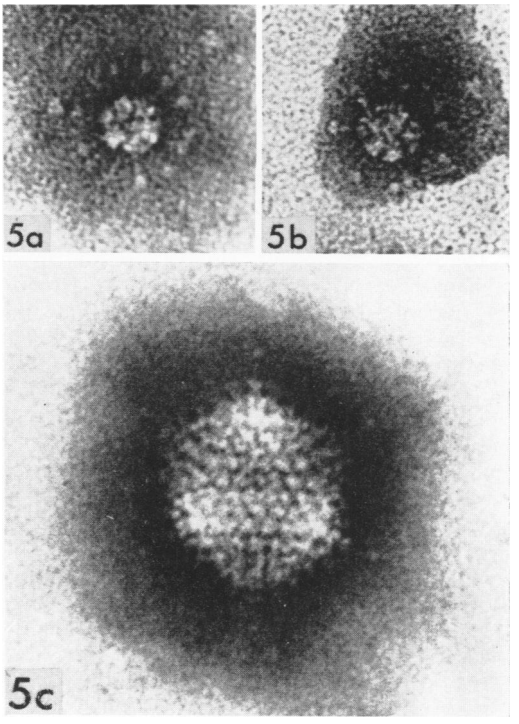

FIG. 5. *Ultrastructure of rapidly sedimenting complete HA (a, b) and a virion of adenovirus type 15 (c) purified by rate zonal and equilibrium centrifugation, respectively.* × *400,000.*

also including projections, was 48 to 53 nm. The latter range of variation in size was determined partly by the orientation of the complete HA.

Electron microscopic examination of virions of types 15 and 9-15 purified by isopycnic banding demonstrated (Fig. 5c) that their vertex capsomers carried projections with ultrastructural characteristics (e.g., length 12 to 13 nm) similar to those of dodecon-associated fibers.

Separation of soluble components of types 9, 15, and 9-15 by anion exchange chromatography. Except on one point, the sequence of elution of different soluble components was similar in principle for all three types of materials. This pattern of elution of the soluble components is illustrated by results from a fractionation of a type 15 material (Fig. 6). Under the conditions of separation, all soluble components were retained by the anion exchanger. Upon introduction of the salt gradient (0 to 0.25 M NaCl) one incomplete HA was eluted immediately. This component was demonstrable in the presence of antisera against members of subgroup III (anti-adenovirus type 6 serum was used in the experi-

ment presented in Fig. 6), as well as against the homologous subgroup (II), e.g., antiadenovirus type 9 serum in the case of type 15. Thus, this component exhibited the same characteristics as the slowly sedimenting incomplete HA, and this identification was verified by zonal centrifugation of early eluting incomplete HA that was recovered from anion exchange chromatography experiments.

The second component eluting from the anion exchanger carried some complete HA activity, the titer of which was slightly increased in the presence of heterotypic antisera. This component was identified with the slowly sedimenting complete HA in zonal centrifugation experiments. Fractionations of types 9 and 9-15 materials occasionally revealed the presence of traces of complete HA activity in a similar position. However, most often the titer of this type of component might have been too low to allow its demonstration. It is also possible that the incomplete hemagglutinins that appeared in proximity to this complete HA in the elution diagrams might have prevented its activity from becoming expressed by competition for receptors on red cells.

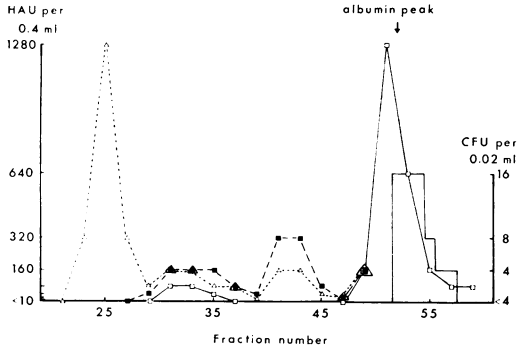

FIG. 6. *Distribution of soluble components of adenovirus type 15 after anion-exchange chromatography on DEAE Sephadex A25 in an 0.04 M Tris-chloride buffer (pH 8.4). All identifiable virus components were retained by the column. They were eluted by introduction of a linear 0 to 0.25 M NaCl gradient. The meaning of different symbols corresponds to those presented in Fig. 2. However, group-specific CF antigen is represented by columns.*

The third component eluted from the anion exchanger (Fig. 6) was a second incomplete HA. This component exhibited a broader reactivity in HE tests than the early eluting incomplete HA. It could be demonstrated by all antisera against heterologous adenovirus serotypes tested. Thus, in contrast to the situation with the early eluting incomplete HA, antisera were active against members of subgroup I (e.g., antiadenovirus type 3 serum used in the experiment in Fig. 6). Zonal centrifugation experiments demonstrated that the late eluting incomplete HA, as was expected, corresponded to the rapidly sedimenting incomplete HA.

The group-specific CF antigen (hexons) and the predominating kind of complete HA of type 15 eluted together. However, there was a slight difference, repeatedly encountered, in their position. The peak of complete HA was recovered immediately before and the peak of hexons immediately after the peak of bovine albumin of normal calf serum that was added to the material in some experiments. The behavior of these two type 15 components was different from that of the corresponding components of types 9 and 9-15. For the latter two serotypes, the group-specific CF antigen was eluted as a separate peak before this complete HA. This has already been described for type 9 (16).

In order to compare more accurately the relative positions of various components in the different materials, the molarity of NaCl required for their elution was determined by conductometric measurements (Conductivity Measurements Bridge PR 9501, Philips Sevenska AB, Stockholm). The results of these measurements are summarized in Table 2. The corresponding components of types 9 and 9-15 eluted at the same molarities. The only possible difference might concern their hexons. There was a trend for the group-specific CF antigen of type 9-15 to elute at slightly higher NaCl molarity than the corre-

TABLE 2. *Molarity of NaCl required for elution of different soluble components of adenovirus types 9, 9-15, and 15 from DEAE Sephadex A25 at pH 8.4 in a 0.04 M Tris-Chloride buffer*

Virus type	Early eluting incomplete HA	Early eluting complete HA	Late eluting incomplete HA	Group-specific CF antigen	Late eluting complete HA	Bovine albumin[b]
9	0.067–0.082		0.104–0.112	0.124–0.133	0.157–0.166	0.140
9–15	0.062–0.085		0.104–0.113	0.129–0.137	0.155–0.163	0.138
15	0.017–0.028	0.030–0.054	0.074–0.086	0.137–0.145	0.122–0.137	0.137

[a] Values are derived from two individual determinations of NaCl concentrations of fractions that contained peak biological activities of different components.

[b] Included as a reference.

sponding component of type 9. However, in none of the individual experiments was the recorded difference significant. The distribution of the peak of early eluting complete HA of types 9, 9-15, and 15 indicated some heterogeneity among components carrying this activity. This is reflected by the fact that the peak activity of this component was recovered over a wider range of NaCl molarity (about 0.02 molar units) than that of other components (0.01 molar units). All adenovirus type 15 components, except hexons, eluted at markedly lower NaCl molarities than the corresponding components of types 9 and 9-15. The early eluting incomplete HA of the former, in point of fact, was barely retained on the column under the conditions of fractionation. Frequently, a portion of this component was recovered in the void volume. The complete HA of type 15, as previously mentioned, eluted immediately before group-specific CF antigen, contrasting with the sequence of the corresponding components in types 9 and 9-15 materials. The hexons of all three serotypes exhibited the smallest difference in the NaCl concentrations required for their elution. However, in all experiments, types 9 and 9-15 hexons eluted immediately before and type 15 hexons immediately after the reference peak of bovine albumin (0.137 to 0.140 M NaCl).

The effect of trypsin on different soluble components of types 15 and 9-15. In order to elucidate further the characteristics of soluble types 15 and 9-15 components, the effect of trypsin treatment on their biological activities was studied. Isolated components to be treated were prepared by anion exchange chromatography, as described above. Table 3 gives the results of one experiment with adenovirus type 15 components. These results are comparable to those obtained in experiments with type 9-15 and, as was published in partly (16), type 9 components.

The activities of the early eluting incomplete and complete hemagglutinins were not inactivated by the proteolytic treatment. Instead, a two- to fourfold increase in their activity was encountered in all experiments. The activity of late eluting incomplete HA, as determined in the presence of an antiadenovirus type 3 serum, was destroyed by the treatment. However, tests that included an antiadenovirus type 6 serum showed an increase in the incomplete HA titer of this component after the treatment. Zonal centrifugation experiments revealed that this change in behavior in HE tests reflected a change from rapidly to slowly sedimenting incomplete HA. Finally, the late eluting complete HA was also degraded into slowly sedimenting incomplete HA by the treatment with trypsin. The titer of group-specific antigen present in the preparations of this complete HA was not significantly affected by the treatment.

Table 3. *Effect of trypsin treatment of some different soluble components of adenovirus type 15*

Type of preparation[b]	Trypsin treatment[c]	Biological activities (HAU/0.4 ml)[a]		
		Complete HA	Rapidly sedimenting incomplete HA[d]	Rapidly and slowly sedimenting incomplete HA[e]
Early eluting incomplete HA	0	<4	<4	256
	+			512
Early eluting complete HA	0	32		
	+	64		
Late eluting incomplete HA	0	<4	128	128
	+		<4	256
Late eluting complete HA	0	160		
	+	<4	<4	64

[a] The absence of a value indicates that no test was performed.

[b] Different components were prepared by anion-exchange chromatography under conditions similar to those presented in Fig. 6.

[c] For details of treatment, see Materials and Methods.

[d] Determined in the presence of an antiadenovirus type 3 serum.

[e] Determined in the presence of an antiadenovirus type 6 serum.

DISCUSSION

In previous studies of adenovirus type 9 (16), a representative of Rosen's subgroup II of adenoviruses, four different soluble components were identified. These were considered to represent a dodecahedral aggregate of 12 pentons [a *dodecon* according to Gelderblom's terminology (2)], pentons, hexons, and fibers. The present study revealed the occurrence of trace amounts of one more soluble component, namely a slowly sedimenting complete HA. In principle, the same kinds of five different soluble components were also identified in preparations of two other members of Rosen's subgroup II, adenovirus types 15 and 9-15.

In the previous study of type 9, fiber components were identified in the form of a slowly sedimenting HIC test-positive material. However, it was pointed out that this component exerted some effect on the sedimentation of rat erythrocytes in the presence of heterotypic antisera against

members of Rosen's subgroup II. The present study revealed that this type of component is an incomplete HA, although it can only bring about a partial agglutination. In HE tests with antisera, this type of incomplete HA can be demonstrated against members of both subgroups II and III, but not of subgroup I. Consequently, either the δ antigen (19) (i.e., the proximal part of fibers) is shared between members of subgroups II and III, or the fibers of members of subgroup III contain an antigen besides the δ antigen which is also present in the corresponding components of members of subgroup II. The marked difference in dimensions of fibers of members of these two subgroups (12) may suggest that the latter hypothesis is the most plausible one. The rapidly sedimenting incomplete HA (pentons) could be demonstrated in HE tests with antisera against members of any of Rosen's subgroups. This implies that the trypsin-sensitive vertex capsomer part of this type of component carries group-specific antigen specificities. Similar to the experience from studies of subgroup III members (18, 27), the fiber components of members of subgroup II were not inactivated by trypsin digestion. Instead, the former showed increased activity and caused a more distinct agglutination of cells after this treatment. This is a phenomenon which should be investigated further.

The fibers were eluted first from anion-exchange chromatography columns. Immediately after this peak of activity, a complete HA was eluted that also was capable of bringing about only a partial agglutination. This type of component was easily demonstrable in preparations of type 15, but occurred only in trace amounts in type 9 and 9-15 materials. It sedimented slightly faster than fibers in zonal centrifugation experiments, whereas in exclusion chromatography experiments the two activities eluted in clearly separate positions. The complete HA was recovered immediately after the void volume, whereas the fibers did not appear until immediately after the peak of 7*S* gamma globulin. This behavior suggests that the former component probably is even more elongated than the isolated fiber. Trypsin treatment had no effect on this type of complete HA. Furthermore, its titer was not increased significantly by the presence of heterotypic antisera. In most tests, the readings could be taken more readily due to an improvement of the bottom patterns, which in turn slightly increased the titer values.

All of the listed properties of this component are shared with those of a new type of soluble complete HA that was identified recently in preparations of adenovirus types 1, 3, 5, 6 (25), and 16 (15). This type of complete HA has been assumed to represent an aggregate of a few (most likely two) fibers forming a complex that is polyvalent with regard to its capacity to interact with receptors on red cells. A simultaneous occurrence of the two kinds of soluble HA, dodecons and fiber polymers, has been demonstrated only in preparations of subgroup II members (15).

The penton-associated incomplete HA of types 9, 9-15, and 15 eluted in anion-exchange chromatography experiments after the fiber incomplete HA (and also the fiber-associated complete HA), but before hexons. This sequence of elution of soluble components corresponds to that described for all members of subgroup III (6, 25, 27), except for the aberrant member, type 4 (24, 31).

Ultrastructural examinations of complete HA of types 15 and 9-15 demonstrated the type of star-shaped structures already demonstrated for some members of subgroup II, types 13, 15, 19 (2), and 9 (16). This type of component, the dodecon, has been interpreted to be composed of 12 pentons plus some extra structure. This was first reported from studies of type 3 (16). In this laboratory, the length of the fibers of both dodecons and virions of the subgroup II members studied (i.e., types 9, 15, and 9-15) has been estimated at 11 to 14 nm. This value is at variance with the values 16 to 19 nm presented by Gelderblom et al. (2) from their studies of types 13, 15, and 19. It is difficult to explain this difference. One finding suggests that the lower figure probably is the more nearly correct one. This is the result of the comparative exclusion chromatography experiments described above of mixtures of fibers. This technique was demonstrated to be an effective means of separating fiber components of varying lengths (12, 15). In the present study, it was found that fibers of all three types studied eluted later than the fibers of type 4, but before those of type 3. Because the lengths of types 3 and 4 fibers have been estimated at 9.5 to 10 nm and 17 to 18 nm, respectively, an interpolation would suggest that the fibers of the members of subgroup II studied are about 13 nm. This value tallies well with that actually found. Future ultrastructural studies of purified fibers and pentons will be of importance to settle conclusively the question about the length of the vertex projections in members of subgroup II.

In its general biological characteristics, the intermediate strain 9-15 exhibits more similarities to type 9 than to type 15. Some facts to be mentioned are (i) that types 9 and 9-15 agglutinate both rat and human type O erythrocytes, whereas type 15 agglutinates only the former; (ii) that only dodecons of the two former types are eluted

from red cells by RDE treatment; (iii) that types 9 and 9-15 materials contain only trace amounts of fiber-associated complete HA and, in addition, relatively less fiber incomplete HA than penton incomplete HA, whereas the opposite holds for type 15 material; and (iv) that the order of elution of soluble components is similar after anion-exchange chromatography of types 9 and 9-15 materials. The order differs, however, from that of type 15 components. The most obvious difference is that hexons of types 9 and 9-15 were eluted before dodecons, whereas the sequence of elution of the corresponding type 15 components is the reverse. This latter finding is at variance with the results presented by Gelderblom et al. (3), although the same group of workers (30) demonstrated an elution of complete HA before hexons in preparations of type 19, another member of subgroup II. The only possible difference between physicochemical properties of the corresponding types 9 and 9-15 components concerns the behavior of their hexons in anion-exchange chromatography experiments. There was a tendency for hexons of type 9-15 to elute somewhat, but not significantly, later than type 9 hexons. Type 15 hexons, in turn, eluted somewhat later than 9-15 hexons. In a recent review (9), it was suggested that an explanation on the structural level for the intermediate position of type 9-15 might be that its capsid contained a mosaic of fibers (or pentons), similar to those of type 9, and hexons related to those of type 15. An identity for type 9-15 pentons and those of type 9 is indicated by the present study. Anion-exchange chromatography behavior of type 9-15 hexons suggests properties intermediate between those of types 9 and 15. A further clarification of those questions probably can be obtained by use of immunological techniques that involve the use of specific antisera against the different components.

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