Soluble Components of Adenovirus Type 8¹

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Soluble components of type 8 adenovirus have been studied. Four different components were isolated by anion-exchange chromatography and purified by further chromatographic procedures, by zonal centrifugation, and by erythrocyte absorption and elution. The four components exhibited the following characteristics. (i) Fiber antigen was trypsin-resistant and functioned as incomplete hemagglutinin (agglutinated rat and human erythrocytes only in the presence of certain types of adenovirus antisera). (ii) The penton was trypsin-sensitive, exerted a cytotoxic effect, and also showed incomplete hemagglutination, being active in the presence of a majority of heterotypic adenovirus antisera studied. (iii) The group-specific hexon antigen reacted in complement fixation reaction and gel precipitation with sera prepared against other types of adenoviruses, besides showing characteristics indicating the presence of a type-specific antigen component. (iv) The soluble complete hemagglutinin was trypsin-sensitive, displayed cytotoxic effect, adsorbed easily to human and rat erythrocytes, and could be eluted from them by means of receptor-destroying enzyme. The three hemagglutinins of adenovirus type 8 proved to be highly unstable, and their demonstration was only successful by using a large quantity of freshly prepared concentrated virus material. Considering these conditions, a method was developed for their concentration and purification.

The antigenic structure of several adenovirus types has recently been elucidated by a number of investigators (4, 5, 20, 22, 24, 25, 34), and yet information concerning the soluble components of adenovirus type 8 is very scarce (1).

Adenovirus type 8 appears to diverge from other adenovirus types in some biological aspects (16); for example, it is markedly reduced in infectivity. On the other hand, it is the causative agent of a characteristic infectious disease, epidemic keratoconjunctivitis (8, 17). On the basis of its deoxyribonucleic acid (DNA) composition it was suggested that adenovirus type 8 may be oncogenic (29), although tumor induction with this type was until now successful only in a single case (33).

Considering these features, it appeared to be of some interest to carry out a detailed study of adenovirus type 8, and, despite the difficulties in its culturing, attempts were made to separate its soluble antigens to compare their characteristics with the known data of other adenovirus types.

MATERIALS AND METHODS

Tissue cultures and virus strains. HEP-2, HeLa, and MAS-A permanent human cell lines, cultured as de-

scribed in previous reports (10, 17, 30), were used for virus propagation and for studying the cytopathic effect (CPE).

The prototype strain of adenovirus type 8, strain "Ijima" (kindly provided by R. Wigand and E. Jawetz), and two strains of adenovirus type 8 isolated from epidemic keratoconjunctivitis cases in Budapest (17) and in Szeged (2) were included in our experiments. The four strains did not show any essential differences.

Infected cells showing complete degeneration were sedimented by low-speed centrifugation, collected in 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (pH 7.6), frozen and thawed five to six times, and disintegrated in a homogenizer (Measuring & Scientific Equipment, London, England). The cell debris was removed by centrifugation, and the virus pool obtained was stored at 4°C. The concentrated virus material was treated in certain cases with fluorocarbon (Arcton 113), and the nonsoluble components were sometimes removed by a three-times-repeated, 1-hr-long run in the ultracentrifuge at $\text{Pi } 5.4$ ($23,000 \times g$). These treatments, however, damaged a considerable part of the soluble components.

Immune sera. Rabbits were injected intravenously with complete virus and intramuscularly with purified components, adding Freund's complete adjuvant to the material, as described earlier (12, 17, 19).

Hemagglutination, incomplete hemagglutination, and hemagglutination-inhibition (HI) techniques. These examinations were performed in the Takátsy micro-titrator (32) by using 0.75% mixed rat or human O

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erythrocytes. The hemagglutination enhancement (HE) titer of the heterotypic adenovirus antisera was determined in chess-board titration, and 2 HE units was used for incomplete hemagglutination.

To study the complete hemagglutinin (HA) in the HI reaction, 8 units of antigen was allowed to incubate with the immune serum for 30 min, and the erythrocytes were added subsequently. To examine the incomplete HA, the antigen was subjected to prior incubation with the appropriate heterologous antiserum for 1 hr before the HI reaction was performed in the same way.

Complement fixation (CF) test. The reaction was performed in the Takátsy microtiter by using 2 units of complement and incubating overnight at 4 C.

Gel-diffusion precipitation. These tests were carried out with the two-dimensional method of Ouchterlony; the procedure was described previously (19).

Ultraviolet (UV) absorption spectrum and protein content determination. The UV absorption spectrum was determined in a Pye Unicam SP 800 spectrophotometer at between 190 and 450 nm. The protein concentration was determined by the procedure of Lowry et al. (15) by using bovine serum albumin as a standard.

Separation by ultracentrifugation. Experiments were carried out in a Superspeed 50 ultracentrifuge (Measuring & Scientific Equipment). To separate the soluble and nonsoluble components, the virus material was centrifuged three times for 1 hr at $Pi\ 5.4\ (23,000 \times g)$ on a CsCl cushion (density, 1.4 g/ml) or was centrifuged for 90 min on a discontinuous CsCl gradient (density, 1.4 and 1.2 g/ml) at $Pi\ 7\ (42,000 \times g)$. Equilibrium centrifugation in CsCl was performed for 24 hr at $Pi\ 13.7\ (83,000 \times g)$. Some of the preparations were subjected to zonal centrifugation for 5.5 hr in a linear gradient of 5 to 20% (w/v) sucrose at $Pi\ 7.5\ (45,000 \times g)$.

Anion-exchange chromatography. Diethylaminoethyl (DEAE)-Sephadex A-50 and A-25 (Pharmacia Fine Chemicals) columns were used to separate the virus components in anion-exchange chromatography. The results were more favorable with the A-25 columns; thus the majority of our experiments were carried out with this exchanger, in 0.04 M Tris-hydrochloride buffer, pH 8.0. The components retained by the column were eluted either stepwise with the same buffer, containing increasing concentrations of NaCl, or by introducing a linear NaCl gradient. Fractions were collected with an LKB fraction collector combined with a Uvicord I, and, in the case of gradient elution, the NaCl content of the fractions was checked in a refractometer.

Enzyme treatments. For trypsin treatment, five times crystallized trypsin (Serva) was added to the material to a final concentration of 0.1%. After incubating the mixture for 1 hr at 37 C, an equivalent quantity of soybean inhibitor (Serva) was added.

For receptor-destroying enzyme (RDE) treatment, the required dilution of standard cholera filtrate (Philips Roxane Inc.), determined by titration, was added to human erythrocytes, which had been previously incubated with HA and subsequently centrifuged. The HA-treated erythrocytes were incubated

for 2 hr with RDE and then removed from the eluate by low-speed centrifugation.

RESULTS

To separate the different soluble components of adenovirus type 8, different techniques were combined. The first step was anion-exchange chromatography.

The fractions eluted from the columns were subjected to the following procedures. The group-specific hexon antigen was determined by gel precipitation or by CF reaction with antisera prepared against the hexon antigens of adenovirus types 5 and 8; the complete HA was demonstrated by hemagglutination with rat erythrocytes; and the two incomplete HA were revealed in the presence of heterologous antisera. As adenovirus type 8 belongs to Rosen's subgroup II (31), it appeared to be necessary to apply a serum of the identical subgroup (adenovirus type 13) and one against a member of subgroup I (type 11).

To study the early CPE, HeLa and MAS-A cells were inoculated with 0.1 ml of the fractions. The UV absorption spectra of the fractions were recorded as well, and their protein content was determined occasionally.

A chromatogram of adenovirus type 8 is presented in Fig. 1. As noted, an incomplete HA was the first to elute, being active with rat and human erythrocytes in the presence of antiadenovirus type 13 serum. The elution of this component started to a slight degree, even without introducing the NaCl, and was finished at a low molarity (0.03 to 0.04 M) of NaCl. The second component eluting was another incomplete HA, which agglutinated the erythrocytes in the presence of both heterologous sera used in the experiments. This component showed a cytotoxic effect and was eluted at a concentration of NaCl of 0.007 M.

The hexon antigen was the third to elute, at a concentration of NaCl of about 0.11 to 0.12 M, and was closely followed by the soluble complete HA, its elution being induced by a concentration of NaCl of approximately 0.17 M. Fractions containing this component exhibited an early CPE. At the end of the chromatographic procedure, the hemagglutination activity increased slightly and a small quantity of CF antigen appeared again, indicating the start of the elution of the non-soluble components.

Four well-separated soluble components were obtained in this manner, and, except for the first one, there were certain overlaps. A detailed study of these components required further purification procedures.

Incomplete HA. The two incomplete HA (designated incomplete HA-1 and incomplete HA-2

FIG. 1. (a) Separation of adenovirus type 8 components by anion-exchange chromatography on a DEAE-Sephadex A-25 column. Different components were eluted by a linear NaCl gradient (dashed line) in 0.04 M Tris-hydrochloride buffer, pH 8.0. The following activities were recorded: $\square$, incomplete HA, determined in the presence of an antiadenovirus serum type 13; $\triangle$, incomplete HA determined in the presence of both antiadenovirus type 11 and 13 sera; $\bullet$, group-specific CF antigen determined by titration with antisera against hexons of type 5 and type 8 adenovirus; $\blacksquare$, complete HA; $\downarrow$, early CPE. (b) Extinction values at 280 nm of the experiment presented in Fig. 1a ($\circ$) and those of an anion-exchange chromatography of HEp-2 tissue culture material performed under identical conditions as in Fig. 1a ($\blacktriangle$).

corresponding to their elution sequence) were purified by chromatography repeated two to three times before further examinations. To determine their hemagglutinating activity, chess-board titration was performed with antisera prepared against the first 16 adenovirus types and against type 5 hexon and fiber antigens to verify which of them reveals the HE property (Table 1). The incomplete HA-1 agglutinated in the presence of the following antisera: type 13 and 15 of Rosen's subgroup II and sera prepared against the complete viruses of types belonging to subgroup III as well as against the fiber of type 5. Incomplete HA-2 displayed activity in the presence of a considerable number of the sera tested, and only those against types 8, 9, and 10 and type 5 antihexon serum failed to show the HE effect.

The isolated and purified incomplete HA both showed extreme lability and their titer decreased considerably, after 5 to 10 days of storage at 4 or -20°C . The same phenomenon could be observed with the complete HA, which will be discussed later. An effort was therefore made to elaborate the quickest possible concentration and purification technique.

TABLE 1. Hemagglutinating activity of the incomplete hemagglutinins of adenovirus type 8 in the presence of different types of antiadenovirus sera

Type of rabbit immune sera	Presence of HE ^a effect for incomplete	
	HA-1	HA-2
Adenovirus 3, 7, 11, 14, 16.....	— ^b	+
Adenovirus 8, 9, 10 ^c	—	—
Adenovirus 1, 2, 4, 5, 6, 12, 13, 15.....	+	+
Adenovirus 5 antihexon.....	—	—
Adenovirus 5 antifiber.....	+	+

^a Hemagglutination enhancement.

^b No effect at 1:20 serum dilution.

^c Hemagglutination-inhibition was observed with complete and incomplete type 8 hemagglutinins.

It is known that the HA of certain adenovirus types, which also agglutinate human erythrocytes, can be eluted from their surface with the help of RDE produced by *Vibrio cholerae* (9, 26). This possibility has been used only with the complete HA thus far, because, as described by several authors (5, 24, 27), it was found that incomplete HA fail to adsorb to the erythrocytes. However, if these HA were incubated with heterologous adenovirus antisera possessing an appropriate HE effect and human or rat erythrocytes were added subsequently, the bulk of them became adsorbed to the cells. Applying human erythrocytes, the incomplete HA, transformed into complete ones, could be recovered successfully by means of RDE. Receptors of rat erythrocytes were resistant to this treatment. This technique offers, besides purification, the concentration of the components to some extent, inasmuch as a volume of enzyme dilution which can be used for elution was smaller than the original quantity of absorbed material.

Table 2 shows the results of hemagglutination obtained in an absorption-elution experiment. In the material originally containing only incomplete HA, a considerable part of the incomplete HA became aggregated in the presence of a heterologous serum, transforming them into complete HA. This complete HA was extracted by two subsequent absorption procedures and was recovered quantitatively in a more concentrated form by treating the erythrocytes with RDE.

Further experiments were carried out with both incomplete HA in linear 5 to 20% sucrose gradients. Similar centrifugation experiments were performed by prior incubation of the components with heterologous serum (Fig. 2 and 3). The upper parts of both figures (a) demonstrate the results obtained in the parallel experiments with-

TABLE 2. Absorption-elution experiment with purified incomplete hemagglutinin (HA)-2 of type 8 adenovirus by using human erythrocytes and receptor-destroying enzyme (RDE)

Treatment	HA units per 0.025 ml of		
	Complete HA	Incomplete HA tested in the presence of serum against	
		Adeno-virus 13	Adeno-virus 11
None	— ^a	64	64
Antiadenovirus type 13 serum	48	64	64
Absorption 1 ^b	24	24	24
Absorption 2	—	4	—
Absorption 3	—	—	—
RDE ^c to cells of absorption 1	96	128	128
RDE to cells of absorption 2	32	48	48

^a Less than 4.

^b In each individual absorption, packed erythrocytes were added to a final concentration of 10%. After incubation for 40 min, cells were removed by centrifugation and the supernatant was tested.

^c A 1:4 dilution of cholera filtrate was added to the packed erythrocytes in a volume corresponding to one-fourth of the original volume of the absorbed material.

out adding heterologous serum, and the lower parts (b) show the results obtained after incubation with heterologous serum. The incomplete HA-2 also contained a small quantity of hexon antigen, its position being indicated in the figures as well.

As shown by Fig. 2 and 3, both incomplete HA, after treatment with heterologous serum, reached even a higher concentration in the gradient because of their aggregation. The connection produced by the heterologous serum seems to be rather unstable, as indicated by the HE effect of the heterologous sera with fractions 3 to 12 in Fig. 3b.

These findings suggest that the incomplete HA-1 corresponds to the fiber of type 8 adenovirus and HA-2 corresponds to the penton, as with other types (21, 27). This is also supported by the response of these components to trypsin treatment. Results of experiments with trypsin treatment are summarized in Table 3. According to these data, the hemagglutinating activity of the incomplete HA-1, corresponding to the fiber, was not diminished by trypsin treatment; in fact, in certain experiments, similarly to other types (20),

FIG. 2. Zonal centrifugation of the incomplete HA-1 of adenovirus type 8 in a linear 5 to 20% sucrose gradient for 5.5 hr at $\text{Pi } 7.5$ ($45,000 \times g$). Fractions were collected dropwise through the bottom of the tube (left). Upper part (a) without heterologous serum; lower part (b) after incubation with antiadenovirus type 13 serum. Symbols correspond to those presented in Fig. 1.

an increase could even be observed. The activity of the penton, however, disappeared in the presence of type 11 serum together with its cytotoxic effect. No changes were found in its hemagglutinating activity and cytotoxic effect after UV irradiation.

Hexon antigen. The group-specific hexon antigen was the third in the sequence of elution. As shown by Fig. 1, this component was contaminated by a certain amount of complete HA. To eliminate the latter, erythrocyte absorption was applied successfully. Thereafter, contamination with incomplete HA-2, corresponding to penton, was also found, with an overlap by the complete HA. The elimination of this factor was accomplished by repeated absorptions, and occasionally heterologous serum was added to the material prior to adding the erythrocytes. The last absorption procedure was followed by three or more subsequent chromatographic treatments, and, if no traces of either HA could be revealed in the fractions, they were considered to contain pure hexon antigen.

Rabbits were immunized with hexon antigen prepared by several erythrocyte absorptions and by five-times-repeated anion-exchange chromatography. The serum reacted satisfactorily with

FIG. 3. Zonal centrifugation of the incomplete HA-2 of adenovirus type 8 under similar conditions as in Fig. 2. Symbols correspond to those presented in Fig. 1, the only difference being that group-specific hexon antigen was tested in gel-diffusion precipitation test (●).

different types of adenovirus strains in gel diffusion and CF tests up to the dilutions 1:128 and 1:1,024, respectively. In neutralization tests, the serum failed to neutralize the CPE of adenovirus types 5, 7, 9, and 10 but neutralized that of the homologous type 8, up to the dilution 1:80.

HI experiments were performed with the serum prepared against the hexon antigen of type 8 (Table 4). The serum failed to inhibit the hemagglutination of three heterologous types, the soluble complete HA and both incomplete HA of the homologous type 8; however, it inhibited partially at a titer of 1:80 the type 8 virion, obtained by CsCl equilibrium centrifugation. This partial reactivity was revealed by the agglutination of two-third of the erythrocytes only at the titer mentioned. Table 4 indicates parallel titers of the sera against the complete adenovirus of homologous types.

Soluble complete HA. The complete HA was

TABLE 3. Effect of trypsin treatment on the incomplete hemagglutinins (HA) of adenovirus type 8

Designation of incomplete HA	Trypsin ^a treatment	HA units per 0.025 ml of		Early cytopathic effect
		Complete HA	Incomplete HA tested in the presence of serum against	
			Adeno-virus 13 Adeno-virus 11	
1	0	— ^b	32	—
	+	—	48	—
2	0	—	64	+
	+	—	128 8	—

^a Incubation with trypsin in a final concentration of 0.1% for 1 hr at 37 C in a water bath.

^b Less than 4.

TABLE 4. Hemagglutination-inhibition (HI) tests with antiadenovirus sera and adenovirus type 8 antihexon immune serum

Adeno-virus type	Antigen prep	Reciprocals of HI titers of immune sera against	
		Homologous crude virus	Type 8 hexon
8	Fiber	80	— ^a
8	Penton	120	—
8	Complete HA ^b	320	—
8	Virion	640	80 partial
9	Crude virus	1,280	—
10	Crude virus	640	—
15	Crude virus	160	—

^a Less than 1:10.

^b Hemagglutinin.

the last in the sequence of elution, its elution usually starting above 0.16 M NaCl and the maximum being about 0.2 M NaCl (Fig. 1). Placed on tissue culture, this component also showed an early CPE and was trypsin-sensitive both in respect to hemagglutinating activity and cytotoxic effect. Results of trypsin treatment are presented in Table 5. Thus, the HA lost its ability to cause complete hemagglutination to a considerable extent, and the majority of it transformed into incomplete HA corresponding to the fiber.

The complete HA was easy to absorb with rat and human erythrocytes and was eluted from the latter with RDE (Table 6). It is noticeable that, while extracting the complete HA, an incomplete HA, covered up by the complete one, can be traced in these fractions besides the hexon contamination. The complete HA of type 8 adenovirus was very unstable, and a four- to eightfold titer decrease could be observed within a few days

TABLE 5. Effect of trypsin treatment on the purified soluble complete hemagglutinin (HA) of adenovirus type 8

Trypsin treatment ^a	HA units per 0.025 ml of			Early cytopathic effect
	Complete HA	Incomplete HA tested in presence of serum against		
		Adenovirus 13	Adenovirus 11	
No	768	768	768	+
Yes	32	1,024	128	—

^a Conditions similar to those presented in Table 3.

TABLE 6. Absorption-elution experiments with fractions of anion-exchange chromatography containing soluble complete hemagglutinin (HA) of adenovirus type 8

Treatment	HA units per 0.025 ml of			Hexon ^a
	Complete HA	Incomplete HA tested in presence of serum against		
		Adeno-virus 13	Adeno-virus 11	
No	256	256	256	+
Absorption 1 ^b	— ^c	96	64	+
Absorption 2	—	96	64	+
RDE to cells of absorption 1 ^d	384	384	384	—

^a Determined in gel-diffusion precipitation tests with homologous and type 5 anti-hexon sera.

^b Absorptions carried out as presented in Table 2.

^c Less than 4.

^d A 1:4 dilution of cholera filtrate added to the packed erythrocytes in a volume corresponding to half of the original volume of the absorbed material.

both in the original fractions and in the RDE eluates. This finding supported the suggestion that this incomplete HA resulted from the decomposition of the complete HA. However, by collecting, concentrating, and submitting the fractions to repeated chromatography after the absorption procedure, the position of this incomplete HA remained unchanged and started to elute at 0.16 M NaCl (Fig. 4).

Results of the zonal centrifugation of an RDE eluate of complete HA (Fig. 5) show likewise the instability of this component. The presence of complete HA equally in both the upper and lower regions of the gradient indicates the possibility that it consists of rapidly and slowly sedimenting

FIG. 4. Recovery of different biological activities of fractions containing complete HA of adenovirus type 8, from which the complete HA had been removed by erythrocyte absorption. The conditions of the anion-exchange chromatography and symbols correspond to those presented in Fig. 1.

FIG. 5. Zonal centrifugation of the complete HA of adenovirus type 8 under similar conditions as in Fig. 2. Symbols as in Fig. 1.

components. The localization of incomplete HA-1 and HA-2 indicates that the complete HA disintegrated as early as during the few hours of centrifugation; this explains the phenomenon that both kinds of incomplete HA are present near the bottom of the tube, localized otherwise after a 5.5-hr run in the upper regions of the tube, as shown in Fig. 2 and 3.

DISCUSSION

Our findings concerning the elution sequence of adenovirus type 8 components in anion-exchange chromatography agree with those of other authors, who studied other members of Rosen's subgroup II (5, 6, 20, 24). A single reference is to be found in the literature dealing with the anion-exchange chromatography of adenovirus type 8 (7). The first successful separation of four different soluble components of this type was carried out in our experiments. The common characteristic of these components, except the hexon, is their extreme instability.

The characteristics of the fiber antigen observed in our experiments, i.e., the elution at a low molarity of NaCl, the trypsin resistance, and the hemagglutinating activity displayed in the

presence of heterologous adenovirus antisera belonging to Rosen's subgroup II and III tally with the characteristics observed with the types 9, 15, 9-15, and 19 of the identical subgroup (5, 20, 24).

It was shown by several investigators (22, 27) that the penton and its aggregate the dodecon are responsible for the early CPE exhibited by adenovirus types of subgroups I and III. Early CPE was also observed in our experiments with type 8 of subgroup II, occurring likewise in the presence of the two soluble components mentioned. This effect of the penton was very unstable and disappeared even before the decay of incomplete hemagglutinating activity (within approximately 7 days). The trypsin sensitivity and the resistance to UV irradiation of the early CPE prove its toxic nature and contradict the possibility that it might be a phenomenon related to virus multiplication.

Our observations on the penton component indicate that every antiserum against the first 16 adenovirus types, except types 8, 9, and 10, enhances its hemagglutination. This supports the theory (21) that the penton of the adenoviruses also contains a group-specific antigen component, localized supposedly in the vertex capsomer.

Besides the lack of HE effect, the HI property could be demonstrated with antisera to types 8, 9, and 10, the titer of the latter two being considerably lower (1:40, and 1:20, respectively). This confirms our previous observations on the cross-reactions among these three types (12, 13, 18). Results obtained in neutralization and HI tests (Table 4) with type 8 antihexon serum indicate the possibility that it is not the hexon component which is responsible for this cross-reaction but the vertex projections. Various data are available with other adenovirus types on the type specificity of hexon and the neutralization or virion HI effect of sera produced against purified hexon (3, 5, 11, 19, 23, 26, 35), whereas other authors report different results (28). Our observations seem to support the previous findings.

The trypsin sensitivity of the complete soluble HA corresponds to that found with type 9 (24), which supports the reasonable suggestion that this component of type 8 is also a polymer of pentons. The results obtained with purified complete HA in zonal centrifugation (Fig. 5) indicate that, although polymers consisting of several pentons, even dodecons, may be present, slowly sedimenting small units, occasionally dimers, may assemble to build this component as well. The localization of the slowly sedimenting incomplete HA is related to the rapid decomposition of the complete HA. Had they been present in the starting material in monomeric form, they would have been localized in fractions 10 to 13, taking the results in Fig. 2 and 3 into consideration. An

eight-times-longer run was required for the identical components of type 9 to move this far from the surface during gradient centrifugation (24). It may be presumed, on the basis of the findings presented in Fig. 5, that the rapidly sedimenting polymers are more unstable, than the slow ones. This is indicated by the fact that the fractions localized in the upper part of the tube, comprising only slowly sedimenting complete HA, which consists supposedly of dimers or trimers, contain only a small quantity, if any, of incomplete HA covered up by the complete one.

The problem of whether the soluble complete HA of type 8 is a dodecon like those of the types studied of subgroup II (4, 20, 24) or a mixture of polymers consisting of different numbers of pentons, is questionable, but it seems worth indicating that slowly sedimenting complete HA (penton or fiber dimers) were found with other adenovirus types as well; these, however, eluted in anion-exchange chromatography already separated from the dodecon (20, 25). Such separately eluting complete HA were not observed in our experiments; thus it might be supposed that the complete HA of type 8 is also a dodecon, which splits in a very short time into smaller polymers of different sizes because of its instability.

The soluble complete HA could be absorbed with human erythrocytes from the solution and subsequently eluted from them by RDE, similarly to certain other adenovirus types (9, 20, 24). This method can be used to purify the complete HA, as described with type 9 (24), and to concentrate it to a certain degree. It was also successfully applied for the purification and concentration of the incomplete HA, by incubating them before the absorption with corresponding heterologous adenovirus antisera. The technique provides the opportunity not only for rapid isolation of the incomplete HA but also for obtaining favorable purification results with the hexon antigen.

The mechanism of the phenomenon which enabled us to develop this method of purification is probably a linkage between the subgroup- or group-specific determinants (ϑ or β) of two or more incomplete HA (23, 27), accomplished by the heterologous antibody, resulting in polymers. The aggregates thus acquiring two or more type-specific antigen determinants (γ), which are supposedly the knobs of the fibers, will be capable of adsorbing onto the surface of erythrocytes, wherefrom they can be eluted by destroying the receptors with RDE.

No explanation was found for the late elution of a part of the pentons (Fig. 4); it is possible that some kind of aggregation causes their elution with the same NaCl concentration as the complete HA or their adsorption to some other virus (or tissue)

component. It is known that the fiber is capable of adsorbing to cell or virus DNA (14), and the fiber is actually one of the constituents of the penton. As no information is available at this time on the component supposedly present inside the dodecahedron (21), a possible role can not be excluded.

Although our experiments were not designed as a detailed examination of the infectivity of adenovirus type 8, the marked instability of the soluble components, some of them playing a part in the adsorption of virus to the cell (21), may perhaps account for the strikingly low infectivity of this type.

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