EVIDENCE FOR THE PRESENCE OF HALF-CYSTINE RESIDUES IN THE CAPSID OF ADENOVIRUS TYPE 7

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1. Introduction

Adenoviruses consist of an internal nucleoprotein core and of an icosahedral capsid constructed from 252 capsomers [1, 2]. Of these, 240 are distributed along the edges and on the faces of the triangles of the icosahedron and are called "hexons". The remaining 12 capsomers, endowed with outward projections, are located on the vertices of the capsid and are called "pentons". Free hexons, pentons and, with some adenovirus serotypes, aggregates of 12 pentons - designated as dodecons - are found in infected cells. Study of the interaction of adenoviruses with sulfhydryl (SH) group reagents [3] suggested that SH groups in the viral capsid play an important role in the maintenance of the three-dimensional structure of the virus and in its reaction with cell receptor sites. Amino acids analyses of adenoviruses revealed the presence of 0.3 to 0.6 mole of half-cystine per 100 moles of amino acids [4]. However, no half-cystine was detected by amino acid analysis of hexons of adenovirus types 2 and 5 [5, 6], and conflicting results were obtained regarding the presence of half-cystine in pentons [6, 8]. In these experiments the adenovirus proteins were hydrolyzed in 5.6 to 6 N HCl alone at conditions which often result in low recoveries of cystine and/or cysteine [9, 10]. The present results show that ^{14}C-cystine (cysteine) is incorporated into the capsomers of adenovirus type 7.

2. Materials and methods

Adenovirus type 7 was grown in human embryonic kidney (HEK) cells. 270 min after infection, 1 μCi/ml of thymidine-methyl-^{3}H or 0.3 μCi/ml of *d,l*-cystine-3-^{14}C was added to the medium. In the latter case, the concentration of unlabeled amino acids in the medium was reduced 10 times, and 40 μl of mercaptoethanol was added to 100 ml of medium. Both isotopes were obtained from International Chemical and Nuclear Corp., Irvine, California. Uninfected cells were labeled in the same way and later processed identically as the infected cell material. Virus particles and free viral components were precipitated from the tissue culture material with 50% saturated ammonium sulfate. The precipitate was resuspended in 0.01 M tris buffer, pH 7.0 (TB). One-ml aliquots were centrifuged for 30 min at 18,000 rpm in the SW65 rotor, using the L-4 preparative ultracentrifuge (Beckman Instruments, Palo Alto, California), in order to pellet the virus particles which were subsequently resuspended in TB. The supernatant fluids were dialyzed overnight against TB and then applied to a column (1.6 × 24 cm) of DEAE cellulose (Serva, Heidelberg, Germany). The elution and titration of hexons, pentons and dodecons were performed as described previously [11]. To facilitate the comparison of elution profiles of radioactivity corresponding to infected and uninfected material, the radioactivity and the titer of the adenovirus components were plotted against the molarity of NaCl of the fractions. Rate zonal centrifugation was performed in linear sucrose gradients as described before [11]. Isopycnic density gradient centrifugation was performed with preformed gradients of CsCl (density range 1.24–1.42 g/cm^3). Isoelectric focusing [2] was performed with the LKB 8101 ampholine column (LKB Produkter AB, Bromma, Sweden) for 48 to 72 hr at 200 V. A carrier ampholyte was used which covered the pH region of 3 to 10.

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Elution of the columns was performed at a speed of 2 ml/min.

Samples for amino acid analysis were hydrolyzed for 24 hr in sealed evacuated ampules in 6 N HCl and 0.2 M dimethyl sulfoxide. Under these conditions, cysteine and cystine are quantitatively converted into cysteic acid [9]. Analysis of amino acids was carried out according to the technique of Moore et al. [13].

For measurement of radioactivity, samples were placed into vials containing 20 ml of scintillation fluid (100 g of naphthalene, 10 g of 2,5-diphenyloxazole, 250 mg of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene, 200 ml of methanol and 1,000 ml of p-dioxane). Radioactivity was measured in a Packard 3375-Tri-Carb liquid scintillation counter.

3. Results and discussion

Adenoviruses have a buoyant density in CsCl solutions of about 1.34 [1], and are conventionally purified and separated from cellular components and free capsomers by isopycnic density gradient centrifugation. Therefore, the occurrence of radioactivity at a density between 1.34 and 1.36 after isopycnic banding of adenovirus type 7 from ^{14}C-cystine-labeled infected cells (fig. 1) serves as evidence for the presence of half-cystine in adenovirus type 7. No radioactivity was recovered at this density range when labeled uninfected material was submitted to the same procedure. When either ^{3}H-thymidine or ^{14}C-cystine-labeled purified adenovirus was submitted to rate zonal centrifugation (fig. 2, top), radioactivity was recovered in a single peak in about the middle of the gradient - as expected for a virus with a sedimentation coefficient of about 800 S [14]. When the same viruses were treated with 30% formamide for 30 min at 36°-a procedure leading to the disruption of the virus into an internal core and into individual capsomers [15] - rate zonal centrifugation resulted in the separation of the ^{3}H-thymidine label (representing internal cores) from most of the ^{14}C-cystine label. The latter stayed on the top of the

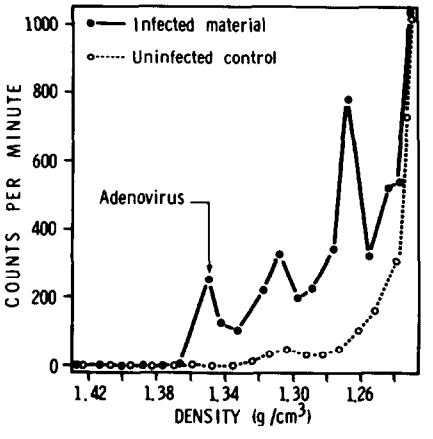

Fig. 1. Isopycnic density gradient centrifugation of resuspended pellets after high speed centrifugation (18,000 rpm for 30 min, rotor SW 65) of ^{14}C-cystine-labeled tissue culture material from adenovirus-infected or uninfected HEK cells.

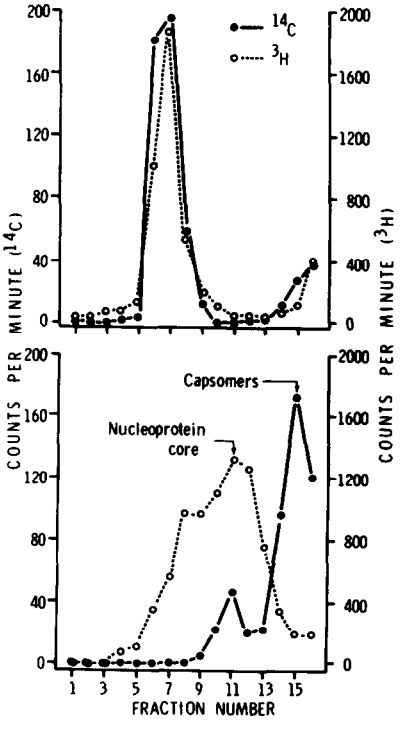

Fig. 2. Distribution of radioactivity in fractions obtained by rate zonal centrifugation of purified labeled adenovirus (top; centrifugation at 18,000 rpm for 30 min, sucrose gradient in 0.075 M phosphate, 0.072 M NaCl, pH 7.2), and of the same virus disrupted by formamide (bottom; centrifugation at the same speed for 60 min in sucrose gradients in 33% formamide). In both cases ^{3}H-thymidine- and ^{14}C-cystine-labeled viruses were centrifuged separately and the results were superimposed on the figure. Fraction 16 = top.

gradient (fig. 2, bottom), as capsomers do under the same conditions of centrifugation [11]. To confirm that most of the ^{14}C-cystine label really represented capsomers, the top fractions (corresponding to fig. 2, bottom) were submitted to rate zonal centrifugation at 65,000 rpm for 2 hr. Under these conditions, both radioactivity and hexons were recovered in a single peak in the middle of the gradient.

To support the finding that cystine (cysteine) was incorporated into the adenovirus capsomers, the incorporation of this amino acid into free hexons and pentons was studied. Hexons, pentons and dodecons were first separated from each other by chromatography on DEAE cellulose (fig. 3). This procedure did not result in sufficient separation of the viral components from cellular contaminants, as is evident from the comparison of the elution profiles of radioactivity corresponding to infected and uninfected material. Hexons and pentons were further purified by rate zonal centrifugation for 2 hr at 65,000 rpm [11]. When hexons purified in this way were submitted to isoelectric focusing, a single peak of radioactivity appeared at pH 4.4, coinciding with the peak of hexon (fig. 4). With ^{14}C-cystine-labeled material from uninfected cells, only traces of radioactivity were recovered in the fractions with pH values corresponding to the peak of hexon. Hexons obtained after electrofocusing were submitted to amino acid analysis. Radioactivity was recovered in a single peak at the position corresponding to the cysteic acid standard (fig. 5). Similar results were obtained with pentons, which, upon isoelectric focusing, were recovered in a pH range of 4.10 to 4.35. Radioactivity corresponding to both hexons and pentons was precipitated in the indirect immunological precipitin test [16] using antiserum against purified adenovirus.

The presented results give qualitative evidence for the presence of half-cystine in adenovirus type 7 capsomers and justify further studies concerning the quantitative distribution of cysteine/cystine in adenovirus polypeptides and their role in intra- and interchain bonds. Recents studies [11] exclude the involvement of S-S bonds between capsomers. However, the cysteine side chain may play a role in the hydrophobic binding between capsomers [17].

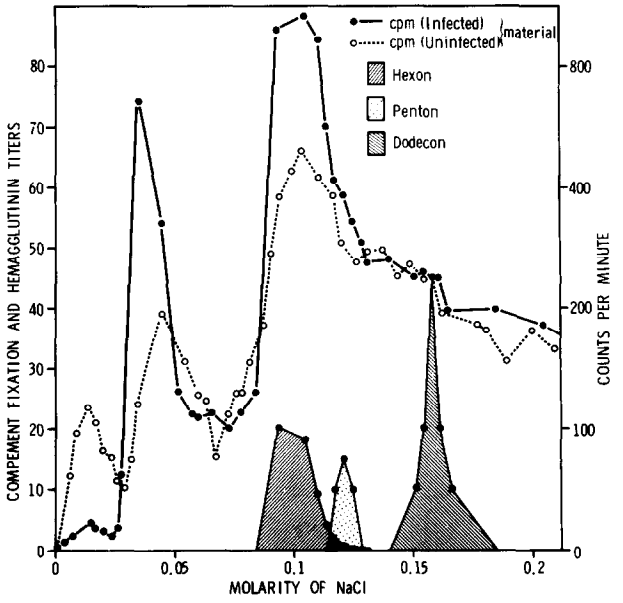

Fig. 3. Distribution of radioactivity, hexons, pentons and dodecons in fractions obtained by chromatography on DEAE cellulose of ^{14}C-cystine-labeled adenovirus-infected and uninfected tissue culture material.

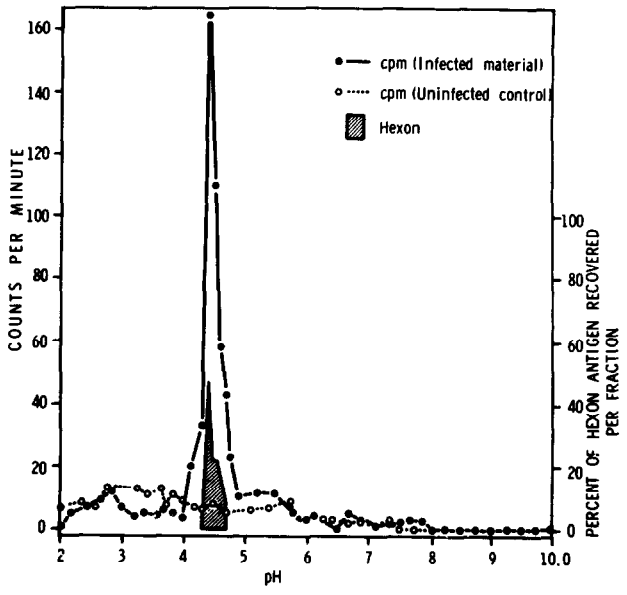

Fig. 4. Isoelectric focusing of ^{14}C-cystine-labeled adenovirus type 7 hexons.

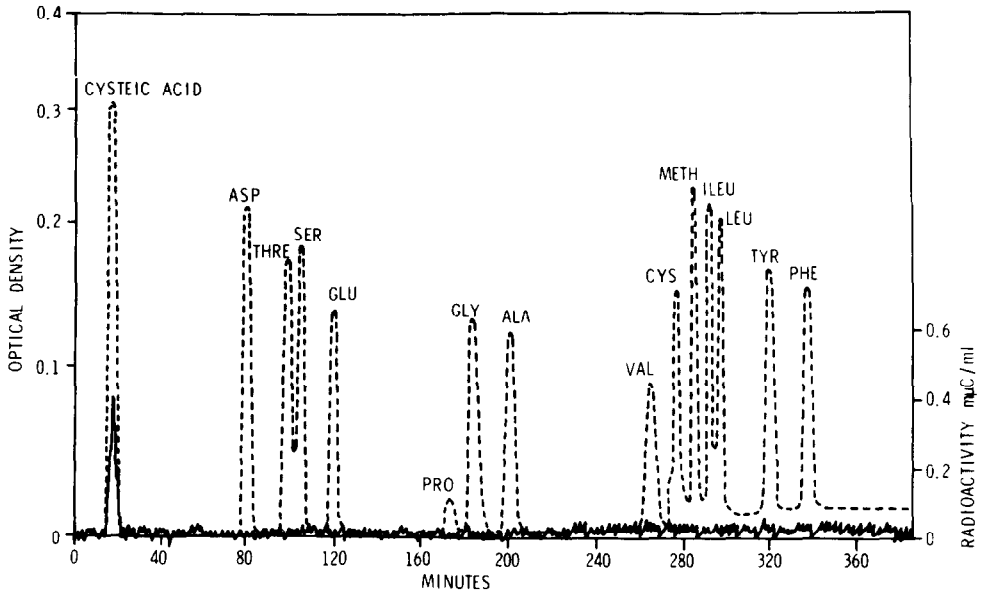

Fig. 5. Distribution of radioactivity (solid line) and ninhydrin-positive material (dashed line) in fractions from amino acid analysis of hexons and a mixture of standard amino acids, respectively.

References

[1] R.W.Schlesinger, Advan. Virus Res. 14 (1969) 2.
[2] E.Norrby, in: Current Topics in Microbiology and Immunology, Vol. 43 (Springer, Berlin, Heidelberg, New York, 1968) p.1.
[3] A.R.Neurath and B.A.Rubin, J. Gen. Virol. 2 (1968) 215.
[4] H.Polasa and M.Green, Virology 31 (1967) 565.
[5] U.Pettersson, L.Philipson and S.Höglund, Virology 33 (1967) 575.
[6] P.A.Boulanger, P.Flamencourt and G.Biserte, European J. Biochem. 10 (1969) 116.
[7] U.Pettersson, L.Philipson and S.Höglund, Virology 35 (1968) 204.
[8] U.Pettersson and S.Höglund, Virology 39 (1969) 90.
[9] R.L.Spencer and F.Wold, Anal. Biochem. 32 (1969) 185.
[10] A.S.Inglis and T.Liu, J. Biol. Chem. 245 (1970) 112.
[11] A.R.Neurath, B.A.Rubin and J.T.Stasny, J. Virol. 2 (1968) 1086.
[12] H.Svensson, Acta Chem. Scand. 15 (1961) 325.
[13] S.Moore, D.H.Spackmann and W.H.Stein, Anal. Chem. 30 (1958) 1185.
[14] W.C.Lawrence and H.S.Ginsberg, J. Virol. 1 (1967) 851.
[15] J.T.Stasny, A.R.Neurath and B.A.Rubin, J. Virol. 2 (1968) 1429.
[16] M.S.Horwitz and M.D.Scharff, in: Fundamental Techniques in Virology, eds. K.Habel and P.Salzman (Academic Press, New York, London, 1969) p. 297.
[17] P.Heitmann, European J. Biochem. 3 (1968) 346.