Identification and characterization of incomplete hepatitis A virus particles

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The range of hepatitis A virus (HAV) particles generated during persistent infection of different cell lines was studied. Buoyant density and sedimentation analyses of cell extracts revealed a uniform profile of empty particles, which banded at 1-305 g/ml and cells. The virion itself usually represented less than 50% of the total mass of virus antigen. A major portion of the antigen was associated with non-infectious, empty particles, which banded at 1-305 g/ml and 1·20 g/ml CsCl, and sedimented in sucrose gradients at 76S and 59S. Empty HAV particles were similar to those of poliovirus with respect to their physical stability and had the characteristic capsid protein content (VP0, VP1 and VP3). An additional RNA-containing particle, probably the provirion, represented only a minor species characterized by a buoyant density of 1·32 g/ml in CsCl and sedimenting at 130S.

Introduction

Hepatitis caused by hepatitis A virus (HAV) has a significant impact on public health in developing countries and affects increasing numbers of travellers to endemic areas, mainly from non-immune populations in industrialized regions. Hence, although hepatitis A is associated with low morbidity and mortality, efforts have been made to produce a vaccine. In the near future, killed-type vaccines based on purified virus derived from HAV-infected cell cultures will be available (André et al., 1990; Sjogren et al., 1988; Flehmig et al., 1989). However, because replication of HAV in cell culture generates a variety of virus particles, the question arises whether extensive purification of mature virus is necessary or whether additional particle species are able to contribute to the immunizing potency of vaccine preparations.

A prerequisite for the investigation of this question would be to have data available that clearly define the range of virus-related particles in infected cell cultures. This would allow isolation of highly purified particle species and elucidation of their respective antigenic and immunogenic properties. However, a review of reports on individual particle types produces a list of particles which differ between authors. For instance, from low density populations some authors detected only infectious particles, whereas others found such particles contaminating large amounts of empty particles (Siegl et al., 1981; Flehmig, 1981; Lemon et al., 1985; Lemon & Binn, 1985; Gauss-Müller et al., 1986; Wheeler et al., 1986a; Heinricy et al., 1987).

Therefore the aim of our investigation was to establish a reference range of HAV particle structures in cell culture, and to characterize the major components of that range in persistent HAV infection and at times when maximal amounts of antigen (Ag) usually are generated. The results define and characterize a major portion of Ag as being associated with empty capsids. Furthermore, the analyses revealed a minor component that most probably consists of provirions.

Methods

Virus. Cells were infected with HAV strain CLF, which originated from an isolated case of hepatitis A in Switzerland (Siegl et al., 1984a). The strain has been adapted to growth in African green monkey kidney (BGM, Vero and BS-C-1), foetal rhesus monkey kidney (FRhK-4) and MRC-5 (human diploid embryonic lung) cells.

Cells. Virus was propagated at 32 °C in BGM, BS-C-1, FRhK-4, MRC-5 and Vero cells in Nunc cell factories for mass production, or in 24-well tissue culture plates (Falcon) for titration of virus infectivity. Cell growth and harvest of infected cultures have been described previously (Siegl et al., 1984a; de Chastonay & Siegl, 1987).

Detection of HAV Ag. HAV Ag was determined by sandwich radioimmunooassay (RIA), as described by Frösner et al. (1977), or ELISA (unpublished results).

 Infectivity assay. Aliquots of fractions of sucrose gradients were extracted with chloroform (1:1), serially diluted 10-fold in medium without serum, and four coverslip cultures per dilution were infected in a 24-well plate (Falcon). Quantification of infectious HAV was performed by in situ RIA after a 21 day incubation as described by Siegl et al. (1984a). Virus titres were calculated according to the method of Reed & Muench (1938).

Purification of virus particles. Cell culture harvests were concentrated by polyethylene glycol (PEG) precipitation (10% PEG 6000, 0·4 m-
sodium lauryl sarcosine (SLS, final concentration 1%) was added. The reaction was stopped by the addition of EDTA (2 mM final concentration). After an additional enzyme treatment (trypsin, 5 mg/ml final concentration) for 1 h at 37 °C, sodium lauryl sarcosine (SLS, final concentration 1%) was added. The suspension was finally incubated with rocking for 1 h at 37 °C.

Isopycnic centrifugation. Virus particles were separated in a caesium chloride/sucrose gradient constructed by layering 3 ml volumes of 1.36, 1.32, 1.30 and 1.28 g/ml CsCl onto a 4 ml cushion of 1.5 g/ml CsCl in a 38.5 ml polyallomer tube (Beckman). The CsCl column was covered with 6 ml 30% sucrose and the purified HAV suspension was added to the top of the gradient. CsCl and sucrose solutions contained TN buffer/0.1% SLS. Gradients were centrifuged in a Beckman SW27.0 rotor at 25000 r.p.m. at 15 °C for 18 h. About 35 fractions were routinely collected from the bottom of the tube and assayed for HAV Ag. Peak Ag fractions were pooled and dialysed against TN buffer/0.1% SLS, concentrated by diafiltration (Centricon 30; Amicon), and rebanded in a self-generating CsCl gradient or analysed by sedimentation in 10 to 30% (w/w) sucrose gradients. Peak Ag fractions of the caesium chloride/sucrose gradient were layered with 1:30 g/ml CsCl/TN buffer/0.1% SLS and centrifuged in a Beckman SW41 rotor at 24000 r.p.m. at 15 °C for 64 h.

Sedimentation analysis. Diafiltered pools, usually 0.2 ml, from caesium chloride/sucrose gradients were layered onto a 10 to 30% (w/w) sucrose (in TN buffer/0.1% SLS) gradient in polyallomer tubes (Siegl & Frösner, 1978) and centrifuged in a Beckman SW41 rotor at 32000 r.p.m. at 15 °C for 105 min.

Detection of HAV RNA by slot blot hybridization. HAV RNA was extracted from fractions of sucrose gradients by treatment with 200 μg/ml proteinase K (E. Merck) in buffer containing 100 mM-Tris-HCl, 12.5 mM-EDTA, 150 mM-NaCl, 1% SDS, pH 7.5 for 30 min at 37 °C. Each sample was then diluted 1:1 with TE buffer (10 mM-Tris-HCl pH 7.5, 1 mM-EDTA). Viral RNA was extracted twice, first with phenol (equilibrated with 100 mM-Tris-HCl, pH 7.5) and then with chloroform/isoamyl alcohol/phenol, 24/1/25 (v/v/v). Samples were diluted with 3 volumes 6-15 M-formaldehyde in 10 x SSC (1 × SSC is 150 mM-NaCl, 15 mM-sodium citrate), incubated for 15 min at 65 °C and applied directly to a nitrocellulose membrane (Amersham; Schuell). The membrane was dried under an infrared lamp for 10 min, then baked for 2 h at 80 °C in a vacuum oven and sealed into a plastic bag until use.

HAV DNA probe cDNA. HAV strain HM-175, from which hybridization probes were derived, was generously provided by Dr John Ticehurst (WRAIR, Washington, D.C., U.S.A.) (Ticehurst et al., 1983). For hybridization with viral RNA we used a HindIII/EcoRI cDNA fragment corresponding to the 2C region of HAV. This fragment was isolated by gel electrophoresis and elution from low molten point agarose, and subsequently radiolabelled with 32P by random-primed DNA synthesis (Amersham).

HAV cDNA-RNA hybridization. Hybridization was performed according to standard procedures (Amersham; Membrane Transfer and Detection Methods, 1985).

Western blot analysis. Individual particle species were purified by isopycnic and subsequent velocity centrifugation as described above. To analyse their protein composition, peak Ag fractions and, in the case of 76S and 59S particles, right and left slope fractions of Ag peaks were pooled from identical Ag profiles. For electrophoretic separation of capped proteins according to Laemmli (1970), fractions were dialysed against stacking buffer, denatured by heat and electrophoretically separated through 12% polyacrylamide gels in a Mini-PROTEAN II cell (Bio-Rad) at a constant voltage of 200 V at 20 °C for 43 min. Electrophoretic transfer onto nitrocellulose membranes (Schleicher & Schuell; BA 85) was performed in transfer buffer in a Mini Trans-Blot cell (Bio-Rad) at a constant 100 V at 4 °C for 30 min, according to the manufacturer's instructions. Detection of HAV capsid proteins was performed with specific rabbit anti-VP1, anti-VP3 and anti-VP0 antisera (a kind gift from Dr V. Gaus-Müller, University of Lübeck, Germany) and gold-conjugated goat anti-rabbit antibodies (Janssen; Auroprobe BLplus and IntenSE BL), according to the manufacturer's instructions. To avoid using sample material for the determination of total protein, individual samples were applied at twofold serial dilutions. After immunodetection of VP1 and/or VP3, diluted samples which produced equally intense VP1 or VP3 signals were analysed at the predetermined dilution with anti-VP0 antibody.

Production and labelling of poliovirus and poliovirus procapsids. Production of poliovirus in HeLa cells, labelling of virus RNA in the presence of [3H]uridine (50 μCi/ml) and harvesting were performed by the method of Siegl & Frösner (1978). Poliovirus procapsids were labelled in the presence of 50 μCi/ml [3H]leucine. Production and isolation procedures have been described previously by Onodera et al. (1986).

Results

Buoyant density and sedimentation rates of various HAV particles

Monolayer cultures of BGM, BS-C-1, FRhK-4, MRC-5 and Vero cells were infected with HAV CLF to investigate possible variation in the range of particles produced by one virus strain in different cell lines. Three weeks after infection cells were harvested and lysed with trypsin, and cell-associated particles were extracted. To obtain optimal resolution of Ags upon subsequent analysis the extraction procedure included treatment with trypsin, DNase I, RNase A and chloroform in various combinations. Extracted virus Ags were separated by buoyant density centrifugation on a caesium chloride/sucrose density gradient and detected by ELISA or RIA with convalescent antibody.

The analysis revealed that, with the exception of BS-C-1 cells (Fig. 1b), infection of all cell lines yielded a nearly identical range of HAV particles (Fig. 1a). Typically, three major Ag peaks were seen at 1.335, 1.305 and 1.20 g/ml CsCl. In an attempt to improve the efficiency of separation of particles from BS-C-1 cells, extensive modifications of various steps of the procedure were tested (not shown). These variations included the use of (i) 1% Triton X-100, (ii) a mixture of detergents (0.1% SDS, 1% sodium deoxycholate, 1% Triton X-100), or (iii) mechanical disruption (in a Dounce homogenizer) under hypotonic conditions and subsequent addition of 1% SB 14 or 2% n-octylglucoside, instead of lysis of cells
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(b)
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10 20 30
Fraction
Fig. 1. Separation of HAV particle species by buoyant density centrifugation on caesium chloride/sucrose density gradients containing 0.1 ~ SLS. (a) Ag profile of HAV particles grown in FRhK-4 cells. (The profiles determined for particles in BGM, MRC-5 and Vero cells were identical and are not shown.) Roman numerals indicate pooled fractions for further analysis. (b) Ag profile of virus particles derived from BS-C-1 cells. Gradients were fractionated from the bottom (fraction 1) and Ag was determined by standard ELISA (a) or RIA (b) using 10 μl of each fraction. Figures indicate buoyant density.

by freezing and thawing. Furthermore, enzyme treatment of cell lysates was varied by the omission of individual enzymes (trypsin or nucleases), or additional extraction of the lysates with Freon. Analyses of density gradients were performed in the presence of 1~ n-octylglucoside or 0.1~ SB 14. However, all these variations resulted in less effective separation of particles and therefore Ags from BS-C-1 cells were not investigated further. All the other virus/cell systems produced similar Ag profiles, but infected FRhK-4 cells regularly produced the highest amount; therefore subsequent experiments were conducted with particles from HAV-infected FRhK-4 cells.

To determine the density values of the major Ags to high precision, pooled fractions from caesium chloride/sucrose density gradients (Fig. 1a) were subjected individually to analytical isopycnic centrifugation in self-generating CsCl density gradients (not shown). Purified, labelled virions (1.34 g/ml) and procapsids (1.31 g/ml) (Onodera et al., 1986) of poliovirus served as internal references. The results showed that Ag from pool I had a density of 1.335 g/ml, Ag from pool III banded as a peak at 1.305 g/ml with a shoulder of greater density and material from pool VII had a density of 1.20 g/ml.

To define further the range of particles produced in infected cells, pooled fractions from the initial caesium chloride/sucrose density gradients were subjected to sedimentation analysis in sucrose gradients (10% to 30% w/w). The Ag profiles obtained revealed the following. (i) Pool I (Fig. 2a) contained mainly Ag that sedimented at 156S. (ii) Pool II (Fig. 2b) material separated into two Ag peaks of about 150S and 66S. In some experiments the peak at 150S resolved into two Ags, sedimenting at 156S and 130S. (iii) Pool III (Fig. 2c) contained some 130S particles and small amounts of 156S particles (visible as a shoulder on the 130S Ag peak), but most of the material accumulated around 66S. (iv) Pool VII material (Fig. 2d) usually sedimented at around 66S. The material from pools II, III or VII that accumulated around 66S in some experiments resolved into asymmetric or shouldered Ag peaks apparently comprising two particle types sedimenting at 76S and 59S (not shown). (v) Ags from fractions located between pools III and VII usually produced a smeared Ag profile with little Ag content extending between 60S and 120S. This region was not investigated further owing to its low Ag content. The various Ags were called (i) virions (156S, 1.335 g/ml), (ii) provirions (130S, about 1.32 g/ml), (iii) 'dense' empty capsids (59S and 76S, 1.305 g/ml) and (iv) 'light' empty capsids (59S and 76S, 1.20 g/ml).

Association of RNA with various types of particles

Particles were analysed for their RNA content by [3H]uridine incorporation studies and for HAV RNA specifically by hybridization analysis. RNA was labelled metabolically 15 days post-infection by treatment of HAV-infected cells with 20 μCi/ml [3H]uridine in the presence of 4 μg/ml actinomycin D for 24 h. Cells were then harvested, antigen was extracted and particles were separated on combined caesium chloride/sucrose density gradients. In parallel assays on individual gradient fractions, the amount of Ag and radioactivity were determined. The results showed that RNA was present in fractions in the density range 1.34 to 1.30 g/ml and was not detectable in fractions at 1.20 g/ml (not shown).

The sensitivity of this method is limited, and pools II and III contained a mixture of particles, so we analysed the RNA content of 156S, 130S, 76S and 59S particles.
Fig. 2. Separation of HAV particles by velocity centrifugation. Pooled fractions (see Fig. 1a) were subjected to sedimentation through linear sucrose gradients (10% to 30% w/w) containing 0.1% SLS. Individual particle profiles were derived from material in (a) 1.335 g/ml CsCl (pool I), (b) 1.31 g/ml (pool II), (c) 1.30 g/ml (pool III) and (d) 1.20 g/ml (pool VII). Gradients were fractionated from the bottom (fraction 1) and antigen was determined for each fraction by standard RIA of a 10 µl aliquot. Poliovirus procapsids (80S) and virions (160S) were used as markers either in the same or parallel gradients. Sedimentation values are geometric means of 15 individual determinations.

individually by cDNA–RNA hybridization after purification on sucrose density gradients. By this method, viral RNA was detected only in 156S and 130S particles (Fig. 3).

Fig. 3. Association of HAV RNA with individual particle species. Aliquots (50 µl) of individual fractions (4 to 26) after velocity sedimentation centrifugation of HAV Ags were analysed for HAV RNA by slot blot hybridization with a 32P-labelled cDNA fragment (Methods). Lanes 1 to 4, pooled fractions I, II, III and VII separated by sucrose density gradient centrifugation, gradients were fractionated from the bottom. Sedimentation values (to the right) indicate the position of individual particle species.

Infectivity of HAV particles

To show that empty particles were not infectious, the infectivity titre of fractions containing individual particle species was determined. The results showed that fractions containing 76S and 59S particles were infectious at titres 104- to 105-fold lower than those in fractions that contained virions. To assess whether this residual infectivity was due to contamination with 156S virus particles during either sedimentation or fractionation of the gradient, or whether 76S and 59S particles were infectious per se, sucrose gradients were screened for the distribution of infectivity. Fig. 4 shows that maximal infectivity was present in fractions containing virions even though Ag could not be detected, whereas fractions containing maximal amounts of viral Ag (76S and 59S) were associated with background infectivity.
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Fig. 4. Infectivity associated with various HAV particle species. Infectivity (TCID<sub>50</sub>) of individual fractions (a) or pools of two fractions (b) from the sedimentation analysis of pool II (a) and VII (b) was determined in parallel to the determination of Ag by standard RIA. Solid bars represent infectivity; □, amount of HAV Ag.

only. These results suggested that the infectivity found in fractions containing 76S and 59S particles is due to residual virions. Owing to the inefficient separation of 130S from 156S particles, it was not certain whether the 130S particles were infectious per se or whether their infectivity was also due to contaminating virions.

Protein content of empty capsids

According to the cascade model of proteolytic processing, cleavage of the structural precursor VP0 to yield VP2 and VP4 accompanies maturation of picornaviruses and a distinguishing feature of empty capsids should be that they contain VP0 not VP2. Therefore, dense empty capsids were characterized by Western blot analysis using rabbit antibodies against HPLC-purified capsid proteins VP1, VP3 or VP0. Fig. 5(a and b) shows that both virions and empty capsids contain VP1 and VP3 (calculated Mr, 33-2K and 27-8K, respectively; observed Mr, 32-2K and 27-4K) at comparable ratios; the peptide precursor VP0 (calculated Mr, 27-3K; observed Mr, 27-6K) was present only in empty capsids (Fig. 5c).

However, the analysis also showed an anti-VP0 antibody-reactive peptide in virions. This peptide had a slightly higher electrophoretic mobility and therefore represents VP2 [27-0K (Wheeler et al., 1986b), apparent Mr, 26-8K], with which anti-VP0 antibodies are able to react. The same analysis performed on light empty particles or individual particle species (76S and 59S) of both densities revealed identical capsid protein patterns (not shown).

Stability of particles

To assess the physical stability of empty particles, pooled fractions, from isopycnic centrifugation, containing 76S and 59S particles were dialysed against TN buffer containing 0-1% SLS, and subsequently subjected to three cycles of freezing and thawing.

Comparison of sedimentation profiles obtained after freezing and thawing of particles with those obtained before treatment revealed that both particle species were sensitive to this treatment (Fig. 6). Antigenic products of ensuing decomposition were found at the top of the gradient (0 to 20S, fractions 27 to 30), but were not further characterized. The addition of 22% sucrose to pooled particles stabilized empty capsids; sedimentation profiles before and after treatment were identical. Provirions and virions were resistant to freezing and thawing irrespective of the presence of sucrose.

Decomposition of 76S and 59S particles was observed when pools of caesium chloride/sucrose density gradient
fractions were stored at $-20\,^\circ\mathrm{C}$ (not shown). However, under these conditions low density, empty capsids (pool VII) apparently were less labile than dense empty particles, most probably because of the higher sucrose content of pool VII. Storage of pools II and III for 1 month at $-20\,^\circ\mathrm{C}$ resulted in a complete loss of detectable Ag corresponding to empty capsids, but again provirions were stable; empty capsids were stable when stored at $4\,^\circ\mathrm{C}$ for 1 month (not shown).

**Discussion**

Standard killed-type vaccine preparations against hepatitis A will be derived from infected cell cultures. Therefore, it is necessary to define the range of virus particles generated during HAV replication in vitro. A review of earlier reports revealed a wide variety of complete and incomplete virus particles; notably a host of different particles of lower density in caesium chloride than the virion have been described. However, due to wide variation in HAV strains, cell lines, isolation procedures and the extent of particle characterization, species are not comparable between different reports. Furthermore, most reports emphasize the existence of infectious particles other than the virion. For instance, Heinricy et al. (1987) reported that in their system all 'low density particles' (1.11 to 1.17 g/ml) were virions associated with cellular debris. A similar conclusion was drawn by Lemon & Binn (1985), who demonstrated that a large amount of non-neutralizable virus (1.14 to 1.18 g/ml in CsCl) was tightly associated with lipid, and Gauss-Müller et al. (1986) described immature virus particles banding at a density of 1.20 g/ml which had a different capsid protein pattern to virions. However, virus particles of that density have not been described for other picornaviruses or in stool specimens from individuals suffering HAV infection. Furthermore, and most interestingly, many reports lack information on incomplete HAV particles, such as empty capsids.

In contrast, our results indicate that infected cell cultures produce empty particles usually exceeding 60% of the total viral Ag, as estimated on the basis of standard immunoassays. The fact that these particles have not been detected by others is surprising because the particle profiles for the variety of HAV strain–cell line pairs reported here are invariant. However, our results with infected BS-C-1 cells indicate that some cells generate particle/cellular component aggregates that may require harsh procedures to separate individual particle species. Such conditions might destroy empty capsid structures.

The Ag profiles in density gradients are clearly dependent on the extraction procedure and enzyme treatment. Treatment of cellular extracts with trypsin for example results in efficient particle separation, whereas omission of this step causes particles to be distributed throughout the gradient. Recent evidence suggests that the epitope on HAV strain HM175 recognized by a
murine neutralizing monoclonal antibody (K3-4C8) may be sensitive to trypsin, whereas other overlapping epitopes are not (Stapelton & Lange, 1988). Recently, we have investigated the antigenic structure of empty capsids and virions of HAV CLF isolated in the presence of trypsin. We did not observe any impairment of epitope recognition by K3-4C8 or differences in its reactivity and that of other antibodies with native virus (Ping et al., 1988; Weitz et al., 1990). Moreover, virions and empty capsids appear to be indistinguishable antigenically, and gross effects of trypsin on the polypeptide composition of the particle were not observed. Therefore, individual particle species derived from HAV CLF apparently were not affected by the enzyme.

Our identification of empty capsids corroborates the results of Siegl et al. (1981), who demonstrated that empty capsids are present in significant amounts in HAV-infected cell cultures, sediment at 74S, and lack infectivity and, from [3H]uridine labelling experiments, viral RNA. However, analysis was confined to HAV antigenic material in a density range of 1.29 g/ml to 1.36 g/ml (in CsCl), and more extensive and precise characterization was not attempted. Furthermore, empty capsids were also observed in stool samples of infected individuals by electron microscopy, but were poorly characterized (for a review see Coulepis et al., 1982). This finding indicates that empty capsids are not only a product of HAV replication in vitro, but also occur in natural infections.

In contrast to earlier reports our results show that two species of non-infectious empty particle, sedimenting at 76S and 59S, are produced in HAV-infected cell cultures, and band at 1-305 g/ml and 1-20 g/ml in CsCl. Only the dense particles are of buoyant density corresponding to the empty capsids found in stool samples (Siegl & Frössner, 1978). The light particles are of different buoyant density, but are identical to dense particles in sedimentation, capsid protein composition, physical stability and antigenic structure (Weitz et al., 1990). Therefore, we suggest that their unusual buoyant density is a result of association with lipid and/or other cellular debris. This association, however, is extremely resistant to various treatments such as extraction with chloroform or Freon and enzymic degradation with trypsin and RNase A.

Our analysis of capsid protein composition, RNA content, sedimentation behaviour and infectivity, characterizes 76S and 59S particles as empty capsids. The physical stability of empty HAV particles corresponds to that reported for poliovirus procapsids, which are sensitive to freezing and thawing, and storage at −20 °C (Onodera & Phillips, 1987), and stabilized in the presence of 22% sucrose. Disintegration of empty HAV capsids upon freezing and thawing may be comparable to the dissociation of poliovirus procapsids. However, since we did not characterize the disintegration products, it remains to be determined whether empty capsids correspond to the morphogenic particle precursor that procapsids are thought to be. The physical lability of empty HAV capsids is in sharp contrast to the observed stability of provirions and the reported stability of the virion (Siegl et al., 1984b). It might indicate, however, that the stability of HAV, unique among the picornaviruses, is dependent on the presence of viral RNA within the particle.

The observation of two forms of empty capsids (76S and 59S particles) is quite common for picornaviruses. Poliovirus, in addition to procapsids, forms 50S to 60S particles (reviewed in Koch & Koch, 1985). Mango virus-infected cells produce two types of empty particle, composed of VP1, VP3 and VP0, which sediment at 53S and 75S (Lee & Colter, 1979). Determination of the M, suggests that the 53S particle represents an incomplete half-shell composed of five 14S subunits and the 75S particle an incomplete shell of 10 14S subunits; the complete capsid has 12 14S subunits. Therefore, by analogy, the 76S particle of HAV might be either a procapsid, as in poliovirus, or an empty capsid lacking a certain number of pentamers (14S), as in Mango virus. Similarly, the 59S particle of HAV might represent a ‘half-shell’ of about five pentamers.

Our results also show that HAV-infected cells contain a particle sedimenting at 130S in sucrose density gradients. To our knowledge, this particle has not been described before and has been operationally called the ‘provirion’. It represents only a minor portion (8%) of the total viral Ag present, as estimated on the basis of Ag profiles in sedimentation analyses, and might have escaped earlier observation. The 130S particle might correspond to the morphogenic 125S proviron of poliovirus, which contains RNase-resistant viral RNA and polypeptides VP1, VP3 and VP0 (Fernandez-Thomas & Baltimore, 1973). Alternatively, it might represent (similar to the poliovirus 135S particle) an uncoating intermediate (lacking VP4) found intracellularly early after poliovirus infection (Gromeier & Wetz, 1990). When particles were harvested (3 to 4 weeks post-infection) HAV-infected cell cultures usually have reached a state of persistent infection. Therefore, an uncoating intermediate could be present in cell culture, assuming that the persistent type of infection involves a process of repetitive uncoating, replication, assembly and uncoating. However, the replicative events in persistent infection of cell cultures by HAV have not been elucidated to an extent that would allow the origin of this particle to be determined. In contrast, Anderson & Ross (1990) have observed provirions and empty particles...
capsids in single-cycle infections which corroborates the results presented here. However, they identify only a single empty capsid of 70S (procapsid) and the sedimentation value of provirions remains unclear.

In summary, our results identify a constant range of HAV particles under various culture conditions. Of these, empty particles represent the main Ag that might be exploited as immunogen in vaccine preparations. Therefore, studies on antigenicity and immunogenicity of empty capsids have been conducted and will be reported elsewhere.

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References


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