Differences in the Physical Properties of Dense and Standard Poliovirus Particles

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SUMMARY

Dense poliovirus particles (DP) differ in buoyant density, sedimentation coefficient and lability from standard poliovirus particles. Dense particles band at a density of 1.44 g/ml in isopycnic CsCl gradients and sediment in sucrose gradients at 220S. However, when DP are centrifuged in sucrose gradients containing 1.5 M-KCl, NaCl or LiCl, two types of particles are observed, one sedimenting at 220S and the other at 160S. Particles sedimenting at 220S are converted into particles sedimenting at 160S by incubation at 37°C in 1.5 M-KCl.

The high buoyant density seems to be correlated with the high lability of DP. Dense particles are extremely labile in isotonic phosphate-buffered saline. Their degradation proceeds through an RNA-containing particle lacking polypeptide VP4, to RNA and empty capsids.

INTRODUCTION

We have isolated a novel poliovirus particle and named it dense poliovirus particle (DP) because it had a higher buoyant density (ρ = 1.44 g/ml) in CsCl gradients than the standard poliovirus particle (ρ = 1.34 g/ml). These dense particles contained the same amount of 35S RNA and protein as standard poliovirus particles. Their protein could be separated on SDS-polyacrylamide gels into four polypeptides indistinguishable in size and relative amount from the polypeptides VP1 to VP4 of standard poliovirus particles (Yamaguchi-Koll, Wiegers & Drzeniek, 1975).

Independently, a 'high density component' was isolated from several vertebrate enteroviruses, i.e. from poliovirus, the bovine enterovirus VG-5-27, Coxsackie B5 virus and swine vesicular disease virus (Rowlands et al. 1975). This high density component had the same buoyant density of 1.44 g/ml in CsCl as DP, irrespective of the virus strain or type of cells used for its propagation. Therefore it seems justified to accept the existence of dense particles as a general phenomenon in the enterovirus group.

Rowlands et al. (1975) determined the average diameter of dense particles to be 28.2 nm ± 0.76, whereas the average diameter of standard virus was found to be 30.1 nm ± 0.74. In attempts to determine the sedimentation coefficient of DP in sucrose gradients we observed a multicomponent sedimentation pattern of these particles. A closer examination of the heterogeneity revealed an unusually high lability of DP in isotonic phosphate-buffered saline (PBS). In order to determine the sedimentation coefficient of DP, it was necessary to centrifuge them in sucrose gradients containing CsCl. During these experiments
it became obvious that dense particles, though homogeneous in CsCl solutions, could be separated into two types of particles when centrifuged in sucrose gradients containing 1.5 M KCl, NaCl or LiCl.

METHODS

**Virus particles.** Poliovirus, type I, strain Mahoney, was used. Dense poliovirus particles, standard poliovirus particles and naturally occurring empty capsids (Maizel, Phillips & Summers, 1967) were prepared as described previously (Drzeniek & Bilello, 1974; Yamaguchi-Koll et al. 1975). Dense particles were stored at −20°C in CsCl solutions.

**Buffers.** PBS: isotonic phosphate-buffered saline (0.12 M NaCl, 0.02 M phosphate, pH 7.2); NT buffer: isotonic tris/HCl (0.1 M NaCl, 0.05 M tris/HCl, pH 7.2).

**Sedimentation in sucrose gradients.** Dense particles and standard poliovirus were kept in stock solutions containing 3 M CsCl. They were diluted into or dialysed against PBS or NT buffer containing the desired concentration of CsCl, KCl, LiCl or NaCl and layered on to separate gradients containing 15 to 30% sucrose and the same salt concentration as the dilution buffer. The samples were centrifuged for 2 h at 162000 g in a SW 40 rotor (Beckman/Spinco) at 4°C and 0.5 ml fractions were collected from the bottom of the tube. The radioactivity was determined in Triton X-100 toluene based scintillation fluid (Drzeniek & Bilello, 1974) in a Beckman LS-250 scintillation spectrometer with appropriate channel settings. When double labelled samples were analysed, disintegrations per min (d/min) were calculated with a program obtained from Beckman Instruments GmbH (München) on a Wang 600 calculator (Wang Laboratories GmbH, Frankfurt).

**Isolation of DP₈ and DP₆.** DP₈ and DP₆ were isolated by centrifuging in sucrose gradients containing either PBS or 1.5 M KCl in NT buffer. Fractions of the 220S peak (DP₈) and 160S peak (DP₆) were used directly or pooled.

**Isopycnic banding of DP₈ and DP₆ in CsCl gradients.** To the appropriate fractions containing DP₈ and/or DP₆, CsCl was added to give a density of 1.35 g/ml. The samples were poured into nitrocellulose tubes and centrifuged for 40 h at 138 000 g in the SW 40 rotor at 6°C. Fractions were collected from the bottom of the tubes and assayed for radioactivity and density.

**Polypeptide analysis.** Virus samples were dialysed against 0.01 M Na-phosphate buffer. Dissociation of dense particles, of standard poliovirus or of naturally occurring empty capsids, and the polyacrylamide gel electrophoresis were done as described by Summers, Maizel & Darnell (1965).

About 10 000 ct/min of 14C-protein hydrolysate-labelled DP₈, DP₆ or empty capsids were coelectrophoresed with about 10 000 ct/min of 3H-leucine-labelled standard poliovirus. Electrophoresis was performed for 10 h at 7.5 mA/gel. Gels were cut into 1 mm slices using an automatic gel-slicer (Model Gilson GF). To each vial containing about 0.3 ml gel/H₂O suspension, 0.3 ml 30% H₂O₂ was added. After incubation at 60°C for 12 h, 0.5 ml H₂O was added followed by 10 ml Triton X-100 toluene based scintillation fluid. The radioactivity was determined in a Packard Liquid Scintillation Spectrometer with appropriate channel settings.

**Ribonuclease treatment.** Fractions of PBS-sucrose density gradients containing 3H-uridine-labelled dense particles or standard poliovirus particles were tested for ribonuclease (RNase)-resistant radioactivity. Each fraction was incubated at 0°C with RNase A at 100 µg/ml. After 1 h, 0.01% bovine serum albumin and 6% trichloroacetic acid were added and the samples were collected on Millipore membrane filters (HAWP25). Filters were washed twice with 5% trichloroacetic acid, dried for 2 h at 60°C and the radioactivity was measured.
Isolation of the ribonucleo-polypeptide complex. In order to demonstrate the ribonucleo-polypeptide (RNPP) complex, 20 μl of dense particles or standard poliovirus particles in 3 M-CsCl were incubated with 200 μl 10 M-urea (in H₂O) at 25°C for 1 h. The samples were diluted with an equal volume of water and layered on to a sucrose gradient (5 to 20% sucrose) containing 5 M-urea in hypotonic PBS (2 × 10⁻³ M-phosphate buffer, pH 7.2, plus 1.2 × 10⁻² M-NaCl). They were centrifuged at 257 000 g at 16°C in the SW 60 rotor (Beckman/Spinco) for 7 h; 0.2 ml fractions were collected and the radioactivity was determined.

RESULTS

Sedimentation coefficient of dense particles

DP (ρ = 1.44 g/ml in CsCl) sedimented as one peak in sucrose gradients containing 1.5 M-CsCl. They sedimented faster than poliovirus particles (Fig. 1a). From the known sedimentation coefficient of poliovirus particles of 160S (Schaffer & Schwerdt, 1959), the sedimentation coefficient of DP was calculated to be 220S ± 10S at isotonic conditions. This value was obtained by extrapolation of data from sucrose gradients containing 0.5, 1.0 and 1.5 M-CsCl. The experiments demonstrated a linear relationship between the sedimentation velocity of dense and standard poliovirus particles at these salt concentrations. All sedimentation coefficients reported in this paper were likewise extrapolated to isotonic conditions.

Despite their homogeneity in CsCl-containing solutions, dense particles separated into two peaks in sucrose gradients containing 1.5 M-KCl, NaCl or LiCl (Fig. 1b). The component which sedimented faster than standard poliovirus sedimented at 220S. It is tentatively called DP₆ because it is more stable in PBS than the very labile DP₅, the other component, which sedimented together with standard poliovirus at 160S.

When DP₆ were incubated for 20 h at 37°C in PBS containing 1.5 M-KCl and centrifuged
Fig. 2. Recentrifugation and isopycnic banding of isolated DP₈ and DP₉. ³H-leucine labelled DP₈ and DP₉ were isolated as described (see Methods). (a) DP₈ (○--○) and DP₈ (△--△) were centrifuged on separate sucrose gradients containing 1.5 M-KCl in PBS after incubation for 20 h at 37°C in 1.5 M-KCl containing PBS. (b) DP₈ (○--○) and DP₉ (△--△) and dense particles obtained directly from a stock containing 3 M-CsCl (□--□) were centrifuged on separate sucrose gradients containing 1.5 M-CsCl in PBS. (c) Isopycnic banding of isolated DP₈ (○--○) and DP₈ (△--△) in CsCl. (●--●) density of the CsCl solution.

on a KCl-containing sucrose gradient, most of the radioactivity sedimented at 160S (Fig. 2a). DP₈, which were kept at 4°C in 1.5 M-KCl for the same period of time sedimented at 220S (not shown). DP₉ treated in the same manner remained unchanged in their sedimentation behaviour in high potassium-containing sucrose gradients (Fig. 2a).

In CsCl-containing sucrose gradients, however, DP₉ always sedimented at 220S (Fig. 2b). Even DP₉ obtained from DP₈ by treatment for 20 h at 37°C in 1.5 M-KCl sedimented at 220S when analysed on a CsCl-containing sucrose gradient (not shown). In all cases DP₈ and DP₉ remained dense, as they band at a buoyant density of 1.44 g/ml in CsCl (Fig. 2c).

From these experiments it is obvious that dense particles sediment with two different sedimentation coefficients, 220S in 1.5 M-CsCl solutions and 220S and 160S in 1.5 M-KCl solutions. When incubated at 37°C in 1.5 M-KCl almost all dense particles sediment at 160S. We therefore suggest that we are dealing with two configurations (DP₈ and DP₉) of the same dense particle (see Discussion).
Dense and standard particles of poliovirus

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Fig. 3. Centrifugation of dense particles in sucrose gradients containing PBS. $^3$H-uridine ($\Delta$) and $^3$H-leucine ($\bigcirc$) labelled dense particles were dialysed overnight against PBS and centrifuged on separate gradients containing PBS as in Fig. 1.

Degradation of dense particles

Dense particles, dialysed overnight against PBS and then centrifuged in isotonic sucrose gradients, revealed four peaks with sedimentation coefficients of approx. 220S, 160S, 80S and 35S (Fig. 3).

The first two peaks were the DP$_s$ and DP$_{sa}$ whereas the 80S peak and the 35S peak resulted from the degradation of dense particles as demonstrated below. When dense particles labelled in the RNA were diluted in cold isotonic PBS and kept in an ice bath for 15 min before centrifuging at 4°C in a sucrose gradient containing PBS, they sedimented at 220S and 160S with only a small proportion (about 5%) of particles at 80S (Fig. 4a). However, after 15 min incubation in PBS at 37°C part of the DP$_s$ and all of the DP$_L$ disappeared. Radioactive RNA was found in the 80S fraction and as free 35S RNA. Some radioactivity was found on top of the gradient (Fig. 4b). When incubation time at 37°C was increased to 10 h (Fig. 4d), the RNA label in the 80S and 35S particles disappeared and radioactivity accumulated on top of the gradient.

On analysing dense particles labelled in the protein, they also showed DP$_s$ and DP$_L$ and only small amounts of 80S particles, when kept in an ice bath prior to centrifugation (Fig. 4a). After incubation in PBS at 37°C, DP$_s$ and DP$_L$ disappeared giving rise to 80S particles (Fig. 4b, c), which after 10 h of incubation at 37°C were slightly degraded (Fig. 4d) and disappeared completely after 24 h (not shown).

These results demonstrate a rapid degradation of DP at 37°C in isotonic buffer into 80S empty capsids and free RNA (35S) through a labile intermediate (Fig. 4b, c), containing RNA and protein, but lacking the polypeptide VP4 (see below). DP$_L$ are degraded faster than DP$_s$. 
Polypeptide composition

To find the polypeptide composition of DP, DP, and the 80S particles, dense particles were separated in isotonic sucrose gradients and analysed after treatment with SDS by polyacrylamide gel electrophoresis (Fig. 5). The results revealed four polypeptides, VP1 to VP4, in DP, and DP, which were indistinguishable in their mol. wt. from the polypeptides of standard poliovirus particles. These polypeptides were present in DP in the same proportions as in standard poliovirus particles. However, 80S particles contained only the polypeptides VP1, VP2 and VP3 (Fig. 5c).

Sensitivity towards RNase

It was of interest to study the state of the RNA in the different components of DP. 3H-uridine-labelled dense particles were separated into DP and DP, on a sucrose gradient containing PBS and treated with RNase A. To avoid thermal degradation, the RNase treatment was done at 0°C with an enzyme concentration of 100 µg/ml. The results from this experiment (Fig. 6a) showed that the RNA of DP and DP, was to a varying extent accessible to the enzyme, whereas standard poliovirus particles were completely RNase resistant under these conditions (Fig. 6b). There was also an indication that the RNA of the DP was less susceptible to the enzyme than the RNA of DP, (Fig. 6a). This result would be consistent with the observation that DP, are more labile in PBS than DP.
Fig. 5. Polypeptide analysis by polyacrylamide gel electrophoresis. (a) DPd, (b) DPp and (c) 80S particles obtained from dense particles and (d) naturally occurring empty capsids labelled with $^{14}$C-protein hydrolysate were coelectrophoresed with $^{3}$H-leucine labelled standard poliovirus (arrows).

Fig. 6. RNase sensitivity of dense particles. All fractions of a PBS-sucrose gradient containing (a) $^{3}$H-uridine labelled dense particles or (b) standard poliovirus particles were assayed for RNase resistant radioactivity. Samples were incubated for 1 h at 0°C in the presence of 100 μg/ml pancreatic RNase (□—□), or in the absence of RNase (○—○).
Failure to detect an RNPP-complex

An RNPP complex containing poliovirus RNA and the structural polypeptide VP1 was isolated after dissociation of poliovirus by concentrated urea (Drzeniek, 1975) and centrifugation in sucrose gradients containing urea in hypotonic salt solutions (Wiegens, Yamaguchi-Koll & Drzeniek, 1976). However, all attempts to isolate the RNPP-complex from DP were unsuccessful. Empty capsids or the appropriate RNA-free polypeptides and free RNA were the only dissociation products.

DISCUSSION

The data showed that DP sedimented as one peak at 220S (extrapolated to isotonic conditions) in sucrose gradients containing 1.5 M-CsCl when compared to standard poliovirus particles sedimenting at 160S at isotonicity (Schaffer & Schwerdt, 1959). In sucrose gradients containing 1.5 M-KCl, dense particles sedimented as two peaks, about 25% at 220S and the remainder at 160S. Incubation of DP₇ for 20 h at 37°C resulted in a conversion to DP₇. However, DP₃ were not visibly converted into DP₇ when kept in 1.5 M-KCl for 20 h at 4°C.

DP₇ were stable in 1.5 M-KCl; they were not converted into DP₃ as long as they were kept in the KCl solution. However, the replacement of 1.5 M-KCl by 1.5 M-CsCl resulted in the immediate conversion of DP₇ into DP₃ sedimenting in CsCl-containing sucrose gradients at 220S. This conversion proceeded at 4°C as well as at 37°C and was so fast that DP₇ were not detected in CsCl-containing gradients.

Under all these conditions, dense particles do not change their buoyant density in CsCl solutions. They always band at a density of 1.44 g/ml even after their conversion into DP₇, which sediment at 160S, i.e. as fast as standard poliovirus particles which band at 1.34 g/ml. Only degradation (see below) can change the buoyant density of DP.

Thus, we are dealing with two types of DP differing in their sedimentation coefficient in CsCl and KCl solutions. Since these two types also differ in their lability in isotonic salt solutions, we named the faster (220S) sedimenting type of dense particles DP₇, because it is more stable than the 160S type, named DP₃ (labile). The experiments further demonstrate that DP are stable in salt solutions containing 1.5 M-CsCl, KCl, NaCl or LiCl, but they are extremely labile at isotonic conditions.

We suggest that the difference in the sedimentation coefficient of these two types of dense particles results from the binding of different cations (Cs⁺ or K⁺) by the core of DP. Recently we have detected a 'core' in poliovirus particles by electron microscopy in 3 M-CsCl solutions (Boublik & Drzeniek, 1976).

Increased binding of caesium ions as the cause of a higher sedimentation coefficient (Korant et al. 1972) or of the higher buoyant density of picornaviruses was already suggested by McGregor & Mayor (1968) and Rowlands, Sangar & Brown (1971).

The instability in isotonic conditions is responsible for the observed heterogeneity of dense particles as revealed by sedimentation velocity analysis in sucrose gradients containing PBS (μ = 0.15). The degradation of dense particles proceeds to empty capsids (about 80S) and RNA (35S) through an RNA-containing particle sedimenting at about 90S and lacking VP₄. This intermediate resembles the particles obtained by heat degradation of poliovirus (Breindl, 1971). The empty capsids and RNA are degraded slowly to smaller components by prolonged incubation. The high lability of DP at isotonic conditions is probably why they remained undetected for so many years although much work has been done on poliovirus particles and their biosynthetic intermediates.
Dense and standard particles of poliovirus

A comparison of data demonstrates that DP ($\rho = 1.44 \text{ g/ml}$) differ in a number of physical properties from standard poliovirus particles ($\rho = 1.34 \text{ g/ml}$), namely in their buoyant density, their sedimentation coefficient and their high lability at isotonic conditions. However, no differences were found between dense and standard poliovirus particles in chemical and structural properties such as the size and amount of RNA and the polypeptides VP$_1$ to VP$_4$ and in their neutralization by type-specific poliovirus antisera (Yamaguchi-Koll et al. 1975). The morphology of dense and standard particles was similar; no differences in the iodination of intact particles were detectable, but dense particles had a slightly smaller diameter (28 nm) than standard poliovirus particles (30 nm) (Rowlands et al. 1975).

We therefore have to consider dense and standard poliovirus particles as two alternative configurations of the poliovirus particle as already suggested by Rowlands et al. (1975). The only indication which we have to explain the existing differences between dense and standard poliovirus particles, is the failure to isolate the RNPP complex (Wiegers et al. 1976) consisting of virus RNA and the structural polypeptide VP$_1$ from DP under conditions in which it was isolated from standard poliovirus particles. This finding suggests differences in the interaction between the RNA and protein of dense and standard poliovirus particles.

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