Distortion of Poliovirus Particles by Fixation with Formaldehyde

(Accepted 2 November 1972)

Cytoplasmic extracts of HeLa cells contain particles which, after fixation, equilibrate in CsCl gradients at densities of 1.40 to 1.44 g/ml (Perry & Kelley, 1966; Miller, 1972) and it has been suggested that they may contain messenger RNA. A possibly analogous particle bearing virus RNA was found in cells infected with poliovirus (Miller, 1972). Isopycnic sedimentation of ribonucleoprotein particles in CsCl solution relies on their prior fixation with formaldehyde (Spirin, Belitsina & Lerman, 1965; Perry & Kelley, 1966) or glutaraldehyde (Baltimore & Huang, 1968): omission of this step results in the dissociation of the complexes into protein and RNA. On the other hand, virus particles are often stable without fixation in CsCl gradients and it is possible, therefore, to examine the effect of fixation on their buoyant density. The buoyant density of newly matured poliovirus particles increases sharply on fixation with formaldehyde (Fenwick & Wall, 1972), and further details of this effect are given here.

HeLa cells growing exponentially in medium BME 405N (Miller, 1967) were centrifuged and resuspended at 2 x 10^6 cells/ml in medium containing the normal amounts of arginine and tyrosine but all other amino acids at 1/40 their normal concentration. The cells (2 x 10^9) were infected with 100 p.f.u./cell of the BRUNHILDE strain of poliovirus, type I, and incubated for 2 h at 37 °C. At that time were added [H]-adenosine (0.25 #Ci/ml) and [14C]-amino acids (Chlorella hydrolysate, 0.025 #Ci/ml, 57 mCi/m-atom, Radiochemical Centre, Amersham, England). After 3 h, an equal volume of medium BME 405N was added and incubation continued for a further 25 h. Virus was extracted and purified by the methods of Boeyé (1965). An additional step consisted in centrifugation of the virus eluted from the Ecteola column on sucrose gradients (28 ml of 15 to 30 % linear sucrose gradient in o.02M-phosphate buffer solution, pH 7.2, in a Spinco rotor SW 25.1 at 44000 rev/min for 4 h at 5 °C). Isopycnic centrifugation in CsCl solution of the purified virus (unfixed or fixed with formaldehyde), collection of the fractions from the gradient and determination of the radioactivity of the doubly labelled samples have been described (Miller, 1972).

Poliovirus was treated with 6 % formaldehyde for 2, 8 or 30 h and then centrifuged in CsCl. The untreated control sample (Fig. 1 a) formed a single band at a density of 1.34 to 1.35 g/ml. The fixed samples showed a second band at 1.43 to 1.44 g/ml (Fig. 1 b, c, d), increasing in amount until, after 30 h of fixation (Fig. 1 d), it contained about 40 % of the total [H] due to virus RNA in the gradient. Such an increase in density might result from a reduction in protein content of the particles from 70 to 61 % (Perry & Kelley, 1966). However, the ratio of [14C] (virus protein) to [H] in the two peaks remained the same throughout, indicating that conversion to the denser form was not due to preferential loss of protein.

A similar experiment to that of Fig. 1 showed that glutaraldehyde treatment (3 % for 24 h in the presence of o.005 M-EDTA) also increased the buoyant density of poliovirus, to 1.42 g/ml.

In order to determine whether formaldehyde had any effect on the sedimentation of the virus particles, the two bands were isolated from a CsCl gradient, dialysed, and centrifuged in sucrose gradients. The 1.35 g/ml fixed particles formed a sharp band closely similar to that
Fig. 1. Effect of HCHO on the density of poliovirus in CsCl solutions. Doubly labelled poliovirus was fixed with 6% neutralized formaldehyde (Miller, 1972). After various times at 4 °C, the samples were centrifuged in CsCl solution. Gradients (b), (c) and (d) contained 2% formaldehyde. •••, [3H] (virus RNA); ○○○○, [14C] (virus protein); ——-, density of CsCl solution. (a) control, unfixed poliovirus; (b), (c) and (d) fixed for 2, 8 or 30 h, respectively.

of unfixed poliovirus (Fig. 2a, b). The 1.44 g/ml particles sedimented in a broad band with a peak of radioactivity very slightly ahead of the less dense particles (Fig. 2c). Thus, the conversion to the denser form was accompanied by an increase in heterogeneity rather than by a clear change in sedimentation rate.

In electron micrographs (Fig. 3) the particles of density 1.44 g/ml appeared larger and more permeable to the negative stain than untreated particles. In some particles the capsid was seriously disrupted.

We conclude that prolonged exposure to formaldehyde loosens the structure of the particle without removing protein. These modified particles have a discrete and higher buoyant density in CsCl than do the infective particles, perhaps because of increased association with Cs+ ions. In this the denser particles resemble the acid-sensitive particles of foot-and-mouth disease (Trautman & Breese, 1962; Brown, Newman, & Stott, 1970) which, with the same proportion of RNA, have a buoyant density in CsCl of 1.43 g/ml. This is increased
Fig. 2. Effect of HCHO on the sedimentation of poliovirus. The two bands isolated from a CsCl gradient (as Fig. 1d) were dialysed and centrifuged on linear sucrose gradients (12 ml of 15 to 30% (w/v) sucrose in 0.02 M-phosphate buffer solution, pH 7.2, in Spinco rotor SW 36 for 2 h at 35,000 rev/min and 4 °C). (a) unfixed poliovirus; (b) 1.35 g/ml band; (c) 1.44 g/ml band.

Fig. 3. Electron micrographs of fixed and unfixed poliovirus particles. The peak fractions from CsCl gradients were dialysed and purified by sucrose gradient sedimentation as described in Fig. 2. The particles were mounted using the pseudoreplica technique, negatively stained with 2% phosphotungstic acid (pH 6.0) and examined with the Siemens-Elmiskop I electron microscope. (a) unfixed poliovirus; (b) fixed 1.44 g/ml particles.

Further to about 1.46 g/ml on treatment with glutaraldehyde (D. V. Sangar & D. J. Rowlands, personal communication.) These effects must be considered when interpreting results with fixed ribonucleoprotein particles from cells infected with picornaviruses. Furthermore, it must be assumed that formaldehyde and glutaraldehyde, often used in fixing specimens for electron microscopy, probably also distort other ribonucleoprotein particles, such as ribosomes.

We are indebted to Mrs M. Vonck for the electron micrographs and to Dr J. Quatacker (Department of Anatomical Pathology, Faculty of Medicine, State University, Ghent,
Short communications

Belgium) for allowing us to use the electron microscope. This work was supported financially from the Fonds voor Kollektief Fundamenteel Onderzoek (Belgium).

Laboratory of Bacteriology and Virology
Faculty of Medicine, State University
De Pintelaan 135, Ghent, Belgium

Dunn School of Pathology
Oxford University, England

REFERENCES


(Received 11 September 1972)