The Polypeptides of Three Rhinoviruses

(Accepted 25 September 1972)

Extensive studies of the proteins of particles of enteroviruses, cardioviruses and foot and mouth disease (FMD) virus (Holland & Kiehn, 1968; Maizel & Summers, 1968; Rueckert, Dunker & Stoltzfus, 1969; Burroughs et al. 1971; Johnston & Martin, 1971; Talbot & Brown, 1972) indicate that these groups of picornaviruses are similar in having three major and one minor polypeptide. Published results on rhinoviruses have been conflicting. Medappa, McLean & Rueckert (1971) found that rhinovirus (RV) IA contained polypeptides similar in size and number to those of ME virus. In contrast, Korant, Lonberg-Holm & Halperen (1970) originally reported that human rhinovirus types 2 and 14 contained only three major polypeptides and thus differed from enteroviruses. However, re-examination of these rhinoviruses by Korant et al. (1972) has shown that they do contain the small polypeptide. There is still disagreement about the mol. wt. of rhinovirus polypeptides and only one report of the relative molar proportions of the four polypeptides (Medappa et al. 1971). In an attempt to resolve these differences, we have examined the composition of rhinovirus types 2, 4 and 5 and compared them with poliovirus type I.

The HGP strain of type 2, 16/60 strain of type 4 and NORMAN strain of type 5 were each derived from purified stock and grown to high titre (10⁸ to 10⁹ p.f.u./ml) in either roller bottles or suspension cultures of rhinovirus-sensitive HeLa cells at 33 °C (Stott & Heath, 1970). Viruses were labelled with [¹⁴C]-amino acids. One hundred million virus-infected cells were cultured in Hanks’s balanced salt solution containing 2% dialysed calf serum and 5 μg/ml actinomycin D. Four to six h after infection 5 μCi of [¹⁴C]-protein hydrolysate (> 45 mCi/milliatom carbon, The Radiochemical Centre, Amersham) were added and after incubation at 33 °C for a further 6 to 10 h the cells were frozen at −70 °C. Poliovirus type I (BRUNHILDE strain) was grown and labelled under similar conditions but incubated at 36 °C.

Viruses were purified by sedimenting at 100000g for 1 h; the pellet was resuspended in 1% SDS in PBS or in 0.25% trypsin and further purified on either a linear 15 to 45% sucrose gradient at 100000g for 3.5 h or a preformed caesium chloride gradient at 100000g for 6 h. Some virus preparations were purified on sucrose and then caesium gradients. Gradient fractions containing virus were pooled and analysed by polyacrylamide gel electrophoresis. Rhinovirus type 5 was also purified by adsorption to and elution from sheep erythrocytes (Stott & Killington, 1972).

Samples for electrophoresis were dialysed overnight against 1000 vol. of 0.01 M-phosphate buffer (pH 7.2) containing 0.5 M-urea, 0.1% SDS and 0.1% mercaptoethanol. Seven and a half, 10 and 12.5% acrylamide gels, containing respectively 0.20%, 0.27% and 0.33% bis acrylamide, and 0.07% ammonium persulphate, 0.23% TEMED, 0.5 M-urea, 0.1% SDS in 0.1 M-phosphate buffer (pH 7.2), were pre-electrophoresed for 30 min at 4 to 5 mA/gel with 0.25% reduced glutathione in the buffer of the upper reservoir. Samples were boiled for 1 min immediately before layering onto the gels. After electrophoresis gels were fixed and stained in 0.1% Coomassie Blue in 40% methanol containing 10% acetic acid. Albumin, ovalbumin, pepsin, myoglobin and insulin were used to calibrate the gels and their position determined with a Joyce-Loebl chromoscan. Radioactivity was determined by slicing frozen gels with a Mickle gel slicer, heating each slice at 60 °C for 2 h with 0.5 ml of Soluene 100.
Fig. 1. Electrophoresis on 11 cm acrylamide gels at 5 mA/gel. (a) Rhinovirus 2; (b) rhinovirus 5; (c) rhinovirus 4. (a) and (b) were run for 19 h on 12·5% acrylamide gels. (c) was run for 17 h on 10% acrylamide gel. ●—●, [14C].

Rhinovirus types 2 and 5 showed two major and one minor peak after electrophoresis for 10 h on gels 6·5 cm long. Under similar conditions poliovirus type 1 gave three major and one minor peak. More prolonged electrophoresis of rhinoviruses 4 and 5 separated the second peak into two, indicating the presence of four polypeptides (VP1, VP2, VP3, VP4) in each virus (Fig. 1). Under similar conditions, the second peak of RV-2 revealed a distinct shoulder (Fig. 1) but could be resolved into two polypeptides by electrophoresis for 24 h in
12.5% acrylamide gels or by scanning stained bands. The estimated mol. wt. of the four polypeptides were between 37,000 and 8000 (Table 1). The method of virus purification and the use of trypsin did not significantly affect the number or mol. wt. of the polypeptides obtained from rhinoviruses 2 or 5.

The proportion of radioactivity in the first peak of RV2 varied considerably for different preparations, suggesting that some loss of VP1 occurred during purification. Calculation of the molar ratios of the polypeptides was therefore unreliable. The percentage of radioactivity in the peaks of RV-5 was consistent irrespective of the virus purification procedure. Since VP's 2 and 3 were not completely separated molar ratio estimations were made using their mean mol. wt. and expressed as the ratios of VP1:VP2 + 3:VP4. As shown in Table 2, the calculated molar ratios were approximately 1:2:0.5.

Our results indicate that rhinoviruses 2, 4 and 5 contain three major and one minor virus particle polypeptides. Together with the findings of Medappa et al. (1971) for types 1A, 2 and 14, and the corrected observations of Korant et al. (1972) for types 2 and 14, our results support the hypothesis that all human rhinoviruses have similar polypeptide composition. Furthermore, this polypeptide composition appears to be common to all picornaviruses so far examined, including enteroviruses (Holland & Kiehn, 1968; Maizel & Summers, 1968; Johnston & Martin, 1971), cardioviruses (Rueckert et al. 1969) and FMD virus (Burroughs et al. 1971; Talbot & Brown, 1972).

Our estimates of the mol. wt. of the virus particle polypeptides are closely similar to those of other workers (Medappa et al. 1971; R. R. Rueckert, personal communication), but significantly lower than those of Korant et al. (1970, 1972). The mol. wt. we have found give an aggregate value of 90,000 to 100,000. Our results for RV-5 suggest molar ratios of 1:1:1:0.5 for the four polypeptides, in contrast to equimolar proportions suggested for RV-1A and 2 (Medappa et al. 1971). If, as has been suggested for bovine enterovirus (Johnston & Martin, 1971) and FMD virus (Talbot & Brown, 1972), rhinoviruses contain 60 chains of each of VP1 to 3 and 30 chains of VP4, the total mol. wt. of protein would be $6.2 \times 10^6$ for RV-5 on
our values. This figure is within the range 5·7 to 6·6 × 10^6 obtained by calculation taking the mol. wt. of rhinovirus RNA as 2·4 to 2·8 × 10^6 and the proportion of protein in the virus particle as 70·2% (Brown, Newman & Stott, 1970; McGregor & Mayor, 1971; Nair & Lonberg-Holm, 1971).

We are grateful to Dr D. A. J. Tyrrell for his interest and encouragement and to Mr G. Heath and Miss G. Howard for their excellent technical assistance. Actinomycin D was the kind gift of Merck, Sharp and Dohme Research Laboratories.

Clinical Research Centre
Watford Road
Harrow, Middlesex HA 1 3 UJ, England

E. J. Stott*
R. A. Killington†

REFERENCES


(Received 15 May 1972)

* Present address: Institute for Research on Animal Diseases, Compton, near Newbury, Berks.
† Present address: Department of Microbiology, University of Leeds, Leeds LS 2 9 NL.