STRUCTURAL POLYPEPTIDES OF THREE RHINOVIRUSES

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Summary

Growth, purification and electrophoretic analysis of three rhinoviruses and an enterovirus are described. It is shown that, with regard to structural polypeptides, the three rhinoviruses differ from other picornaviruses, and from each other. However, all three rhinoviruses resemble one another in possessing structural polypeptides of molecular weights 33,000 and 30,000 in similar proportions.

Introduction

Rhinoviruses differ from other picornaviruses in their higher buoyant density in CsCl and their lability when exposed to pH 5 and below (7). Their other biochemical properties have received little attention. We have begun a comparative study of several rhinoviruses to determine whether the various serotypes may have structural properties common to the whole group, and how they are related to other picornaviruses. Initially we will describe the structural polypeptides comprising the virions of three rhinoviruses, and compare them with those of a representative enterovirus, echovirus 12.

Materials and Methods

Growth and purification of virus stocks

Echovirus 12 was grown in monolayer cultures of the LLCMK2 line of rhesus monkey kidney cells, using Eagle's minimal essential medium. Virus was labeled (manuscript in preparation) with ^{14}C amino acid mixtures purchased from New England Nuclear Corp., and purified by isopycnic CsCl gradient centrifugation as previously described (1).

Equine rhinovirus obtained from Dr. R. R. Grunert of Stine Laboratory, Newark, Delaware, was grown, labeled, and purified in a manner similar to echovirus 12.

A substrain of rhinovirus type 2 HGP was obtained from Dr. Grunert and was plaque purified after seven passages. Type 14 strain 1059 was obtained from Dr. W. L. Davies of Stine Laboratory. These were propagated and assayed with HeLa cells ("Rhino-HeLa cells", Flow Laboratories, Rockville, Md.) which were grown in monolayers, in modified McCoy's 5a Medium containing 10 percent calf serum. One percent fetal calf serum was employed during infection at 34.5°C, and labeling was with radioactive L-amino acid mixtures.

Rhinovirus-infected cells were broken either by freeze-thawing and Dounce homogenization, or by disruption in the presence of "Freon" 113.* Concentration was by centrifugation in Spinco rotors at 200,000 x g for 1-3 hours.

The virus pellets were resuspended and then sedimented through a sucrose gradient. Finally the virus preparations were banded isopycnically in CsCl gradients of average density 1.4 g/ml.

Gel electrophoresis

The virus from the CsCl gradients was dialyzed against 0.01M phosphate buffer, pH 7.2. After dialysis, the virus preparations were reconstituted to contain 0.1% sodium dodecyl sulfate (SDS) and 0.1% 2-mercaptoethanol, and then immersed in a boiling water bath for one minute. The preparations were then electrophoresed through 10% polyacrylamide gels containing 0.1% SDS (6) by the application of 5 mAmpere per gel for 17-20 hours. Gels were then either stained for protein with 0.25% Coomassie blue in 50% methanol containing 10% acetic acid (8), or fractionated in a crushing device (2). In the latter case, the fractions were counted in Bray's solution and counted by liquid scintillation.

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Results

In the CsCl gradients the buoyant density of rhinovirus 14 was 1.385 g/ml., while rhinovirus 2 and equine rhinovirus had buoyant densities of 1.40 g/ml. The density of echovirus 12 was, as previously reported (1) 1.33 g/ml.

Polypeptide patterns of the four viruses are shown in Figure 1. The gels were calibrated by electrophoresing bovine serum albumin, ovalbumin, pepsin, trypsin, pancreatic ribonuclease and cytochrome C, all obtained from Worthington Biochemical Co.

The polypeptide patterns of the rhinoviruses are different from the enterovirus, ECHO 12. Echovirus 12 contains four polypeptides of molecular weights (MW) 37,000; 30,000; 25,000; and 7,600. This resembles closely the patterns reported for

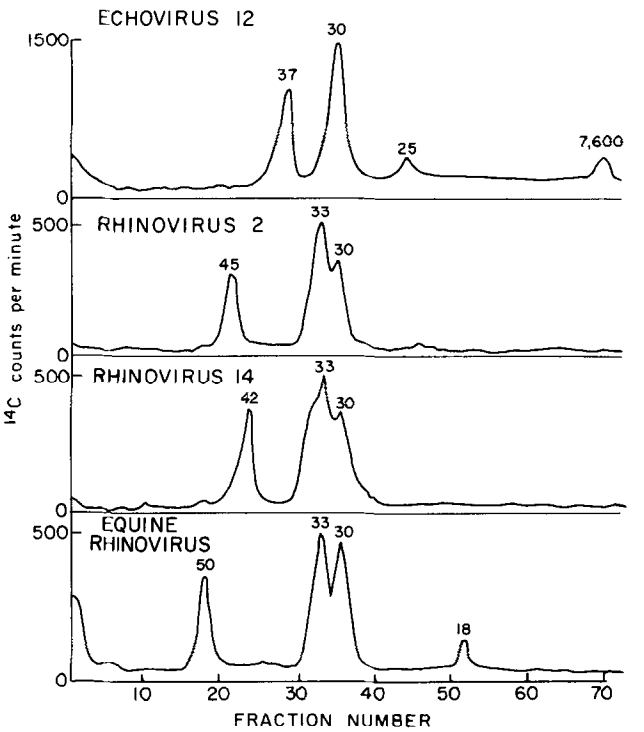

Figure 1. Electrophoresis (left to right) of virus structural polypeptides. Gels were of 10% polyacrylamide, containing 0.1% SDS. Duration was 19 hours at 5m Amp per gel. The numbers on the various peaks refer to the estimated molecular weights in thousands.

poliovirus (3), ME virus (5), and Mengovirus (4), the other picornaviruses studied to date.

The rhinoviruses differ from all these other picornaviruses in that none of the three possesses a small polypeptide of MW 10,000 or less, which is postulated to be located within the capsid, associated with the viral RNA (3, 4, 5). Also, the rhinoviruses contain larger polypeptides than any reported in other picornaviruses. Rhinovirus 14 contains a 42,000 MW polypeptide, rhinovirus 2 has a 45,000 MW polypeptide, and the equine rhinovirus contains the largest polypeptide so far found in any picornavirus, one with a MW of 50,000. The equine rhinovirus contains additionally an 18,000 MW polypeptide.

These data show that the rhinoviruses differ from each other, as well as from the other picornaviruses. However, all three show a striking similarity by possessing polypeptides with MWs of 33,000 and 30,000. Heating the preparations in 1% mercaptoethanol altered neither the number nor the migration of the polypeptides, indicating that partial dimerization due to disulfide linkages was not a factor in the patterns. Staining with Coomassie blue of gels containing non-radioactive virus polypeptides confirmed the size distribution and number observed by liquid scintillation counting of labeled polypeptides.

Conclusion

Gel electrophoresis of three rhinoviruses has shown that their capsids are composed of several polypeptide species. Thus, no small RNA animal virus has yet been found to contain fewer than three sizes of structural polypeptides.

The similarity of structural polypeptide patterns of both enteric and Colombia SK group picornaviruses to each other suggests certain intrinsic functional similarities. The rhinoviruses differ in that none contains detectable amounts of the small polypeptide, 10,000 MW or less, reportedly associated in

the other picornaviruses with viral RNA. This suggests that principles of virion construction may be somewhat different in rhinoviruses from other picornaviruses.

The data indicate that at least three rhinoviruses resemble one another closely in possessing structural polypeptide species of MW 33,000 and 30,000. No such similarity in both polypeptide size distribution and relative proportions has been observed with other picornaviruses. It is tempting to suggest that certain features peculiar to the rhinoviruses, particularly acid lability and increased buoyant density, are directly related to their structural polypeptides.

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