Purification and Characterization of Equine Infectious Anemia Virus

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With 6 Figures

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Summary

EIA virus was purified from equine fetal kidney cell cultures by PEG precipitation, two sucrose-gradient sedimentations (5–30 per cent) and (25 to 60 per cent) centrifugation, using the immunodiffusion test to follow the procedure. Purified EIA virus had a density (20°C) of 1.162 and a sedimentation constant of S20w = 656. Electron microscopy revealed a particle of about 100 nm in diameter with a very flexible but usually spherical shape. The dense core may be at various locations inside the membrane bound particle.

Introduction

Equine infectious anemia (EIA) is a widespread disease of horses which occurs in several forms: acute, subacute, chronic, or inapparent. A characteristic of all forms, however, is the persistence of a viremia which is usually lifelong. There have been very few attempts at the purification and characterization of this virus primarily because of the lack of adequate means of propagating sufficient quantities of the virus and assaying it accurately. These difficulties were alleviated somewhat by Kobayashi and Kono (6) who found that EIA virus propagated in equine leukocyte cultures. However, the lack of a convenient method of measuring virus propagation in these cultures and the presence of high concentration of serum required for growth and maintenance of leukocyte cultures complicated the purification of EIA virus. Nevertheless, Nakajima and co-workers (11, 15) and Kono et al. (7) have reported initial characterization of EIA virus grown in equine leukocyte culture, and isolated from infected horse serum.

More recently Malmquist et al. (9) described an isolate of EIA virus which grew in monolayer cultures of an equine dermal cell line and primary equine embryonic spleen. The virus, however, did not produce a cytopathic effect in
these cultures, and its growth was followed by determining the levels of extractable precipitating antigen or by subinoculation and observation for cytopathic effect in equine leukocyte cultures. Nakajima and Usui (14) extracted a precipitating antigen, subsequently shown to be identical (12) to that described by Coggins and Norcross (2), from EIA virus by treatment with ether. Malmquist et al. (9) precipitated EIA virus from culture fluids with polyethylene glycol, extracted the viral antigen with ether, and assayed the antigen using a radial diffusion technique. It has since been noted in this laboratory that the virus, adapted by Malmquist and co-workers to grow in equine dermal cells and fetal spleen, propagates equally well in other equine tissue culture systems including primary equine fetal lung and kidney cell cultures.

This paper describes the purification of EIA virus grown in roller bottle cultures of equine dermal or fetal kidney cells using an immunodiffusion test (2) to follow indirectly the purification procedure. Initial attempts to characterize the virus are also described.

Materials and Methods

Virus

Monolayers of equine dermal or primary fetal kidney (EFK) cells in roller bottles were infected with Malmquist-adapted EIA virus1 (9). Growth medium consisted of Eagle’s basal medium, 0.5 per cent lactoalbumin and 10 per cent fetal calf serum; serum content was reduced to 2 per cent in the maintenance media. The EIA virus had no visible effects on the cultures, and maintenance fluid containing virus was harvested every 5 days as long as the cells remained attached to the bottle. Virus was precipitated from culture fluids with 8 per cent (w/v) polyethylene glycol (PEG) as described by Malmquist et al. (9), and resuspended in 8 per cent PEG and phosphate buffered saline (PBS) prior to storage at minus 70°C until further purified. The Malmquist-adapted virus was infectious for a susceptible pony and was reisolated in EFK cell culture.

Infectivity Assays

Tittrations of virus infectivity were made in EFK cell culture using a fluorescent antibody (FA) technique as described by Crawford et al. (3) or by examining for viral antigen as was done by Malmquist (9). It was not possible to determine infectivity end-points directly. At higher dilutions of the virus inoculum, viral immunofluorescent and precipitating antigen was only detectable when the infected cultures were subpassed after an initial incubation period of 5 days. The percentage of cells showing immunofluorescence increased as infected cells were subcultured, but never more than about 40 per cent of the cells exhibited detectable antigen.

Immunodiffusion Test

The immunodiffusion test (ID) used for the detection of antigen was a modification of that described by Coggins and Norcross (2) and Malmquist et al. (9). As reported by Malmquist et al. (9), purified virus did not react in the ID tests, but when treated with 20 per cent ether at room temperature for 30 minutes, a soluble precipitating antigen was released which reacted strongly in the ID test. Thus, the amount of precipitating antigen extractable from a purified virus preparation was an indirect measure of the amount of virus. A seven-well pattern in 0.8 per cent agarose containing holes 5 mm in diameter and 2 mm apart were used in plastic diffusion plates with lids. Antiserum was placed in the center well and control antigen was added to 3 of the

1 Obtained from Dr. W. A. Malmquist as persistently infected equine dermal cells. (National Animal Disease Center, U.S.D.A., Ames, Iowa).
2 Immuno-Plate commercially available from Hyland, Costa Mesa, Calif.