JOURNAL OF VIROLOGY, Nov. 1972, p. 1082-1085

Vol. 10, No. 5
Printed in U.S.A.

Physical Properties of Lactic Dehydrogenase-Elevating Virus and Its Ribonucleic Acid

MARGO B. DARNELL AND PETER G. W. PLAGEMANN
Department of Microbiology, The University of Minnesota Medical School, Minneapolis, Minnesota 55455

Received for publication 25 August 1972

Infectious lactic dehydrogenase-elevating virus propagated in primary cultures of mouse peritoneal macrophages in the presence of ^{3}H-uridine and isolated by isopycnic centrifugation was found to have a density of 1.12 g/cm^3. Ribonucleic acid extracted from the virus by treatment with sodium dodecyl sulfate was single stranded with a sedimentation coefficient of approximately 48*S*.

The lactic dehydrogenase-elevating virus (LDV), a small, enveloped virus, was originally isolated from mice as a contaminant of serially transplanted mouse tumors (22). Mice infected with LDV show a 5- to 10-fold elevation in their plasma levels of several enzymes including lactic dehydrogenase (19). In vitro, LDV replicates only in primary cultures of normal mouse tissues (2, 5, 17, 27). Cultures rich in peritoneal macrophages, however, seem to yield the highest amounts of infectious virus, but virus replication in these cultures occurs only transiently (2, 5, 6, 27). Although LDV has been extensively studied biologically, few of its physical properties have been well characterized, and no direct evidence is available on the nature of its nucleic acid. The replication of LDV in mice affects the immune system (20, 23) and also, possibly, tumor formation (25), making this an extremely interesting virus for further investigation.

The pool of LDV used for infecting cell cultures was prepared by collecting plasma from 20 adult random-bred Swiss mice 24 hr after infection. Plasma was obtained by the orbital bleeding technique. The pooled plasma was diluted fivefold with McCoy medium supplemented with 15% (v/v) fetal calf serum (FCS). The solution was filtered through a membrane filter (HA type, 450-nm pore diameter, Millipore Corp.) and stored at −70 C. The final solution contained 10^8 median infectious dose units (ID_{50}) per ml. Virus concentrations were estimated by titration in mice as described previously (16).

Cultures of peritoneal macrophages were prepared by a procedure modified from that of Virolainen and Defendi (26). The peritoneal cavities of adult random-bred Swiss mice which had been injected intraperitoneally 72 hr previously with 2 ml of a 1% (w/v) starch solution were rinsed with 5 ml of McCoy medium containing 0.5 units of heparin per ml. The cells obtained were washed with fresh medium containing no heparin, resuspended in McCoy medium supplemented with 15% (v/v) FCS, and seeded in 25 cm^2 plastic Falcon flasks. One-day-old cultures were infected with approximately 20 ID_{50} of LDV per cell. After virus adsorption, McCoy medium supplemented with 5% (v/v) FCS was added. Two hours later, 30 μCi of ^{3}H-uridine per ml (26 Ci/mmole; Schwarz/Mann) was added per flask. The culture fluid was harvested 24 hr after infection, clarified at 10,000 × *g* for 20 min, and immediately layered in 5-ml amounts onto linear 0.5 to 1.5 M sucrose gradients made in a solution (B12) composed of 0.1 M NaCl, 0.05% (v/v) mercaptoethanol, 10 mM tris(hydroxymethyl)aminomethane (Tris)-chloride (*p*H 7.4), and 1 mM ethylenediaminetetraacetic acid (EDTA). The gradients were centrifuged in a SW27 rotor in an L-3 Beckman ultracentrifuge at 22,000 rev/min at 4 C for 14 hr. One-milliliter fractions were collected from the gradients with an ISCO gradient fractionator (model 183) which was attached to a continuously recording spectrophotometer and a fraction collector. Samples of the fractions were analyzed for radioactivity in acid-insoluble material (15) and infectivity. The density of gradient fractions was measured directly by weighing 100-μliter samples.

LDV had a density of 1.12 g/cm^3 as indicated by both infectivity titrations and radioactivity determinations (Fig. 1A). When either uninfected macrophage cultures were grown in the presence of ^{3}H-uridine or infected cultures were labeled with ^{3}H-thymidine (30 μCi/ml, 10 Ci/mmole; Schwarz/Mann) for 24 hr and the culture fluids were analyzed, no radioactivity was recovered in acid-insoluble material in the bottom half of the gradients.

For comparison, the C-type virus associated

with L cells (10) was labeled with ^{3}H-uridine in a similar manner and centrifuged on a companion gradient. In agreement with a previous report (10), the L cell virus was found to have the characteristic density of 1.16 g/cm^3 of C-type virions (Fig. 1B). We also labeled Newcastle disease virus (NDV), grown in chicken embryo fibroblasts, vesicular stomatitis virus (VSV), and Sindbis virus, grown in L cells with ^{3}H-uridine, and determined their densities by centrifugation through sucrose density gradients. In agreement with previous reports (4, 7, 9, 14) their densities were found to be 1.19, 1.21, and 1.18 g/cm^3, respectively.

The virus containing fractions from the different gradients were pooled, and the viruses were precipitated with absolute ethanol at −20 C in the presence of unlabeled Novikoff rat hepatoma ribosomal ribonucleic acid (RNA). The pellets were resuspended each in 1 ml of 10 mM Tris-chloride (*p*H 7.4) containing 1% (w/v) sodium dodecyl sulfate (SDS) and incubated at 37 C for 5 min with repeated vigorous mixing. Then the samples were layered onto 0.15 to 0.9 M sucrose gradients made in a solution (B6) composed of 0.05 M NaCl, 10 mM Tris-chloride (*p*H 7.4), 10 mM EDTA, and 0.5% (w/v) SDS. The gradients were centrifuged in an SW27 rotor in an L-3 Beckman ultracentrifuge at 22,000 rev/min at 20 C for 9 hr. Fractions from the gradients were analyzed for radioactivity in acid-insoluble material.

The sedimentation coefficients of LDV RNA and of the various other viral RNAs were estimated by the method of Martin and Ames (11) by using 29*S* and 18*S* Novikoff rat hepatoma ribosomal RNAs as markers. As indicated by the data in Fig. 2, an RNA was released from LDV which sedimented as 48*S*. This sedimentation coefficient was approximately the same as that of NDV RNA and slightly greater than those of the RNAs of Sindbis virus and VSV (Fig. 2A and B).

Upon heating or treatment with dimethylsulfoxide (DMSO) the 60 to 70*S* RNAs of C-type viruses are converted to more slowly sedimenting forms (3). RNA molecules which are composed of a continuous polynucleotide chain show no such change in their sedimentation coefficient after the same treatment. Samples of LDV RNA and from the RNA L cell virus were heated (see legend, Fig. 3) and then quickly chilled. Another sample of LDV RNA was incubated with ribonuclease A (Sigma Chemical Co.). The samples were then analyzed by gradient centrifugation. The LDV RNA was only slightly degraded by the heat treatment (Fig. 3A), whereas the RNA of the L cell C-type virus was completely converted to slower sedimenting forms (Fig. 3B). The observation that LDV RNA was completely digested by ribonuclease (Fig. 3A) while unaffected by heating is consistent with the conclusion that LDV contains a single-stranded RNA molecule composed of a continuous polynucleotide chain. A molecular weight of approximately 5 × 10^6 daltons has been calculated for this RNA by the method of Spirin (24).

Results from preliminary experiments have

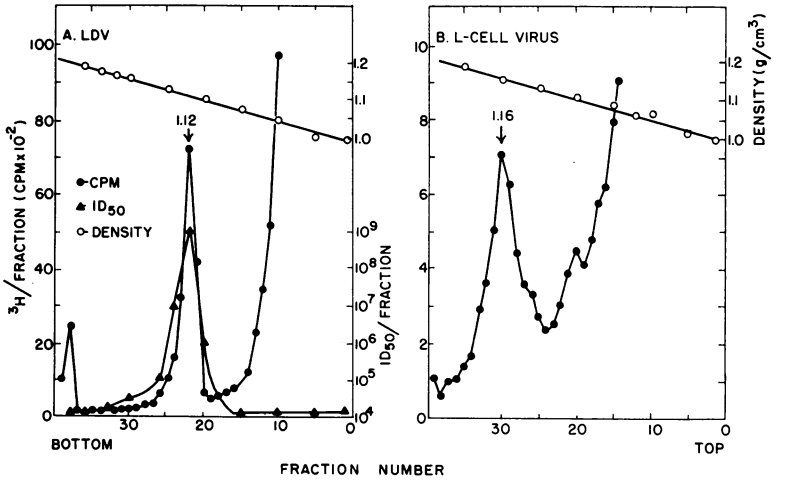

FIG. 1. *Isopycnic sucrose density gradient centrifugation of (A) LDV and (B) C-type virus from L cells. The viruses were labeled with ^{3}H-uridine as described in the text. Samples of 5 ml of culture fluid were centrifuged through linear 0.5 to 1.5 M gradients of sucrose in B12 in a SW27 rotor at 22,000 rev/min at 4 C for 14 hr. Samples of the gradients were analyzed as indicated for density, infectivity, and radioactivity in acid-insoluble material.*

shown that LDV, unlike NDV and VSV (8), does not possess a detectable virion-associated RNA polymerase activity. LDV also differs from these viruses in its density, and size (1).

LDV has a diameter of 40 to 50 nm (1, 12) and thus is similar in size to many encephaloviruses. LDV also seems to resemble these viruses in its general morphology (1, 12), its apparent

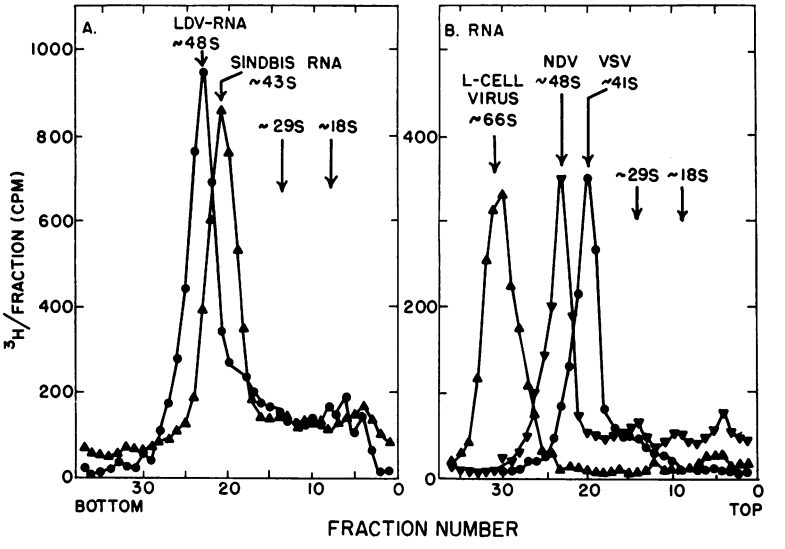

FIG. 2. *Zone centrifugation in sucrose density gradients of ^{3}H-uridine labeled RNA extracted from (A) LDV and Sindbis virus and (B) VSV, NDV, and L cell virus. The RNAs were released from the ^{3}H-uridine-labeled viruses by treatment with SDS and centrifuged together with unlabeled Novikoff rat hepatoma ribosomal RNA through linear 0.15 to 0.9 M gradients of sucrose in B6 in a SW27 rotor at 22,000 rev/min at 20 C for 9 hr. The gradients were analyzed for absorbancy at 260 nm, and gradient fractions were analyzed for radioactivity in acid-insoluble material.*

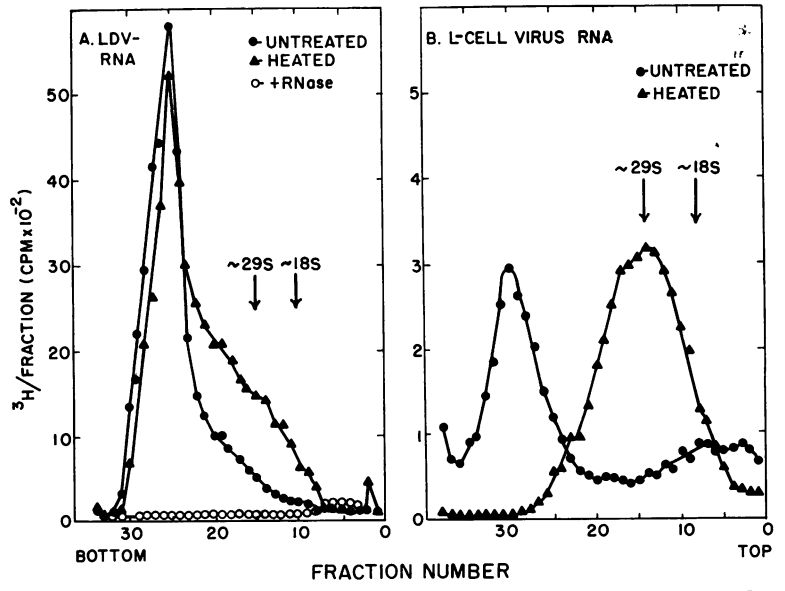

FIG. 3. *Zone centrifugation in sucrose density gradients of ^{3}H-uridine-labeled (A) LDV RNA and (B) L cell virus RNA with and without prior treatment with heat or ribonuclease. Samples of 1 ml of viral RNA in B12 containing 0.1% (w/v) SDS were incubated for 25 sec at 100 C and then quickly chilled in ice. A sample of LDV RNA was incubated in B12 supplemented with 1% (w/v) sodium deoxycholate and 15 μg of ribonuclease A per ml for 30 min at 37 C. All samples were analyzed by sucrose density gradient centrifugation as described in the legend of Fig. 2.*

lack of a virion-associated RNA polymerase, and the nature of its RNA; however, the density of LDV in sucrose (1.2 g/cm^3) is much lower than the densities so far reported for encephaloviruses. The involvement of an insect vector in the transmission of LDV has not as yet been investigated.

The density of LDV has been found to be 1.12 g/cm^3 in many independent experiments in the present study. This value is significantly lower than the density of 1.16 to 1.17 g/cm^3 previously reported by other investigators for LDV (12, 21). The reason for this discrepancy is not clear but, conceivably, the virus used in our experiments contained a relatively greater proportion of lipid. Our isolation procedure is gentle, rapid, and does not include freezing of the preparation and, therefore, may better preserve the intact virus particle. We have occasionally observed a 1.16 g/cm^3 density particle in culture fluids harvested from LDV-infected cultures when the cells were incubated in media containing 0.5% (v/v) of a pancreatic autolysate (18). This particle was also found to be infectious. Present experiments are directed toward elucidating the mechanism by which virions of different densities are produced and understanding the biochemical processes involved in the replication of LDV.

This work was supported by Public Health Service research grants 07250 and AI 00090 from the National Institute of Allergy and Infectious Diseases. We thank John Erbe for excellent technical assistance.

LITERATURE CITED

1. DuBuy, H. G., and M. L. Johnson. 1965. Some properties of the lactic dehydrogenase agent of mice. J. Exp. Med. **122**: 587–600.
2. DuBuy, H. G., and M. L. Johnson. 1966. Studies on the *in vivo* and *in vitro* multiplication of the LDH virus of mice. J. Exp. Med. **123**:985–997.
3. Duesberg, P. H. 1968. Physical properties of Rous sarcoma virus RNA. Proc. Nat. Acad. Sci. U.S.A. **60**:1511–1518.
4. Evans, M. J., and D. W. Kingsbury. 1969. Separation of Newcastle disease virus proteins by polyacrylamide gel electrophoresis. Virology **37**:597–604.
5. Evans, R. 1967. Replication of Riley's plasma enzymes elevating virus in tissue culture: the importance of the cellular composition. J. Gen. Virol. **1**:363–374.
6. Evans, R. 1970. Further studies on the replication of the lactate dehydrogenase-elevating virus (LDH virus) in mouse peritoneal macrophage cultures. Proc. Soc. Exp. Biol. Med. **133**:831–836.
7. Fuscaldo, A. A., H. G. Aaslestad, and E. J. Hoffman. 1971. Biological, physical, and chemical properties of Eastern equine encephalitis virus. I. Purification and physical properties. J. Virol. **7**:233–240.
8. Huang, A. S., D. Baltimore, and M. A. Bratt. 1971. Ribonucleic acid polymerase in virions of Newcastle disease virus: comparison with the vesicular stomatitis virus polymerase. J. Virol. **7**:389–394.
9. Huang, A. S., J. W. Greenawalt, and R. R. Wagner. 1966. Defective T particles of vesicular stomatitis virus. I. Preparation, morphology, and some biologic properties. Virology **30**:161–172.
10. Kindig, D. A., R. Karp, and W. H. Kirsten. 1968. Further characterization of L-cell virions. Proc. Nat. Acad. Sci. U.S.A. **59**:1103–1109.
11. Martin, R. G., and B. N. Ames. 1961. A method for determining the sedimentation behavior of enzymes. Application to protein mixtures. J. Biol. Chem. **236**:1372–1379.
12. Notkins, A. L. 1971. Enzymatic and immunological alterations in mice infected with lactic dehydrogenase virus Amer. J. Pathol. **64**:733–746.
13. Notkins, A. L., and S. J. Shochat. 1963. Studies on the multiplication and the properties of the lactic dehydrogenase agent. J. Exp. Med. **117**:735–747.
14. Pfefferkorn, E. R., and H. S. Hunter. 1963. Purification and partial chemical analysis of sindbis virus. Virology **20**:433–445.
15. Plagemann, P. G. W. 1968. Mengovirus replication in Novikoff rat hepatoma and mouse L cells: effects on the synthesis of host-cell macromolecules and virus-specific synthesis of ribonucleic acid. J. Virol. **2**:461–473.
16. Plagemann, P. G. W., K. F. Gregory, H. E. Swim, and K. K. W. Chan. 1963. Plasma lactic dehydrogenase elevating agent of mice: distribution in tissues and effect on lactic dehydrogenase isozyme patterns. Can. J. Microbiol. **9**: 75–86.
17. Plagemann, P. G. W., and H. E. Swim. 1966. Propagation of lactic dehydrogenase-elevating virus in cell culture. Proc. Soc. Exp. Biol. Med. **121**:1147–1152.
18. Plagemann, P. G. W., and H. E. Swim. 1966. Replication of mengovirus. I. Effect on synthesis of macromolecules by host cell. J. Bacteriol. **91**:2317–2326.
19. Plagemann, P. G. W., M. Watanabe, and H. E. Swim. 1962. Plasma lactic dehydrogenase-elevation agent of mice: effect on levels of additional enzymes. Proc. Soc. Exp. Biol. Med. **111**:749–754.
20. Proffitt, M. R., C. C. Congdon, and R. L. Tyndall. 1972. The combined action of Rauscher leukemia virus and lactic dehydrogenase virus on mouse lymphatic tissue. Int. J. Can. **9**:193–211.
21. Riley, V., H. A. Campbell, J. D. Loveless, and M. A. Fitzmaurice. 1964. Density gradient centrifugation and molecular sieve studies of lactic dehydrogenase-elevating virus-like agents. Proc. Amer. Ass. Cancer Res. **5**:53.
22. Riley, V., F. Lilly, E. Huerto, and D. Bardell. 1960. Transmissible agent associated with 26 types of experimental mouse neoplasms. Science **132**:545–547.
23. Snodgrass, M. J., D. S. Lowrey, and M. G. Hanna, Jr. 1972. Changes induced by lactic dehydrogenase virus in thymus and thymus-dependent areas of lymphatic tissue. J. Immunol. **108**:877–892.
24. Spirin, A. S. 1963. Some problems concerning the macromolecular structure of ribonucleic acids. Prog. Nucl. Acid Res. **1**:301–345.
25. Turner, W., P. S. Ebert, R. Bassin, G. Spahn, and M. A. Cirigos. 1971. Potentiation of murine sarcoma virus (Harvey) (Maloney) oncogenicity in lactic dehydrogenase-elevating virus-infected mice. Proc. Soc. Exp. Biol. Med. **136**:1314–1318.
26. Virolainen, M., and V. T. Defendi. 1967. Dependence of macrophage growth *in vitro* upon interaction with other cell types. *In* V. Defendi and M. Stoker (ed.), Growth regulating substances for animal cells in culture. Wistar Inst. Symp. Monogr. No. 7, p. 67–85.
27. Yaffe, D. 1962. The distribution and *in vitro* propagation of an agent causing high plasma lactic dehydrogenase activity. Cancer Res. **22**:573–580.