Incorporation of Labeled Precursors into the RNA and Proteins of Lactic Dehydrogenase Virus

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Lactic dehydrogenase virus was grown in primary mouse embryo cells and labeled with ³H-uridine and ³H-amino acids. Concentrated and purified virus was banded by isopycnic centrifugation in sucrose gradients, and infectivity and radioactivity were found to correspond at a density of 1.17 g/cm³. The extracted viral RNA was resolved by electrophoresis in polyacrylamide-agarose mixed gels, and the mol wt was estimated to be 6.0 × 10⁶.

The biological properties of lactic dehydrogenase virus (LDV) have been well characterized (7), but there is still relatively little information about its chemical and physical properties. That LDV is an RNA virus was demonstrated by extracting an infectious nucleic acid from the virus and showing that it could be inactivated by RNase, but not by DNase (5). Recent studies on the replication of LDV in primary mouse embryo (PME) cells showed that actinomycin D markedly inhibited viral replication if administered during the early phase of the infection, but cytosine-β-D-arabinofuranoside and 5-fluorodeoxyuridine had no effect on the replication of LDV (13). These findings showed that new DNA synthesis was not required for viral replication, but suggested that some unknown host function dependent on intact, cellular DNA might be needed to initiate the replication of LDV in PME cells. The present investigation was undertaken to substantiate that LDV is an RNA virus and to determine the buoyant density of isotopically labeled virions and the mol wt of LDV nucleic acid.

LDV was grown in PME monolayers and assayed for infectivity in mice as described previously (9, 13). Two strains of LDV were used; one was originally isolated in our laboratory (N strain) (9), and the other was kindly provided by Herman DuBuy of the National Institute of Allergy and Infectious Diseases (3). Except where indicated, the N strain was used in all experiments. Cells that had been in culture for 24 to 48 h in Falcon flasks (75-cm² surface) were infected with LDV at a multiplicity of infection of 10. After adsorption at 37°C for 2 h, the monolayers were washed twice and

processed in one of two ways. To label the viral nucleic acid, the monolayers were incubated for 8 h with 10 ml of Eagle minimum essential medium (MEM) containing penicillin (100 U/ml), streptomycin (100 µg/ml), and 5% dialyzed calf serum. The medium was then aspirated, the monolayers were washed, and 10 ml of MEM without calf serum, but containing 10 µCi of ³H-uridine per ml (20 Ci/mmol; Schwarz/Mann), was added. To label the viral proteins, the monolayers were incubated for 8 h with 10 ml of MEM that contained neither leucine nor tyrosine. The medium then was aspirated, the monolayers were washed, and 10 ml of MEM without calf serum, leucine, or tyrosine, but containing 10 µCi of ³H-leucine per ml (40 Ci/mmol) and 10 µCi of ³H-tyrosine per ml (25 Ci/mmol; New England Nuclear Corp.) was added. At 20 h after infection (12 h after adding the labeled ³H-uridine or ³H-amino acids), the culture medium was collected, clarified at 1,000 × g for 10 min, and centrifuged at 13,000 × g for 15 min. The supernatant fluid was centrifuged at 78,000 × g for 2 h in a Spinco type-30 rotor. The pellet was suspended in 6.0 ml of MEM and centrifuged at 105,000 × g for 2 h in a Spinco type-40 rotor. The supernatant fluid was aspirated, the pellet was suspended in 1.0 ml of TNE buffer (0.01 M Tris [pH 7.3], 0.1 M NaCl, and 0.001 M EDTA), and stored at -70°C. This procedure resulted in a 200- to 300-fold concentration of the virus.

Prior to use, the partially purified, labeled virus was thawed and sonically treated at 1 A for 5 s to break up aggregates. The material (0.3 ml) was layered on 5.0 ml of a 10 to 60% preformed sucrose gradient in cellulose nitrate tubes. The tubes were centrifuged at 100,000 × g for 20 h in a Beckman SW-39L rotor. Fractions

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(0.3 ml) were collected from the bottom of each tube, samples were weighed to determine the density and assayed for infectivity, and the acid-precipitable radioactivity (8) was counted in a Packard liquid scintillation spectrometer.

LDV-RNA was extracted from the fractions that banded in sucrose at 1.16 to 1.18 g/cm³. Fifty microliters of the pooled fractions were extracted with 1% sodium lauryl sulfate and phenol. The mixture then was placed on a 2% polyacrylamide gel fortified with 0.5% agarose (1). Electrophoresis was carried out at 200 V × 75 mA for 2 h. Cellular RNA (45S, 28S, and 18S) from duck embryo cells were used as markers. At the end of 2 h, the gel was washed and sectioned, and 1-mm slices were counted for radioactivity.

Preliminary experiments revealed that virus obtained from the plasma of infected mice had a density in sucrose of approximately 1.17 g/cm³. Figure 1A shows that virus obtained from infected PME cultures pulsed with ³H-uridine also had a density of approximately 1.17 g/cm³. The mean density calculated from several experiments was 1.174 ± 0.013 g/cm³. The correspondence of the peak of radioactivity with the peak of infectivity indicates that the ³H-uridine had become incorporated into the RNA of the virion. Materials prepared in the same way from

uninfected PME cultures or from infected cultures labeled with ³H-thymidine failed to show a peak of radioactivity at 1.17 g/cm³ (data not shown).

On occasion, a peak of radioactivity was observed between 1.10 and 1.13 g/cm³ (Fig. 1A and B) and at 1.24 g/cm³ (Fig. 1B). If, however, each fraction was treated with 10 μg of RNase A per ml (Worthington Biochemical Corp.) for 30 min at 37 C, the peaks at 1.10 to 1.13 g/cm³ and at 1.24 g/cm³ were markedly reduced (Fig. 1B). Moreover, as seen in Fig. 1C, if the cultures were pretreated with 0.1 μg of actinomycin D per ml for 1 h prior to infection with LDV, neither peak was observed. The titer of infectious virus, however, was reduced by over 99% (13).

To see whether there was any difference in the density of virus obtained at different times after infection, cultures were pulsed with ³H-uridine from 6 to 12 h, 12 to 18 h, and 18 to 24 h after infection. Virus collected at the end of each of these intervals was analyzed on a 10 to 60% preformed sucrose gradient. In each case the peak of radioactivity was approximately 1.17 g/cm³ (data not shown). Similarly, a density of 1.17 g/cm³ was obtained with the two different strains of LDV used in our experiments (Fig. 1A and B).

FIG. 1. Isopycnic density gradient centrifugation of lactic dehydrogenase virus (LDV) labeled with ³H-uridine. Partially purified virus was layered on preformed 10 to 60% sucrose gradients. The tubes were centrifuged at 100,000 × g for 20 h in a Beckman SW-39L rotor. Fractions were collected from the bottom of each tube, and samples were weighed, assayed for infectivity, and counted for acid-precipitable radioactivity. Symbols: (—●—) ³H-uridine; (---○---) infectivity; (—○—) density; and (—▲—) incorporation determined after treatment of samples from each fraction with RNase (10 μg/ml) for 30 min at 37 C. The vertical arrows represent the density at 1.17 g/cm³. The N strain of LDV was used in Fig. 1A and C; the DuBuy strain was used in Fig. 1B. In Fig. 1C, virus was prepared from cultures that had been treated with 0.1 μg of actinomycin D per ml for 1 h at 37 C prior to infection.

FIG. 2. Polyacrylamide gel electrophoresis of ^3H -uridine-labeled RNA from lactic dehydrogenase virus (LDV). LDV-RNA was extracted with sodium lauryl sulfate and phenol from the viral fractions that banded on a sucrose gradient of 1.16 to 1.18 g/cm^3 . Cellular RNAs (45S, 28S, and 18S) from duck embryo cells served as markers. Slices (1 mm) were counted for radioactivity. The 45S and 28S RNA markers were found at 25 and 35 mm, respectively, in this gel (arrows).

The mol wt of the RNA from LDV was estimated by its mobility after electrophoresis in agarose-polyacrylamide composite gels. RNA was extracted from virus that had banded on sucrose at 1.16 to 1.18 g/cm^3 . A single peak migrated more slowly than the 45S RNA marker (Fig. 2). Treatment of the RNA with RNase prior to electrophoresis eliminated the band (data not shown). By extrapolation from the known mol wt of the marker RNAs (10), the mol wt of LDV-RNA was estimated at 6×10^6 . This corresponds with the estimates obtained from zonal sedimentation (2) and radiation inactivation data (12).

The proteins of the virion were labeled by incubating infected cultures with ^3H -leucine and ^3H -tyrosine. Approximately 0.7 ml of concentrated virus was centrifuged on the top of a 20 to 34% sucrose gradient with a 60% sucrose cushion at $130,000 \times g$ for 4 h. The fractions at 1.16 to 1.18 g/cm^3 were pooled and analyzed in the usual manner on a 10 to 60% gradient. The ^3H -amino acid-labeled virus banded as a distinct peak at approximately 1.17 g/cm^3 (Fig. 3).

Earlier experiments (7) and those reported here show that LDV can be labeled with ^3H -uridine and ^3H -amino acids and that the virus bands in sucrose at approximately 1.17 g/cm^3 . Several lines of evidence support the contention that the peak at 1.17 g/cm^3 represents labeled virus. First, independent determinations by Riley (11) indicate that virus obtained from the plasma of infected mice bands at approximately 1.17 g/cm^3 as measured by infectivity. Second, both the viral strain used in our laboratory and that obtained from DuBuy (3) band at about 1.17 g/cm^3 when measured by either infectivity or radioactivity. Third, although on occasion we found a small peak of radioactivity between 1.10 and 1.13 g/cm^3 , this usually could be eliminated by treatment with RNase. Moreover, virus obtained from cultures treated with actinomycin D showed no peak of radioactivity at 1.10 to 1.13 g/cm^3 . Finally, virus labeled with ^3H -amino acids also banded at 1.17 g/cm^3 .

Recently, Darnell and Plagemann (2) reported that LDV had a density in sucrose of 1.12 g/cm^3 , but on occasion they observed particles with a density of 1.16 g/cm^3 . Their methods of

FIG. 3. Isopycnic density gradient centrifugation of lactic dehydrogenase virus labeled with ^3H -leucine and ^3H -tyrosine. Partially purified virus was placed on a 20 to 34% sucrose gradient which had been layered over a 60% sucrose cushion. After centrifugation at $130,000 \times g$ for 4 h, the fractions at 1.16 to 1.18 g/cm^3 were pooled, dialyzed, and concentrated. This material then was recentrifuged on a 10 to 60% sucrose gradient as described in the legend to Fig. 1. Symbols: (●—) ^3H -amino acids; (○—) density.

viral preparation, purification, and density gradient centrifugation were somewhat different from ours; nonetheless, the marked difference in the density of LDV observed in the two laboratories is hard to explain solely on a technical basis. An alternative explanation is related to the fact that their virus was grown in peritoneal macrophage cultures, whereas ours was grown in PME cells. Theoretically, at least, this could lead to differences in the lipid content of the envelope and result in particles with a different density. This is consistent with a similar approximation of the mol wt of LDV-RNA in both laboratories. Darnell and Plagemann (2) observed a sedimentation coefficient of 48S in a sucrose gradient corresponding to the value of 6×10^6 daltons as determined in our studies by using gel electrophoresis.

Because of the lack of sufficient information on the physical and chemical properties of LDV, it has been difficult to classify LDV with respect to other viruses. The data reported here on the density of LDV and the mol wt of its RNA, together with previous information on the size, morphology, and ether sensitivity of the virion (7), suggest that LDV fits best in the Togavirus group (4). However, the mol wt of LDV-RNA appears to be higher than that of other togaviruses, and the DNA-dependent RNA step involved in the replication of LDV in PME cells (13) is not characteristic of this group. Definitive classification awaits more detailed studies on the morphogenesis of LDV and the properties of its nucleic acid and proteins.

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