In Vitro Radiolabeling Procedure Which Labels the Proteins of Newcastle Disease Virions with Carbon-14

JANIS McMILLEN* AND R. A. CONSIGLI

Division of Biology, Kansas State University, Manhattan, Kansas 66506

Received for publication 18 August 1975

A convenient and gentle method was used for in vitro radiosotopic labeling the proteins of Newcastle disease virus. This method, which utilizes [14C]formaldehyde and sodium borohydride, labels all of the proteins of purified Newcastle disease virus. Virions labeled in this manner retained biological and biophysical integrity.

A convenient method used for identifying and characterizing virion proteins has been that of in vitro radiolabeling. Radiiodination has been successfully used to label the surface proteins of several RNA-containing enveloped viruses (1, 9, 11, 12, 15, 18). Although high specific activities can be achieved with this method, the half-life of the radioactive proteins is short. When feline leukemia virus is initially disrupted, all of the virion proteins can be labeled by reductive alkylation using [3H]formaldehyde (14). A recent report (6) describes the use of radioactive acetic anhydride to label the proteins of intact avian myeloblastosis virus. However, it was not determined whether the virus remained infectious after this procedure.

During the course of studies with Newcastle disease virus (NDV), it was desirable to have both infectious virions and the proteins radioactively labeled to a higher specific activity than can be obtained after in vivo radiolabeling in embryonated eggs. We previously reported the in vitro radiosotopic labeling of all the proteins of polyoma virus using [14C]formaldehyde and sodium borohydride (4). This report describes the successful application of that technique to an enveloped virus (NDV), resulting in the labeling of all virion proteins while retaining biological activity of the virus.

MATERIALS AND METHODS

Virus propagation and purification. The Roakin strain of NDV was used throughout these studies. Virus stocks were prepared as described (5). Virus was purified from infected allantoic fluid by centrifuging the allantoic fluid over 20% sucrose in an SW27 rotor at 25,000 rpm for 90 min. The pelleted virions were resuspended in TE buffer [0.002 M ethylenediaminetetraacetic acid, 0.002 M tris(hydroxymethyl)aminomethane, pH 7.4] and centrifuged to equilibrium in a glycerol-potassium tartrate gradient, and the virus band was passed over a Sephadex G-50 column (8). The purified virus was stored at −70 C until used for in vitro labeling.

In vitro labeling of NDV. Purified virus was dialyzed overnight at 4 C against 0.1 M sodium borate, pH 9.0. This virus was then radioactively labeled using [14C]formaldehyde and sodium borohydride, as described previously (4), except that the labeling was carried out at 4 C for 7 h. At this time, the labeled virus and the unlabeled control virus were each centrifuged to equilibrium in a glycerol-potassium tartrate gradient as above. Fractions (0.5 ml) were collected and assayed for infectivity (PFU), hemagglutinating ability (HA), radioactivity, and buoyant density.

Preparation of NDV radioactively labeled in vivo. Ten-day-old embryonated eggs were infected with 80 HA units of NDV/egg. After a 2-h adsorption period, the embryo and yolk sac were removed, leaving the chorioallantoic membrane attached inside the shell. Five milliliters of Dubbecco modified Eagle medium, with a 10% concentration of amino acids and supplemented with 7 μCi each of [14C]arginine and [14C]lysine per ml of medium, was added to each egg. The eggs were sealed with Saran Wrap and rotated in a humidified CO2 incubator at 37 C. After 72 h, the membranes and medium were harvested and the virus was purified as described above.

Polyacrylamide gel electrophoresis. Virus samples were prepared for electrophoresis by boiling in sodium dodecyl sulfate and 2-mercaptoethanol as described elsewhere (3). These virus preparations were electrophoresed on 12.5% sodium dodecyl sulfate-polyacrylamide gels using the discontinuous buffer system of Laemmli (2) and on slab gels (13). Electrophoresis was carried out at a current of 30 mA/slab until the tracking dye had reached the bottom of the gel. After staining and destaining, the gel was dried in vacuo and bands were located by autoradiography on Kodak medical X-ray film. Molecular weights were determined by the method of Weber and Osborn (16).

Other methods. Hemagglutination assays were carried out at ambient temperature using 0.75% guinea pig erythrocytes. Infectivity was determined by plaque assay (5). Radioactivity was measured in toluene-Triton X-100 scintillation fluid using a Beckman LS233 liquid scintillation counter. Buoyant den-
sity was determined by weighing samples of the gradient fraction. Neuraminidase assays were performed according to the method of Webster and Campbell (17), using influenza virus as the standard.

RESULTS AND DISCUSSION

Biological and biophysical properties of in vitro labeled NDV. This investigation was undertaken to determine if the [14C]formaldehyde-sodium borohydride procedure for in vitro labeling could be successfully applied to an enveloped virus, namely NDV. It was of interest to determine whether all the proteins of the virus could be labeled, and also if the virus would remain infectious and intact after this labeling procedure.

Figure 1 demonstrates the results of equilibrium sedimentation of NDV on a glycerol-potassium tartrate gradient after in vitro labeling of the virus. As can be seen, the peaks of radioactivity, HA, and infectivity all coincide. These results show that this in vitro labeling method does not disrupt or alter NDV since all of the radioactivity was present in the peak of infectious virions. When an unlabeled control virion preparation was centrifuged to equilibrium in a glycerol-potassium tartrate gradient, similar results were obtained with respect to HA and infectivity (data not shown).

Further evidence that this in vitro labeling method is suitable for NDV is presented in Table 1. At the termination of the in vitro labeling reaction, the labeled virions and unlabeled control virions were assayed for hemagglutination activity, infectivity, and neuraminidase activity. As can be seen, there was no loss of infectivity, HA titer, or neuraminidase activity in the in vitro labeled sample. Buoyant density determinations from the glycerol-potassium tartrate gradient reveal that the peak fraction of the labeled and unlabeled virion preparations have similar buoyant densities. Average specific activity from three different in vitro labeled preparations was $10^6$ to $2 \times 10^6$ counts/min per mg of protein.

Electrophoretic comparison of in vitro and in vivo labeled NDV proteins. A comparison was made of the autoradiograms of sodium dodecyl sulfate-polyacrylamide gels of NDV labeled in vivo and in vitro (Fig. 2). Radioactive bands of both preparations are coincident. Further, in each virus preparation, all bands that stained with Coomassie blue were radioactive and there were no bands of radioactivity that did not stain with Coomassie blue. Using the nomenclature assigned to paramyxovirus proteins by Scheid and Choppin (10), the following radioactive proteins (with their molecular weights) were resolved on each gel: HN, hemagglutinating and neuraminidase glycoprotein, 70,000; F0, precursor to fusion glycoprotein, 64,000; F1, fusogenic protein, 35,000; M, matrix protein, 22,000; P, phosphoprotein, 17,000; L, RNA-dependent RNA polymerase, 90,000; and N, nucleoprotein, 22,000; and P, phosphoprotein, 17,000.

Table 1. Biological and biophysical properties of NDV at various steps in the in vitro labeling procedure

<table>
<thead>
<tr>
<th>Virus sample</th>
<th>HA/ml*</th>
<th>PFU/ml</th>
<th>Neuraminidase activity</th>
<th>Buoyant density (g/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDV in TE buffer</td>
<td>12,800</td>
<td>$0.4 \times 10^6$</td>
<td>0.088</td>
<td>1.194</td>
</tr>
<tr>
<td>NDV after in vitro labeling</td>
<td>12,800</td>
<td>$1.0 \times 10^6$</td>
<td>0.100</td>
<td>1.195</td>
</tr>
</tbody>
</table>

* HA expressed as the reciprocal of the last dilution giving positive hemagglutination.

Neuraminidase activity expressed as optical density at 540 nm per 10 µg of viral protein.

Buoyant densities determined on the peak (counts per minute) fraction after centrifugation of the samples in a glycerol-potassium tartrate gradient.
of the nucleocapsid protein (NP) and membrane protein (M) are somewhat smaller than previously reported (10). This minor difference could be a function of increased gel strength (12.5%) or an intrinsic difference of the NDV strain used in this study. The protein marked C, with a molecular weight of 42,000, may represent a cleavage product of the nucleocapsid subunit, as previously suggested (7). A protein migrating at 15,000 to 17,000 molecular weight, designated S, has not previously been reported. This protein band is consistently seen in gel profiles of virions prepared from unlabeled and in vivo labeled allantoic fluid and chicken embryo tissue culture. It may represent a protein unique to the strain of NDV used.

The data presented in this report demonstrate that an in vitro labeling procedure of reductive alkylation using [14C]formaldehyde and sodium borohydride labels all of the proteins of NDV. Further, the virus remains fully infectious, retaining both hemagglutinating and neuraminidase activities. This method is quick, simple, and inexpensive and relatively high specificity can be obtained. It is suggested that the application of this method to other enveloped viruses should be successful.

ACKNOWLEDGMENTS

We express appreciation to H.C. Minocha for kindly performing the neuraminidase assays.

This paper is contribution no. 1263 from the Agricultural Experiment Station, Kansas State University.

LITERATURE CITED


