Enzymatic Radioiodination of the Envelope Proteins of Avian Myeloblastosis Virus¹

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Purified avian myeloblastosis virus was iodinated by the lactoperoxidase method. Disruption of the labeled virions and chromatography of the viral proteins showed that radioiodine was associated only with the glycoproteins of the viral envelope. Reaction of the radiolabeled virus with antiviral antibody followed by mild detergent treatment and subsequent recovery of immune complexes showed this technique to be useful for the isolation of viral envelope antigens.

Lactoperoxidase-catalyzed iodination of cells and viruses has been demonstrated to be surface specific (6, 8-11). This method enables one to investigate the precise location of proteins and polypeptides in cells and viruses, since only those proteins which are exposed to the external environment will be iodinated under the conditions of the experiment.

Much experimental evidence points to the fact that there are two glycoproteins, m1 and m2, associated with the envelope of avian leukosis viruses (3). The remainder of the viral proteins, gs1-4, are presumed to be located in the interior of the viral envelope. The experiments reported here were designed to test this hypothesis as well as to provide a model study for more complex investigations of the surface proteins of normal and RNA tumor virus-infected chicken embryo cells.

Additionally, the surface specificity of this radiolabeling procedure should enable one to readily isolate surface antigens from cells and viruses. Experiments were designed to test this possibility by using avian myeloblastosis virus (AMV) as a model system, and the results are reported below.

MATERIALS AND METHODS

Virus. AMV was produced by intraperitoneal inoculation of day-old chicks (SPAFAS) with 0.1 ml of infectious plasma. Onset of disease was determined by microscope examination of stained peripheral blood smears. Blood was taken from acutely leukemic chicks by cardiac puncture, the plasma was separated by centrifugation, and virus was isolated from the fresh plasma by differential centrifugation as previously described (5).

Iodination. Lactoperoxidase was purchased from

Sigma Chemical Co. (St. Louis, Mo.). The enzyme-catalyzed iodination reaction was carried out as described by Marchalonis (7). Briefly, virus was purified from 2 to 5 ml of plasma, resuspended in a 0.3-ml volume of phosphate-buffered saline (0.4 mM phosphate, 0.14 M NaCl, pH 7.4), and mixed with 2 μ liters of 0.0002 M KI. To this mixture were added 10 μ liters of lactoperoxidase (6.25 μ g), 10 μ liters of ¹²⁵I, usually 10 to 50 μ Ci, and 10 μ liters of 8.8 mM H₂O₂. After incubation for 10 min at 25 C, 0.5 ml of 5 mM cysteine was added to stop the reaction.

This mixture was then layered on 4.5 ml of 7.5% (wt/vol) sucrose and centrifuged at 100,000 $\times g$ for 30 min in a Beckman L2-65B ultracentrifuge. The supernatant fluid was decanted, and the pellet was dissolved in a solution of 8 M guanidine-hydrochloride (Gu-HCl), 2% mercaptoethanol, 0.01 M EDTA, and 0.05 M Tris-hydrochloride (pH 8.5) by heating at 60 C for 1 h.

For comparative purposes purified virus which had been disrupted with 1% sodium deoxycholate (DOC) was iodinated by the lactoperoxidase method. In these experiments unreacted iodide was removed by exhaustive dialysis. The solution of labeled virus (0.5 ml) was made 8 M with respect to Gu-HCl by the addition of solid salt.

For radioiodination by the KI-NaNO₂ method (2), 150 μ liters of 1 N HCl was added to a small test tube followed, in order, by 25 μ liters of 0.002 M KI, 10 μ liters of radioactive iodide, 25 μ liters of NaNO₂, 200 μ liters of borate-buffered saline (pH 8.1), and 150 μ liters of 1 N NaOH. This reaction mixture was added dropwise to the DOC-disrupted virus. Unreacted iodide was removed by exhaustive dialysis, and the radiiodinated preparation was prepared for column chromatography as described previously for the viral preparations labeled by the lactoperoxidase procedure.

Immunological procedures. The rabbit antiserum prepared against density gradient-purified AMV has been described previously (4). It is capable of reaction with both internal and envelope antigens of AMV. Radiolabeled intact virus was reacted with 0.2 ml of immune serum overnight at 4 C. The resulting precip-

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itate was washed five times with 1 ml of ice-cold phosphate-buffered saline, and then 1 ml of 0.1% DOC was added. This concentration of detergent has previously been found to have little significant effect on antibody-antigen reactions but is capable of disrupting the viral envelope (R. Fritz, unpublished data). The remaining precipitate was then washed with ice-cold phosphate-buffered saline, and dissolved in 8 M Gu-HCl. In one experiment the immunoglobulin G (IgG) fraction of the rabbit antiserum was separated and labeled with ^{125}I and used in place of the whole antiserum in order to follow immune complex formation. This was done to be certain that complete dissociation occurred when the immune complexes were treated with 8 M Gu-HCl under reducing conditions.

Chromatography. The radiolabeled virus preparations were chromatographed on Biogel A-5m (Bio-Rad Laboratories, Richmond, Calif.) by the procedure described by Fleissner (3). Effluent fractions (0.9 ml) were collected and assayed for radioactivity in a Packard autogamma spectrometer. All values were corrected for instrument background (60 to 70 counts/min).

RESULTS

Figure 1 illustrates the elution pattern of radiolabeled proteins when intact virus was radioiodinated and then disrupted and chromatographed on Biogel A-5m equilibrated with 6 M Gu-HCl. Only two peaks of radioactivity corresponding to the envelope glycoproteins, m1 and m2, were observed. Glycoprotein m1 exists as an aggregate in 6 M Gu-HCl and has a molecular weight of at least 100,000, whereas m2 exists as a monomer with a molecular weight of 70,000 (3). Under our chromatographic conditions, m1 would be expected to elute at the void volume of the column (V_0 = fraction 48), whereas m2 would be expected to elute at a V_0/V_r of approximately 1.3. This corresponds to fractions 60 to 64, and it is in this region that a small peak of radioactivity is consistently observed. In several experiments no other significant peaks of radioactivity were observed when

FIG. 1. Gel filtration of AMV proteins in the presence of 6 M Gu-HCl and 0.01 M dithiothreitol. Radioiodination of virus was done prior to disruption for chromatography. Fraction size was 0.9 ml.

fresh unfrozen virus was radioiodinated. If once-frozen and thawed preparations of virus were labeled, the integrity of the virion was sufficiently altered to allow iodine to penetrate the envelope and react with internal polypeptides. Sodium dodecyl sulfate polyacrylamide gel electrophoretic analysis of the peak eluting at fractions 60 to 64 showed this protein to have a molecular weight of 70,000 in accordance with the value reported for m2 by Fleissner (3). In addition, hamster anti-group-specific antigen immune serum was unable to precipitate radioactivity from either peak shown in Fig. 1.

The pattern of radioactivity eluted from the column when DOC-disrupted virus was labeled by the lactoperoxidase method was more complex than that of the intact virus (Fig. 2). At least five peaks of radioactivity were observed. These peaks correspond to both the envelope glycoproteins and the lower-molecular-weight internal polypeptides, since hamster anti-group-specific immune serum was capable of precipitating radioactivity from the peaks eluting at fractions 75, 83, and 100, but not those corresponding to the envelope glycoproteins m1 and m2. Since in these experiments relatively little radioiodine was incorporated into m2 as compared with m1, the KI- NaNO_2 method of iodination was used to radioiodinate DOC-disrupted virus. The results of this experiment indicated that m2 in disrupted viral preparations was apparently refractory to labeling by the lactoperoxidase method, since relatively greater amounts of radioactivity were incorporated when the KI- NaNO_2 method was employed (Table 1).

FIG. 2. Gel filtration of AMV proteins in the presence of 6 M Gu-HCl and 0.01 M dithiothreitol. Radioiodination of viral proteins was done after disruption of the virus with 1% DOC. Fraction size was 0.9 ml.

TABLE 1. *Relative incorporation of radioactivity into envelope glycoproteins of AMV after iodination by various methods*

Treatment of virus	Labeling method	Counts/min m1 ^a counts/min m2
None	Lactoperoxidase	5.5
1% DOC	Lactoperoxidase	16.4
1% DOC	KI-NaNO ₂	5.3

^a Molar ratio of m1 to m2 was estimated to be 2.5 (3).

Results of the experiments designed to investigate the feasibility of direct isolation of viral surface antigens by antiviral antibody are shown in Fig. 3. In this experiment, virus was radiolabeled with ¹²⁵I and the IgG fraction of the rabbit immune serum was labeled with ¹³¹I. The elution pattern of the products of the reaction shows m1 and m2 to be present. The two peaks of ¹³¹I radioactivity correspond to rabbit IgG light and heavy chains which were reduced and separated during incubation of the immune complexes in 8 M Gu-HCl and mercaptoethanol. Thus, it appears that this method may afford a simple and rapid means of isolation of viral and cell surface antigens. In a similar manner, it has been possible to isolate labeled internal polypeptides by using hamster anti-group-specific antigen immune serum, and this procedure has been utilized for the isolation of viral polypeptides from oncornavirus-infected cells (12).

Two other conclusions may be drawn from these data. First, since the minor peak of radioactivity (m2) elutes well ahead of the rabbit IgG heavy chain, this protein has a molecular weight in excess of 50,000 and, there-

fore, is m2 and not a smaller internal protein. Second, since the viral envelope proteins were isolated by using specific antibody, labeled lactoperoxidase (molecular weight equals 78,000) was not responsible for the peak seen at V_0/V_o of 1.3.

DISCUSSION

These experiments were designed to answer several questions. First, is the lactoperoxidase iodination procedure surface specific in the case of AMV, and, as a corollary to this question, are the glycoproteins m1 and m2 the only proteins in the virion exposed to the external environment? The results shown in Fig. 1 and 3 answer these questions in a positive manner. Only m1 and m2 were labeled under the conditions employed.

When DOC-disrupted virus was radioiodinated by the enzyme method, labeling of several proteins was noted. However, it was noteworthy that m2 was found to incorporate much less radioactivity relative to m1, regardless of whether intact or disrupted virus was iodinated. Fleissner has estimated that the ratio of m1 to m2 in the closely related MC29 avian leukosis virus to be 2.5 (3). Assuming that a similar ratio exists with AMV, this does not explain the more than fivefold difference in radioactivity in these two proteins. Indeed, when disrupted virus was radioiodinated by the enzyme method, the relative amount of radioactivity found in the m1 fraction was much increased. Since the increased amount of radioactivity found in this fraction after labeling of disrupted virus might have been due to the formation of aggregates of m1 with labeled internal proteins, disrupted virus was labeled by the KI-NaNO₂ method. In this case the ratio of counts per minute in m1 to counts per minute in m2 was the same as when intact virus was iodinated by the lactoperoxidase method. Thus, it appears that m2 is refractory to iodination catalyzed by lactoperoxidase after detergent disruption of the virus. The possibility exists that after such treatment m2 may form a complex with other polypeptides or lipids and be largely unavailable for iodination by the large lactoperoxidase-iodine complex. These data also indicate that the relatively low incorporation of radioiodine into m2 when intact virus was iodinated is not due to a differential exposure of the two glycoproteins at the virion surface, but is due to an increased resistance of m2 to iodination.

A second question to be answered by this study was the feasibility of using the lactoperoxidase iodination procedure as a first step in the

FIG. 3. Gel filtration of immune complexes of AMV proteins (●) and rabbit antiviral IgG (■) in the presence of 6 M Gu-HCl and 0.01 M dithiothreitol. Radioiodination of virus was done prior to disruption with 0.1% DOC. Fraction size was 0.9 ml.

direct isolation of viral or cell surface antigens by antibody. A similar attempt has been successfully made in using this technique for the isolation and characterization of immunoglobulins and other surface markers from the surface of lymphocytes (1, 11). In our studies we have shown that, by reaction of radioiodinated virus with antibody followed by disruption of the virus under mild conditions, the glycoproteins m1 and m2 are the sole labeled viral proteins eluted from the Gu-HCl column. Since these are known to contribute to the type specificity of the virion, it appears that this method may be suitable for such studies with virus-transformed cells.

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