Labeling of Antibodies with \(^{3}\)H-Acetate

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Antibodies can be labeled with \(^{3}\)H-iodoacetate at pH 9.8 without significant loss of biological activity, and the labeled antibody can be used to detect viral antigens.

Radioimmunoassays have been extensively used for detection of small amounts of \(^{125}\)I-labeled antigen (4). Recently, this method has been used for detecting hepatitis B antigen (9) and for quantitating immunoglobulins (1, 2). We were interested in extending the versatility of the radioimmunoassay by labeling with tritium, a nuclide with a much longer half-life than \(^{125}\)I, and labeling the antibodies (immunoglobulin G) which can usually be obtained in larger quantities than the antigen. The labeling reagent used was iodoacetic acid, an alkylating agent which has been shown to react with sulfhydryl groups, methionine, histidine, and lysine at pH 9.0 or higher (3). In the present study, we report that antibodies and other biologically interesting proteins can be labeled with \(^{3}\)H-acetate without significant loss in biological activity, and that the labeled antibody can be used to detect viral antigens.

The critical step common to all radioimmunoassays is the separation of the unbound isotopically labeled reactants, whether antigen or antibody, from the bound, labeled reagent. As a model system, we chose Costelytra zealandica iridescent virus (CzIV) as an antigen. It is a large insect icosahedral virus with a 130-nm diameter having a sedimentation coefficient of 2,100S (6). This makes it possible to quantitatively remove the bound, labeled antibody from the unbound antibody by a relatively short centrifugation. Since the antigen and any associated antibody can be removed from the reaction, there is no problem of soluble complexes.

Viruses were purified by sucrose gradient centrifugation, and viral concentrations were determined by their extinction at 260 nm (6). Antisera to two serologically distinct insect iridescent viruses, CzIV and Chilo iridescent virus (CIV), were prepared in rabbits by intramuscular injection of virus mixed with complete Freund adjuvant (5). Rabbit immunoglobulins were purified by ammonium sulfate fractionation and labeled with iodoacetic acid-2-\(^{3}\)H (Radiochemical Center, Amersham). The labeling was carried out by use of a modification of the fluorescein isothiocyanate procedure (8). This consisted of the following: to 1 to 2 mg of immunoglobulin in 0.1 M Na\(_2\)HPO\(_4\) was added 200 \(\mu\)Ci of \(^{3}\)H-iodoacetate in distilled water, the pH adjusted to 9.8 with 0.04 N NaOH, bringing the total reaction volume to 2.0 ml. The solution was incubated in the dark at 56 C for 30 min. The reaction was stopped by layering the solution on a Sephadex G-50 column (30 by 1.5 cm) and eluting with phosphate-buffered saline. The first fractions eluted were precipitated by adding enough solid ammonium sulfate to make the resulting solution one-third saturated. The precipitates were collected by centrifugation and redissolved in phosphate-buffered saline. Specific activity was determined by using an absorbance at 280 nm of 1.8/mg of protein and by counting a sample by liquid scintillation; specific activities of 700 dpm/\(\mu\)g of protein were routinely obtained. The labeling reaction was markedly dependent on pH and temperature, being more efficient at higher pH values and temperature. For example, a specific activity of 12,400 dpm/\(\mu\)g was obtained at pH 11.5 and 70 C for 30 min. However, under these conditions the biological activity of the antibody, tested by double diffusion in agar gels (5) or by antibody binding assays, was found to be completely destroyed.

The antibody binding assays were carried out in acid-washed 18- by 75-mm polycarbonate tubes with varying amounts of antigen or antibodies. The reaction volume was made up to 1.0 ml and incubated at 37 C for 30 min, and then antibody-virus complexes were removed by centrifugation at 40,000 \(\times\) \(g\) for 20 min. The supernatant fluid or the resuspended pellet, or
both, were counted by liquid scintillation. It was found that counting the supernatant gave reproducible results, and the percent bound antibody was calculated by comparison with a control without virus. When a constant amount of ¹H-labeled antibodies (15 µg) was reacted with varying amounts of antigen, a saturation curve was obtained with 50% binding of the total counts (Fig. 1). The fact that as much as one-half of the total labeled immunoglobulins present in the rabbit serum could be bound by the virus is a further indication that the labeling conditions do not significantly destroy the biological activity of the immunoglobulins. To rule out the possibility that this was a nonspecific binding of proteins to the surface of the virus, a competition experiment was carried out. Unlabeled homologous and unlabeled heterologous antibodies were added to a constant amount of labeled antibody and virus. The heterologous antibody used was prepared against CIV, a serologically unrelated iridescent virus (5). By using the saturation amounts of 15 µg of ¹H-labeled antibody and 25 µg of CzIV, increasing amounts of unlabeled antibodies were added. It can be seen (Fig. 2) that the binding of radioactivity is virus specific, since increasing the amounts of unlabeled homologous antibodies decreases the amount of label bound (60%), whereas increasing the unlabeled heterologous antibodies has little effect. The slight depression of binding (12%) with 80 µg of heterologous antibodies could be explained by the presence of some serological relatedness not previously detected by immunodiffusion (5).

These results indicate that the labeling of immunoglobulins with ¹H-iodoacetic acid is technically feasible, does not destroy biological activity, and is sensitive enough to make serological comparisons. Tritium as a labeling nuclide has several advantages, since no special precautions are necessary for its use, no specialized equipment is necessary, and the labeled reagent can be stored for long periods without significant loss in specific activity. Other proteins have been labeled by using ¹H-iodoacetic acid; these include the hemagglutinating antigen of influenza virus, a fungal toxin from Mortierella wolfii, and intact iridescent virus particles. There was no detectable loss of biological activity, indicating that these labeling conditions may have wide applicability. Although, in the present antigen-antibody study, the bound antibody could be readily separated by centrifugation, unbound antibody can also be separated by using the "sandwich" solid-phase radioimmunoassay (7).

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FIG. 2. Specificity of the ¹H-labeled antibody and antigen reaction. Each assay contained 15 µg of ¹H-labeled antibody and 25 µg of CzIV, to which was added varying amounts of either unlabeled homologous antibody (△) or unlabeled heterologous antibody (○). Total reaction volume was 1.0 ml. The results are expressed as percentage of maximal binding in the absence of added unlabeled antibody. Heterologous antibodies were prepared against CIV.

FIG. 1. Binding of ¹H-labeled antibody by virus. Each assay contained 15 µg of antibody (700 dpm/µg) with varying amounts of CzIV in a total volume of 1.0 ml. After incubation at 37 C for 30 min in 18- by 75-mm polycarbonate tubes, the reaction mixture was centrifuged at 40,000 × g for 20 min. The amount of radioactivity bound was obtained by subtracting the amount of radioactivity remaining in the supernatant fluid.

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