chloroacetic acid precipitability (10%), and we show this to be the result of formation of aggregates with low immunoreactivity. This study emphasizes (a) the importance of removing both aggregated and degraded material from intact tracer to obtain label optimally suitable for use in radioimmunoassay, and (b) the unsuitability of using trichloroacetic acid precipitability as an indication of immunoreactivity.

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Improved Method of Purifying Some Radiolabeled Glycopeptide Hormones

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We describe a simplified method for purifying radiolabeled glycopeptide hormones (thyrotropin, lutropin, follitropin, and human choriogonadotropin), which results in preparations of over 95% immunoreactivity. This method is based on competitive elution from columns of the plant lectin, concanavalin A, and is applicable to glycopeptide hormones derived from several species.

Additional Keyphrases: concanavalin A–Sepharose • exploiting glycopeptide binding to lectins • purification on Sephadex compared

Purity of the radiolabeled hormone used as antigen is most important for optimal sensitivity and specificity of radioimmunoassays and radioreceptor assays. Our laboratory has optimized methods of radioiodination and purification of several peptide hormones, including small peptides (1).

In 1975 Catt and Dufau published a method for extracting and purifying unlabeled and iodinated human choriogonadotropin by affinity chromatography (2). We have applied this technique to the four related glycopeptide hormones from several species. We report herein this practical technique for high-quality labeling and purification of radioiodinated thyrotropin, follitropin, lutropin, human choriogonadotropin, and choriogonadotropin β-subunit, and show how this technique has improved the quality of these assays.

Materials and Methods

Iodination procedure. Glycopeptide hormones labeled included highly purified bovine and human thyrotropin; human follitropin, lutropin, and choriogonadotropin, as well as the beta subunit of choriogonadotropin; and rabbit lutropin (AFP-558 B) and follitropin (AFP-538-C), all obtained from the National Pituitary Agency.

All hormones were radioiodinated by a modification of the Chloramine T method (3). We have compared enzymatic (glucose oxidase) iodination to use of Chloramine T and find they are equally efficacious if each is optimized.

To minimize damage of peptides during the iodination reaction, we reduced the amount of Chloramine T to 2.5 μg. Twenty microliters (2 μg) of hormone was pipetted into each of several vials, frozen, and stored at −20 °C until it was to be iodinated. The iodination was done at 4 °C. Reagents were added to the vial as follows: 50 μL of 0.5 mol/L phosphate buffer, pH 7.5; 1 mCi (about 2.5 μL) of carrier-free Na125I; and finally, 2.5 μg of Chloramine T in 5 μL of phosphate-buffered saline, pH 7.4 (0.01 mol/L sodium phosphate, 0.15 mol/L sodium chloride). The reaction mixture was gently mixed and allowed to react for 45 s; the reaction was then stopped by adding 300 μL of bovine serum albumin solution (10 g/L), in phosphate-buffered saline. The reaction mixture was transferred immediately to a previously prepared concanavalin A–Sepharose column for purification.

Purification of iodinated hormone. A column of concanavalin A–Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ 08854), 8.5 × 50 mm, was prepared at room temperature, washed with 10 g/L bovine serum albumin in phosphate-buffered saline. The iodination mixture was placed on top of the column; free iodine, bovine serum albumin, and damaged peptides were eluted with 60 mL of phosphate-buffered saline. The iodination mixture was placed on top of the column; free iodine, bovine serum albumin, and damaged peptides were eluted with 60 mL of phosphate-buffered saline. Fractions of 3 mL were collected in tubes that each contained 50 μL of 10 g/L bovine serum albumin in phosphate-buffered saline. After the damaged peptides, iodinated albumin, and free iodine were eluted, the labeled hormone, bound to the affinity column, was eluted with 60 mL of phosphate-buffered saline.

References


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Fig. 1. Elution patterns of (A) radiiodinated human thyrotropin (hTSH), (B) human follitropin (hFSH), and (C) human lutropin (hLH) on concanavalin A-Sepharose. Methyl-alpha-D-glucopyranoside (M-α-D) was added at the point indicated. On the left ordinate is shown counts per minute (cpm); on the right, percent of counts bound to antibody at both excess antiserum and antiserum dilution used in assay tubes. On the abscissa is shown tube or fraction number; each tube contained 3 ml.

saline containing 0.2 mol/L methyl α-D-glucopyranoside. After purification, the percentage of binding activity for different labeled hormones was determined by radioimmunoassay (5) or radioreceptor assay (6). Each elution fraction was tested for immunoprecipitability as follows: A mixture of 100 µL of labeled hormone, 100 µL of appropriately diluted rabbit antibody, and phosphate-buffered saline was incubated for 24 h at 7°C as already described (4). Antibody-bound and free hormone were separated by use of goat anti-rabbit gamma-globulin. Binding in the radioreceptor assay was tested for human choriogonadotropin according to the method of Catt et al. (6). Radioligand receptors were prepared by using testes from Wistar rats, as already published (7).

Results

The elution patterns for human thyrotropin, human follitropin, and human lutropin from a concanavalin A-Sepharose column are shown in Figure 1 (A, B, and C, respectively). The first peak, eluted with phosphate-buffered saline, contained free iodine, damaged hormone, and labeled bovine serum albumin. When eluted radioactivity decreased and had become consistent, methyl α-D-glucopyranoside was added, and immediately eluted labeled hormone, corresponding to the second peak of radioactivity. The percentages of immunoprecipitable activity, determined by use of both the working dilution of the antiserum and antibody, are shown (right ordinate). By using excess antibody, in all cases over 95% of the labeled hormone was precipitable with excess antiserum. Generally, antibody binding was greatest in the third or fourth fractions after adding methyl α-D-glucopyranoside (tubes 23 and 24). Nonspecific binding ranged from 4.2 to 2.7%, the lowest being observed in the second or third fraction of labeled hormone. Figure 2 shows data for choriogonadotropin and choriogonadotropin beta subunit (D and E, respectively). Similar elution profiles were obtained for each of these peptides. The nonspecific binding for choriogonadotropin, tested in the radioreceptor assay with 10 g/L bovine serum albumin, was exceptionally low; 1.2%.

Figure 3 illustrates a comparison of the commonly used purification on Sephadex (8) with the concanavalin A purification for human choriogonadotropin. For the former, the binding activity (at working dilution of antisera) of Fraction A of the organic peak was only 17%, with a nonspecific binding of 3.6%. This fraction was repurified by chromatography on concanavalin A-Sepharose. The result was an increase in binding activity to 63%, at working antiserum dilution, with nonspecific binding remaining at 3.0%. For labeled hormones prepared in this manner in our assay systems, the minimal detectable dose for the radioimmunoassays decreased by 50 to 90% of that of the same hormones purified on Sephadex columns (e.g., choriogonadotropin 2 to 0.2 ng, rabbit lutropin 1 to 0.1 ng).

Discussion

Concanavalin A-Sepharose affinity chromatography has become a reliable and practical method for separating and
purifying many glycopeptides. The selective binding activity of glycopeptides to this lectin is related to the presence of specific carbohydrate moieties in the molecules. Concanavalin A binds D-mannopyranosyl, D-glucopyranosyl, D-fructofuranosyl, and D-arabinofuranosyl groups (9). Thus glycopeptides containing these sugars may be purified by the procedure we describe. By use of other plant lectins, of course, other glycoproteins presumably may be similarly purified. This purification procedure has the advantage of being easily standardizable, less time consuming, and more reproducible than the usual purification by Sephadex gel filtration. Our data indicate that concanavalin A purification of labeled glycopeptide hormones results in a considerable improvement in the quality of radioimmunoassays and radioreceptor assays.

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