also useful to monitor treatment of these patients with the new therapeutic agents currently used.

References

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Carbodiimide Used in Coupling Triiodothyronine Antibody to Carboxymethyl-Cellulose Powder for Solid-Phase Radioimmunoassay

To the Editor:

Protein antigens and antibodies coupled to materials such as Sephadex, Sepharose, and cellulose are widely used in radioimmunoassay and related techniques. Proteins are usually coupled to the solid phase by the cyanogen bromide activation method. However, handling the toxic cyanogen bromide requires special facilities. Sasaki et al. (1) reported coupling antibodies to discs of carboxymethyl-cellulose by using carbodiimide. We have used this method to couple antibody to carboxymethyl-cellulose powder and have developed a solid-phase assay for triiodothyronine (T3).

One milliliter of rabbit antisera to T3 was treated with 180 mg of Na2SO3 to precipitate the globulin fraction. The precipitate was washed and redissolved in phosphate coupling buffer (200 mmol/L, pH 7.4), then used for coupling to cellulose. We suspended 100 mg of carboxymethyl-cellulose (CMC-11, Whatman Ltd.) in 3 mL of coupling buffer, added 1 mL of the purified antibody, and stirred the mixture for 72 h. During this time, 50 mg of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride was used thrice, at 24-h intervals. The antibody-coupled cellulose was filtered and washed with the coupling buffer to remove unbound proteins. Finally, the cellulose was suspended in barbital assay buffer (80 mmol/L, pH 8.6, containing, per liter, 1 g of sodium azide and 2 g of bovine serum albumin). We determined the extent of antibody binding to the cellulose by estimating the binding of [125I]T3 to both the supernate and the cellulose powder. Coupling was maximum in 72 h. We studied the effect of storage on the stability of the immunoadsorbent by estimating the antibody leaking into the supernate, using [125I]T3 as before. No antibody leaked from the cellulose during two months.

This immunoadsorbent was used in a solid-phase radioimmunoassay of T3. We arrived at the optimum amount of immunoadsorbent required for the assay by setting up a titer curve for the immunoadsorbent, using [125I]T3 in solution (3 mCi/μg, 0.4 mCi/mL, containing 1 g of 8-anilino-1-naphthalene sulfonic acid per liter). Standard T3 solutions, in concentrations ranging from 0 to 4 μg/L, were prepared in T3-depleted normal human serum. The assay was set up by adding, sequentially, appropriate volumes of assay buffer, 50 μL of T3 standard (or sample to be assayed), 0.1 mL of [125I]T3 and 0.1 mL of antibody–cellulose suspension, mixing, and incubating at 37 °C for 1 h. The tubes were then centrifuged and the supernates discarded. The radioactivity bound to the cellulose pellet was counted and the counts used for plotting the standard curve.

This carbodiimide condensation procedure is relatively simple for the preparation of solid-phase antibody. However, the concentration of carbodiimide used is critical; too-high concentrations can decrease the sensitivity of the assay. The reduction in sensitivity of the assay is due to inter- and intra-molecular coupling of antibodies. Figure 1 shows the standard curves for the same antibody with a dextran-charcoal type of assay and two batches of immunoadsorbent prepared as described here. In the first case (upper standard curve) 150 mg of carbodiimide was added at once and the reaction was carried out for 72 h. A significantly decreased sensitivity of the assay was noticed in this case. In the second case, 150 mg of carbodiimide was added in three portions; on using this cellulose powder, we found the sensitivity of the assay was comparable with that of the dextran-charcoal type of assay.

The amount of gamma-globulin taken up by the cellulose powder was 16 mg/g of cellulose, as estimated by the Folin–Ciocalteu method, higher than reported in the earlier paper disc method. Our procedure of preparing immunoadsorbent can also be used for preparation of affinity chromatography beds for purification of antigens and antibodies; work in this direction is in progress.

Reference

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Figure 1: Standard curve with immobilized antibody from two different batches compared with a dextran-coated charcoal assay.