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Use of Affinity Chromatography in Developing Acridinium Ester-Labeled Antibodies for an Immunometric Assay of Parathyrin

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Use of Affinity Chromatography in Developing Acridinium Ester-Labeled Antibodies for an Immunometric Assay of Parathyrin, *David S. Hage,*[1] *Bob Taylor,*[2] *Pat Schryver,*[2] *and Pai C. Kao*[2] ([1] Dept. of Chemistry, Univ. of Nebraska, Lincoln, NE 68588-0304; and [2] Dept. of Lab. Med., Mayo Clinic/Foundation, Rochester, MN 55905)

In developing an immunometric assay of intact parathyrin (parathyroid hormone, PTH), we found that affinity chromatography is a useful tool in purifying and optimizing the labeling conditions for acridinium ester-labeled antibodies. We noted this in labeling polyclonal anti-(1-44 PTH) antibodies with *N*-hydroxysuccinimide acridinium ester (London Diagnostics, Eden Prairie, MN). The labeled antibodies were prepared according to published procedures, with 2.5 μg of acridinium ester per 50 μg of antibody and a reaction time of 15 min (*1*).

The labeled antibodies were initially purified by size-exclusion chromatography on a Sephadex G-25 column, as described in the literature (*1,2*). However, we found that this purified label had relatively low specific binding in the PTH assay. To test whether a substantial amount of antibody had been inactivated or denatured as a result of the labeling process, we compared the activity of labeled antibodies purified by the Sephadex G-25 column with the activity of those purified on an affinity column containing PTH 1-44 immobilized on cyanogen bromide-activated Sepharose 4B. A 0.7 cm (i.d.) × 4 cm affinity column was used with an application buffer of pH 7.4 phosphate buffer, 0.10 mol/L, containing 1 g of bovine serum albumin, 1 mL of Triton X-100, 9 g of sodium chloride, and 0.2 g of sodium azide per liter. Nonretained components were washed from the affinity column with a pH 5.0 phosphate buffer, 0.10 mol/L, and the retained antibodies were eluted in pH 2.3 HCl or pH 2.3 glycine buffer, 0.10 mol/L, containing 1 mL of Triton X-100 and 9 g of sodium chloride per liter.

The labeled antibodies purified by the affinity and Sephadex G-25 columns were compared by using equal counts of each in the assay of intact PTH. The calibration curves obtained are shown in Figure 1. Both types of labeled antibodies had similar background counts, but the response

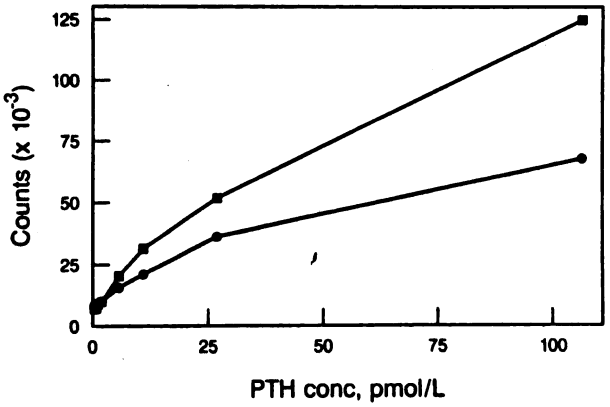

Fig. 1. Calibration curves for an intact PTH immunometric assay involving acridinium ester-labeled antibodies purified by affinity chromatography (■) or by a Sephadex G-25 column (●)

obtained with the label purified by the Sephadex G-25 column was only half that of the affinity-purified label. To examine the reason for this, we applied to the affinity column a portion of the Sephadex G-25-purified label. Protein assays and chemiluminescent measurements of the retained and nonretained fractions revealed that >50% of these labeled antibodies could not bind to the immobilized PTH fragments.

We also used the affinity column to monitor the production of active labeled antibodies; decreasing the amount of acridinium ester dramatically improved the amount of active labeled antibodies obtained. When 2.5 μg of acridinium ester per 50 μg of antibody was used, 72% of the chemiluminescent species in the reaction mixture was eluted without binding to the affinity column. At 0.625–1.25 μg of acridinium ester per 50 μg of antibody, this was reduced to 30%, with the remainder of the counts appearing in the active antibody fraction. The labeling reaction time was less crucial. For example, at 1.25 μg of acridinium ester per 50 μg of antibody, there was no apparent decrease in the relative amount of active labeled antibodies over reaction times of 5–15 min. Indeed, the actual time required for labeling could be very short, with the reaction being almost 75% complete in only 5 min.

In summary, affinity chromatography was found to be useful in the purification of acridinium ester-labeled antibodies, particularly for removing denatured antibodies from the prepared label and for monitoring the amount of active labeled antibodies produced.

References

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