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Improved Recovery of a Radiolabeled Peptide with an Albumin-Treated Reversed-Phase HPLC Column, David S. Hage,¹ Robert L. Taylor,² and Pai C. Kao² (¹ Dept. of Chem., Univ. of Nebraska, Lincoln, NE 68588-0304; and ² Dept. of Lab. Med., Mayo Clinic/Foundation, Rochester, MN 55905)

Reversed-phase high-performance liquid chromatography (RP-HPLC) is an important tool in the purification of radiolabeled peptides and proteins for immunoassay. However, for some proteins and peptides it is difficult to achieve reproducible behavior in RP-HPLC because of the low recovery of these compounds. Factors that can be varied to improve recovery include the strength or pH of the mobile phase, the chain length and spacing of groups on the reversed-phase support, and the flow rate or steepness of the elution gradient (1-5).

In this work, we found that pretreating reversed-phase columns with albumin can also lead to improved recovery of a radiolabeled peptide. This was noted in the purification of ¹²⁵I-labeled [Tyr⁰]1-34 parathyroid hormone (parathyrin)-related peptide (PTHrP). The [Tyr⁰]1-34 PTHrP was obtained from Peninsula Labs (Belmont, CA). The peptide was labeled by mixing 50 μL of 0.50 mol/L phosphate buffer, pH 7.4, containing 2 μg of [Tyr⁰]1-34 PTHrP, with 1 μg of Iodo-Gen (Pierce, Rockford, IL), adding 2000 μCi of Na¹²⁵I, and reacting for 5 min. The radiolabeled peptide was initially purified on a 7 mm (i.d.) × 15 cm Bio-Gel P-2 size exclusion column (Bio-Rad, Richmond, CA), with 0.1 mol/L acetic acid containing 1 g of bovine serum albumin (BSA) and 1 mL of Triton X-100 per liter as the eluent. The label was stored at -20 °C until use. Incorporation of ¹²⁵I into the peptide by this procedure was 34 (± 5)% (n = 6).

Before using it in a PTHrP immunoassay (6), we further purified the labeled peptide by injecting 150 μL of the label preparation onto a 4.1 mm (i.d.) × 30 cm Versapack C₁₈ column (Alltech, Deerfield, IL). The label was eluted from the column at 1 mL/min by using a 40-min gradient from 100% water containing trifluoroacetic acid (TFA), 1 g/L, to a 60:40 (by vol) acetonitrile:water mixture, also containing 1 g of TFA per liter. A typical chromatogram obtained under these conditions is shown in Figure 1a. The major peak at 29 min was identified as radiolabeled [Tyr⁰]1-34 PTHrP, in agreement with results reported by the manufacturer for the unlabeled peptide under similar chromatographic conditions. The activity of this peak was tested by combining the label in this fraction with various dilutions of RAS 6151N rabbit anti-(1-34 PTHrP) antibodies from Peninsula Labs. An average of 60% binding occurred with a 40 000-fold antibody dilution.

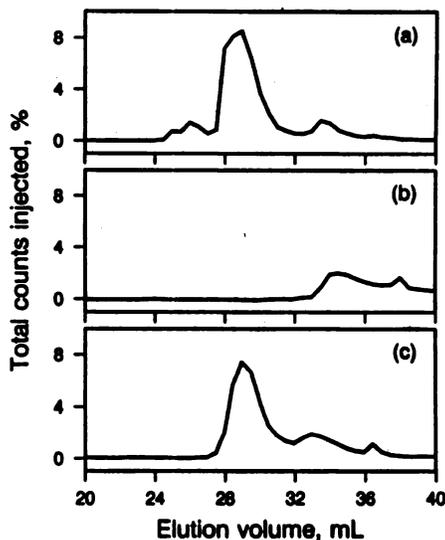


Fig. 1. Chromatograms for ¹²⁵I-labeled [Tyr⁰]1-34 PTHrP injected onto (a) an old C₁₈ column, (b) a new C₁₈ column, and (c) a new C₁₈ column after treatment with BSA

The results in Figure 1a were obtained with a column that had already been used in >300 protein and peptide purifications. When we replaced this with a new column from the same manufacturer, there was a dramatic decrease in recovery of the labeled peptide (Figure 1b), as shown by the absence of the 1-34 PTHrP peak at 29 min. Such behavior might be caused by strong or irreversible binding of peptide (5). We suspected this was the case because no samples had previously been injected onto this column.

To remove strong or irreversible binding sites from the column, we applied a 10 g/L BSA solution at 1 mL/min for 3 h. Excess BSA and reversibly bound BSA were eluted from the column through the use of several gradient cycles. When radiolabeled 1-34 PTHrP was later injected onto the BSA-treated column, a chromatogram was obtained similar to that seen on the old column (Figure 1c). Fractions collected from the peak eluting at 29 min gave 42% binding with a 40 000-fold dilution of the anti-(1-34 PTHrP) antibody, confirming the presence of active 1-34 PTHrP label. Identical results were obtained on this column for at least a year and 50 purifications of the 1-34 PTHrP label, indicating stable column behavior.

In summary, we obtained better recovery and more reproducible chromatographic behavior for labeled 1-34 PTHrP with an albumin-pretreated reversed-phase column than with a new, untreated reversed-phase column. This treatment is simple and inexpensive and should be generally applicable for use in the purification of other radiolabeled peptides and proteins.

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