Improved Recovery of a Radiolabeled Peptide with an Albumin-Treated Reversed-Phase HPLC Column, David S. Hage, Robert L. Taylor, and Pai C. Kuo (Dept. of Chem., Univ. of Nebraska, Lincoln, NE 68588-0304; and 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 [Tyr1]-34 parathyroid hormone (parathyrin)-related peptide (PTHrP). The [Tyr1]-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 [Tyr1]-34 PTHrP, with 1 μg of Iodo-Gen (Pierce, Rockford, IL), adding 2000 μCi of Na[125]I, 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 [125]I 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 labeled preparation onto a 4.1 mm (i.d.) × 30 cm VersaPack C18 column (Alltech, Deerfield, IL). The column 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 50:50 (by vol) acetonitrile:water mixture, also containing 1 g of TFA/L per liter. A typical chromatogram obtained under these conditions is shown in Figure 1a. The major peak at 29 min was identified as radiolabeled [Tyr1]-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 [Tyr1]-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.

References
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Long-Term Storage of Salivary Cortisol Samples at Room Temperature, Yu-Ming Chen,1 Nitza M. Cintrón,2 and Peggy A. Whiston2,3 (1)Krug Life Sciences, 1290 Hercules, Suite 120, Houston, TX 77068; (2)NASA/Johnson Space Center Biomed. Operations and Res. Branch, Mail Code SD4, Houston, TX 77068; (3)author for correspondence)

Salivary cortisol concentration is an excellent indicator of the plasma cortisol concentration (1). Collection of saliva samples for the measurement of cortisol during space flight provides a simple, noninvasive technique for studying the change in adrenal function during the body’s adaptation to weightlessness. However, during space flight the availability of refrigerator space is limited. Therefore, we examined several methods to determine the best way to preserve saliva samples at room temperature.

HPLC analysis (2) of a cortisol standard added to saliva and the direct radioimmunoassay (RIA) (3) of endogenous cortisol in saliva samples exhibited similar fractional decreases in cortisol concentrations as a function of time at room temperature (data not shown). The HPLC analysis in our laboratory indicated that the cortisol in the saliva was not being enzymatically converted into cortisone (4). We then tested several preserving agents, including acetic acid, ascorbic acid, boric acid, and citric acid. According to RIA, after 16 weeks of storage, the tubes containing acetic acid or citric acid (each at 10 g/L) lost <20% of the total cortisol, whereas the tubes without preservatives lost >90% of the cortisol.

The Salivette (Sarstedt, Inc., Newton, NC), a saliva-collection device containing a cotton swab, is used routinely for collecting saliva samples in space flight experiments. Citric acid-treated Salivettes were examined for their ability to preserve cortisol in saliva stored at room temperature. Sixteen subjects each provided three saliva samples: two saliva samples were collected with untreated Salivettes and the third was collected with a citric acid-treated Salivette. One of the untreated Salivettes was stored frozen as the control and the remaining untreated Salivette and the citric acid-treated Salivette were stored at room temperature for four to six weeks. The control salivary cortisol concentration was 6.3 (SD 4.4) µg/L (n = 16). The average (SD) cortisol concentration in the samples stored at room temperature was 4.3 (3.4) and 6.4 (3.6) µg/L in the untreated and the citric acid-treated Salivettes, respectively. The citric acid-treated Salivette not only preserved >85% of the salivary cortisol, but also gave results that correlated well (r = 0.95) with those for the freezer-stored samples (Figure 1). In conclusion, the salivary cortisol was stable in the presence of citric acid, 10 g/L, or preserved in a citric acid-treated cotton swab for as long as six weeks at room temperature.

References

Serum Carnitine Quantification, Jan Cekova1,2 and Karel Kithier1 (1)Demon Clin. Labs. at Detroit Med. Center, and 2Dept. of Pathol., Wayne State Univ. School of Med., Detroit, MI 48201)

Most methods used for carnitine quantification are based on the transfer of an acetyl group from acetyl coenzyme A (AcCoA) to carnitine by use of the enzymatic carnitine acetyltransferase (CAT; EC 2.3.1.7); either a radiochemical or colorimetric method can be used. We have modified the colorimetric method of Wieland et al. (1), which is based on the reaction of 5,5'-dithiobis-2-nitrobenzoate (DTNB) with free CoA released during the acetyl transfer (2), and compared our results with those obtained by the original method (1).