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Some Recommendations to Improve the Quality of a Radioimmunoassay for Human Parathyrin

To the Editor:

Many laboratories are able to establish a great variety of radioimmunoassays (RIAs) to measure human parathyrin (hPTH), because different fragments of the PTH molecule are now commercially available. Some features encountered in our laboratory during the development of a RIA with midregion specificity for the PTH molecule could be valuable as hints for other laboratories working in the field.

A labeled fragment lacking the N-terminal sequence of the bovine PTH (bPTH) molecule was used as tracer in our RIA system. This tracer, \(^{125}\text{I}\)-bPTH 37–84 (Immuno Nuclear Co., Stillwater, MN), was further purified by gel filtration through Ultrigel AcA-202 before use in the assay as previously described (1). This purification step produced a tracer that increased the antiserum titer by two-fold (from 1:1000 to 1:2000, final diln.) as compared with the titer with the tracer as supplied by the manufacturer (Figure 1A).

The antiserum, \(^{125}\text{I}\)-spring-78, was kindly provided by the European PTH Study Group. It was produced in a goat with an extract of human parathyroid adenoma that consisted predominantly of C-terminal fragments as the immunogen (2). This antiserum appeared to recognize mainly some sequence between amino acids 34 and 65 of the hPTH molecule.

The standard used in the assay was the International Reference Preparation (IRP) bPTH for immunoassay, 71/524, or the (N.I.B.S.C., London, England) Research Standard hPTH for immunoassay, 75/549, since both reacted similarly in our RIA system.

We tested the assay conditions, to optimize the quality of our RIA. The assay buffer used contained, per liter: 100 mmol of sodium borate, 4 g of bovine serum albumin, and 500 000 int. units of aprotinin (Trasylol), pH 8.4. Changes in buffer pH between 7.5 and 8.6 did not improve the quality of the assay.

Initially, we used a total incubation time of five days. To shorten this period, we added polyethylene glycol (PEG 6000; Sigma Chemical Co., St. Louis, MO) to give a final concentration of 20 g/L. We then could shorten assay incubation time to 42 h with no loss in sensitivity (Figure 1B), a finding in agreement with others (3).

In summary, when one is setting up a new specific RIA for measuring hPTH, such considerations can lead to better assay performance. Our finding that a further purification by gel-filtration of a commercial tracer permitted a higher antiserum titer could be of especial interest when an antiserum with low titer is being used. The observation that PEG decreases the assay incubation time, in agreement with a previous report (3), could be of particular value in the clinical laboratory routine.

References


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