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Bolton A E & Hunter W M. The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent: application to the radioimmunoassay. *Biochemical J.* 133:529-39, 1973.
[Med. Res. Council, Radioimmunoassay Team, Edinburgh, Scotland]

A method is described for labelling proteins to high specific activities with ^{125}I in which the protein is treated with ^{125}I-labelled 3-(4-hydroxyphenyl) propionic acid N-hydroxysuccinimide ester, resulting in the conjugation of the radioiodinated phenyl moiety to free amino groups in the protein by amide bonds. [The *SCI*® indicates that this paper has been cited in over 1,190 publications since 1973.]

Anthony E. Bolton
Department of Biochemistry
North East London Polytechnic
London E15 4LZ
England

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"In 1970, I joined what was shortly to become the MRC Radioimmunoassay Team in Edinburgh, headed by Bill Hunter. I was introduced to the complexities of the radioimmunoassay (RIA) only to discover what an unreliable method it was at this time, principally because of problems of tracer preparation. For months before I arrived, some assays had been completely unusable for this reason, although the problem was sporadic. My task was to develop an alternative radioiodination system for proteins to supplement the chloramine-T oxidation method of Hunter and Greenwood,[1] then the only widely used method available.

"To avoid exposure of sensitive proteins to oxidising agents, Hunter suggested conjugating them to an ^{125}I-containing group. We met J. Rudinger, a peptide chemist from ETH Zurich, and a profitable collaboration ensued. The Zurich group synthesised a series of active ester derivatives which we tested as routes for incorporating ^{125}I into proteins. The N-hydroxysuccinimide ester of p-hydroxyphenyl propionic acid,[2] which reacts with epsilon amino sidechains of lysine residues of proteins, showed promise. This had the additional advantage of altering different amino acid residues from tyrosine and histidine substituted in direct oxidative iodination.

"There followed a long period of development of the method at the end of which we approached a UK company about the commercial exploitation of the method; we were told there would be no market. We submitted the manuscript for publication. It was rejected on the grounds that new methods must be shown to be better in practise than those existing, not just new and theoretically better. At that point in time, this was not easy to prove—the sporadic problems of the Hunter-Greenwood method had spontaneously and unaccountably been resolved. Fortunately, one of our battery of antisera bound preferentially to tracer prepared by the new method—inclusion of these new data into the paper enabled its publication. Some time later, we heard that an American manufacturer wished to market the radioiodinated compound as 'Bolton-Hunter reagent.'

"I think the widespread use of this method, reflected in its high citation rate, results from the commercial availability of the Bolton-Hunter reagent, making this a simple method to use. I have prepared tracers of unstable proteins by this technique, e.g., in the RIA of the platelet-specific antigen PF4.[3] There are some proteins where the biological activity is retained after labelling by this but not direct oxidative iodination methods, and some peptides lack tyrosine and/or histidine. For these, conjugation labelling methods are necessary. Alternative active ester derivatives have been described since;[4] presumably these would have been developed independently under the pressure of a need for such methods.

"Rudinger sadly died in the mid-1970s. Hunter is now retired."

1. **Hunter W M & Greenwood F C.** Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature* 194:495-6, 1962. (Cited 4,315 times.)
2. **Rudinger J & Ruegg U.** Preparation of N-succinimidyl 3-(4-hydroxyphenyl) propionate. *Biochemical J.* 133:538-9, 1973. (Cited 35 times.)
3. **Bolton A E, Ludlam C A, Pepper D S, Moore S & Cash J D.** A radioimmunoassay for platelet factor 4. *Thromb. Res.* 8:51-8. 1976. (Cited 115 times.)
4. **Langone J J.** Radioiodination by use of the Bolton-Hunter and related reagents. *Meth. Enzymology* 73:112-27. 1981.

