*Journal of Chromatography*, 363 (1986) 424–427
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 781

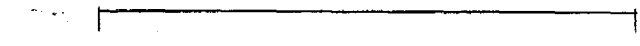
## Note

# Radioiodination of somatostatin analogues employing Sep-Pak rapid sample purification and label assessment by high-performance liquid chromatography

PETER DJURA and RONALD M. HOSKINSON*
*C.S.I.R.O., Division of Animal Production, P.O. Box 239, Blacktown, N.S.W. 2148 (Australia)*
(Received May 12th, 1986)

Somatostatin is a 14-amino-acid peptide first isolated in 1973 by Brazeau and co-workers[1]. Interest in this peptide stemmed from its ability to inhibit the secretion of a number of metabolic hormones, including growth hormone, thyroid-stimulating hormone (TSH) and insulin[2]. There has been a continuing interest in methods for radioiodinating derivatives of somatostatin so that the radioligand could be used in hormone assays, receptor binding studies and measurement of somatostatin-specific antibody in serum. A number of reports of labelling somatostatin analogues have employed the Hunter–Greenwood[3] procedure using metabisulphite to terminate the process and gel filtration for purification[4,5]. The instability of these analogues in the presence of metabisulphite had previously been suspected[6] and some radioiodination procedures substituted a competitive substrate such as bovine serum albumin[7,8] or tyrosine[9] to terminate the radioiodination reaction.

We report here studies on the modification of the Hunter–Greenwood labelling technique for radioiodination of somatostatin analogues (Fig. 1, $Tyr^0$, $Tyr^1$, $Tyr^{11}$), the rapid purification of these peptides with Sep-Pak reversed-phase cartridges[10] and the use of high-performance liquid chromatography (HPLC)[11] to monitor the purity of radiolabelled peptide. The purity of radioiodinated somatostatin prepared by this method is compared with that of two commercially available labels. Degradative effects of metabisulphite on $^{125}I$[$Tyr^0$]-somatostatin is also assessed.




| | |
|---|---|
| Somatostatin | $R_1$=H; $R_2$=Ala; $R_3$=Phe |
| [$Tyr^1$]-somatostatin | $R_1$=H; $R_2$=Tyr; $R_3$=Phe |
| [$Tyr^0$]-somatostatin | $R_1$=Tyr; $R_2$=Ala; $R_3$=Phe |
| [$Tyr^{11}$]-somatostatin | $R_1$=H; $R_2$=Ala; $R_3$=Tyr |

Fig. 1. Structures of somatostatin analogues.

0021-9673/86/$03.50 

## EXPERIMENTAL

### *Instrumentation*

Chromatography was conducted using Waters Assoc. equipment consisting of a Model 510A and Model M45 pumping system controlled by a Model 660 gradient programmer and monitored using a Model 481 Lambda-Max variable-wavelength detector set at 273 nm. Samples were injected through a Model U6K injector onto a Nova-Pak $C_{18}$ analytical column.

Separation of peptides was performed using a gradient elution of 0–40% solvent B over 50 min with a flow-rate of 1 ml/min. Solvent A comprised 0.1% trifluoroacetic acid (TFA) in acetonitrile–water (20:80) and solvent B was 0.1% TFA in 100% acetonitrile. All HPLC radioactive fractions were collected using a Pharmacia Frac-100 set at an 0.5-min collection interval and monitored with a Packard Model 5260 auto gamma scintillation spectrometer.

### *Chemicals and reagents*

Somatostatin, [$Tyr^0$]- and [$Tyr^1$]-somatostatin were obtained from Peninsula Laboratories. Carrier-free $Na^{125}I$ was purchased from Amersham Corp. and [$Tyr^{11}$]-somatostatin was from Bachem. Chloramine-T and sodium metabisulphite were obtained from Sigma. HPLC-grade acetonitrile and Sep-Pak cartridges were from Waters Assoc. UV-grade TFA was obtained from Merck and redistilled from glass prior to use.

### *Methods*

Radioiodinations were performed by the method of Hunter and Greenwood[3] with the modification that dilution with distilled water was used to replace metabisulphite quenching in order to inhibit competitive reactions with chemically sensitive peptides. The peptide (10 $\mu$g in 10 $\mu$l of 0.05 *M* phosphate buffer, pH 6.8), 100 $\mu$l of phosphate buffer and 5 $\mu$l (*ca.* 0.5 mCi) carrier-free $Na^{125}I$ were added to a small stoppered fusion tube (2.0 cm × 0.5 cm). The reaction was initiated by the addition of 5 $\mu$l of chloramine-T (1 mg/ml) and allowed to proceed at room temperature for 0.5 min with occasional agitation. Reactions were "terminated" prior to Sep-Pak loading by the addition of distilled water (500 $\mu$l) followed by direct application to a preactivated Sep-Pak cartridge via a glass pipette. The cartridge was then washed with distilled water (10 ml) to remove salts and any unreacted iodine. This was followed by a wash with 0.1% TFA in acetonitrile–water (10:90) (5 ml) and final elution of the radiolabel using 0.1% TFA in acetonitrile–water (60:40). These eluants were applied manually with a 10-ml disposable syringe at a flow-rate of *ca.* 10 ml/min. The radioactivity of each eluted volume was monitored using an Ortec Model 776 gamma counter. The entire labelling reaction and Sep-Pak purification was completed in less than 30 min. The above procedure was performed in a fume hood and all manipulations of radioactive samples were carried out with appropriate lead shielding.

The purity of a 10-$\mu$l sample of the eluted peptide was assessed using the above-mentioned HPLC conditions (see *Instrumentation*). Fractions were collected every 0.5 min, radioactivity monitored and profiles thereof superimposed on the corresponding elution profile of unlabelled peptide.

## RESULTS AND DISCUSSION

Our initial attempts at radioiodinating somatostatin analogues using the Hunter–Greenwood procedure yielded a heterogeneous mixture that was not well resolved from $Na^{125}I$ during gel filtration. The heterogeneity might be expected from the reductive effects of metabisulphite used to terminate the iodination.

Here we report that a five-fold dilution to "terminate" effectively a radioiodination procedure followed by rapid Sep-Pak elution yields substantially homogeneous radioiodinated peptides. We chose a modification of the Hunter–Greenwood technique due to its overall simplicity and reproducibility in yielding a highly effective label for our antibody immunoassay.

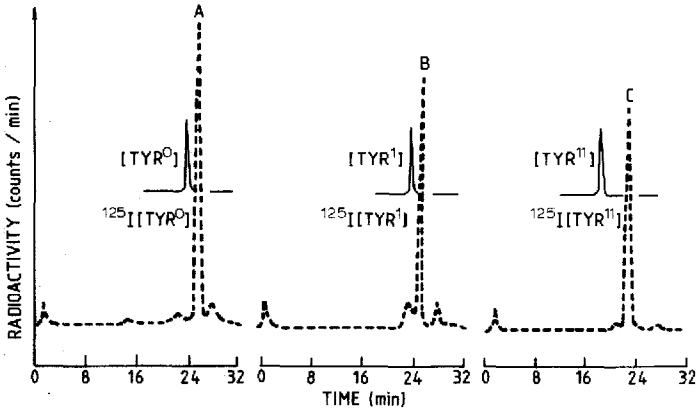



Fig. 2. HPLC elution profiles of radiolabelled somatostatin analogues prepared by the modified Hunter–Greenwood technique after employment of rapid Sep-Pak purification.

The radioactive profiles of several somatostatin analogues are shown in Fig. 2. Unreacted $Na^{125}I$, which would elute within the first 8 min, is minimal. The monoiodo species[12], being the major peak, accounts for more than 80% of the applied radioactivity, whereas chemically modified and the diiodo species account for less than 15% of the residual. Over 80% of the radioactive counts applied to a Sep-Pak cartridge were eluted, with greater than 95% recovery of radioactivity when applied to HPLC.

In a standard Hunter–Greenwood iodination reaction, the time interval between addition of metabisulphite to the reaction and the point at which it separates from the radioiodinated peptide on a gel filtration column may approach 15 min, ample time for significant reductive changes to occur. In order to determine the effect of metabisulphite on these radioligands we treated the $^{125}I[Tyr^0]$ analogue (10 $\mu$l), which had previously been shown to be relatively homogeneous (see Fig. 2), with a small aliquot of metabisulphite (*ca.* 1 $\mu$g). The HPLC profiles of the time-dependent metabisulphite effect show quite clearly that $^{125}I[Tyr^0]$-somatostatin is readily modified to several more polar components (Fig. 3).

The use of Sep-Pak cartridges in the purification of radiopharmaceuticals has,

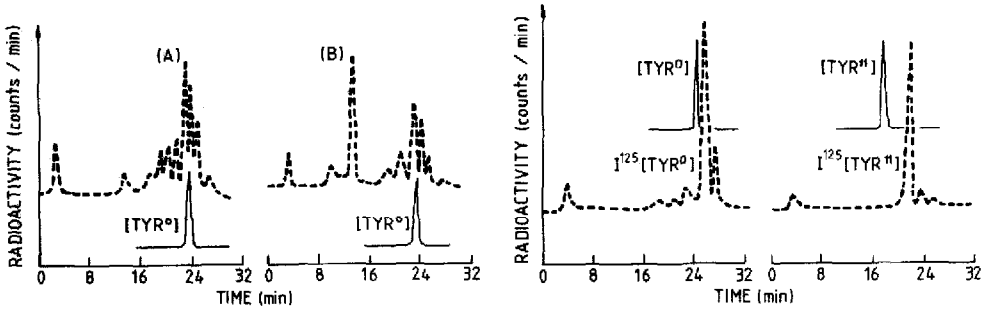



Fig. 3. Degradative effects of sodium metabisulphite on $^{125}$I[Tyr$^0$]-somatostatin. Aliquots of the Sep-Pak-purified label (10 $\mu$l) were treated with 3 $\mu$l of metabisulphite solution (1 mg/5 ml), allowed to stand at room temperature for (A) 5 or (B) 15 min and then assessed by HPLC.

Fig. 4. HPLC of commercially available $^{125}$I[Tyr$^0$]- and $^{125}$I[Tyr$^{11}$]-somatostatin.

over the past few years, gained increasing attention. Kilbourn *et al.*[13] used Sep-Pak cartridges in their purification of compounds containing carbon-11. Miller *et al.*[14] employed Sep-Pak reversed-phase cartridges in their initial purification of radioiodinated Leu-enkephalin and angiotensin, then used HPLC to separate the mono- and diiodo derivatives.

The advantages of using Sep-Pak $C_{18}$ reversed-phase cartridges are that they provide a rapid and efficient means of removing unreacted Na$^{125}$I, they are inexpensive, readily disposable and easy to handle under fume hood conditions, thereby minimizing exposure of the user to volatile $^{125}I_2$. We have employed Sep-Pak $C_{18}$ reversed-phase cartridges as an alternative to gel filtration in the purification of a number of radiolabelled peptides. The modified Hunter–Greenwood labelling technique, along with the rapid Sep-Pak purification described above, produces a radiolabel which is comparable in purity to commercially available radioligands (*cf.* Figs. 2 and 4).

## REFERENCES

1 P. Brazeau, W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier and R. Guillemin, *Science (Washington, D.C.)*, 179 (1973) 77.
2 A. V. Schally, D. H. Coy and C. H. Meyers, *Annu. Rev. Biochem.*, 47 (1978) 89.
3 W. M. Hunter and F. C. Greenwood, *Nature (London)*, 194 (1962) 495.
4 T. Yamada, D. Marshak, S. Basinger, J. Walsh, J. Morley and W. Stell, *Proc. Natl. Acad. Sci. U.S.A.*, 77 (1980) 1691.
5 C. McIntosh, R. Arnold, E. Bothe, H. Becker, J. Köbberling and W. Kreutzfeldt, *Gut*, 19 (1978) 655.
6 Y. C. Patel and S. Reichlin, *Endocrinology*, 102 (1978) 523.
7 V. Harris, J. M. Conlon, C. B. Srikant, K. McCorkle, V. Schusdziarra, E. Ipp and R. H. Unger, *Clin. Chim. Acta*, 87 (1978) 275.
8 J. Gerich, K. Greene, M. Hara, R. Rizza and G. Patton, *J. Lab. Clin. Med.*, 93 (1979) 1009.
9 G. Aguilera and D. S. Parker, *J. Biol. Chem.*, 257 (1982) 1134.
10 D. D. Gay and R. A. Lahti, *Int. J. Pept. Protein Res.*, 18 (1981) 107.
11 O. von Stetten and R. Schlett, *J. Chromatogr.*, 254 (1983) 229.
12 B. H. Frank, M. J. Beckage and K. A. Willey, *J. Chromatogr.*, 266 (1983) 239.
13 M. R. Kilbourn, D. D. Dischino, C. S. Dence and M. J. Welch, *J. Liq. Chromatogr.*, 5(10) (1982) 2005.
14 J. J. Miller, G. S. Schultz and R. S. Levy, *Int. J. Pept. Protein Res.*, 24 (1984) 112.