Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **XLI**, 255-259 (1998)

ELECTROPHILIC RADIOIODINATION OF TYROSINE DERIVATIVES

FARAH K. and FAROUK N.

Labelled Compounds Dept., Hot Laboratories Centre,
Atomic Energy Authority, P.O.Code 13759,
Cairo, Egypt.

SUMMARY

A comparative study on the electrophilic radioiodination of L-tyrosine, L-α-methyl tyrosine and L-tyrosine methyl ester has been carried out using chloramine-T (CAT) and iodogen as oxidizing agents to generate electrophilic radioiodine. Optimization of the radioiodination conditions has been performed resulting in high labelling yields within short reaction times at room temperature. Radiochromatograms also revealed side product impurities at longer reaction time and higher oxidizing agent concentration. Maximum yields of 93%, 90% and 78% were obtained in case of CAT, while 87%, 88% and 53% were obtained in case of iodogen for L-3-[^{131}I] iodotyrosine, L-3-[^{131}I] iodo-α-methyl tyrosine and L-3-[^{131}I] iodotyrosine methyl ester respectively.

Key Words: L-tyrosine, L-α-methyl tyrosene, L-tyrosine methyl ester, chloramine-T, iodogen, elctrophilic radioiodination, iodine-131

INTRODUCTION

Radioiodinated compounds prepared by the oxidative radioiodination procedures were originally developed for labelling proteins. The condition for the labelling is the presence of tyrosine ring in the molecule. In some cases, histidine or tryptophan rings can also be labelled. Electrophilic radioiodine can be generated by a variety of oxidizing agents, the most commonly used being chloramine-T and iodogen (1).

Amino acids are the precursors of neurotransmitters and other important metabolic intermediates in the central nervous system. Tyrosine is biologically important in the synthesis of thyroid hormones, catecholamines and melanin (2). Since in proteins, tyrosine is predominantly labelled (3), this compound was chosen as a model substrate. Radioiodinated α-methyl tyrosine exhibits a four-fold higher uptake than that of the non-methylated iodotyrosine (4), resists enzymatic deiodination and is used in brain SPECT (5). Radioiodinated tyrosine and α-methyl tyrosine have been prepared by different techniques (6-11). Tyrosine methyl ester is a prosthetic group which is used to label organic molecules which do not have activated aromatic groups or may not be stable to harsh oxidizing conditions (12).

In this work, the factors affecting the labelling yields using CAT and iodogen as oxidizing agents were studied to obtain the optimum conditions for the preparation of the labelled compounds.

CCC 0362–4803/98/040255–05$17.50

Received 12 May 1997
Revised 27 December 1997

EXPERIMENTAL

Materials:

L-tyrosine (Sigma), L-α-methyl tyrosine (Aldrich), L-tyrosine methyl ester (Aldrich), chloramine-T (Aldrich) and iodogen (Pierce Chem. Co) were used without further purification.

^{131}I is no-carrier-added Na ^{131}I in 0.1 N NaOH locally produced in the ARE reactor by dry distillation from irradiated TeO_2, act. conc. (50mCi/ml). All other reagents and solvents used were of analytical grade.

Labelling Technique:

In a reaction vial containing an appropriate amount of the substrate in 0.1M phosphate buffer solution, (KH_2PO_4 and K_2HPO_4), a suitable amount of radioiodide (50μCi) is added, followed by the addition of chloramine-T solution of the desired concentration in a total reaction volume of 500 μl. The reaction is allowed to proceed for a chosen interval of time, after which the reaction is terminated by the addition of 50μl aqueous $Na_2S_2O_5$ solution (30 mg/ml) to ensure that all the unreacted iodine is in a reduced form before chromatographic analysis. Iodogen is insoluble in water; 5 mg of iodogen were dissolved in 5ml chloroform and a volume containing the desired amount of iodogen is placed in the reaction vial. The solvent is allowed to evaporate forming a solid thin film on the wall of the reaction vial. To this coated vial, a solution of the substrate and radioiodine were added and the volume completed to 500μl. After a chosen interval of time, the reaction is quenched by the addition of 50μl $Na_2S_2O_5$.

The products were analyzed on Whatman No 3 paper chromatography using a mixture of n-butanol: acetic acid : water (4:1:1, V/V/V) as developing solvent. The R_f for L-3-[^{131}I] iodotyrosine, L-3-[^{131}I] iodo-α-methyl tyrosine and L-3[^{131}I] iodotyrosine methyl ester are 0.5, 0.7 and 0.9 respectively, while iodide remains near the origin. Separation of the labelled products were also achieved by HPLC on RP-18 column (250x4mm) Lichrosorb, Merck, eluted with 0.02M sodium acetate:ethanol (9:1, V/V) at a flow rate of 0.5ml/min. The yield is calculated as the ratio of the radioactivity of the labelled product to the total radioactivity. The reported yields are the mean value of 2 experiments.

RESULTS AND DISCUSSION

pH dependence:

The effect of the pH of the medium on the labelling yield of L-3-[^{131}I] iodotyrosine was investigated from pH 1 to 11. Fig.(1) shows a maximum yield at pH 7 where tyrosine is ionized to the anion and iodide is oxidized to electrophilic species. The presence of phenolic hydroxyl group facilitates the iodination since the o-position of tyrosine is activated to electrophilic attack owing to the electron donation of the neighboring hydroxyl group. The change in the labelling yield is attributed to changes in the ionization of the carboxyl, the amino and the phenolic hydroxy OH groups with change in pH.

Time dependence:

The labelling yields were determined at different time intervals for L-tyrosine, Fig.(2). Labelling was carried out from 0.1M phosphate buffer pH 7 using the ratio (1:5, w/w) oxidant:substrate. The reaction is fast; the optimum reaction time for L-tyrosine is 10 min in case of CAT and 15min in case of iodogen. Similar results for L-α-methyl tyrosine indicate a reaction time of 5 min and 10 min, and for L-tyrosine methyl ester, a reaction time of 20 min and 30 min. Thus in iodogen method, the

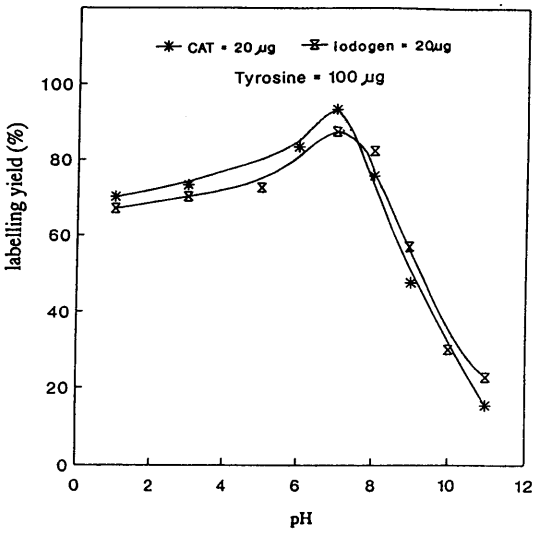

Fig.(1): Effect of pH on the labelling yield of L-3-[^{131}I] iodotyrosine
(0.2 mg/ml tyrosine + 0.04 mg/ml oxidizing agent + 50 µCi Na ^{131}I in 0.5 ml buffer).

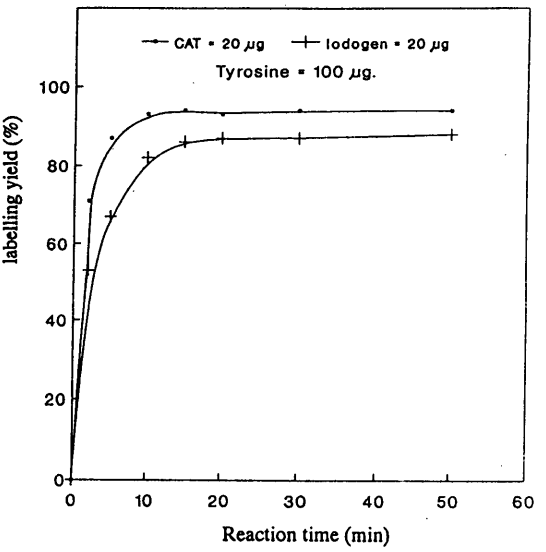

Fig.(2): Effect of reaction time on the labelling yield of L-3-[^{131}I] iodotyrosine
(0.2 mg/ml tyrosine + 0.04 mg/ml oxidizing agent + 50 µCi Na ^{131}I in 0.5 ml phosphate buffer, pH 7).

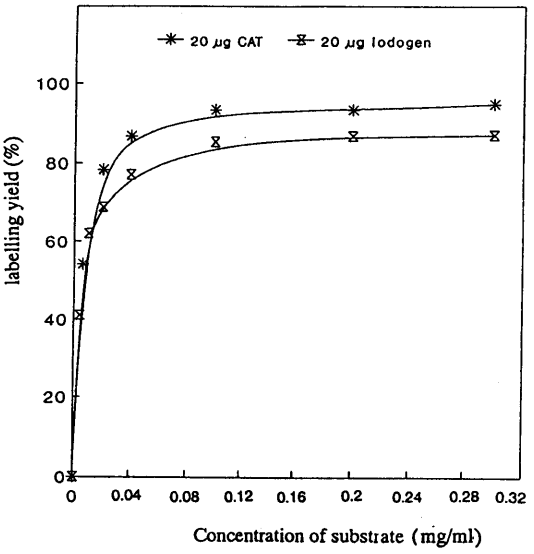

Fig.(3): Variation of the labelling yield of L-3-[^{131}I] iodotyrosine with the concentration of substrate
(x mg/ml tyrosine + 0.04 mg/ml oxidizing agent + 50 µCi Na ^{131}I in 0.5 ml phosphate buffer, pH 7).

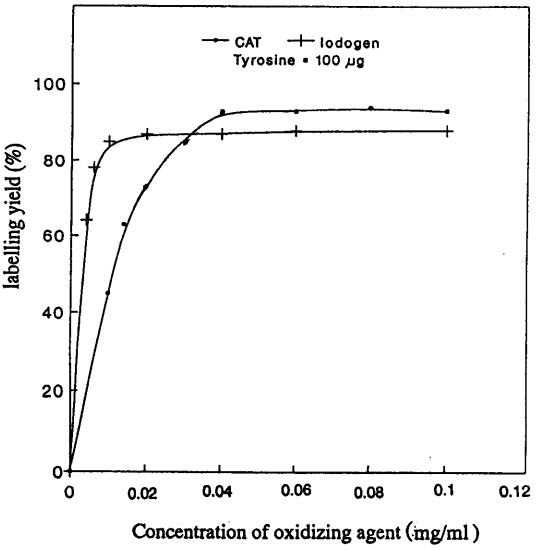

Fig.(4): Variation of the labelling yield of L-3-[^{131}I] iodotyrosine with the concentration of oxidizing agent
(0.2 mg/ml tyrosine + x mg/ml oxidizing agent + 50 µCi Na ^{131}I in 0.5 ml phosphate buffer, pH 7).

oxidation and iodination proceed slowly and the reaction time is increased. It can be observed that the ester of tyrosine has iodination rate smaller than the parent non-esterified compound.

Substrate conc. dependence:

Fig.(3) shows the variation of the labelling yield of L-3-[^{131}I] iodotyrosine with the concentration of substrate at constant CAT or iodogen concentrations. Data indicate that the yield increases with increase in substrate conc. at low values and further increase in the concentration of substrate has no significant effect on the yield. 100 μg of substrate was chosen and kept constant in all other experiments.

Oxidizing agent conc. dependence:

The effect of the variation in the concentration of chloramine-T or iodogen on the labelling yield of L-3-[^{131}I] iodotyrosine was investigated, Fig.(4). Results indicate that an increase in the oxidizing agent conc. increases the yield to a maximum and further increase in the oxidant concentration has no effect on the yield in the range studied and at the optimum reaction time. No side products were observed in this range of concentration. It was found that increasing the concentration of CAT or iodogen to 100μg (0.2mg/ml) and the reaction time to 60 min leads to considerable decrease in the yield of L-3-[^{131}I] iodotyrosine. The effect is more pronounced in the case of CAT. Chloramine-T is highly reactive and high concentrations from CAT cause oxidative side reactions (14). Iodogen gives lower yields but with less oxidative damage (15).

In fact, there is a certain ratio of oxidant/substrate which achieves good iodination. Maximum yields of 93%, 90% and 78% were obtained for L-3-[^{131}I] iodo-tyrosine, L-3-[^{131}I] iodo-α-methyl tyrosine and L-3-[^{131}I] iodotyrosine methyl ester respectively at a ratio (1:5, w/w) CAT:substrate while maximum yields of 87%, 88% and 53% were obtained at a ratio (1:5, w/w) iodogen:substrate. Thus the concentration of oxidizing agent and the reaction time must be carefully optimized to obtain high radiochemical yield and purity of the labelled compound.

KI carrier dependence:

Various amounts of KI carrier were mixed with Na ^{131}I and added to the reaction mixture containing 100 μg substrate and 20 μg CAT. The labelled yield increased with increase of KI reaching a maximum yield of 97% L-3-[^{131}I] iodotyrosone, 95% L-3-[^{131}I] iodo-α-methyl tyrosine and 88% L-3-[^{131}I] iodotyrosine methyl ester at 10^{-3}μg, 10^{-1}μg and 1 μg, KI, respectively. Further increase in KI carrier lead to decrease in the yield due to competition between radioactive and inactive iodide. Thus high CAT concentration over iodide is necessary to achieve an efficient electrophilic substitution.

REFERENCES

1) Hunter R. - Proc. Soc. Exp. Biol. Med. 133: 989 (1970).
2) Kloss G. and Leven M. - J. Nucl. Med. 4:179 (1979).
3) Krohn K.A., Knight L.C., Harwig J.F. and Welch M.J. - Biochem. Biophys. Acta 490: 497 (1977).
4) Coenen H.H. and Stockin G. - Radioaktive Isotope in Klinik und Forschung 18:402 (1988).
5) Langan K.J., Coenen H.H. and Rossen N. - J. Nucl. Med. 31:281 (1990).
6) Kloss G. and Leven M. - J. Nucl. Med. 4:179 (1979).

7) Baldas J. and Colmanet S.F. - Int. Symposium on Radiopharmacology, Sydney, Australia, 24-26 Aug. (1989).
8) Knust E.J., Dutshka K. and Machulla H.J. - J. Radioanal. Nucl. Chem., Letters, 144: 107 (1990).
9) Trivedi M.A. and Carnochan P. - J. Labelled Compd. Radiopharm. 33:61 (1993).
10) Krummeich C., Holschback M. and Stocklin G.- Appl. Radiat. Isot. 45:929 (1994).
11) Guhlke S. and Biersack H.J. - Appl. Radiat. Isot. 46:177 (1995).
12) Seevers R.H. and Counsell R.E. - Chem. Rev. 82:575 (1982).
13) Ganguli P.C. and Hunter W.M. - Radioimmunoassay Methods, Edingburgh Churchill, Livingstone, p. 54 (1971).
14) Petzold G. and Coenen H.H. - J. Labelled Compd. Radiopharm. 18:1319 (1981).
15) Salisburry J.G. and Graham J.M. - Biochem. J. 194:351 (1981).