PREPARATION OF NO-CARRIER-ADDED 4-[^{131}I]IODOANTIPYRINE USING CHLORAMINE-T

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SUMMARY

The blood flow reagent 4-iodoantipyrine, labelled with ^{131}I or ^{125}I, was prepared by allowing aqueous antipyrine to react with either no-carrier-added $^{131}I^-$ or $^{125}I^-$ in the presence of chloramine-T. Purification of 4-[^{131}I]iodoantipyrine was performed by reversed-phase liquid chromatography and resulted in a 99±1% radiochemically pure product in an overall 90% yield. The use of iodogen for oxidation of the radioiodine was also investigated.

Key words: 4-[^{131}I]Iodoantipyrine, 4-[^{125}I]Iodoantipyrine, Chloramine-T, Iodogen, No-Carrier-Added $^{131}I^-$ and $^{125}I^-$, Reversed-Phase Liquid Chromatography.

INTRODUCTION

Radioisotopically labelled 4-iodoantipyrine (4-IAP), a nonpolar, highly diffusible compound with a high lipophilicity, has been shown to be a suitable tracer for studying regional cerebral blood flow (1-13). It has been labelled with ^{131}I, ^{125}I, ^{123}I, ^{14}C, and ^{11}C (14-22). Recently, several radioisotopic methods using 4-IAP labelled with ^{14}C, ^{123}I, and ^{131}I have shown that double tracer autoradiography allowed precise local assessment of regional cerebral blood flow and glucose utilization (6-12).

Our previous work involving surface catalysed iodinations (19,22) indicated that 4-^{131}IAP was readily prepared by reaction of antipyrine (AP) with oxidized forms of $^{131}I^-$. Since chloramine-T (C-T) is an excellent reagent for oxidation, we have investigated its use to label AP with no-carrier-added $^{131}I^-$ or $^{125}I^-$. The use of iodogen (24) as an oxidant was also studied. This paper presents a

0362-4803/86/050479-07$05.00

Received February 1, 1985
Revised April 23, 1985

method using C-T for rapid preparation of 4-^{131}IAP and subsequent purification by reversed-phase liquid chromatography.

MATERIALS AND METHODS

General.

Therapeutic Na^{131}I solution was purchased from Syncor, Inc., Miami, Florida. The formulation, claimed to be "carrier-free", was supplied in phosphate buffered sodium chloride containing up to 0.16% sodium thiosulphate at a pH of 7.5-9.0, adjusted with NaOH. Reductant free Na^{125}I was from Dupont NEN Products. Antipyrine (Aldrich Chemical Co.); chloramine-T (Eastman Kodak Co.); and Iodogen (1,3,4,6-tetrachloro-3 α, 6 α-diphenylglycouril, Sigma Chemical Co.) were used without further purification. 4-IAP was synthesized according to a literature method (15).

All solvents for high performance liquid chromatography (HPLC) were high purity grade obtained from Burdick and Jackson Laboratories and degassed ultrasonically under vacuum before use. Separations were performed using an Altex Model 330 liquid chromatograph equipped with an Altex Model 155-40 variable wavelength UV detector operated at 240 nm. Specific activity determinations were performed at 190.5 nm to increase the limit of detection for 4-IAP (22). Eluates were continuously monitored for radioactivity using a NaI(Tl) detector with a Nuclear Data Model 60A Multichannel Analyzer operating in the multichannel scaling mode. The conditions for HPLC spearations are shown in Table I. System B was used for preparative separation.

Labelling Procedure.

Analytical experiments were performed as follows: To either 100 μl of water or ethanol containing an appropriate amount of antipyrine, either Na^{131}I (0.5-1.0 μl, 50-100μCi/μl) or Na^{125}I (3 μl, 20 μCi/μl) was added. A solution of aqueous chloramine-T (4 μl, 70 μg/μl) was then added resulting in a 10^{-2} M concentration. After 2 min the reaction was stopped by the addition of aqueous $Na_2S_2O_3$(25 μl, 30mg/ml). Ten microliter samples were removed for HPLC analysis using solvent system A. Iodogen (20 μl, 10 mg/ml) in methylene chloride was

Table I. Conditions for High Performance Liquid Chromatographic Separations Using a C-18 Reversed-Phase Column[a].

	Retention time (min)[b]	
Compound	System A	System B
$^{131}I^-$	1.5	2.0
Antipyrine	5.3	11.2
4-Iodoantipyrine	10.3	15.0

a) Alltech C-18 column (10μ, 25 cm x 4.6 mm i.d.) equipped with a Analytichem C18 guard column (40μ, 3 cm x 4.6 mm i.d.).

b) The mobile phases were: System A - H_2O/CH_3CN(75:25) at 2.0 ml/min; System B- H_2O/MeOH(75:25) at 2.0 ml/min.

coated on the bottom of a 12x75mm tube by evaporation of solvent (24). To this was added at room temperature 50 μl of water containing AP (0.5 mg/ml) and either Na^{131}I (3 μl, 1 μCi/μl) or Na^{125}I (3 μl, 20 μCi/μl). Samples were removed after 2-15 min for analysis.

The preparative procedure was performed as follows: Antipyrine in water (10 μl, 5mg/ml, 0.27 μmol) and chloramine-T in water (5 μl, 70 mg/ml, 1.24 μmol) were added to 90 μl of the Na^{131}I solution. After 5 min the reaction was stopped by the addition of aqueous $Na_2S_2O_3$(25 μl, 30mg/ml, 3.0 μmol). The reaction mixture was injected onto the HPLC column using solvent system B and the 4-^{131}IAP collected in a 50 ml conical vessel. The solvent was reduced to ∿2 ml under vacuum.

RESULTS AND DISCUSSION

The reaction of either $^{131}I^-$ or $^{125}I^-$ with antipyrine (AP) in the presence of chloramine-T (C-T) or iodogen was investigated. The results shown in Table II indicate that the reaction using $^{131}I^-$ and C-T was rapid in both ethanol and water. When the reaction time was limited to 2 min, a higher yield of 4-^{131}IAP was obtained in water, as compared to ethanol, at lower AP concentrations and higher carrier-added I^- concentrations (Table II). The addition of large

amounts of carrier iodide significantly reduced the yield of 4-^{131}IAP (~50%). This confirms a previous investigation of Linhart and co-workers (16) who prepared 4-^{131}IAP using antipyrine, C-T and carrier KI (220mg). Experiments performed at C-T concentrations of 10^{-3} M and 10^{-4} M using no-carrier-added $^{131}I^-$ and an AP concentration of 5 mg/ml indicated little variation in yields (97-99%). The reaction of no-carrier-added $^{125}I^-$ with aqueous AP using C-T gave essentially the same results as those experiments using $^{131}I^-$.

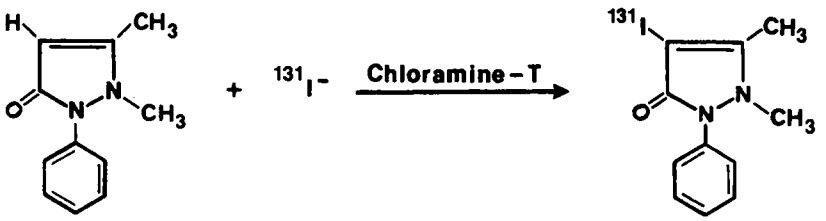

Table II. Yield of 4-^{131}IAP Under Various Conditions[a].

AP, mg/ml	Solvent	Carrier I^-, mg/ml	Percent 4-^{131}IAP
0.05	H_2O	NCA[b]	97-99
0.5	H_2O	NCA	95-98
5.0	H_2O	NCA	98-99
5.0	H_2O	0.1	98-99
5.0	H_2O	1.0	43-54
0.05	Ethanol	NCA	57-59
0.5	Ethanol	NCA	98
5.0	Ethanol	NCA	97-99
5.0	Ethanol	0.01	98-99
5.0	Ethanol	0.1	84
5.0	Ethanol	1.0	0.5-2.0

a) Reactions were allowed to proceed for 2 min. Chloramine-T concentration was 10^{-2}M.

b) NCA = no-carrier-added.

The use of iodogen for oxidation of the radioiodine was investigated. The maximum yield of 4-^{125}IAP or 4-^{131}IAP that could be obtained was 50% in 2 min;

no further increase in yield was observed with extended reaction time. Surprising, the iodogen appeared to decompose AP, resulting in a product which would interfere with the HPLC purification of 4-IAP.

Preparative experiments were developed so that purification could be performed using an analytical reversed-phase C-18 column. Keeping the injection volume to less than 130 μl resulted in adequate separation of the 4-^{131}IAP from AP and $^{131}I^-$. The results of two preparative experiments are shown in Table III. As much as 90% of the injected activity was recovered as 4-^{131}IAP.

Table III. Results from HPLC Purification of 4-^{131}IAP[a].

Starting Activity, mCi	Purified Product, mCi[b]	Radiochemical Purity, %	Specific Activity[c] mCi/μmol
1.84	1.70	100.0	256±11(∿ 1100)
4.60	4.45	98.0	697±25(∿ 1000)

a) Experiments performed using two different lots of Na^{131}I solution.

b) Activity obtained off the HPLC.

c) Specific activity is at end of synthesis. The figures in parentheses represent the specific activity calculated back to the time of calibration of the Na^{131}I solution.

The mass of 4-IAP in the product was determined by HPLC using solvent system A (Table I) by comparison to suitable 4-IAP standards of known mass. Although the original Na^{131}I solutions were claimed to be carrier-free, the final product was not carrier-free (Table III). An independent HPLC method (25) indicated that the carrier I^- was introduced from the original Na^{131}I solutions.

An examination was also made of the decomposition of purified 4-^{131}IAP. The results indicated that the decomposition rate was greater at higher specific activities, higher concentrations of activity per volume, and higher storage temperature. This confirms previous results (15,20).

Radioiodinated 4-IAP has been prepared by iodine for iodine exchange with 4-IAP, either in solution (17,18) or in a high temperature melt (21); by iodine for bromine exchange in 4-bromoantipyrine in solution at low pH (18); and by

iodine for hydrogen exchange with AP on a silica gel surface (19-22). The latter two methods produce a no-carrier-added product. Purification has been accomplished by ion exchange (17,18), preparative thin layer chromatography (19) or dry column chromatography (20,21).

The advantages of the method reported here are several. An adjustment of pH is not necessary to achieve maximum yield as is required in most of the above methods (17-22). The reaction is rapid at room temperature and consistently results in high yield. The reaction can be performed with no-carrier-added or small amounts (0.1 mg/ml) of carrier-added iodide. The presence or absence of reductant in the radioiodine solution appears to have no effect. HPLC purification can be performed on an analytical column and completely separates the 4-IAP from radioiodide, AP, and C-T. The entire procedure, including synthesis and purification, can be performed in less than 60 min and should be applicable to the incorporation of all isotopes of radioiodine.

ACKNOWLEDGEMENT

The authors express their appreciation to Ms. Zohra Khatib and Ms. Jan Holle for aiding in manuscript preparation.

REFERENCES

1. Talso P.J., Lahr T.N., Spafford N., Ferenzi G. and Jackson H.R.O. - J. Lab. Clin. Med. 46: 619 (1955).
2. Sapirstein L. and Hanucek G. - Am. J. Physiol. 193: 272 (1958).
3. Oldendorf W.H. and Kitano N. - Neurology 15: 994 (1965).
4. Uszler J.M., Bennett L.R., Mena I. and Oldendorf W.H. - Radiology 115: 197 (1975).
5. Sakurada O., Kennedy C., Jehle J., Brown J.D., Carbin G.L. and Sokoloff L. - Am. J. Physiol. 234: H59 (1978).
6. Jones S.C., Lear J.L., Greenberg J.H. and Reivich M. - Acta Neurol. Scand. 60 (Suppl. 72): 202 (1979).
7. Ginsberg M.D., Busto R., Boothe T.E. and Campbell J.A. - Brain Research 230: 165 (1981).

8. Mies G., Niebuhr I. and Hossmann K.-A. - Stroke 12: 581 (1981).
9. Lear J.L., Jones S.C., Greenberg J.H., Fedora T.J. and Reivich M. - Stroke 12: 589 (1981).
10. Mies G., Niebuhr I. and Hossmann K.-A. - Eur. Neurol. 20: 188 (1981).
11. Furlow T.W., Martin R.M. and Harrison L.E. - J. Cereb. Blood Flow Metab. 3: 62 (1983).
12. Lucignani G., Nehlig A., Blasberg R., Patlak C.S., Anderson L., Fieschi C., Fazio F. and Sokoloff L. - J. Cereb. Blood Flow Metab. 5: 86 (1985).
13. Ginsberg M.D., Lockwood A.H., Busto R., Finn R.D., Campbell J.A., and Boothe T.E. - Stroke 12: 745 (1981).
14. Suner A.A., Desales G.N.B. and Mitta A.E.A. - Radiochim. Acta 14: 157 (1970).
15. Dimattio J. and Hochwald G.M. - Stroke 3, 446 (1972).
16. Linhart J., Sedmera P. and Benes J. - Radiochem. Radioanal. Lett. 18: 29 (1974).
17. Robinson, Jr. G.D. and Lee A.W. - J. Nucl. Med. 17: 1093 (1976).
18. Robinson, Jr. G.D. and Lee A.W. - Int. J. Appl. Radiat. Isot. 30: 365 (1979).
19. Boothe T.E., Campbell J.A., Djermouni B., Finn R.D., Gilson A.J. and Ache H.J. - Int. J. Appl. Radiat. Isot. 32: 153 (1981).
20. Diksic M. and Kodery B. - J. Label. Compd. Radiopharm. 20: 339 (1983).
21. Shiue C.-Y. and Wolf A.P. - J. Label. Compd. Radiopharm. 20: 1363 (1983).
22. Boothe T.E., Finn R.D., Vora M.M., Emran A.E. and Kothari P.J. - Int. J. Appl. Radiat. Isot. 35: 1138 (1984).
23. Campbell J.A., Finn R.D., Boothe T.E., Djermouni B., Ginsberg M.D., Lockwood A.H., Gilson A.J. and Ache H.J. - J. Nucl. Med. 22: 538 (1981).
24. Fraker P.J. and Speck Jr., J.C. - Biochem. Biophys. Res. Commun. 80: 849 (1978).
25. Boothe T.E. - unpublished results.