Iodination of Docosahexaenoic Acid by Lactoperoxidase and Thyroid Gland in vitro: Formation of an Iodolactone

J.M. BOEYNAEMS[1], **J.T. WATSON**[2], **J.A. OATES** and **W.C. HUBBARD**, Department of Pharmacology, Vanderbilt University, Nashville, TN 37232

ABSTRACT

In the presence of iodide, hydrogen peroxide and lactoperoxidase, docosahexaenoic acid (22:6ω3) was converted into iodinated compounds. The major product was identified as 5-iodo-4-hydroxy-7, 10,13,16,19-docosapentaenoic acid, γ-lactone, on the basis of ^{125}I incorporation, mass spectrometry, chemical modifications and proton nuclear magnetic resonance spectroscopy. Iodolactonization of docosahexaenoic acid occurred in the rat thyroid in vitro and was inhibited by the peroxidase inhibitor methimazole. These data indicate that formation of an idolactone constitutes one pathway of docosahexaenoic acid metabolism which could be expressed in tissues containing an iodide peroxidase.

INTRODUCTION

The lack of information concerning the metabolism of docosahexaenoic acid (22:6ω3) contrasts with the growing knowledge of the multiple metabolic transformations of arachidonic acid (20:4ω6). Docosahexaenoic acid is an important constituent of mammalian phospholipids; it is more abundant than arachidonic acid in the retina of several animal species (1,2), in the human cerebral cortex (3) and in ram spermatozoa (4). In the phospholipids of bovine thyroid, the amounts of arachidonic acid and docosahexaenoic acid are comparable (5). We have recently observed that the rat thyroid transforms arachidonic acid into an iodo-δ-lactone (6). In this report, we show that the major product of lactoperoxidase-catalyzed iodination of docosahexaenoic acid is an iodo-γ-lactone and that the same transformation occurs in the rat thyroid in vitro.

MATERIALS AND METHODS

Materials

Docosahexaenoic acid (purity >99%) was obtained from Nu-Chek-Prep, Elysian, MN. ^{125}I (15.8 mCi/μg) was purchased from Amersham, Arlington Heights, IL. Lactoperoxidase (EC 1.11.1.7) from milk was obtained from Sigma Chemical Co., St. Louis, MO.

Incubation of Docosahexaenoic Acid with Lactoperoxidase

Lactoperoxidase (2.9 μg/ml or 0.17 purpurogallin units/ml), docosahexaenoic acid (0.21 mM), (^{125}I) KI (0.4 mM, 0.5 μCi/ml) and H_2O_2 (0.26 mM) were stirred in phosphate buffer (0.1 M, pH 7.4) for 30 min at room temperature. After addition of sodium thiosulfate, the reaction mixture was extracted with 2 vol of ethyl acetate.

Incubation of Thyroid Lobes

White male Sprague-Dawley rats weighing around 250 g were sacrificed by ether inhalation and the thyroid lobes were incubated for 40-min periods, under air, at 37 C and with constant shaking (80 rpm). Nine lobes (70 mg wet wt) were incubated in 20 ml of Krebs-Ringer phosphate medium containing glucose (8 mM), ^{125}I (0.1 μCi/ml), KI (5 or 25 μM) and docosahexaenoic acid (0, 4, 21 or 107 μM). The incubation medium was then extracted with 2 vol of ethyl acetate.

Liquid Chromatography

Silicic acid chromatography was performed in 1.5-cm-diameter columns packed with a slurry in chloroform of Porasil A (35 to 75-μ particles; Waters Associates, Milford, MA), (column height: 18 cm). Elution was done with chloroform (85 ml) and then methanol (30 ml). Reversed phase-high pressure liquid chromatography (RP-HPLC) was done on a μBondapak C_{18} column (3.9 × 300 mm, 10-μ particles, Waters Associates). The injector (Model U6K) and the pump (Model 6000 A) were from Waters Associates. The samples were injected after dissolving in 100 μl methanol. Elution was done with methanol/water (80:20, v/v) at a flow rate of either 1 or 2 ml/min.

Chemical Modifications and Derivatizations

Alkali treatment: 100 μg of material was dissolved in 3 ml THF to which 2 ml of 0.5 M NaOH in water was added; after 3 hr at room temperature, the reaction mixture was diluted

[1]Present address: Institute of Interdisciplinary Research, School of Medicine, Free University of Brussels, 2 rue Evers, 1000 Brussels, Belgium.

[2]Present address: Dept. of Biochemistry, Michigan State University, E. Lansing, MI 48824.

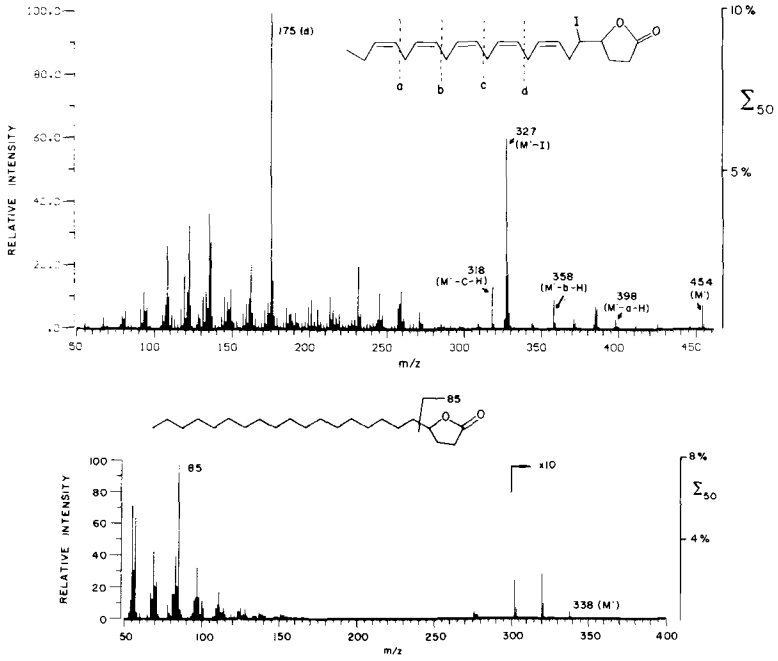

FIG. 1. Top: mass spectrum (12 eV) of the major product of lactoperoxidase-catalyzed iodination of docosahexaenoic acid (peak A of Fig. 2). Bottom: mass spectrum (70 eV) of hydrogenated compound A. The scale on the right (Σ_{50}) represents the intensity of % of the total ion current above m/z 50.

with water and extracted with 2 vol of ethyl acetate. Catalytic hydrogenation: 10 μg of material (ester or lactone) was dissolved in 0.5 ml ethanol to which 1 mg platinum oxide was added; hydrogen gas was bubbled for 2 min, after which the reaction mixture was diluted with water and extracted with diethyl ether. Methyl esters were prepared by reaction with excess ethereal diazomethane; trimethylsilyl ethers were obtained by reaction with excess bis-trimethylsilyl-trifluoro-acetamide (BSTFA) in pyridine.

Mass Spectrometry

Mass spectra were obtained either on a LKB 9000 magnetic instrument operated at 12 eV, using sample introduction by direct inlet probe (Fig. 1, top) or on a Hewlett-Packard combined gas chromatograph-quadrupole mass spectrometer (Model 5982A) operated at 70 eV (Fig. 1, bottom).

RESULTS

Incubation of docosahexaenoic acid with lactoperoxidase in the presence of hydrogen peroxide and iodide resulted in the formation of several iodinated products, which were resolved by silicic acid column chromatography (Fig. 2). One compound (A) largely predominated and was obtained in a 20–40% yield (3 experiments). RP-HPLC of compound A on a μBondapak C_{18} column (solvent: methanol/water, 80:20, v/v) revealed a single peak of ^{125}I

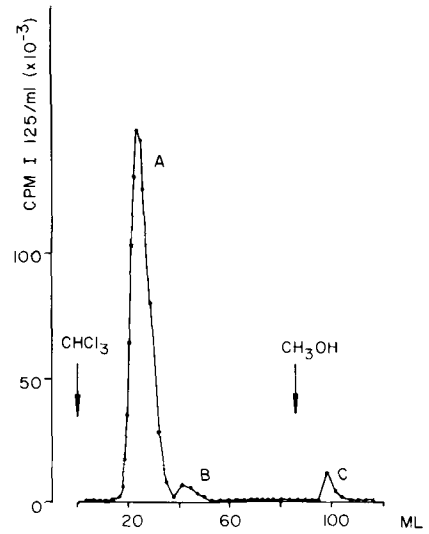

FIG. 2. Silicic acid column chromatography of the products of docosahexaenoic acid iodination catalyzed by lactoperoxidase.

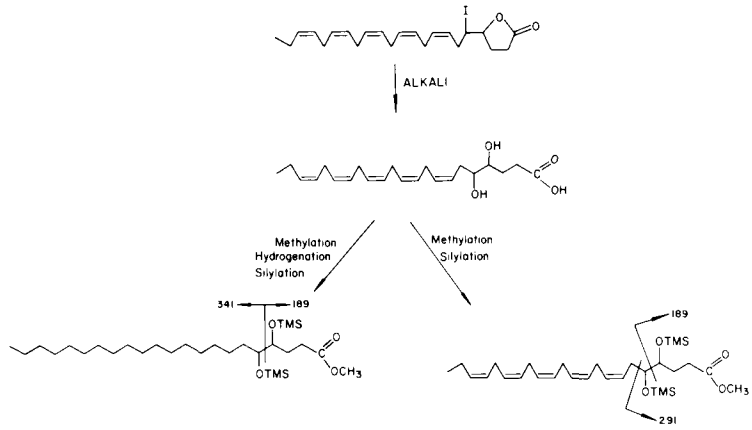

FIG. 3. Degradation of compound A (Fig. 2) at alkaline pH; gas chromatographic-mass spectrometric analysis of the main product. The major fragmentation pattern under electron impact is described.

radioactivity; the retention vol was 26 ml as compared to 23 ml for the iodolactone derived from arachidonic acid (6). Gas chromatographic analysis of compound A (underivatized) showed a single peak with an equivalent chain length C-25.7 (vs fatty acid methyl esters on OV-1). The electron ionization mass spectrum showed a molecular ion at m/z 454, the expected molecular weight of 5-iodo-4-hydroxy-docosapentaenoic acid, γ-lactone, and a prominent peak at m/z 327 (M-127), produced by the loss of iodine which is a typical fragmentation of alkyl iodides (7) (Fig. 1, top). The base peak at m/z 175 corresponds to the fragment represented by d in Fig. 1 (top). Many of the smaller peaks apparently result from the loss of various vinyl moieties together with a distal hydrogen: 390 (M-a-H), 358(M-b-H), 318(M-c-H). After catalytic hydrogenation, a peak with an equivalent chain length of C-23.6 (OV-1) was observed during gas chromatography. The mass spectrum showed a molecular ion at m/z 338 and a base peak at m/z 85, typical of γ-lactones (8) (Fig. 1, bottom). The decrease of the retention time caused by the hydrogenation and the shift from a major fragment ion at m/z 327 to a molecular ion at m/z 338 is consistent with the saturation of 5 double bonds and the substitution of hydrogen for iodine.

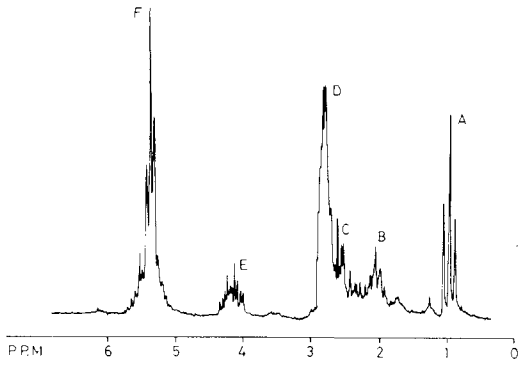

FIG. 4. Proton magnetic resonance spectrum of compound A. The spectrum was recorded on a JEOL FX-90Q Fourier transform spectrometer operated at 90 MHz. The sample was dissolved in deuterochloroform and tetramethylsilane was used as internal reference. The correspondence between the peaks of the spectrum and the protons of the molecule is as follows: A (0.97 ppm), 3H, C_{22}; B (2.07 ppm), 4H, C_3 and C_{21}; C (2.6 ppm), 2H, C_2; D (2.8 ppm), 1OH, C_6, C_9, C_{12}, C_{15} and C_{18}; E (4.2 ppm), 2H, C_4 and C_5; F (5.4 ppm), 10H, C_7, C_8, C_{10}, C_{11}, C_{13}, C_{14}, C_{16}, C_{17}, C_{19} and C_{20}.

Treatment with alkali degraded compound A. The mass spectrometric analysis indicated that the major product is a 4,5 diol (Fig. 3), presumably resulting from iodine displacement by hydroxyl anion and hydrolysis of the lactone ring. Since compound A was identified as a γ-lactone, iodine must be bound to C_5.

The proton magnetic resonance spectrum of compound A was consistent with the proposed structure of 5-iodo-4-hydroxy-docosapentaenoic acid, γ-lactone (Fig. 4). It also indicated that the configuration of remaining double bonds was not changed as compared to the precursor docosahexaenoic acid. The covalent structure of the major product of lactoperoxidase-catalyzed iodination of docosahexaenoic acid is thus 5-iodo-4-hydroxy-7,10,13,16, 19-docosapentaenoic acid, γ-lactone. The absolute configuration of the chiral centers (C_4 and C_5) has not been determined.

The release of this iodolactone by rat thyroid lobes incubated in vitro was measured

by the incorporation of ^{125}I followed by RP-HPLC purification. The recovery of this procedure was high (85%) and the retention volumes highly reproducible: 25.8 ± 1.2 ml (mean ± SD, n = 16). An excellent correlation between the results obtained by this method and those provided by a gas chromatographic-mass spectrometric (GC-MS) assay has been demonstrated in the case of the release of 6-iodo-5-hydroxyeicosatrienoic acid, δ-lactone by the rat thyroid (6). Figure 5 shows a typical RP-HPLC chromatogram of iodinated products released by the rat thyroid in the absence and the presence of exogenous docosahexaenoic acid. The nature of the iodinated components eluted in peaks I and III was not investigated. GC-MS analysis of component II revealed the existence of a peak having a retention time and a mass spectrum identical to those of 5-iodo-4-hydroxy-docosapentaenoic acid, γ-lactone, obtained by the lactoperoxidase reaction. The amounts of iodolactone released depended on the concentration of both iodide and docosahexaenoic acid (2 experiments, Fig. 6). Methimazole (200 μM) completely inhibited the release of the iodolactone.

DISCUSSION

The existence of iodinated lipids in cells has been reported, but their structures have not been fully characterized. Olefin diiodides were detected in thyroid lipids of dogs on a high iodine intake by use of proton nuclear magnetic resonance spectroscopy (9,10). In other studies, it has been shown that lactoperoxidase-catalyzed iodination of intact cells labels not only cell surface proteins, but also several classes of membrane lipids (11–16). The mechanism of these iodinations is presumed to involve either addition of iodine to double bonds or substitution of iodine to hydrogen in unsaturated and saturated fatty acids, respectively. In this report, we have shown that docosahexaenoic acid can be iodinated by intact cells by a third mechanism: iodolactonization.

The formation of 5-iodo-4-hydroxy-7,10,13,16,19-docosapentaenoic acid, γ-lactone, in the rat thyroid is likely to involve the activity of the thyroid peroxidase, since it is blocked by methimazole and mimicked by lactoperoxidase. There is, however, no evidence for a direct interaction between docosahexaenoic acid and the peroxidases. The role of these enzymes might be restricted to the oxidation of I^- into a reactive species, possibly I^+ (17,18), which

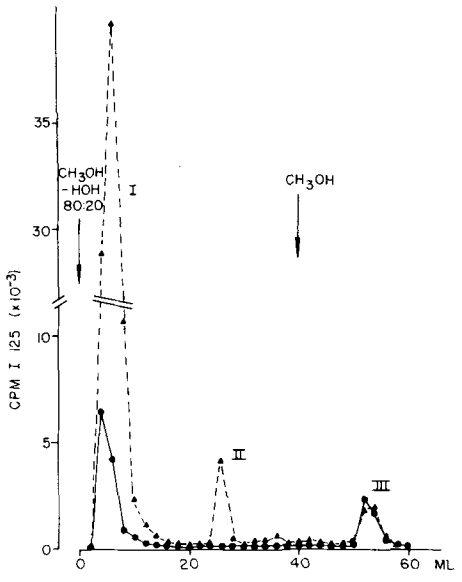

FIG. 5. RP-HPLC chromatogram of iodinated products released by the rat thyroid in vitro. —•—: control; ---▲---: docosahexaenoic acid (107 μM). Incubation and chromatography were performed as described in Methods.

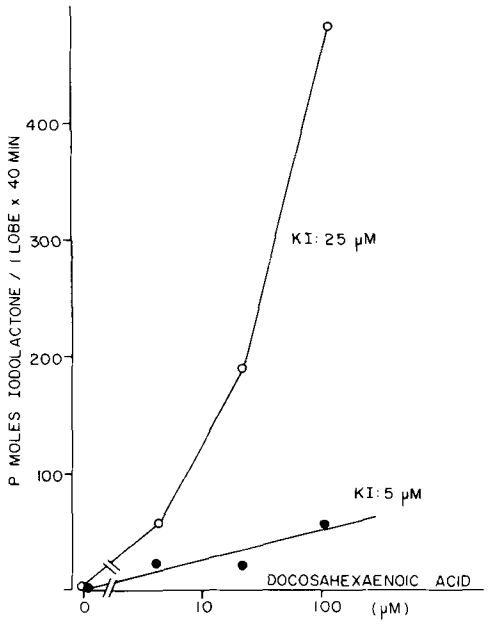

FIG. 6. Dependence of iodolactone formation by the rat thyroid on the concentrations of docosahexaenoic acid and iodide. The incubation of the thyroid lobes and the RP-HPLC of the incubation medium extract were performed as described in Methods. To determine the amounts of iodolactone released, the total molar amount of iodide added was multiplied by the ratio between the ^{125}I radioactivity in peak II (Fig. 5) and the total radioactivity added.

would then react with docosahexaenoic acid by a purely chemical process, analogous to the well known reaction of alkaline iodolactonization of $\beta, \gamma, \gamma, \delta$ and δ, ϵ unsaturated carboxylic acids (19–21). This transformation might be of general biological significance, since docosahexaenoic acid is abundant in phospholipids, e.g., in the thyroid (5), and since, in addition to thyroid and mammary glands, salivary glands (22), polymorphonuclear leukocytes (23) and oocytes (24) contain an iodide peroxidase.

The possible biological activity of 5-iodo-4-hydroxy-7, 10,13,16, 19-docosapentaenoic acid, γ-lactone, is presently unknown. It might be the product of scavenging by docosahexaenoic acid of excess iodine formed by the thyroid peroxidase or play a role as mediator of the inhibitory actions of excess iodide on the thyroid (25, 26).

ACKNOWLEDGMENTS

We are grateful to Dr. J.G. Hardman for his continuous support and valuable discussions and to D. Reagan who performed the NMR analysis. This work was supported by NIH grants GM-15431 and BRSG-RR-05424 and by Public Health Service International Research Fellowship TW 02685. J.M. Boeynaems is Fellow of the Fogarty International Center and Aspirant of the Fonds National de la Recherde Scientifique (Belgium). J.A. Oates is the J. and M. Werthan Professor of Investigative Medicine.

REFERENCES

1. Anderson, R.E., and M.B. Maude, Arch. Biochem. Biophys. 151:270 (1971).
2. Anderson, R.E., Exp. Eye Res. 10:339 (1970).
3. Sun, G.Y., J. Lipid Res. 14:656 (1973).
4. Jones, R., and T. Mann, Proc. R. Soc. Lond. 193:317 (1976).
5. Lagrou, A., W. Dierick, A. Christophe and G. Verdonk, Lipids 9:870 (1974).
6. Boeynaems, J.M., and W.C. Hubbard, J. Biol. Chem. 255:9001 (1980).
7. Budzikiewicz, H., C. Djerassi and D.H. Williams, in "Mass Spectrometry of Organic Compounds," Halden-Day, San Francisco, 1976, pp. 429–441.
8. Budzikiewicz, H., C. Djerassi and D.H. Williams, in "Mass Spectrometry of Organic Compounds," Halden-Day, San Francisco, 1976, pp. 205–208.
9. Zanger, M., and J.L. Rabinowitz, J. Org. Chem. 40:248 (1975).
10. Rabinowitz, J.L., M.D. Zanger, and V. Podolski, Biochem. Biophys. Res. Commun. 68:1161 (1976).
11. Morrison, M., G.S. Bayse and R.G. Webster, Immunochemistry 8:289 (1971).
12. Butters, T.D., and R.C. Hughes, Biochem. J. 150:59 (1975).
13. Podulso, J.R., and P.E. Braun, J. Biol. Chem. 250:1099 (1975).
14. Mersel, M., A. Benenson and F. Doljanski, Biochem. Biophys. Res. Commun. 70:1166 (1976).
15. Schlager, S.I., J. Immunol. 123:2108 (1979).
16. Benenson, A., M. Mersel, A. Pinson and M. Heller, Anal. Biochem. 101:507 (1980).
17. Taurog, A., in " Handbook of Physiology," edited by S.R. Geiger, Am. Phys. Soc., 1974, pp. 101–132.
18. Morrison, M., and G.R. Schonbaum, Ann. Rev. Biochem. 45:861 (1976).
19. Arnold, R.T., and K.L. Lindsay, J. Am. Chem. Soc. 75:1048 (1953).
20. Van Tamelen, E.G., and M. Shamma, J. Am. Chem. Soc. 76:2315 (1954).
21. Corey, E.J., H. Nirva and J.R. Falck, J. Am. Chem. Soc. 101:1586 (1979).
22. Alexander, N.M., J. Biol. Chem. 234:1530 (1959).
23. Klebanoff, S.J., J. Exp. Med. 196:1063 (1967).
24. Foerder, C.A., and B.M. Shapiro, Proc. Natl. Acad. Sci. USA 74:214 (1977).
25. Nagataki, S., in "Handbook of Physiology," Vol. III. edited by S.R. Geiger, Am. Phys. Soc., 1974, pp. 329–344.
26. Van Sande, J., G. Grenier, C. Willems and J.E. Dumont, Endocrinology 96:781 (1975).

[Received August 8, 1980]