SYNTHESIS OF [^{11}C] PLATELET-ACTIVATING FACTOR (PAF) ANALOGS FOR IN VIVO IMAGING OF PAF RECEPTORS

Sasaki, T.[a], Karasawa, K.[b], Ishiwata, K.[a], Satoh, N.[b], Ishii, S.[a], Ogawa K.[c], Nozaki, T.[c], Setaka, M.[b], Nojima, S.[b] and Senda, M.[a]

[a]Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-cho, Itabashi, Tokyo , [b]Faculty of Pharmaceutical Sciences, Teikyo University, Kanagawa 199-01, and [c]Faculty of Hygienic Sciences, Kitasato University, Kanagawa 228 (Japan).

SUMMARY

1-O-Hexadecyl-2-O-N,N-dimethylcarbamoyl-sn-glycero-3-phosphocholine[choline methyl-^{11}C] ([^{11}C]dimethylcarbamoyl-PAF) and 1-O-Hexadecyl-2-O-acetyl-sn-glycero-3-phosphocholine[choline methyl-^{11}C] ([^{11}C]C_{16}-PAF) were synthesized as follows; Each of non-labeled dimethylcarbamoyl-PAF and C_{16}-PAF was treated with sodium benzene thiolate to derive their desmethyl-precursors containing a dimethylphosphoethanolamine at sn-3. ^{11}C-Labeled dimethylcarbamoyl-PAF and C_{16}-PAF were synthesized by methylation of the respective desmethyl-precursors using [^{11}C]CH_3I. The radiochemical yield of methylation in [^{11}C]dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF was about 15 and 10 % (decay corrected), respectively. The lower yield of [^{11}C]C_{16}-PAF compared with that of [^{11}C]dimethylcarbamoyl-PAF was attributed to hydrolysis of the 2-acetyl group of [^{11}C]C_{16}-PAF during methylation. To study the stability to enzymatic hydrolysis, [^{11}C]dimethylcarbamoyl-PAF or [^{11}C]C_{16}-PAF was incubated with mouse plasma at 37 ℃.

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[^{11}C]Dimethylcarbamoyl-PAF remained intact for 60 min. On the other hand, almost all the radioactivity of [^{11}C]C_{16}-PAF was converted into [^{11}C]C_{16}-lyso-PAF in 5 min.

These observations indicate that [^{11}C]dimethylcarbamoyl-PAF can be a suitable probe for in vivo imaging of PAF receptors.

Key words: ^{11}C-PAF, ^{11}C-PAF Analogs, Synthesis, PAF-receptors, PET

INTRODUCTION

Platelet-activating factor (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine, PAF) is a class of phospholipids, and it has a potent biological activity (1). PAF has been suggested to be a mediator of various inflammatory and allergic reactions (1). Recently, interesting roles of PAF in stroke and brain injury and presence of PAF receptors in brain have been reported (2). It is also reported that the level of PAF precursor (lyso-PAF) is high in the hippocumpus and that the high sensitivity of hippocumpus to ischemia could be due to the binding of PAF to its receptors in this brain region (3). For the purpose of in vivo imaging for PAF receptors in brain and other tissue, synthesis of ^{11}C-PAF was planned. However, it is probably impossible to image the PAF receptors in vivo by using ^{11}C-native PAF, because native PAF is easily hydrolyzed by PAF acetylhydrolase in plasma and tissues (4, 5). Karasawa and co-workers raised antibody to PAF by injection of conjugate between synthetic PAF analogs with substituents at the sn-2 position and bovine serum albumin into rabbits. They have developed a radioimmunoassay for measuring the very low concentrations of PAF in plasma and other tissues (6-8). This synthetic hapten ,1-O-hexadecyl-2-O-N,N-dimethylcarbamoyl-sn-glycero-3-phosphocholine (dimethylcarbamoyl-PAF), has a resistance to PAF acetylhydrolase and also has a similar level of biological activity as the native PAF (8). We tried ^{11}C-labeling of dimethylcarbamoyl-PAF which was expected to be resistant to metabolism in vivo. Its desmethyl-precursor containing a dimethylphosphoethanolamine at sn-3 was labeled with [^{11}C]CH_3I.

This paper gives the conditions suitable for labeling and for purification of the [^{11}C]dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF, together with a comparison of their stability to enzymatic hydrolysis in vitro.

EXPERIMENTAL

Synthesis of desmethyl-precursors

Synthesis of desmethyl-precursors of [^{11}C]dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF were carried out by the modified method of Stoffel W. (9, 10)(Fig 1). That is, each 50 mg of non-labeled dimethylcarbamoyl-PAF synthesized by the method of Karasawa et al.(11), and C_{16}-PAF purchased from Bachem Co. Ltd., was dissolved in 10 mL of freshly distilled dioxane, and 10 mg of sodium benzene thiolate was added to the solution. The reaction mixture was heated under a stream of nitrogen at 95 ℃ for 60 min with stirring. The reaction was quenched by adding 10 mL of an ice-cold 2N-HCl solution into a cooled reaction mixture. The reaction product was extracted with 50 mL of chloroform and dried over Na_2SO_4 before the solvent was evaporated to dryness under vacuum. The residue was dissolved in a small portion of eluting solution and applied to preparative HPLC system equipped with a pump LC-9A, a UV detector SPD-6AV (Shimadzu) and a silica column (Megapak SIL, 10 mm I.D. x 250 mm, Nihon Bunko Co. Ltd). The desmethyl-precursors were eluted with 5 mL/min of mobile phase (isopropanol:hexane:water=110:100:20; V/V) and monitored for absorbance at 205 nm. Purified desmethyl-precursors corresponding to dimethylcarbamoyl-PAF and C_{16}-PAF were confirmed by TLC (silica gel 60A; Whatman Co. Ltd., solvent system; chloroform:methanol:water= 100:50:20; V/V) and ^{1}H-nuclear magnetic resonance spectrum (JEOL, GSX-400V.; ^{1}H-NMR; 400 MHZ, solvent: $CDCl_3$). Chemical purities of desmethyl-precursors were determined using an analytical HPLC (Finepak SIL, 4.6 mm x 250 mm; Nihon Bunko Co. Ltd.) eluted with the same eluting solution as preparative HPLC at a flow rate of 1.5 mL/min.and monitored for absorbance at 205 nm

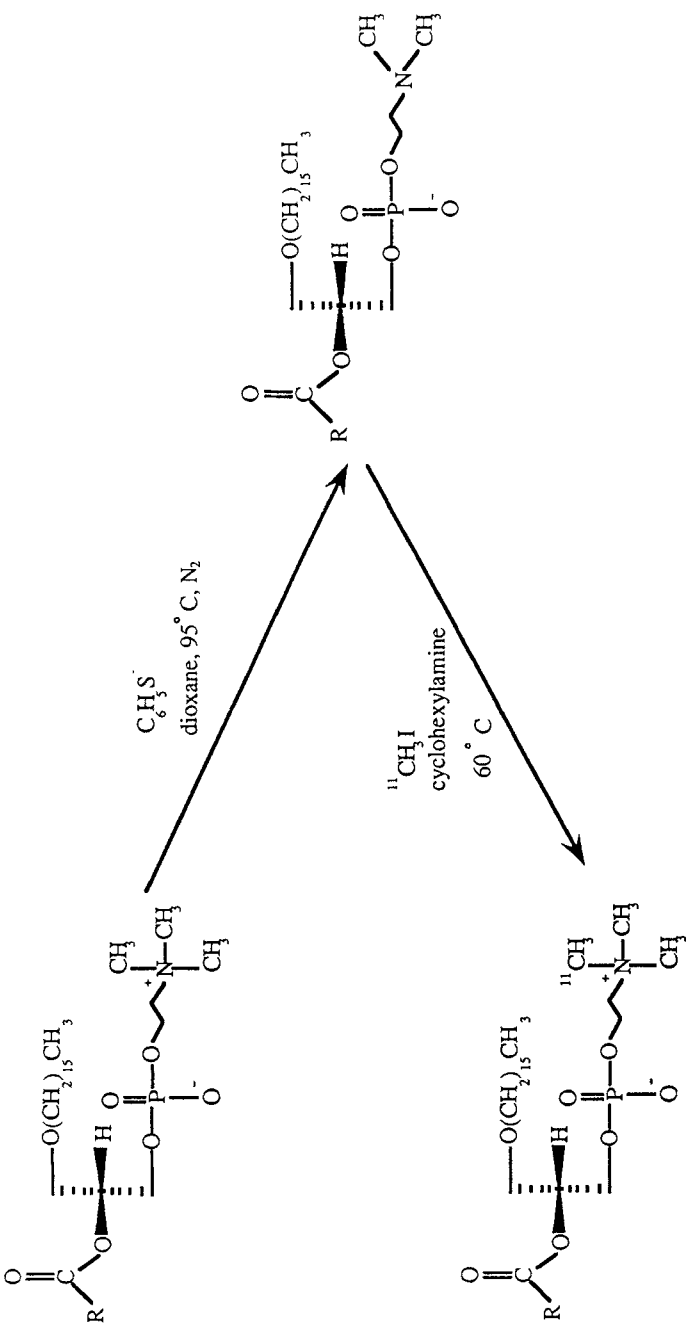

Fig. 1. Reaction scheme for the desmethylation and ^{11}C-labeling of dimethylcarbamoyl- PAF ; R=$(CH_3)_2N$ and C_{16}-PAF ; R=CH_3.

Synthesis of [^{11}C]dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF

[^{11}C]Dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF were synthesized as shown in Fig. 1. [^{11}C]CO_2 was produced according to the procedures previously described (12). The concentrated [^{11}C]CO_2 in liquid argon cooled copper tube was introduced into the first reaction vessel containing a solution of $LiAlH_4$ (10 μmol; Aldrich Co. Ltd.) in 100 μL of tetrahydrofuran (THF, Aldrich Co. Ltd.). The first vessel was heated and THF was removed under N_2. Then, 0.5 mL of HI (Wako Pure Chemical Industries Co. Ltd.) was added to the same vessel which was heated at 120℃ , and $^{11}CH_3I$ was transferred into a second vessel with N_2 at 150 mL/min. The second vessel contained desmethyl-precursor (1 mg) and cyclohexylamine (1-10 μL) in 100 μL of absolute methanol. The second vessel was then heated at 60 ℃ for 10 min in an aluminum heat block.

Reaction products were analyzed using an analytical HPLC system equipped with a pump LC-9A, a UV detector SPD-6AV (Shimadzu), a radiodetector (Radiomatic A-200; Canberra Co. Ltd.) and a silica column (Finepak SIL, 4.6 mm I.D. x 250 mm; Nihon Bunko Co. Ltd.) eluted with 1.5 mL/min of eluting solution (isopropanol:hexane:water= 110:100:20; V/V). [^{11}C]Dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF were purified by a preparative HPLC system equipped with a pump LC-9A, a UV detector SPD-6AV (Shimadzu) and a silica column (Megapak SIL,10 mm I.D. x 250 mm, Nihon Bunko Co. Ltd). The column was eluted with 10 mL/min of eluting solution (isopropanol:hexane:water= 110:100:20; V/V) and monitored for both radioactivity and absorbance at 205 nm. Peaks of [^{11}C]dimethylcarbamoyl-PAF, [^{11}C]C_{16}-PAF and [^{11}C]C_{16}-lyso-PAF in analytical and preparative HPLC were confirmed by comparison with the corresponding non-labeled authentic samples. To determine the mass of [^{11}C]dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF, absorbance of UV detector (205 nm) in the preparative HPLC was pre-calibrated with corresponding mass of cold dimethylcarbamoyl-PAF and C_{16}-PAF. Each of [^{11}C]dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF

specific activity was calculated from determined radioactivity by a dose calibrator (Capintec) and mass.

[^{11}C]Dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF thus obtained were evaporated to dryness and solubilized with 0.25% human serum albumin (HSA) containing 0.9 % NaCl.

Stability of [^{11}C]dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF in mouse plasma

Each of [^{11}C]dimethylcarbamoyl-PAF (3.7 MBq, 0.23 μg) and [^{11}C]C_{16}-PAF (3.7 MBq, 0.40 μg) in 0.25 % HSA containing 0.9 % NaCl, was incubated with 0.5 mL of mouse plasma at 37 ℃ for 5, 30 or 60 min. The incubation was stopped by adding 1.25 mL of 2 % acetic acid containing methanol and 0.625 mL chloroform. Then, 0.625 mL of chloroform and 0.625 mL of water were added. The mixture was centrifuged at 600xg for 5 min, and the radioactivity in organic, aqueous and precipitate fractions was counted. During all experiments, the extraction efficiency in an organic layer of added radioactivity was 91 % ([^{11}C]dimethylcarbamoyl-PAF) and 81 % ([^{11}C]C_{16}-PAF), respectively. The radioactivity in an organic layer was analyzed by the analytical HPLC as described above. Radioactivity applied to this HPLC was quantitatively recovered (>98 %).

RESULTS AND DISCUSSION

Desmethyl-precursors of dimethylcarbamoyl-PAF and C_{16}-PAF were synthesized using sodium benzene thiolate, and yields of demethylation were 46 and 53 %, respectively. The following NMR spectra ($CDCl_3$) of desmethyl-dimethylcarbamoyl-PAF (a) and desmethyl-C_{16}-PAF (b) indicated the structures of the sn-3-dimethylethanolamine derivatives. (a): δ ppm 5.05 (quintet, 1H, -CH-), 4.20 (broad multiplet, 2H, -P-O-CH_2-), 4.05 (multiplet, 2H, -CH_2-O-P-), 3.60 (quintet, 2H, -CH_2-N^+), 3.45 (multiplet, 2H, -CH_2-O-), 2.92 (singlet, 6H, $(CH_3)_2$-N-), 2.83 (singlet, 6H, $^+N(CH_3)_2$), 1.26 (singlet, 28H, -$(CH_2)_{14}$-) and 0.86 (triplet, 3H, -CH_3), (b): δ ppm 5.17 (quintet, 1H, -CH-), 4.20

(broad multiplet, 2H, -P-O-CH_2-), 4.04 (multiplet, 2H, -CH_2-O-P-), 3.59 (quintet, 2H, -CH_2-N^+), 3.45 (multiplet, 2H, -CH_2-O-), 2.84 (singlet, 6H, $(CH_3)_2$-N-), 2.08 (singlet, 3H, -CO-CH_3), 1.25 (singlet, 28H, -$(CH_2)_{14}$-) and 0.88 (triplet, 3H, -CH_3). Their Rf values determined by TLC were as follows: desmethyl-dimethylcarbamoyl-PAF (0.19), desmethyl-C_{16}-PAF (0.21),dimethylcarbamoyl-PAF (0.10) and C_{16}-PAF (0.12). Chemical purities of both desmethyl-precursors determined by an analytical HPLC were >99 %. These desmethyl-derivatives existed as HCl-salts. Therefore, it is necessary to liberate the amines from the HCl-salts for the methylation with [^{11}C]CH_3I. We screened HCl-salts liberating agents in situ. The yield of methylation was increased in presence of the HCl-salts liberating agents, in order of cyclohexylamine>2,2,6,6-tetramethylpiperidine>BaO (data not shown). We chose cyclohexylamine as an HCl-salts liberating agent. Effects of cyclohexylamine content on the yield of methylation were studied in Fig. 2. The radiochemical yields of [^{11}C]dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF both were increased with amounts of added cyclohexylamine and reached the maximum at 10 μL of cyclohexylamine. However, the yield decreased with further amounts of cyclohexylamine. Reaction products of methylation were subjected to analytical HPLC (Fig. 3). The addition of cyclohexylamine to the reaction mixture produced by-products eluting at retention time of 2.2 min and trapped in the HPLC column. The radioactivity trapped is considered to be a complex of cyclohexylamine and [^{11}C]CH_3I. In the case of [^{11}C]C_{16}-PAF, one more by-product, [^{11}C]C_{16}-lyso-PAF, was observed (retention time 14.5 min) and its radiochemical yield also reached the maximum with 10 μL of added cyclohexylamine. The radiochemical yields of dimethylcarbamoyl-PAF and C_{16}-PAF in the presence of 10 μL of cyclohexylamine were 15.2 and 10.5 % (decay corrected), respectively. It is probable that the reason for the lower yield of [^{11}C]C_{16}-PAF compared with [^{11}C]dimethylcarbamoyl-PAF was attributable to the formation of [^{11}C]C_{16}-lyso-PAF by hydrolysis of the sn-2-acetyl group in [^{11}C]C_{16}-PAF or its desmethyl-precursor during the methylation at 60 ℃. under an alkaline condition with

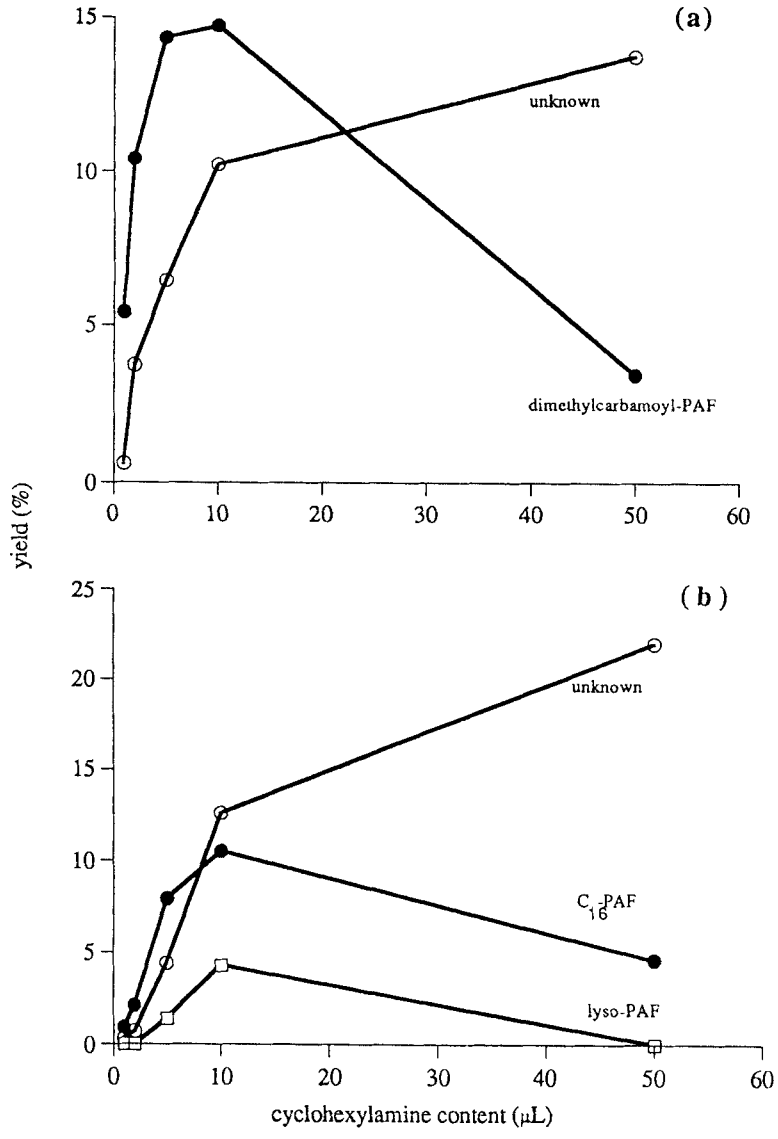

Fig. 2. Radiochemical yield of [^{11}C]dimethylcarbamoyl-PAF (a) and [^{11}C]C_{16}-PAF (b) v.s. amounts of cyclohexylamine. [^{11}C]Labeled dimethylcarbamolyl-PAF and [^{11}C]C_{16}-PAF was synthesized by methylation of the respective 1 mg of desmethyl-precursors with [^{11}C]CH_3I and 0~50 μL of cyclohexylamine in 100 μL of methanol for 10 min at 60 ℃.

cyclohexylamine. The reaction products were purified by preparative HPLC system and radioactivities of [^{11}C]dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF were eluted at 11.3 and 8.4 min, respectively. The retention times of desmethyl-dimethylcarbamoyl-PAF and desmethyl-C_{16}-PAF in this preparative HPLC were 5.4 and 3.3 min, respectively.

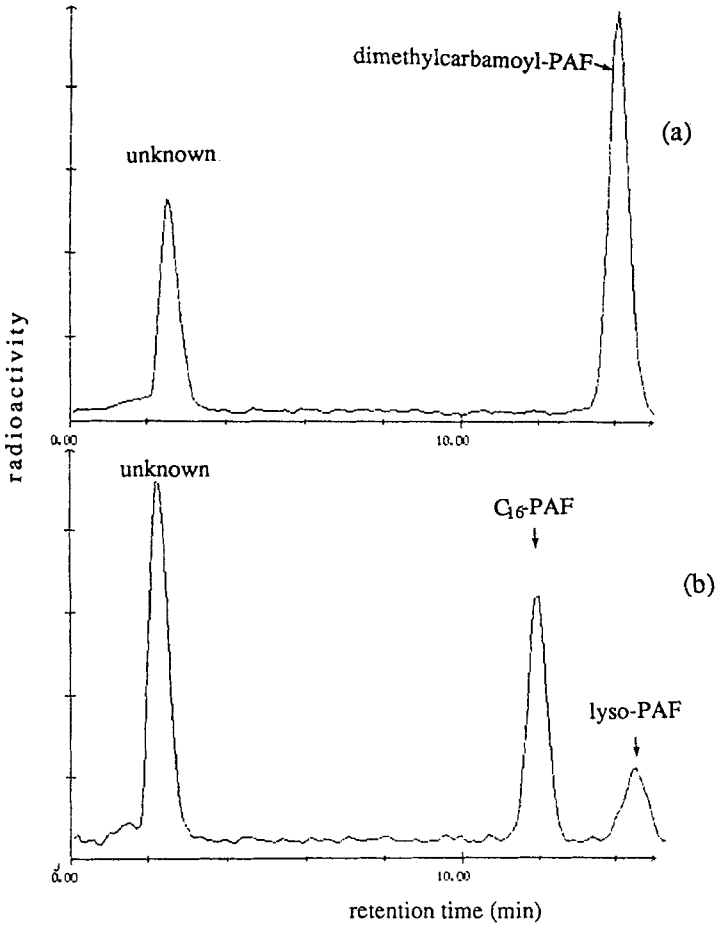

Fig. 3. Analytical radio-HPLC of [^{11}C]dimethylcarbamoyl-PAF (a) and [^{11}C]C_{16}-PAF (b) reaction mixtures.

Finally, [^{11}C]dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF with specific activity of 40-60 GBq/μmol were obtained at 35 min from the EOB (30 μA beam of 14.1 MeV protons for 30 min). Radiochemical purities of both [^{11}C]dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF were >98 %.

Comparison of metabolic stability between [^{11}C]dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF in the plasma is shown in Fig. 4. The [^{11}C]dimethylcarbamoyl-PAF remained intact for 60 min. In contrast, almost all of the [^{11}C]C_{16}-PAF radioactivity was converted rapidly to [^{11}C]C_{16}-lyso-PAF.

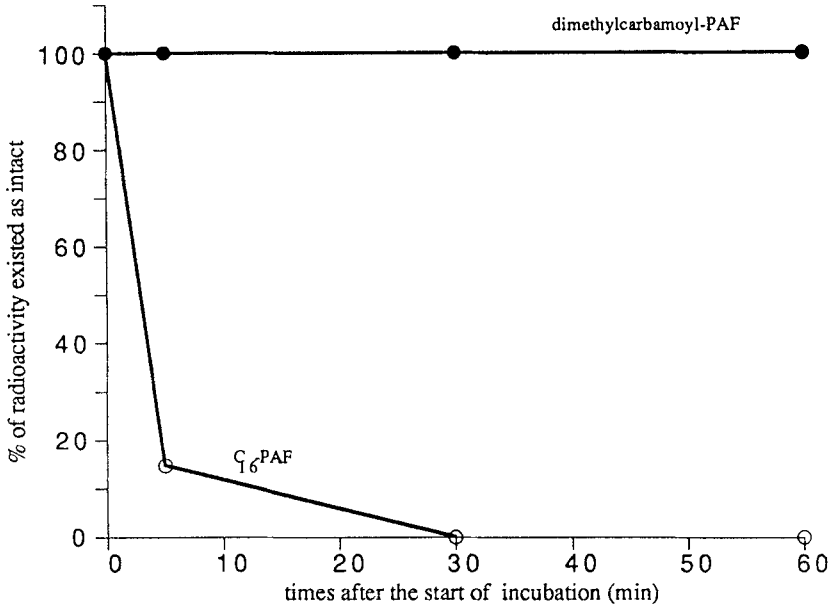

Fig. 4. Stability of [^{11}C]dimethylcarbamoyl-PAF and [^{11}C]C_{16}-PAF in mouse plasma. Plasma (500 μL) was incubated with [^{11}C]dimethylcarbamoyl-PAF (3.7 MBq, 0.23 μg) or [^{11}C]C_{16}-PAF (3.7 MBq, 0.40 μg) at 37 ℃ for 5~60 min. The incubation mixture was extracted with a solvent of methanol:chloroform:water:acetic acid=2.5:2.5:1.25:0.12; V/V. The chemical forms of the radioactivity in the organic layer was checked by radio-HPLC.

These observations indicated that the [^{11}C]dimethylcarbamoyl-PAF is expected to be more stable to enzymatic hydrolysis than [^{11}C]C_{16}-PAF *in vivo* and it can be a suitable probe for *in vivo* imaging of PAF receptors.

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