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Research Article

Synthesis of (4-[^{18}F]fluorophenyl)triphenylphosphonium as a potential imaging agent for mitochondrial dysfunction

Zhen Cheng, Murugesan Subbarayan, Xiaoyuan Chen and Sanjiv Sam Gambhir*

Department of Radiology and Bio-X Program, Molecular Imaging Program at Stanford (MIPS), Stanford University, Stanford, CA, 94305, USA

Summary

Tetraphenylphosphonium (TPP) cation is able to function as a molecular probe for monitoring mitochondrial disease. The F-18 labeled TPP, (4-[^{18}F]fluorophenyl) triphenylphosphonium (^{18}FTPP), was therefore developed as a PET radioligand for *in vivo* molecular imaging of mitochondrial dysfunction. ^{18}FTPP was synthesized via direct nucleophilic substitution of no-carrier-added [^{18}F]fluoride with the precursor 4-nitrophenyltriphenylphosphonium. After purification by HPLC, the average radiochemical yield was determined to be 10–15% and the specific activity was >500 Ci/mmol at the end of synthesis. The total synthesis time was within 60 min, and the radiochemical purity of the ^{18}FTPP was above 95%.

Key Words: phosphonium; F-18; mitochondria; imaging

Introduction

It has been recognized that a wide range of diseases, including cancers, diabetes, heart failure, AIDS, degenerative diseases, and the pathophysiology of aging are associated with mitochondria dysfunction.[1–3] It has also become widely accepted in the past few years that mitochondria play a key role during apoptosis.[4] Cumulative evidence reveals that dramatic changes of mitochondrial membrane potential ($\Delta\psi_m$) are associated with these mitochondrial diseases.[1–4] For instance, the difference in $\Delta\psi_m$ between normal epithelial cells

*Correspondence to: Sanjiv Sam Gambhir, Molecular Imaging Program at Stanford, Department of Radiology and Bio-X Program, Stanford University, 318 Campus Dr., Clark Center E-150, Stanford, CA 94305, USA. E-mail: sgambhir@stanford.edu

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and many carcinoma cells is at least 60 mV,[5] and the loss of $\Delta\psi_m$ is an early event in cell apoptosis caused by pro-apoptotic agents.[6] Therefore, to develop drugs based on the changes in $\Delta\psi_m$ may serve as a novel effective approach for disease treatment.[7–10] Furthermore, the development of radiolabeled mitochondriotropic molecular probes may provide a powerful tool to investigate the role of mitochondria in the pathophysiology and treatment of cancer using a non-invasive imaging technology such as positron emission tomography (PET).

It has been known for decades that lipophilic cations such as the rhodamine-123 (Rh123) and tetraphenylphosphonium (TPP) salts, can penetrate the plasma and mitochondrial membranes and selectively accumulate in mitochondria, because of the negative inner mitochondrial transmembrane potential (−120 to −170 mV, negative inside).[7–11] Many phosphonium and other lipophilic cations have been evaluated as anticancer drugs, as well as carriers to deliver passenger molecules selectively to mitochondria of cancer cells, for manipulation of these cells.[12–18] We and several other laboratories have shown that ^{3}H-labeled phosphonium analogs can function as a molecular probe for selective accumulation in certain cancer cells.[19–21]. Several radiolabeled phosphonium cations, [^{11}C]methyltriphenylphosphonium,[22] 3-[^{18}F]fluoropropyl and 4-[^{18}F]fluorobenzyltriarylphosphonium,[23–24] have been synthesized and evaluated as mitochondrial targeting agents.

Recently, the mitochondria targeting ability of a library of phosphonium analogs were studied in our laboratory using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). It was found that (4-[^{19}F]fluorophenyl)triphenylphosphonium exhibited 80% of the TPP uptake in glioma C6 cells *in vitro*, which is much higher accumulation than that of methyltriphenylphosphonium and butyltriphenylphosphonium (unpublished data). Having achieved good tumor accumulation and pharmacokinetic properties of [^{3}H]-TPP,[21] we pursued further to label tetraphenylphosphonium with Fluorine-18 which may be considered useful as a novel molecular 'voltage sensor'. We report in the present study the radiosynthesis of (4-[^{18}F]fluorophenyl)triphenylphosphonium.

Results and discussion

The radiosynthesis of (4-[^{18}F]fluorophenyl)triphenylphosphonium (^{18}FTPP) via a one-step procedure is shown in Figure 1. The precursor for radiosynthesis, 4-nitrophenyltriphenylphosphonium (1, NO_2TPP) was first prepared by following the procedure reported by Rieke *et al.*[25] The diazonium chloride intermediate was formed by slowly adding hydrochloric acid to 4-nitroaniline at 0°C. The formation of NO_2TPP was obtained by the addition of triphenylphosphine. Extraction with aqueous solution and precipitation with sodium iodide (NaI) afforded pure precursor NO_2TPP which was

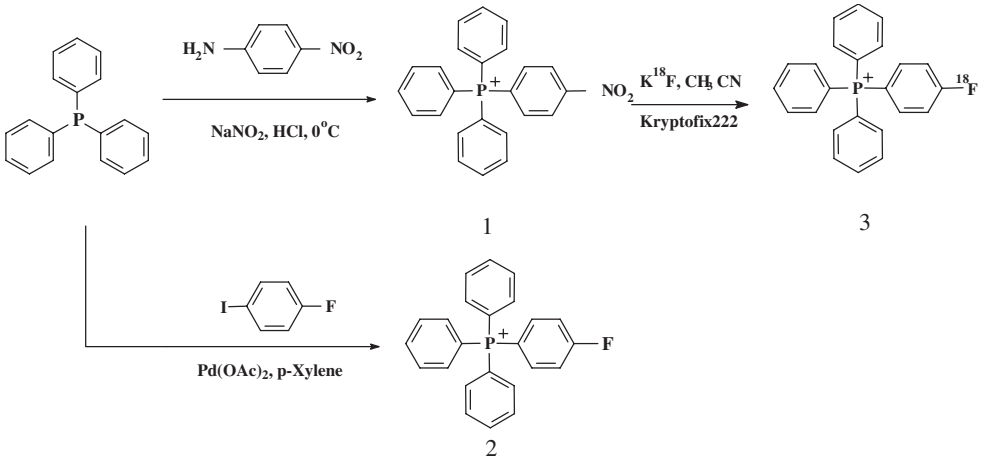

Figure 1. Synthetic scheme for (4-[$^{18/19}$F]fluorophenyl)triphenylphosphonium cation

confirmed by analytical HPLC, ESI-MS and NMR. ^{18}FTPP was synthesized by nucleophilic substitution reaction of NO_2TPP and no-carrier-added [^{18}F]fluoride in the presence of potassium carbonate (K_2CO_3) and phase transfer agent Kryptofix 222. After heating for 15 min at 120°C in the mineral oil bath, the reaction solution was cooled and injected onto the semi-preparative HPLC column to separate the labeled product. Under the HPLC gradient described in the 'Experimental' section, the retention time of ^{18}FTPP and un-reacted NO_2TPP were 26.0 and 24.5 min, respectively, which made the separation of these two species feasible. Analytical HPLC analysis revealed that the radiolabeled product exhibited identical retention time with a fully characterized cold (4-[^{19}F]fluorophenyl)triphenylphosphonium (FTPP) standard. The radiochemical purity of ^{18}FTPP determined by analytical HPLC was above 95%, and radiochemical yield for ^{18}FTPP was 10–15% at end of synthesis (EOS) with specific activity 576–715 Ci/mmol.

Experimental

No-carrier-added [^{18}F]Fluoride was obtained from PETNET Pharmaceuticals, Inc. (Palo Alto, CA). All other reagents were purchased from Sigma-Aldrich Chemical Co. ESI-MS were performed by Vincent Coates Foundation Mass Spectrometry Laboratory, Stanford University. NMR spectra were recorded on a Varian 600 MHz instrument. Melting points were determined on an Electrothermal's TA 9100 digital melting point instrument (Barnstead/Thermolyne, Dubuque, IA). HPLC was performed on a Dionex Summit® HPLC system (Dionex Corporation, Sunnyvale, CA) equipped with a 170U 4-Channel UV-Vis absorbance detector and radioactive detector (Carroll & Ramsey Associates, model 105S, Berkeley, CA). UV detection

wavelengths are 225 and 280 nm for all the experiments. Both semi-preparative (Zorbax SB-C18, 9.4 mm × 250 mm) and analytical (Dionex Acclaim®120 C18, 4.6 mm × 250 mm) RP-HPLC columns were used. The mobile phase was solvent A, 100 mM ammonium formate (HCO_2NH_4), and solvent B, acetonitrile. CRC-15R PET dose calibrator (Capintec Inc., Ramsey, NJ) was used for all radioactivity measurements.

Synthesis of 4-nitrophenyltriphenylphosphonium iodide

NO_2TPP was prepared as a precursor for radiosynthesis of ^{18}FTPP by the method reported previously[25]. Briefly, 2.8 g (0.02 mol) 4-nitroaniline was mixed with 10 ml concentrated hydrochloric acid at 0°C. Then $NaNO_2$ (0.02 mol, 1.38 g) dissolved in cold 10 ml H_2O was added to the 4-nitroaniline dropwise with stirring, followed by adding 5.6 g sodium acetate (NaOAc) dissolved in water (20 ml). Triphenylphosphine (5.6 g) dissolved in 80 ml ethyl acetate was then added dropwise to the reaction vial at 0°C within 60 min. After incubation at room temperature for 5 min, the reaction solution was acidified by adding concentrated HCl (2 ml). The aqueous layer was then washed twice using ether, and the ethyl acetate layer was extracted twice with water. The aqueous solutions were then combined and NaI (2.2 g in 2 ml H_2O) was added to triturate the final product. The purity of 4-nitrophenyltriphenylphosphonium iodide was over 95% as confirmed by analytic HPLC (analytic column, flow rate: 1 ml/min, elution protocol starts with 95% A for 3 min, followed by a linear gradient to 15% A over 30 min, retention time: 23.4 min). Yield: 6.34 g (62%), pale brown solid; m.p. >200°C (lit[25] 228°C); from the HMBC (multiple-bond CH correlation, 600 MHz, $CDCl_3$) experiment, we observe two aromatic hydrogens (8.57–8.58 ppm) coupled with C-2 and C-6 (127 ppm) carbons as doublet and two other aromatic hydrogens (8.09–8.10 ppm) coupled with C-5, C-7 (137 ppm) as doublet of doublet with integral values 1:1 accordingly (the carbon connected to NO_2-group is numbered as 1, and the other carbons in the same benzene ring are numbered clockwise as 2–6, the same numbering system for carbon is applied for FTPP). This indicates that NO_2-group is in para position relative to phosphorus atom. The rest of protons are combined in 7.6–8.1 ppm interval with integral value of 15 protons, thus it support the total integral ratio of 19 protons for compound NO_2TPP. ESI-MS (high-resolution): m/z calculated: 384.1153 ($C_{24}H_{19}NO_2P$), found: 384.1153.

Synthesis of (4-fluorophenyl)triphenylphosphonium formate

^{19}FTPP was synthesized according to the method reported for synthesis of (4-bromophenyl)triphenylphosphonium (Figure 1).[26] Triphenylphosphine (1.76 g, 6.72 mmol), 4-fluoro-iodobenzene (1.49 g, 6.72 mmol) and $Pd(OAC)_2$

(75 mg, 0.33 mmol, 5 mol%) in *p*-xylene (40 ml) were mixed together and stirred at 130–140°C for 2 h. The color changed from yellow to brown and a white precipitate formed. The solvent was removed *in vacuo*. 100 mg residue was dissolved in CH_3OH and purified by HPLC using semi-preparative column (flow rate: 3 ml/min; elution protocol starts with 95% A for 3 min, followed by a linear gradient to 15% A over 30 min, retention time: 23.8 min). The purity of the product was analyzed by analytical HPLC (analytic column, flow rate: 1 ml/min, same gradient as that for NO_2TPP analysis, retention time: 24.1 min, >95% purity). White solid, m.p. >200°C; three experiments, ^1H NMR, HMBC and HSQC (heteronuclear single quantum correlation) (600 MHz, $CDCl_3$) were also performed for confirmation of the structure of FTPP. From these experiments, we observe two protons (7.42–7.54, multiplet) coupled with C-2 and C-6 (118 ppm) carbons and two other aromatic hydrogens (7.68–7.75 ppm, multiplet) coupled with C-3 and C-5 (138 ppm), which proves that fluorine-group is in para position relative to phosphorus atom. The rest of protons are in the range of 7.58–7.67 ppm (multiplet, six protons), 7.76–7.84 (multiplet, six protons), and 7.85–7.96 ppm (triplet, three protons), supporting the total integral ratio of 19 protons for compound 2. ESI-MS (high-resolution): m/z calculated: 357.1208 ($C_{24}H_{19}FP$), found: 357.1217.

Radiosynthesis and HPLC purification of (4-[^{18}F]fluorophenyl)triphenylphosphonium cation

[^{18}F]fluoride was added to a pyrex glass reaction vessel containing 200 µl 25 mM potassium carbonate and Kryptofix 2.2.2. (3.0–4.0 mg) dissolved in 300 µl CH_3CN. The solution was evaporated at 120°C by bubbling nitrogen gas and the residue was dried by azeotropic distillation with acetonitrile (3×0.5 ml). To this anhydrous residue was added a solution of NO_2TPP (1–1.5 mg) in dry DMSO (0.32 ml). The reaction mixture was heated for 15 min at 120°C in an oil bath. The solution was cooled and injected onto a semi-preparative HPLC column (the flow rate was 3 ml/min, with the mobile phase starting from 28% solvent B (CH_3CN) and 72% solvent A (0.1 M HCO_2NH_4 in water) (0–3 min) to 48% solvent B and 52% solvent A at 33 min, then going to 85% solvent B and 15% solvent A (33–36 min), maintaining this solvent composition for another 3 min (36–39 min) and returning to initial solvent composition by 42 min). Pure [^{18}F]FTPP, eluted off the column with a retention time of 26 min, was collected in a small round bottle. The product was dried in a rotary evaporator and was made isotonic with sodium chloride and passed through a 0.22 mm membrane filter into a sterile multidose vial. The product was found to be >95% radiochemically and chemically pure as determined by analytical HPLC (same gradient as used for semi-preparative HPLC; flow rate: 1.0 ml/min). The retention time for [^{18}F]FTPP under

analytical HPLC conditions was 25.5 min. Radiochemical yield ranged between 10 and 15% (EOS; corrected for decay). Thus starting typically with 100 mCi of [^{18}F]-fluoride ion, about 7 mCi of product, ready for injection, was routinely obtained in 60 min.

Conclusion

In conclusion, radiosynthesis of 4-[^{18}F]fluorophenyl)triphenylphosphonium in high radiochemical purity was achieved through a one-step reaction. The yield and specific radioactivity are sufficient for further biological evaluation of ^{18}FTPP as a molecular probe for imaging mitochondrial dysfunction.

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