Proceedings of the II Symposium on Positron Emission Tomography, Kraków, September 21–24, 2014

Synthesis of Endogenous Compounds Labeled with ^{11}C for Positron Emission Tomography

K. Kilian* and A. Pękal
Heavy Ion Laboratory, University of Warsaw, L. Pasteura 5a, 02-093 Warsaw, Poland

Among the β^+ emitting isotopes, produced in medical cyclotrons, carbon-11 is promising radionuclide for labeling of biologically active compounds and tracking their distribution in living organism. Therefore aminoacids, carboxylic acids, fatty acids and amines have been widely used in assessing and staging of brain tumors (^{11}C-methionine), prostate cancer (^{11}C-choline, ^{11}C-acetate) and neurological disorders (^{11}C-labeled neurotransmitters). This paper reviews recent achievements in synthesis of endogenous substances labeled with ^{11}C and presents our experience in ^{11}C-methionine synthesis. Proposed protocol is a reliable tool for routine manufacturing in clinical applications and animal studies.

DOI: 10.12693/APhysPolA.127.1475
PACS: 87.57.un

1. Introduction

Positron emission tomography (PET) proved its effectiveness as a powerful molecular imaging tool, providing unique opportunity to real-time monitoring of biological processes *in vivo*. It is accompanied with increasing demand for selective and specific radiotracers. Among the β^+ emitting isotopes, produced in medical cyclotrons, carbon-11 is the most convenient radionuclide for labeling of biologically active compounds and tracking their distribution in living organism. Although ^{18}F with $t_{1/2} = 109.8$ min is routinely applied as tracer in commercially available radiopharmaceuticals, carbon-11 ($t_{1/2} = 20.4$ min) labeled molecules are attractive because labeling does not alter the molecular structure and biochemical properties. Thus the ^{11}C-labeled radiotracers are indistinguishable from their stable forms in metabolic processes so labeled compound is incorporated in metabolic processes and follows the same path as its natural equivalent. Moreover, using the position-specific labeling approach, imaging of metabolites and metabolic paths of selected functional groups expands the biological information on molecular level.

Several endogenous PET radiopharmaceuticals were developed and successfully applied in clinics to visualize molecular pathways and pathologic changes. The primary cause of the intensive research for compounds alternative to ^{18}F-flurodeoxyglucose (^{18}FDG) was limitation in imaging of low grade tumors and areas with high background glucose metabolism [1].

Therefore other groups: aminoacids, carboxylic and fatty acids, amines [2] have been widely used in assessing and staging of brain tumors (^{11}C-methionine) [3], prostate cancer (^{11}C-choline, ^{11}C-acetate) [4] and neurological disorders (^{11}C-labeled neurotransmitters, agonists, antagonists and modulators) [5].

*corresponding author; e-mail: kilian@slcj.uw.edu.pl

The aim of this paper is to review recent achievements in synthesis of endogenous substances labeled with ^{11}C and to present our experience in ^{11}C-methionine synthesis.

2. ^{11}C manufacturing

^{11}C with 20.3 min half-life is routinely produced in medical cyclotrons via ^{14}N(p,α)^{11}C reaction. The target gas is $^{14}N_2$, but depending on target gas composition two chemical forms of final product are available: $^{11}CO_2$ from irradiation of nitrogen mixture with 1–2% of O_2 or $^{11}CH_4$ from nitrogen with 1–5% hydrogen addition. Final activities depend on target pressure and cyclotron performance, but reach 1500–3000 mCi (for $^{11}CH_4$ and $^{11}CO_2$ respectively, in average) after 30 min irradiation [6]. $^{11}CO_2$ and $^{11}CH_4$ are transformed to the more reactive agents and the most common are methyl iodide (CH_3I) and methyl triflate (CH_3OTf), used in labeling by methylation. $^{11}CH_3I$ is prepared by iodination of CH_4 at elevated temperature. Methyl iodide can be prepared in gas phase ("dry method"), where $^{11}CO_2$ is collected in cryogenic trap or molecular sieve, than converted to $^{11}CH_4$ by reaction with H_2 on nickel catalyst and finally $^{11}CH_4$ is iodinated by molecular I_2 to $^{11}CH_3I$.

$$^{11}CO_{2(g)} \xrightarrow{-165^{\circ}C} {}^{11}CO_{2(s)} \xrightarrow[425^{\circ}C]{H_2,\,Ni} {}^{11}CH_4 \xrightarrow[750^{\circ}C]{I_2} {}^{11}CH_3I$$

Alternative method for methyl iodide synthesis is reduction of $^{11}CO_2$ to $^{11}CH_4$ by $LiAlH_4$, followed by iodination with HI ("wet method").

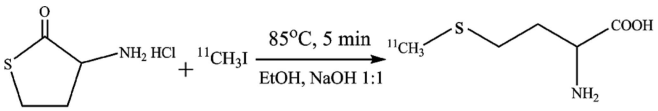
Comparing these two methods, wet method profits from shorter synthesis time, resulting in higher yield and final activity. However, corrosive HI and noticeable content of natural CO_2 in $LiAlH_4$ result in decrease of specific activity, introduction of carrier ^{12}C atoms and reducing of system resistance to chemical corrosion.

Dry method profits from elimination of corrosive and carrier adding chemicals, leading to higher specific activity of produced methyl iodide, which is an advantage in neurological imaging with receptor-interacting radiopharmaceuticals. Practically, wet method is preferred in

high-throughput clinical diagnostics, when dry method is chosen for preclinical studies, due to its flexibility and short recovery time for subsequent synthesis. Among the ^{11}C labeling precursors, ^{11}C methyl iodide is the most frequently employed agent for methylation of carbanions and heteroatoms but other ^{11}C forms are used in specific applications. Direct reaction with $^{11}CO_2$ is commonly used for ^{11}C acetate and fatty acids synthesis [7], recently transition metal mediated ^{11}C carboxylation with ^{11}CO has been dynamically developing [8]. For some application, requiring high chemical reactivity or ambient reaction conditions, $^{11}CH_3I$ could be converted in one-reaction step to very reactive methyl triflate ($^{11}CH_3OTf$) by reaction with silver triflate at elevated temperature [6].

3. Endogenous compounds

Although ^{18}FDG showed its domination in imaging of glucose consuming (high-grade) tumors, detailed studies indicated some limitations. Imaging of particular organs (brain, heart, urinary tract) or specific histotypes (neuroendocrine, low-grade tumors) with ^{18}FDG suffers from reduced sensitivity or indistinct separation from the background. ^{11}C synthons have found significant role in labeling of endogenous substances, which overcame ^{18}FDG limitations. ^{11}C aminoacids have been found as effective markers of excessive transport and proliferation in tumor [2]. Among them, ^{11}C methionine got the leading position, due to reproducible and effective manufacturing method from L-homocysteine thiolactone.



Three different strategies were applied. First, standard "bubbling" method, where gaseous $^{11}CH_3I$ was passed through ethanolic solution containing precursor [9]. Second was conducted on solid support, where precursor is immobilized on suitable resin [10] and third, "loop" method, where the labeling is conducted in Teflon tube and directly injected to the purification system [11]. All methods provided ^{11}C methionine according to the pharmaceutical regulation and established imaging with ^{11}C methionine as a gold standard for visualization of glicomas in neurooncology [3, 12, 13] and supplementary in adenocarcinoma [14] or in radiation therapy planning [15]. ^{11}C acetate is an important endogenous substrate in the Krebs cycle and takes part in metabolism of fatty acids and cholesterols. Shows alternative to glycolysis, energy distribution from lipids metabolism, which is used for myocardium activity imaging and general evaluation of circulation system [16]. Even more promising application is imaging of prostate cancer [4], due to overexpression of fatty acids synthetases and increased uptake of acetates in cancer cells. Additionally low urine excretion favors ^{11}C acetates in delineation of tumor, comparing to ^{18}FDG, which is physiologically concentrated in bladder.

^{11}C acetate is synthesized in reaction of the Grignard reagents: methylmagnesium bromide (CH_3MgBr) or chloride (CH_3MgBr) with gaseous $^{11}CO_2$, transferred from cyclotron. No chemical conversion of target gas is needed and direct carboxylation is applied. As for ^{11}C methionine synthesis by bubbling $^{11}CO_2$ to precursor solution [17] or loop synthesis [18] were reported. For final purification, single use cartridges are usually used, which reduces time of synthesis even to 5 min which results in high average radiochemical yield, in range 65–80% [18, 19].

^{11}C acetate synthesis protocol could be easy adopted for fatty acids which are synthetized by direct carboxylation of respective, long-chained Grignard reagents [7]. The most commonly used ^{11}C-palmitate is applied for myocardial fatty acids metabolism and perfusion studies.

4. Quality control of ^{11}C radiopharmaceuticals

Quality control of ^{11}C methionine and ^{11}C acetate is performed according to respective monographs in European Pharmacopoeia [20]. Protocols include chemical and nuclidic identification, chemical and radiochemical purity and microbial tests (sterility and endotoxins) as for intravenous preparation.

An important issue to consider in ^{11}C quality control is short half-life. Thus some parameters such a sterility, bacterial endotoxins, radionuclidic purity and residual solvents content could be a subject of parametrical release.

Identification and purity tests are conducted by high performance liquid chromatography (HPLC) with dedicated set of detectors. Routinely radiometric detector is connected in-line with spectrophotometric (UV-VIS) or conductivity detector.

For ^{11}C methionine chemical purity is determined with UV and radiometric detectors, where ^{11}C methionine and cold by-products (L-homocysteine thiolactone, homocysteine) are quantitatively determined. Other chemical purity tests cover enantiomeric purity, determined by chiral separation and residual organic solvents, usually ethanol, acetone and acetonitrile. In this case content of ethanol could be elevated, because ^{11}C methionine is sensitive to radiolysis by its own radiation and ethanol in concentration up to several percent is added as radicals scavenger [21].

For ^{11}C acetate, the same setup as for ^{18}FDG could be used, containing radio HPLC with UV detector and 0.1 M sodium hydroxide as mobile phase. Due to well recognized reaction path, leading to ^{11}C acetate synthesis, chemical tests cover only acetate content and residual solvents determination.

5. ^{11}C-methionine synthesis

$^{11}CO_2$ was produced by the $^{14}N(p,\alpha)^{11}C$ reaction with 16.5 MeV proton beam at GE Pettrace 840 cyclotron (General Electric, Uppsala, Sweden). The target was gaseous N_2 (6.0), containing 5% O_2 (5.5) in high pressure

(170 psi) target body (GE, Uppsala, Sweden). ^{11}C was produced with total activity *ca.* 1000 mCi (37 GBq) after 10–15 min irradiation with 25–30 μA beam current. ^{11}C-methionine was synthesized via ^{11}C methylation from L-cysteine thiolactone (ABX, Radeberg, Germany) in solution using the bubbling method. Target gases were passed through cryogenic trap, where $^{11}CO_2$ was deposited at –165 °C. Then $^{11}CO_2$ was converted to methane (H_2/Ni, 400 °C) and ^{11}C-methyliodide was synthesized by passing the $^{11}CH_4$ over iodine in a triplicate loop at an elevated temperature (720 °C) and trapping on a Porapak column (dry method).

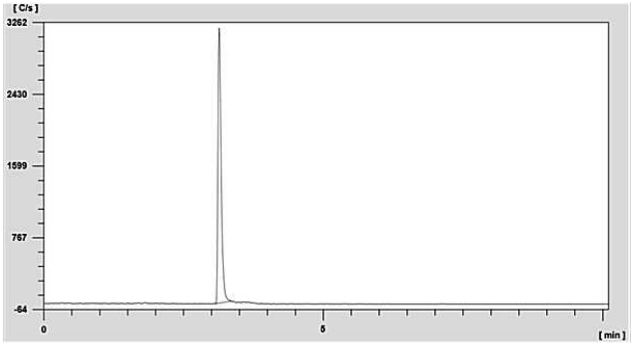


Fig. 1. Radiochromatogram of ^{11}C-methionine (t_r = 3.07 min).

Thermically desorbed $^{11}CH_3I$ was bubbled into the reactor with L-homocysteine thiolactone (2 mg) in a 300 μL solution of 2:1:1 (v/v) 1 M NaOH, ethanol and water at ambient temperature (85 °C, 5 min). The product was then purified by semipreparative HPLC (C18 column, 0.05 M NaH_2PO_4 + 2% EtOH as mobile phase) with a total wet-synthesis time of 20 min. Radiochemical yield was 21.3 ± 4.6% (not corrected), total synthesis time, including activity transfer, gas phase iodination of CH_4, labeling, purification and final formulation was 27–32 min. ^{11}C was identified by recording the principal γ-peak at 511.5 ± 0.3 keV and determination of half-life (20.5 ± 0.3 min). ^{11}C-methionine was confirmed, comparing the retention times of standards, observed in the reference chromatogram with retention time of the principal signal in the radiochromatogram. Radiochemical purity was determined by HPLC with radiometric detection, where the peak of ^{11}C-methionine was observed at 3.07 min, with no other signals recorded (Fig. 1). The average content of ^{11}C-methionine was higher than 99%. Enantiomeric purity was assessed by chiral HPLC. L- and D-isomers were identified by HPLC with UV and radiometric detectors (Fig. 2).

The percentage of L-^{11}C-methionine was 91.6 ± 0.4%. Produced ^{11}C-methionine formulations were tested and the mean value of the ethanol concentration was 33 ± 2 g/L. No traces of acetone or acetonitrile were detected. pH, sterility and endotoxin test, as well as other parameters were according to the quality criteria for ^{11}C-methionine in [20].

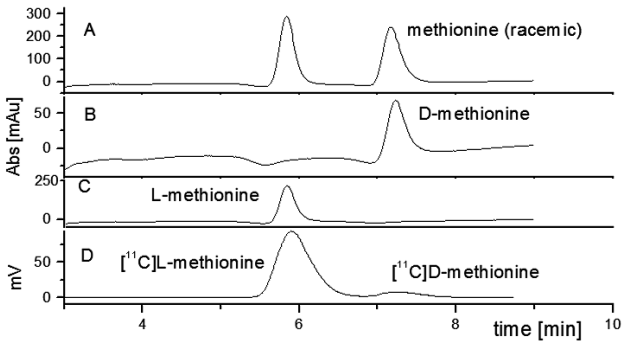


Fig. 2. Certified reference standards (CRS) and sample chromatograms for enantiomeric purity evaluation. (A) UV spectra of racemic methionine CRS, (B) UV spectra of D-methionine CRS, (C) UV spectra of L-methionine CRS, (D) radiochromatogram of 11C-methionine sample.

6. Conclusions

Endogenous substances labeled with ^{11}C are attractive PET biomolecules, due to involvement in real metabolic processes *in vivo*. Established methods of ^{11}C production, well recognized labeling reactions, leading to radiopharmaceuticals applied for clinical oncology and neurology imaging make ^{11}C chemistry complementary to fluorine-18 applications. Unaffected biochemical properties lead to better understanding the molecular background of diseases and could help in finding the most effective diagnostics and treatment. In this work the "bubbling" method of ^{11}C-methionine synthesis as an alternative way to SPE synthesis was presented. All the impurities were efficiently determined, then eliminated in the purification process and the final product was free from main radionuclidic and chemical impurities. Proposed ^{11}C-methionine synthesis is a reliable tool for routine manufacturing in clinical applications and animal studies.

Acknowledgments

Heavy Ion Laboratory gratefully acknowledges funding for the equipment from the Ministry of Health and from European Regional Development Fund under the Innovative Economy Program (POIG.02.02.00-14-024/08-00) project: Center of Preclinical Studies and Technology (CePT). The authors wish to thank AAA Polska for assistance in $^{11}CO_2$ production.

References

[1] J.W. Fletcher, B. Djulbegovic, H.P. Soares, B.A. Siegel, V.J. Lowe, G.H. Lyman, R.D. Samson, D. Delbeke, M. Gorman, A.F. Shields, E. Coleman, R. Wahl, J.Ch. Paschold, N. Avril, L.H. Einhorn, W.W. Suh, *J. Nucl. Med.* **49**, 480 (2008).

[2] B. Långrström, F. Karimi, Y. Wantanabe, *J. Label Comp. Radiopharm.* **56**, 251 (2013).

[3] C. Pötzi, A. Becherer, C. Marosi, G. Karanikas, M. Szabo, R. Dudczak, K. Kletter, S. Asenbaum, *J. Neurooncol.* **84**, 305 (2007).

[4] S. Albrecht, F. Buchegger, D. Soloviev, H. Zadi, H. Vees, H.G. Khan, A. Keller, A.B. Delaloye, O. Ratib, R. Mirabell, *Eur. J. Nucl. Med. Mol. Imaging* **34**, 185 (2007).

[5] M. Thambisetty, K.A. Gallardo, J. San Liow, L.L. Beason-Held, J.C. Umhau, A.K. Bhattacharjee, M. Der, P. Herscovitch, J.L. Rapoport, S.I. Rapoport, *J. Cereb. Blood. Flow. Metab.* **32**, 676 (2012).

[6] J. Andersson, P. Truong, Ch. Halldin, *Appl. Radiat. Isot.* **67**, 106 (2009).

[7] B.H. Mock, C. Brown-Proctor, M.A. Green, B. Stelle, B.E. Glick-Wilson, Q. Zheng, *Nucl. Med. Biol.* **38**, 1135 (2011).

[8] S. Kealey, A. Gee, P.W. Miller, *J. Label Comp. Radiopharm.* **57**, 195 (2014).

[9] Xi. Shao, R. Hoareau, A.C. Runkle, L.J.M. Tluczek, B.G. Hockley, B.D. Henderson, P.J.H. Scott, *J. Label Comp. Radiopharm.* **54**, 819 (2011).

[10] N.A. Gomzina, O.F. Kuznetsova, *Russ. J. Bioorg. Chem.* **37**, 191 (2011).

[11] V. Gomez, J.D. Gispert, V. Amador, J. Llop, *J. Label Comp. Radiopharm.* **51**, 83 (2008).

[12] A.H. Jacobs, A. Thomas, L.W. Kracht, H. Li, C. Dittmar, G. Garlip, N. Galldiks, J.C. Klein, J. Sobesky, R. Hilker, S. Vollmar, K. Herholz, K. Wienhard, W.D. Heiss, *J. Nucl. Med.* **46**, 1948 (2005).

[13] B. Pirotte, S. Goldman, N. Massager, P. David, D. Wikler, A. Vandesteene, I. Salmon, J. Brotchi, M. Levivier, *J. Nucl. Med.* **45**, 1293 (2004).

[14] M. Hasebe, K. Yoshikawa, S. Ohashi, S. Toubaru, K. Kawaguchi, J. Sato, J. Mizoe, H. Tsujii, *Mol. Imaging Biol.* **12**, 554 (2010).

[15] M. Matsuo, K. Miwa, O. Tanaka, J. Shinoda, H. Nishibori, Y. Tsuge, H. Yano, T. Iwama, S. Hayashi, H. Hoshi, J. Yamada, M. Kanematsu, H. Aoyama, *Int. J. Radiat. Oncol. Biol. Phys.* **82**, 83 (2012).

[16] I. Grassi, C. Nanni, V. Allegri, J.J. Morigi, G.C. Montini, P. Castellucci, S. Fanti, *Am. J. Nucl. Med. Mol. Imaging* **2**, 33 (2012).

[17] S. Le Helleix, F. Dolle, B. Kuhnast, *Appl. Radiat. Isot.* **82**, 7 (2013).

[18] D. Soloviev, C. Tamburella, *Appl. Radiat. Isot.* **64**, 995 (2006).

[19] D. Le Bars, M. Malleval, F. Bonnefoi, C. Tourvieille, *J. Label Comp. Radiopharm.* **49**, 263 (2006).

[20] European Pharmacopoeia, 8th ed. (8.0), L-Methionine ([11C]methyl) injection and Sodium [11C]Acetate injection.

[21] T. Fukumura, R. Nakao, M. Yamaguchi, K. Suzuki, *Appl. Radiat. Isot.* **62**, 1279 (2004).