Procurement and Biological Significance of Pure \(^{11}\)C-Glucose


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Biological significance of \(^{11}\)C-glucose purity has been a controversial matter. In order to understand the contribution of impurities contained in the photosynthetic \(^{11}\)C-glucose, its analysis and biodistribution in mice was studied. Studies indicated the interference of impurities, particularly \(^{11}\)C-fructose in the injectate, with the quantitative evaluation of glucose metabolic rate in brain. Furthermore, results obtained offered a good basis for search of simpler and easier separation methodology. Namely, good selection and combination of commercially available high performance liquid chromatography (HPLC) column provided \(^{11}\)C-glucose with high radiochemical purity. The radiochemical yield achieved was 6-10\% at 75 min after the end of bombardment including the HPLC purification. The imaging study by positron computed tomography in a rabbit showed the suitability of \(^{11}\)C-glucose as a radiopharmaceutical for the diagnosis of the brain function.

Key Words: carbon-11-glucose, photosynthesis, carbon-11-fructose, high performance liquid chromatography, positron computed tomography, biodistribution, radiochemical purity

1. Introduction

D-glucose is the sole energy source of the brain. Therefore, \(^{11}\)C-glucose and various \(^{12}\)C and \(^{18}\)F labeled analogs, such as \(^{11}\)C-2-deoxyglucose, 2-[\(^{18}\)F]fluoro-2-deoxyglucose, 3-[\(^{18}\)F]fluoro-3-deoxyglucose and 3-[\(^{11}\)C]methylglucose, have been used for the study of brain metabolic function\(^{11-7}\), namely, with the accessibility of positron computed tomographic diagnosis. However, the use of these analogs requires the correction in the tracer model (glucose), to adjust for differences in transport properties and enzyme affinities\(^{5,9}\). On the other hand, the use of \(^{11}\)C-glucose, a better tracer to serve those purposes\(^{10,11}\), has been limited. Photosynthetic production of \(^{11}\)C-glucose, the easiest and fastest method so far reported\(^{12-16}\), suffers from an efficient procedure to purified it from other carbohydrates, often rationalized as contributing factor for low metabolic rate\(^{17,18}\).

In this study, in order to provide an understanding of the biological significance of \(^{11}\)C-glucose purity, effect of the impurities contained in the photosynthetic \(^{11}\)C-glucose on the diagnosis was investigated. Mice biodistribution studies offered a good basis for search of simpler and easier separation methodology. Good selection and combination of commercially available high performance liquid chromatography (HPLC) column, provided \(^{11}\)C-glucose with high radiochemical purity, readily applicable in routine production, tested by analytical chromatography and animal biodistribution studies.

2. Materials and Methods

2.1 \(^{11}\)C Production

\(^{11}\)C was produced via \(^{14}\)N(p, a)\(^{11}\)C reaction with 11.3 MeV protons on a target of nitrogen gas by an ultra compact cyclotron (Sumitomo CYPRIS Model 325). A stream of nitrogen
gas was passed through the target at a rate of 200 ml/min and the produced $^{11}$C$_2$O$_2$ was trapped on Molecular Sieve 4A for 20–40 min.

2.2 $^{11}$C-glucose preparation

2.2.1 Photosynthesis

The photosynthesis was performed using the techniques of Lifton, et al. and Monma, et al. with several modifications.

The diagram for the photosynthesis system is shown in Fig. 1. Spinach leaves (1 g) were placed in the reaction vessel 1 and evacuated. $^{11}$C$_2$O$_2$ trapped on Molecular Sieve 4A was heated at 250°C and the desorbed $^{11}$CO$_2$ was flushed out with a stream of nitrogen gas into the vessel 1. Photosynthesis was activated by the illumination with a 100 W halogen lamp, while maintaining the temperature at 30–35°C. After 10 min, 10 ml of 90% ethanol was added to the reaction vessel 1 and boiled at 100°C for 8 min. The resulting solution was transferred to reaction vessel 2 and 2 ml of 2N hydrochloric acid was added. Additional rinsing of the vessel 1 with 90% ethanol (2 ml) was also transferred to the vessel 2. Then, hydrolysis of sucrose and evaporation of ethanol were simultaneously performed by boiling the mixture for 8 min at 100°C under a stream of nitrogen gas. After cooling, the hydrolysate was passed through a C-18 reverse-phase liquid chromatography SEP-PAK cartridge (Waters Associates) and two ion-exchange columns (AG3–X4 and AG50W–X8). The column of ion-exchange resin was eluted with distilled water and a mixture of $^{11}$C-glucose and $^{11}$C-fructose was obtained. This solution was sterilized by passage through a 0.22 μM Millipore filter. Automation of these procedures was achieved by on line attachment of timer, limit switch, photo-level sensor and NaI detector.

2.2.2 HPLC separation (preparative HPLC)

The solution obtained under Method 2.2.1, was evaporated to a volume of approximately 500 μl, which was then injected onto an Aminex HPX-87C column (7.8×300 mm). The mobile phase was water at 85°C with a flow rate of 1 ml/min. Fractions corresponding to glucose (6.6 min) and fructose (8.2 min) were collected. Then, $^{11}$C-glucose and $^{11}$C-fructose eluted, were diluted with saline to an appropriate volume for injection. Detection involved both the refractive index and the radioactivity.

2.3 Radiochemical analysis (Analytical HPLC)

The analytical method employed was HPLC. HPLC was carried out with 3.9×300 mm column of μBondapak Carbohydrate and a mix-
ture of 85% acetonitrile and 15% water was used for the elution at a flow rate of 2 ml/min. The retention times of glucose, fructose and sucrose were 9.6 min, 7.0 min and 20.0 min, respectively.

2.4 Animal experiments
Male mice (ddy) weighing about 25 g, were intravenously injected with a solution containing 1.1-1.5 MBq (30-40 µCi) of the sample. At appropriate time intervals, the mice were killed and autopsied. Sample of blood was collected by cardiac puncture and excised organs were weighed and collected in plastic counting tubes. The 11C activity was determined in all organs using a well scintillation counter, with automated decay correction device.

The positron tomographic brain images in a normal rabbit were obtained using the positron computed tomography (CT) at Kyoto University Hospital, Positologica III[19]. Positologica III consists of four rings with 192 BGO detectors in each ring. The spatial resolution is 7.6 mm in reconstructed images. An albino rabbit, weighing 2.5 kg, was anesthetized by injection of sodium pentobarbital and 44 MBq (1.2 mCi) of 11C-glucose was injected intravenously. Serial tomograms were taken from the injection time to 40 min.

3. Results
3.1 11C-glucose preparation
Following the photosynthetic condition described under the Method, the chromatographic analysis of the alcoholic extract (analytical HPLC) (Fig. 2(a)), showed the presence of sucrose (Fraction 7) (45%), a compound eluted at 33 min (Fraction 8) (45%) and small amount of other materials (1, 2, 3) (10%). Hydrolysis of the alcoholic extract (100°C) revealed the unhydrolyzable character of Fraction 8 (Fig. 2(b)). However, this fraction was removable by adsorption on a cationic exchange resin (AG50W-X8). Radiochromatogram of this eluate revealed 85-90% of the radioactivity at the 11C-glucose and 11C-fructose (Fraction 5 and 6) (Fig. 2(c)). Since fructose tended to be more degradative on the hydrolysis step, a heating at 120°C for 5 min eliminated its presence but generated other product hard to be removed.

For the separation of glucose from fructose, selection of an Aminex HPX-87C column, using water at 85°C as the mobile phase, gave a very satisfactory separation of glucose (Fig. 3). The fraction corresponding to 11C-glucose was radiochemically pure as detected by analytical HPLC (Fig. 2(d)). In further effort to separate these two sugars, an ion-exchange method was examined. A preparative column (AG50W-X8) in Ca2+ form and eluted with Ca(OH)2 (5×10^-3 M) gave excellent result but Ca2+ ion complicated the final purification.

Radiochemical yield obtained on each step of the photosynthesis and purification is shown in Table 1. In a typical experiment, using 15 GBq (400 mCi) of 11CO2 as the starting radio-
Column: Aminex HPX-87C: 7.8×300 mm,
Mobile phase: Water, Flow rate: 1 ml/min.
(1, 4, 5, 6 and 7): unknown, (2): Glucose,
(3): Fructose.
Fig. 3 Preparative HPLC of $^{11}$C-labeled purified
hydrolyzed product.

Table 1 Preparation of $^{11}$C-glucose (Radiochemical
yield registered at each step)

<table>
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<th>Step</th>
<th>Yield (%)</th>
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<tbody>
<tr>
<td>(a)</td>
<td>$^{14}$CO$_2$ in vessel 1</td>
</tr>
<tr>
<td>(b)</td>
<td>$^{14}$CO$_2$ incorporated into spinach leaves</td>
</tr>
<tr>
<td>(c)</td>
<td>$^{14}$C-sugar extract</td>
</tr>
<tr>
<td>(d)</td>
<td>$^{14}$C-glucose/fructose</td>
</tr>
<tr>
<td>(e)</td>
<td>Purified $^{14}$C-glucose</td>
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activity for the photosynthesis, 480 MBq (13 mCi) of glucose–fructose mixture was registered.
After the separation by the preparative HPLC, 100 MBq (2.8 mCi) of pure $^{11}$C-glucose was
obtained (radiochemical yield 9%). The complete procedure from the end of bombardment (EOB) to the production of pure $^{11}$C-glucose required 75 min. The specific activity achieved was of the order of 19 GBq/mmol (0.5 Ci/mmol).

3.2 in vivo biodistribution

The in vivo biodistribution of radioactivity in mice after injection of each of $^{11}$C-glucose, $^{11}$C-fructose and the solution obtained after hydrolysis, are shown in Fig. 4.

Figure 4(a) shows the biodistribution of purified $^{11}$C-glucose (fraction 2 of Fig. 3) which displayed the analytical chromatographic pattern shown in Fig. 2(d). Brain radioactivity remained almost constant until 20 min and then declined slowly. The brain uptake was the highest in the organs studied from 10 min to 60 min after injection. Pancreas showed an increasing incorporation as a function of time, while in all other organs, clearance of radioactivity was observed.

Figure 4(b) shows a biodistribution of the fraction 3 collected from the preparative column (Fig. 3), containing $^{11}$C-fructose as a large part of the total radioactivity (90%) but with some contamination of $^{11}$C-glucose (5%) and unknown materials (5%) (Fig. 2(e)). The most striking feature observed, was the low initial radioactivity of brain with a steep rise after 20 min. The value reached at 20 min was considerably high and remained constant over the

Fig. 4 Biodistribution in mice of $^{11}$C-glucose (a), $^{11}$C-fructose (b), and solution obtained after hydrolysis (c). Each point is a mean value for 3–4 animals.
next 10 min. All the organs studied, except for the brain and pancreas, showed very high values at the initial sampling point of 5 min but then the radioactivity cleared rapidly. Relatively high uptake of radioactivity in the kidney was noticeable.

Figure 4(c) shows the biodistribution of a solution obtained after hydrolysis, containing 25% $^{11}$C-glucose, 20% $^{11}$C-fructose and 55% unknown compounds, corresponding to chromatographic pattern described Fig. 2(b). High kidney uptake with radioactivity retention over 60 min period was observed. Radioactivity in other organs cleared slowly. Brain uptake was lower as compared with other two samples with faster clearance.

Imaging studies of pure $^{11}$C-glucose were performed on a normal rabbit with positron CT. Figure 5 shows sequential positron tomograms of head. Although large radioactivity in the sinus was observed at the initial stage, whole brain radioactivity increased along with the time and a clear image of brain was achieved at 30–40 min after injection.

4. Discussion

Purity of photosynthetically produced $^{11}$C-glucose has been often discussed$^{12,14,15,20,21}$, but no work has assessed the effect of those contaminants on the $^{11}$C-glucose utilization.

On this basis, the data gathered in mice (Fig. 4), clearly showed the complicity of impurities on the biological interpretation of $^{11}$C-glucose data, if used as a radiotracer for cerebral functional imaging. The mice biodistribution pattern of $^{11}$C-glucose obtained was closely related to published $^{11}$C-glucose distribution studies$^{20}$. The interference of $^{11}$C-fructose or other contaminants on $^{11}$C-glucose was established. In particular, the great difference in brain uptake was noticeable. The slower $^{11}$C-fructose accumulation might be due to lower brain uptake index of fructose$^{20}$ and its requirement to be converted to glucose in liver, kidney and small intestine$^{24}$, before its brain utilization. As a consequence, those organ activity involved in the interconversion (liver and kidney) showed very high accumulation followed by fast metabolic clearance.

Quantification of glucose metabolism, an estimation highly desirable in clinical diagnosis as for brain, calls for the production of pure $^{11}$C-glucose. Various stages of the preparation, hydrolysis and purification, were tested. Temperature of the alcoholic extract hydrolysis eliminated the interference of fructose but generated other impurities hard to be removed. Selection of a commercially available Aminex HPX–87C column gave satisfactory separation of $^{11}$C-glucose from other impurities. Particularly the use of water as eluant appeared suitable for clinical studies. As described under Method, the presented procedure is simple and easy to prepare pure $^{11}$C-glucose, in only 75 min, with a radiochemical yield of 6–10% and a specific activity of 19 GBq/mmol (0.5 Ci/mmol). Automatic on line production of $^{11}$C-glucose generated an easy to handle radiopharmaceutical for positron CT imaging studies, as shown in Fig. 5, with great suitability for the evaluation of brain functional studies.

References

要 旨

高純度\(^{11}\text{C}\)-グルコースの合成とその純度の体内動態に与える影響

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高純度\(^{11}\text{C}\)-グルコースを光合成法により合成し, さらにその純度が体内動態に与える影響について検討した。\(^{11}\text{C}\)-グルコースへの他の\(^{11}\text{C}\)標識光合成生成物の混入はそのマウスにおける体内動態に大きく影響し, \(^{11}\text{C}\)-グルコースを用いる体内代謝状態の定量的評価を難しくすることが認められた。とくに, \(^{11}\text{C}\)-グルコースの混入が脳機能の評価に大きく影響することが示され, その結果, 高純度\(^{11}\text{C}\)-グルコースの合成の必要性が強く認識された。高純度\(^{11}\text{C}\)-グルコースの合成は高速液体クロマトグラフィによる分離法を用いることにより成功し, 合成開始後75分で放射化学的収率6-10%で得られた。さらに, ウサギを用いるポジトンコンピュータ断層撮影での検討により, 高純度\(^{11}\text{C}\)-グルコースの脳機能診断薬としての有用性が認められた。