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Direct labelling of peptides with 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) ☆

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ABSTRACT

The study describes the use of [^{18}F]FDG as ^{18}F building block for the direct labelling of various aminooxy-functionalised peptides via chemoselective oxime formation.

Radiolabeled peptides have become important radio-pharmaceuticals in nuclear medicine for molecular imaging and therapy of tumors. Despite the advantageous nuclear and physical properties of short-lived positron emitter fluorine-18 (^{18}F) like its ease of production at high specific activity, the low β^{+} energy (0.64 MeV) and the favorable half-life (109.8 min), ^{18}F labelling of peptides implies several challenges. Direct incorporation of ^{18}F at high specific activity as [^{18}F]fluoride into peptides is hampered due to the harsh reaction conditions encountered during [^{18}F]fluoride labelling reactions, albeit recent reports discuss direct labelling of peptides with readily available cyclotron-produced [^{18}F]fluoride. However, most ^{18}F-labelling of peptides is usually accomplished by means of prosthetic groups, also referred to as bifunctional labelling agents.

The used prosthetic groups differ in their complexity of synthesis and conjugation to the peptide. The synthesis of prosthetic group usually requires laborious and time-consuming multi-step synthesis sequences starting from cyclotron-produced no-carrier-added [^{18}F]fluoride. Conjugation of the prosthetic group to peptides can either be accomplished via acylation, amidation and imidation of amine groups, alkylation of thiol groups, photochemical conjugation and chemoselective reactions like formation of oximes and hydrazones[1], or more recently, click chemistry.[2a–d]

2-[^{18}F]Fluoro-2-deoxy-D-glucose ([^{18}F]FDG) is the most important radiotracer for positron emission tomography (PET) imaging in nuclear medicine. The highly efficient radiosynthesis makes this radiotracer available in almost every PET center worldwide. However, despite the convenient automated synthesis and almost unlimited availability of [^{18}F]FDG there are only very few examples using [^{18}F]FDG as building blocks for the synthesis of ^{18}F-labeled compounds. To date, no higher molecular weight compounds like peptides have been labelled with [^{18}F]FDG directly. Besides the convenient availability of [^{18}F]FDG, introduction of [^{18}F]FDG as a sugar moiety into peptides holds additional advantages as it is a powerful means of improving pharmacokinetics radiolabeled peptides. Carbohydration of peptides leads to reduced lipophilicity of small radiolabeled peptides and thus to a dramatic reduction of hepatobiliary excretion in favor of renal excretion.[3a–f] Moreover, tumor uptake of various ^{18}F-labeled peptides could also be improved by glycosylation. The use of [^{18}F]FDG as a ^{18}F building block relies on its mutarotation in aqueous solutions leading to the formation of a mixture of α/β anomers (Fig. 1).

The isomerization process between both anomers proceeds through formation of an acyclic aldehyde form, enabling the reaction with aminooxy groups to give the corresponding oximes. The presence of glucose as a competitive aldehyde reagent in the used [^{18}F]FDG solution (50–70 μg/mL) may limit the scope of the reaction. The presence of glucose in the radiopharmaceutical formulation of [^{18}F]FDG is inevitable due to the production process of [^{18}F]FDG.[4,5]

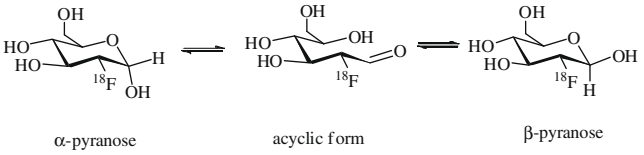

Figure 1. Mutarotation of [^{18}F]FDG.

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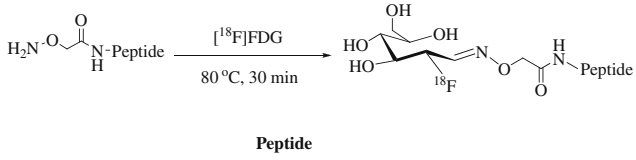
However, the fluorine substituent in the α-position of the carbonyl group in [^{18}F]FDG is expected to further enhance the reactivity of the carbonyl carbon in comparison to glucose. This should allow the preferred reaction of [^{18}F]FDG with aminooxy groups in the presence of glucose.

Based on our recent results upon the synthesis and application of a [^{18}F]FDG-based prosthetic group ([^{18}F]FDG-MHO),[6] in this study we describe the direct conjugation of [^{18}F]FDG to peptides through chemoselective oxime formation with various aminooxy-functionalised monomeric, dimeric, and tetrameric neurotensin NT(8–13) derivatives (Fig. 2).[‡]

First, the principle feasibility of using [^{18}F]FDG as a prosthetic group for direct peptide labelling was studied with aminooxy-functionalised monomeric NT(8–13). Recent studies on the ^{18}F labelling of peptides have clearly demonstrated that the labelling efficiency of peptides with various ^{18}F prosthetic groups largely depends on the amount of used peptide.[1]

The coupling reaction was performed in a mixture of aminooxy-functionalised monomeric NT(8–13) (2.5 mg/mL and 12 mg/mL) in methanol and aqueous [^{18}F]FDG solution at 80 °C for 30 min. The desired FDG-coupled peptide could be obtained in radiochemical yields of 63% and 80%, respectively, as determined by radio-HPLC analysis. Hence, the use of as little as 2.5 mg/mL of peptide gave sufficient radiochemical yields. The aminooxy-aldehyde coupling reaction involving hexoses is known to result in the formation of various isomers.[6,7] Likewise, reaction of aminooxy-functionalised NT(8–13) monomer with [^{18}F]FDG as a hexose led also to the formation of different isomers as confirmed by radio-HPLC analysis of the reaction mixture (Fig. 3).

The different peaks at 23.9 min, 24.5 min, and 25.6 min correspond with the expected formation of an acyclic oxime isomer (Fig. 2) and cyclic pyranose and furanose isomers (not shown). The peaks were collected and reinjected separately. The resulting radio-HPLC traces of the individual peaks revealed the same peak



Peptide

—Arg-Arg-Pro-Tyr-Ile-Leu-OH

—Glu—Arg-Arg-Pro-Tyr-Ile-Leu-OH
Glu—Arg-Arg-Pro-Tyr-Ile-Leu-OH

—Glu—Arg-Arg-Pro-Tyr-Ile-Leu-OH
Arg-Arg-Pro-Tyr-Ile-Leu-OH

Glu—Arg-Arg-Pro-Tyr-Ile-Leu-OH
Arg-Arg-Pro-Tyr-Ile-Leu-OH

Figure 2. Direct peptide labelling with [^{18}F]FDG.

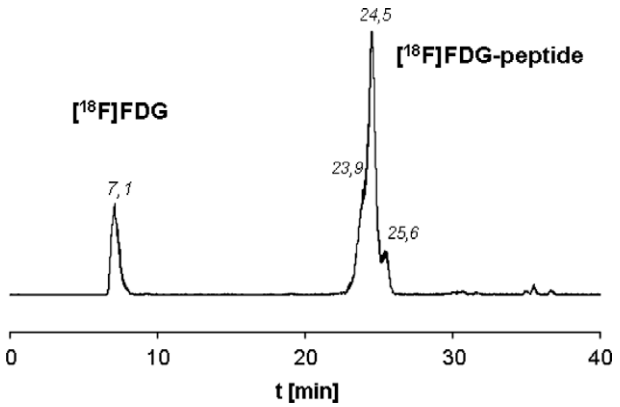


Figure 3. Radio-HPLC trace of the reaction mixture.

Table 1
Direct radiolabelling of aminooxy-functionalised NT(8–13) derivatives with [^{18}F]FDG

Peptide	Radiochemical yield of product[a] (%)	
	2.5 mg/mL	7.5 mg/mL
Monomer	63	80
Dimer	15	88
Tetramer	5	8

[a] Radiochemical yield was determined by radio-HPLC of aliquots taken from the reaction mixture after 30 min at 80 °C representing the percentage of cross-coupled product present in the reaction mixture.

pattern as observed in the original reaction mixture, indicating the formation of isomers. The formation of isomers was further proved by MALDI-TOF MS analysis of collected peaks obtained from the preparation of the reference compound with [^{19}F]FDG. All three collected peaks at 24.2, 25.0, and 26.2 min showed the same mass (m/z = 1055.1 $[M+H]^+$) of the FDG-coupled product with monomeric NT(8–13).

The corresponding UV-trace of the reaction mixture for the preparation of [^{18}F]FDG-coupled monomeric NT(8–13) showed a peak at 23.4 min, which was collected and analyzed by MALDI-TOF MS. The determined peak at 1053.3 $[M+H]^+$ corresponds with the expected glucose-based compound. The [^{18}F]FDG-labeled compound eluting at 24.5 min and the glucose–peptide conjugate eluting at 23.4 min could be separated by semi-preparative HPLC, leading to radiochemically and chemically pure [^{18}F]FDG-coupled product.

After showing the principle feasibility of labelling peptides directly with [^{18}F]FDG, the scope of the reaction was further studied with dimeric and tetrameric aminooxy-functionalised NT(8–13) as more complex peptides. The reaction was performed with different concentrations of both peptides (2.5 mg/mL and 7.5 mg/mL). The results are summarised in Table 1.

The data presented in Table 1 show that direct labelling of small aminooxy-functionalised peptides with the readily available radiopharmaceutical [^{18}F]FDG proceeds in sufficient radiochemical yields (63%) with reasonably small amounts of peptide (2.5 mg/mL). The use of structurally more complex dimeric and tetrameric peptides resulted in a significant decrease of radiochemical yield at low peptide concentrations (2.5 mg/mL). The use of a higher peptide concentration (7.5 mg/mL) led to satisfactory high radiochemical yield of 88% in the case of dimeric peptide, whereas no improvement was achieved with tetrameric peptide. In contrast to the utilization of small monomeric NT(8–13) derivative, efficient separation of the corresponding glucose-based dimeric and tetrameric peptides was not possible. This observation suggests that more complex and larger aminooxy-functionalised peptides seem to be not suitable for the direct radiolabelling with [^{18}F]FDG aimed at the synthesis of ^{18}F-labelled peptides at high effective specific activity.

In summary, the reaction of the readily available radiopharmaceutical [^{18}F]FDG with aminooxy-functionalised peptides is a convenient and simple method for the rapid and efficient radiolabelling of small peptides. However, application of more complex and larger peptides seems to be limited by the requirement of using large amounts of peptide to achieve sufficient radiolabelling yields, and separation difficulties of the corresponding glucose-peptide conjugate.

The almost unlimited availability of [^{18}F]FDG as the most important PET radiotracer makes this approach especially attractive for research groups without a complete PET center.

References and notes

†. While this work was in progress, two reports describing a similar approach have been published (Namavari, M.; Cheng, Z.; Zhang, R.; De, A.; Levi, J.; Hoerner, J.K.; Yaghoubi, S.S.; Syud, F.A.; Gambhir, S.S. *Bioconjug. Chem.* **2009**, *20*,

432. Hultsch, C.; Schottelius, M.; Auernheimer, J.; Alke, A.; Wester, H.-J. *Eur. J. Nucl. Med. Mol. Imaging.*, in press.)

‡. Peptide syntheses were performed on peptide synthesizer (Syro I, MultiSynTech, Germany) using standard Fmoc chemistry according to literature procedure.[8] All peptides were purified with HPLC and analyzed with MALDI-TOF MS. After lyophilization, the chemical purity of all peptides was greater 95%.Typical procedure for labelling of aminooxy-functionalised peptides with [^{18}F]FDG. [^{18}F]FDG solution[9] (5–20 MBq, 25 μL) was added to an Eppendorf vial containing a solution of monomeric peptide (0.2–0.5 mg; 0.22–0.56 μmol) in MeOH (50 μL). The mixture was heated at 80 °C for 30 min. Aliquots of the reaction mixture were injected onto a HPLC column (Zorbax 300SD C18, 9.4 × 250 mm, 5 μm) and eluted at a flow rate of 2 mL/min using CH_3CN with 0.04% TFA (A) and water with 0.05% TFA (B) as eluents. The following gradient was used: 0 min 10% A, 5 min 20% A, 22 min 20% A, 30 min 25% A. The radiolabelled product eluted between 24 and 26 min.

1. Wester, H.-J.; Schottelius, M. *Ernst Schering Res. Found. Workshop* **2007**, *62*, 79. and references therein.
2. (a) Marik, J.; Sutcliffe, J. L. *Tetrahedron Lett.* **2006**, *47*, 6881; (b) Glaser, M.; Arstad, E. *Bioconjug. Chem.* **2007**, *18*, 989; (c) Li, Z. B.; Wu, Z.; Chen, K.; Chin, F. T.; Chen, X. *Bioconjug. Chem.* **2007**, *18*, 1987; (d) Ramenda, T.; Bergmann, R.; Wuest, F. *Lett. Drug Des. Discovery* **2007**, *4*, 279.
3. (a) Leisner, M.; Kessler, H.; Schwaiger, M.; Wester, H.-J. *J. Label. Compd. Radiopharm.* **1999**, *42*, 549; (b) Haubner, R.; Wester, H.-J.; Weber, W. A.; Mang, C.; Ziegler, S. I.; Goodman, S. L.; Senekowitsch-Schmidtke, R.; Kessler, H.; Schwaiger, M. *Cancer Res.* **2001**, *61*, 1781; (c) Schottelius, M.; Wester, H.-J.; Reubi, J. C.; Senekowitsch-Schmidtke, R.; Schwaiger, M. *Bioconjug. Chem.* **2002**, *13*, 1021; (d) Schottelius, M.; Reubi, J. C.; Eltschinger, V.; Schwaiger, M.; Wester, H.-J. *J. Med. Chem.* **2005**, *48*, 2778; (e) Schottelius, M.; Rau, F.; Reubi, J. C.; Schwaiger, M.; Wester, H.-J. *Bioconjug. Chem.* **2005**, *16*, 429; (f) Wester, H.-J.; Schottelius, M.; Poethko, T.; Bruus-Jensen, K.; Schwaiger, M. *Cancer Biother. Radiopharm.* **2004**, *19*, 231.
4. Hamacher, K.; Coenen, H. H.; Stöcklin, G. *J. Nucl. Med.* **1986**, *27*, 235.
5. Füchtner, F.; Steinbach, J.; Mäding, P.; Johannsen, B. *Appl. Radiat. Isot.* **1996**, *47*, 61.
6. Wuest, F.; Berndt, M.; Bergmann, R.; van den Hoff, J.; Pietzsch, J. *Bioconjug. Chem.* **2008**, *19*, 1202.
7. Ramsay, S. L.; Freeman, C.; Grace, P. B.; Redmond, J. W.; MacLeod, J. K. *Carbohydr. Res.* **2001**, *333*, 59.
8. Hultsch, C.; Pawelke, B.; Bergmann, R.; Wuest, F. *Bioorg. Med. Chem.* **2006**, *14*, 5913.
9. 2-[^{18}F]Fluoro-2-desoxy-D-glucose ([^{18}F]FDG) was prepared in the PET-Center Rossendorf according to the procedure developed by Füchtner et al.[5]