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Original article

MODIFICATION OF [^{18}F]FDG BY THE FORMATION OF A HYDRAZONE BOND

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ABSTRACT

Purpose: The [^{18}F]-fluorodeoxyglucose ([^{18}F]-FDG) is known to be one of the most used radiopharmaceuticals for positron emission tomography. [^{18}F]-FDG allows the assessment of glycolytic activity, which is more enhanced in tumor cells than in normal cells. It is also used in the assessment of heart and neurological diseases. The aim of our work is to follow the possibility of modifying [^{18}F]-fluorodeoxyglucose and to develop an indirect radiofluorination procedure applicable under standard clinical conditions.

Material/Methods: In the clinic of nuclear medicine at the University Hospital Sta. Marina-Varna, for routine clinical purposes, [^{18}F]-FDG is produced by the nucleophilic method of fluorination, using mannose triflate as a precursor. In addition to being used as a universal radiopharmaceutical, [^{18}F]-FDG may be involved as a prosthetic group in biorthogonal reactions. [^{18}F]-glycosylation by oxime or hydrazone formation is a chemoselective method for indirect radiofluorination of sensitive molecules. The process can improve the pharmacokinetics and stability of the labeled compounds in the blood.

Results: We developed a method for modifying fluorine-deoxyglucose by forming a hydrazone bond with bifunctional tetrazine {3-[4-(6-phenyl-[1,2,4,5]-tetrazine-3-yl)-phenoxy]-propyl}-hydrazine) (Tz). The progress of the process and the product obtained were monitored by radio TLC. The radiolabeled tetrazine product will be used for future biorthogonal click reactions with trans-cyclooctene under physiological conditions.

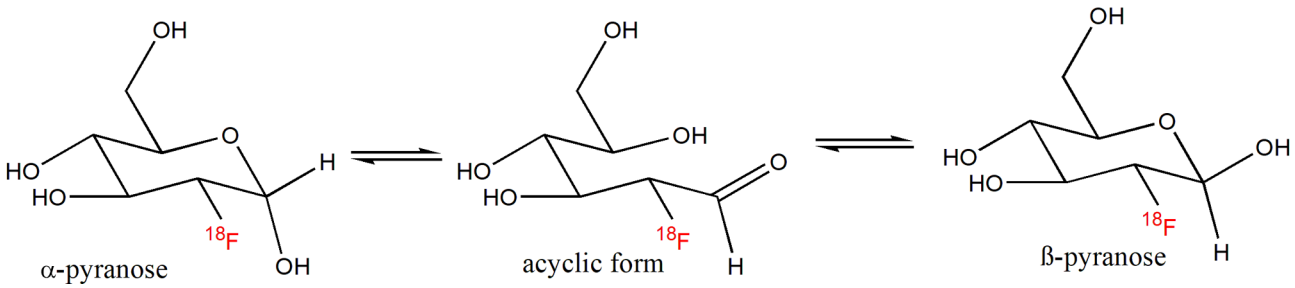
Keywords: Radionuclide ^{18}F, PET-CT, [^{18}F]-FDG, prosthetic group, hydrazone formation, click chemistry, tetrazine, bioorthogonal reaction,

INTRODUCTION

The use of molecular imaging agents plays an important role in the advancement of medical procedures and is an important part of ongoing research. At the forefront of molecular imaging is the use of positron emission tomography (PET-CT), which relies on a biomarker labeled with a short-lived positron-emitting radionuclide [1]. ^{18}F is one of the attractive radionuclides used in positron emission tomography. Most often, for the purposes of radiopharmaceutical synthesis, ^{18}F is obtained in a cyclotron by proton bombardment of ^{18}O enriched water by the reaction ^{18}O(p,n)^{18}F. ^{18}F is a positron emitter, and its half-life is about 110 minutes [2]. Its incorporation into organic molecules can have a significant effect on the physicochemical properties of the compound [3]. The most widely used PET radiopharmaceutical in most Nuclear Medicine Centers is ^{18}F-deoxyglucose ([^{18}F]-FDG). The [^{18}F]-FDG is an analog of glucose and enters cells by the same mechanisms. Cancer cells use more glucose than normal cells [4]. The use of PET-CT with [^{18}F]-FDG plays an important role in the planning of radiation therapy in pathologies such as lung cancer, head and neck cancer, colon cancer, Hodgkin's lymphoma [5]. Apart from being a universal PET radiopharmaceutical, ^{18}F-FDG can also be used as a prosthetic group for indirect labeling of biomolecules such as peptides, proteins and others under relatively mild reaction conditions [6].

The use of a prosthetic group overcomes the impossibility of radiofluorination of certain compounds by nucleophilic ^{18}F-substitution of a suitable leaving group. The development of ^{18}F prosthetic groups makes extensive use of the concept of click chemistry [7]. In recent years, various strategies for chemoselective reactions for ^{18}F labeling have been successfully developed, facilitating the availability of new PET radiopharmaceuticals. Glycosylation of biomolecules such as peptides or proteins may improve in vivo pharmacokinetics and stability in the blood [8]. The use of [^{18}F]-FDG as an ^{18}F-containing building block is based on the equilibrium observed in aqueous solutions between the cyclic and acyclic forms.

Fig. 1. Equilibrium of glucopyranose in aqueous solution

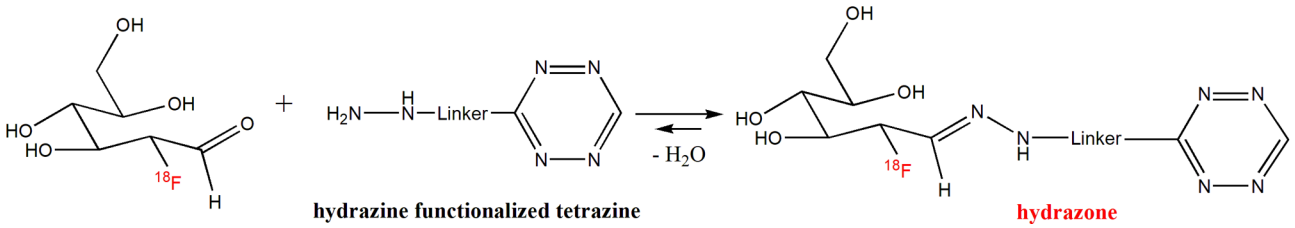


The formation of hydrazone and oxime bonds between á-nucleophiles (hydrazines, alkoxyamines) and carbonyl compounds are convenient and widely used in many fields of study. While the reagents are simple, a significant drawback is a relatively slow reaction at neutral pH. A strategy for accelerating these reactions is described using bifunctional buffer compounds that not only control the pH but also catalyze the reaction [9]. Due to the easy availability of [^{18}F]-FDG, peptide labeling using this molecule as an aldehyde is an attractive technique that does not require derivatization [10]. A pH of about 4.5 is usually advantageous for the formation of oximes and hydrazones. However, many biological applications require this ligation to take place under physiological conditions, which is a challenge due to the slow reaction rate at neutral pH and low concentrations of the reactants [11]. A strategy for accelerating these reactions using bifunctional buffer compounds that not only control pH but also catalyze the reaction is described [9].

The hydrazines react more slowly with aldehydes than aminooxy derivatives, making them less common click partners in ^{18}F-tagged peptides [7]. In terms of conjugation yields, hydrazone formation is as effective a method as oxime formation [12].

We develop a method for modifying [^{18}F]-fluorodeoxyglucose by forming a hydrazone bond with bifunctional Tz. A general scheme of the reaction can be seen in Figure 2.

Fig. 2. Modification of [^{18}F]-FDG by hydrazone conjugation



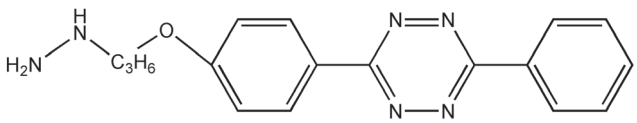
The radiolabeled tetrazine product will be used for future biorthogonal click reactions with trans-cyclooctene under physiological conditions. This is an opportunity to develop new and more highly specific radiopharmaceuticals for the diagnosis and targeted therapy.

The highly efficient reaction between tetrazines and trans-cyclooctene is a fast and pure biorthogonal method of conjugation [15]. In addition, the reaction proceeds without a catalyst, which was beneficial for easier purification of the final radioactive indicator [7]. The conjugation of tetrazine with trans-cyclooctene shows promising rates of the synthesis process, making this click reaction concept very suitable for ^{18}F labeling as well as for in vivo use in living systems [13].

MATERIAL AND METHODS

The [^{18}F]-fluorodeoxyglucose is produced in the Clinic of Nuclear Medicine at the University Hospital "St. Marina" – Varna (Bulgaria) for clinical routine purposes, using a nucleophilic method for radiofluorination and acid hydrolysis. The [^{18}F^{-}] anion is produced in a BG-75 biomedical cyclotron by bombarding ^{18}O-enriched water with the ^{18}O(p,n)^{18}F nuclear reaction [14]. We are using a portion of the [^{18}F]-FDG produced to develop and optimize a methodology for modifying a bifunctional tetrazine derivative that will provide a clickable agent for radiofluorination of bioactive trans-cyclooctene structures. We use the formation of hydrazone with [^{18}F]-FDG as a method to modify the molecule. The used hydrazine functionalized tetrazine ({3-[4-(6-phenyl-[1,2,4,5]-tetrazine-3-yl)-phenoxy]-propyl}-hydrazine) was synthesized on order from the organic synthesis group at Sofia University St. Kliment Ohridski, part of the current project group. The purified and NMR-H characterized compound was used for further synthesis. The structure of Tz used is shown in Figure 3.

Fig. 3. Structure of the tetrazine used (Tz)



{3-[4-(6-Phenyl-[1,2,4,5]tetrazin-3-yl)-phenoxy]-propyl}-hydrazine

The process of hydrazone formation occurs at an excess of Tz (10:1) relative to [^{18}F]-FDG. The temperature and pH of the medium are varied, with the best result obtained at a temperature of 70-75°C and a pH of about 4. The applied radioactivity is in the range between 5 and 25 MBq. Heating was carried out in a thermostated water bath. *p*-Methoxyaniline (Cat.) was used as a catalyst. A 50 mM aqueous solution of the catalyst was prepared and acidified with acetic acid to pH 4.0 - 4.2. From the resulting solution, 0.5 ml was taken and transferred to the reaction vessel and was added 0.2 ml of [^{18}F]-FDG. The reaction mixture was heated for 15 min at the appropriate temperature. Then 0.1 ml of bifunctional tetrazine solution dissolved in acetonitrile was added and heat was repeated one more time.

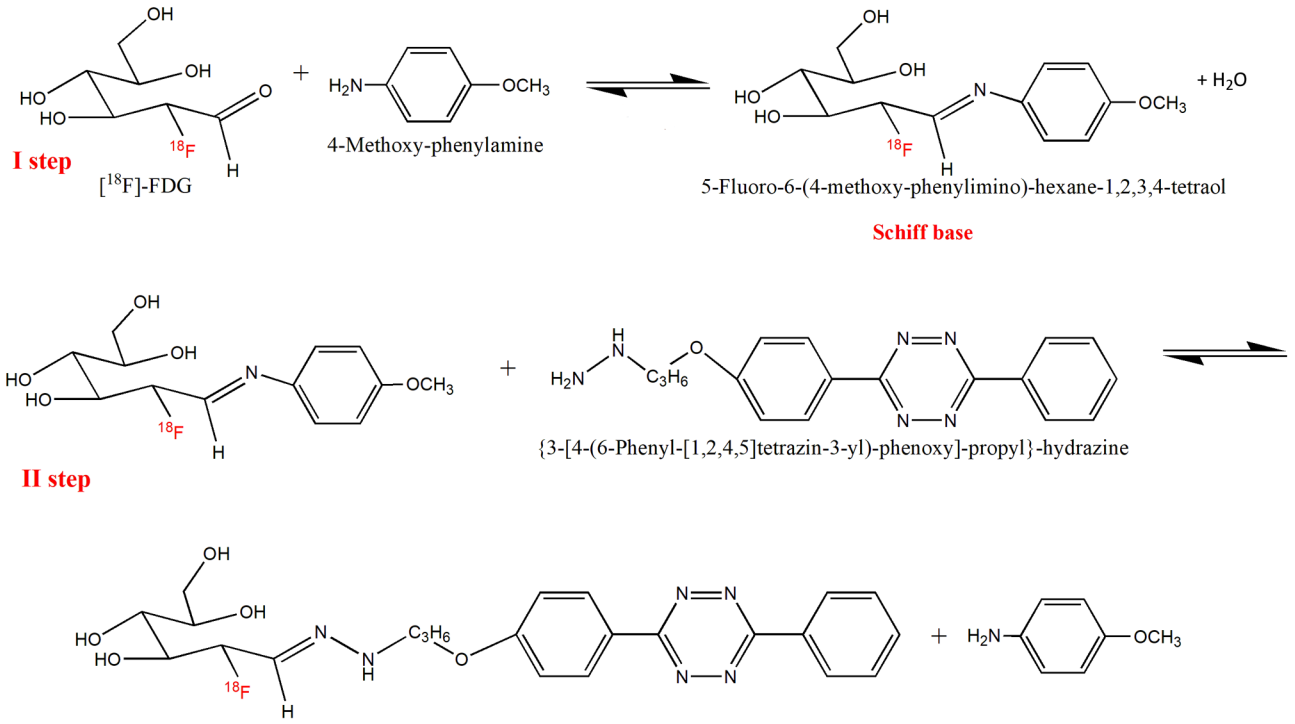
The progress of the process and the product obtained were monitored by radio TLC. Ethyl acetate is used as the eluent.

RESULTS AND DISCUSSION

The effect of important factors such as catalyst, pH and temperature on the radio chemical yield (RCY) was comprehensively studied. The reaction between Tz and [^{18}F]FDG was ran under standard condition (1:10 = [^{18}F]FDG : Tz) for 30 minutes and the resulting radiochemical yield determined by radio TLC was RCY=1.5 %. In order to increase the radiochemical yield, two catalysts p-methoxyaniline and aniline at a concentration of 50 mM were used as a medium to carry out the indicated reaction. The following results were observed: RCY=10.5% in the presence of p-methoxyaniline and RCY=5.4% using aniline. The reason for such low yields was high pH = 9.3 and series of experiments with different pH were run We selected p-methoxyaniline (Cat.) as the reaction medium and acetic acid was used to adjust the pH to the following values: 3.3; 4.2; 5.2; 6.1 and 7.3. The same reactions conditions were used (1:10 = [^{18}F]FDG : Tz; 22°C; Cat.) and RCY= 15.4 % for pH=3.3; RCY= 20.3 % for pH=4.2; RCY= 14.2 % for pH=5.2; RCY= 12.4 % for pH=7.3 were calculated. Optimization of pH leaded to increasing of the yield two time but still too low for beneficial radiolabeling.

The hydrazone formation reaction with [^{18}F]-FDG in the presence of catalyst proceeds in two steps. In the first step, [^{18}F]-FDG was activated and interacts with the catalyst, forming a Schiff base. After the addition of the bifunctional tetrazine, the Schiff base was rapidly converted to the hydrazone product (fig. 4). In both steps the most important was presents of acyclic form of glucopyranose and effect of temperature would be significant over RCY.

Fig. 4. Reaction steps of hydrazone formation between [^{18}F]-FDG and tetrazine



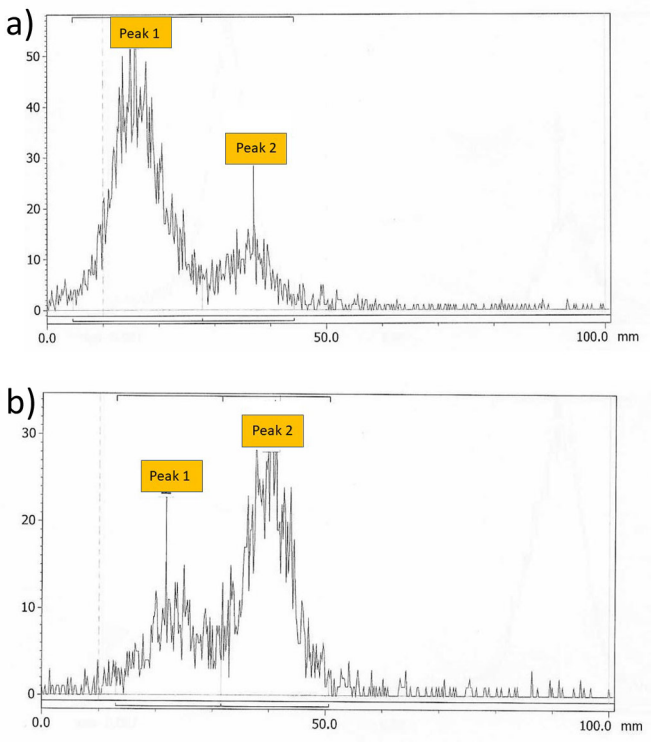
Keeping in mind reaction scheme for hydrazone formation and equilibrium of glucopyranose in aqueous solution was ran additional experiments were run where first [^{18}F]FDG and Cat mixture was heated (22°C; 30°C ;50°C; 70°C; 90°C) for 15 min. and then Tz was added and heated (22°C; 30°C ;50°C; 70°C; 90°C) for 15 min. Applauded changes according reaction scheme gave following results: RCY = 20.3 % for 22°C (fig. 6 a); RCY = 34.3 % for 30°C; RCY = 54.7 % for 50°C; RCY = 68.3 % for 70°C (fig. 6 b); RCY = 61.2 % for 90°C.

As was mentioned above the progress of the performed experiments were monitored by TLC and the same algorithm for interpretation was used as followed: after spotting the samples onto TLC plates and subsequent elution, the following distribution of reaction components was observed: unlabeled tetrazine (1) and catalyst (4) moved to the front, unreacted [^{18}F]FDG (2) remained at the start, and the resulting product was recorded as another colored spot off the start (3). Figure 5 presents a chromatogram with the distribution of the individual substances. The best RCY was obtained at 70°C.

Fig. 5. Chromatogram with the distribution of the individual substances: 1- Tz; 2 - [^{18}F]FDG, 3 - [^{18}F]FDG - Tz (product); 4 – Cat.; 5 – Tz.

Radio-TLC data ware used to confirm the formation of a hydrazone between [^{18}F]-FDG and Tz with and to determine the radiochemical yields. Figure 6 presents radio-chromatograms of syntheses carried out at different temperatures.

Fig. 6. Radio-chromatograms of the performed syntheses: peak 1– unreacted [^{18}F]FDG, peak 2–product; **a)**T= 22°C, pH=4.2; RCY =20.3% **b)** T=70°C, pH=4.2 ; RCY=68.8% .



Facile method for modification of fluorodeoxyglucose by forming a hydrazone bond with bifunctional Tz was developed where used procedure was simple and applicable for standard clinical laboratory settings and labeling was done at a not very high temperature and for a relatively short time, considering the half-life of ^{18}F.

CONCLUSIONS

Based on the experiments, the following conclusions can be drawn: 1) The hydrazine functionalized tetrazine ({3-[4-(6-phenyl-[1,2,4,5]-tetrazine-3-yl)-phenoxy]-propyl}-hydrazine) was successfully modified under mild reaction conditions; 2) At T=70°C and pH 4, a radiolabeled product is obtained with the best radiochemical yield. 3) The hydrazone formation with [^{18}F]-FDG is a practical method for indirect fluorination; 4) The develop method is fully applicable for standard clinical laboratory settings.

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