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HYNIC a bifunctional prosthetic group for the labelling of peptides with ^{99m}Tc and ^{18}FDG

Sepideh Khoshbakht[1] · Farzad Kobarfard[2] · Davood Beiki[3] · Omid Sabzevari[4] · Mohsen Amini[5] · Faramarz Mehrnejad[6] · Kimia Tabib[7] · Soraya Shahhosseini[8,9]

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Abstract With regard to high reactivity and chemoselectivity of HYNIC towards carbonyl of acyclic form of ^{18}FDG and its stable complexes with ^{99m}Tc, in this study, LIKKPF as the model peptide was conjugated with HYNIC and labelled with ^{99m}Tc (RCP >90 %) and ^{18}FDG for the first time. The RCP of >70 % was achieved for labelling with ^{18}FDG, in the presence of glucose (50–250 μg/mL). Our results showed the high potential of HYNIC conjugated peptides for labelling with ^{99m}Tc and ^{18}FDG as ^{18}F-fluorinated prosthetic group, to be clinically accepted for the radiolabelling of peptides.

Keywords HYNIC · Hydrazone bond · ^{18}FDG · ^{18}F-fluorinated prosthetic group · ^{99m}Tc

Abbreviations

FDG	2-Flouro-2-deoxy-D-glucose
PET	Positron emission tomography
SPECT	Single photon emission computed tomography
HPLC	High performance liquid chromatography
TLC	Thin layer chromatography
HYNIC	6-Hydrazinonicotinamide
BFCAs	Bifunctional chelating agents

✉ Soraya Shahhosseini
soraya.shahhosseini@gmail.com; s_shahoseini@sbmu.ac.ir

Sepideh Khoshbakht
khoshbakhtsepideh@yahoo.com

Farzad Kobarfard
kobarfard@sbmu.ac.ir

Davood Beiki
beikidav@sina.tums.ac.ir

Omid Sabzevari
omid@tums.ac.ir; omidsabz@yahoo.com

Mohsen Amini
moamini@sina.tums.ac.ir

Faramarz Mehrnejad
mehrnejad@ut.ac.ir

Kimia Tabib
kimia.tabib@gmail.com

[1] Department of Radiopharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

[2] Department of Pharmaceutical Chemistry, School of Pharmacy, Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Vali-e Asr Ave., Niayesh Junction, P.O.Box 14155-6153, Tehran, Iran

[3] Research Center for Nuclear Medicine, Tehran University of Medical Sciences, Tehran, Iran

[4] Department of Toxicology & Pharmacology, Faculty of Pharmacy, and Toxicology and Poisoning Research Centre, Tehran University of Medical Sciences, Tehran, Iran

[5] Department of Medicinal Chemistry, and Drug Design and Development Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

[6] Department of Life Science Engineering, Faculty of New Sciences & Technologies, University of Tehran, P.O.Box: 14395-1561, Tehran, Iran

[7] Department of Pharmaceutical Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[8] PET/CT Unit, Ferdous Nuclear Medicine Center, Dr Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[9] Department of Pharmaceutical Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Vali-e Asr Ave., Niayesh Junction, P.O.Box 14155-6153, Tehran, Iran

LC–MS	Liquid chromatography–mass spectrometry
TFA	Trifluoroacetic acid
Tricine	*N*-[tris(hydroxymethyl)methyl]glycine
HOBT	*n*-Hydroxy benzotriazole
DIC	Diisopropylcarbodiimide
DIPEA	Diisopropylethylamine
DMF	Dimethylformamide
TIS	Triisobutylsilane
MEK	Methyl ethyl ketone
EDDA	Ethylenediamine diacetate
Fmoc	9-Fluoroenylmethoxycarbonyl
RCP	Radiochemical purity
RCY	Radiochemical yield
MW	Molecular weight

Introduction

The basis of peptide receptor radionuclide scintigraphy (PRRS) is the over expression of peptide receptors in pathologic conditions, e.g., as cancer. As specific molecular probes used for receptor imaging and tumour targeting, radiolabelled peptides have a huge impact on the diagnosis and treatment of diseases. In the future, more effective selective imaging and therapeutic agents based on radiopeptide radiopharmaceuticals are expected [1].

For imaging studies, peptides can be labelled with several radionuclides such as ^{99m}Tc, ^{111}In, ^{68}Ga, ^{64}Cu, $^{123/124}$I. For routine clinical SPECT imaging, ^{99m}Tc is the radionuclide of choice. This is because it has good radiation physical characteristics [IT, 140 keV (90), 6 h] and is available through an inexpensive ^{99}Mo/^{99m}Tc generator. Furthermore, it is well developed and has varied chemistry [2]. For routine clinical PET imaging, ^{18}F is the radionuclide of choice due to its low energy of positron (0.69 MeV, 97 % β^+, 3 % EC). Additionally, it has the highest potential resolution for PET imaging, a low tissue radiation dose and a half life of 109.8 min. This makes it possible for a more complex synthesis with sufficient quantities to be delivered to centres far from its production facility [3].

The radiolabeling of peptides with ^{99m}Tc can be directly achieved through amino acids. Alternatively, it can be indirectly achieved through BFCAs, e.g., HYNIC. Since HYNIC-peptide conjugate is easily labelled with ^{99m}Tc, using a number of co-ligands with high stability, HYNIC is one of the most widely used BFCAs for the labelling of peptides with ^{99m}Tc [4–6].

Direct fluorination of peptides with ^{18}F is not possible. This is because of the harsh reaction conditions such as high temperature, pH, solvents and a large amount of peptide. Thus, indirect methods, which utilize fluorinated prosthetic groups, have been developed. The preparation of most fluorinated prosthetic groups is a multi-step procedure and is time-consuming. Furthermore, the final product usually needs to be purified by HPLC with a low yield. In addition, the reaction between peptide and fluorinated prosthetic group is not selective and a peptide precursor should be protected [7–15]. To avoid side reactions, chemoselective reactions, such as oxime and hydrazone bond formation, were introduced. Previous studies show that unprotected peptide precursors, functionalized with aminooxy or hydrazine, form a stable oxime or hydrazone bond with ^{18}F-fluorinated aldehyde as a prosthetic group in aqueous media [16–18]. In recent years, Human serum albumin (HAS) and unprotected peptides functionalized with HYNIC, such as RGD, octreotide and substance p, were fluorinated with ^{18}Fluorobenzaldehyde with high stability via hydrazone formation [19–21]. For oxime bond formation, the reagent Eei-Aoa-NHS was introduced to incorporate aminooxy at N-terminal end of peptide for oxime bond formation. In a simple one step method, ^{18}FDG was applied to fluorinate aminooxy peptides, through oxime bound formation [22–24].

The future approach is a fast, easy and efficient method for large scale production of ^{18}F labelled peptides for clinical routine application at nuclear medicine centres. ^{18}FDG has high potential as ^{18}F-fluorinated prosthetic group. This is due to the availability of ^{18}FDG in most PET centres and the presence of aldehyde group in acyclic form of ^{18}FDG, as a result of mutarotation in aqueous solutions.

Our aim is to functionalize peptide for labelling with ^{18}FDG and ^{99m}Tc for PET and SPECT imaging studies. With regard to high reactivity and chemoselectivity of HYNIC toward carbonyl for hydrazone bond formation and its stable complexes with ^{99m}Tc, HYNIC-conjugated peptides was considered for the labelling of peptides with ^{18}FDG and ^{99m}Tc. In this study, LIKKPF as a model peptide was synthesized and conjugated with HYNIC. The HYNIC conjugated peptide was labelled with ^{99m}Tc using $Na^{99m}TcO_4$ and ^{18}F using ^{18}FDG. Here, we report the direct labelling of a HYNIC functionalized peptide with ^{18}FDG. Moreover, the stability, labelling efficiency and radiochemical purity of ^{99m}Tc and ^{18}F radiolabeled peptide were determined and compared.

Materials and methods

For this study, the amino acids and resin were obtained from Bachem (Bubendorf, Switzerland). Coupling reagents, HOBT and DIC were purchased from Sigma-Aldrich (St. Luis, MO, USA). Cold FDG [^{19}FDG] and Succinimidyl-N-Boc-HYNIC were purchased from ABX

advanced Biochemical compounds GmbH (Radeberg, Germany). All of the chemicals, solvents and reagents were of analytical quality and used without further purification. Silica gel 60 F_{254} pre-coated aluminium sheets from Merck were used for TLC. Using normal serum, $^{99m}TcO_4^-$ was eluted from a $^{99}Mo/^{99m}Tc$ generator (Pars-isotope, Tehran, Iran). Furthermore, ^{18}FDG was provided from a routine in-house synthesis at the PET/CT Unit, Ferdous Nuclear Medicine centre, Dr Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences (Tehran, Iran). Additionally, ^{18}FDG was prepared by TracerLab MX_{FDG} (GE Medical Systems Benelux s.a) with specification according to the European Pharmacopeia. SepPak Plus C-18 was purchased from Waters Corporation (Milford, USA). The distribution of radioactivity on TLC was determined using a TLC Scanner Mini-Scan, MS.1000. This was equipped with flow count B-FC-1000 and gamma detector MS3200 (Bioscan, Washington, USA). Mass-Spectra was recorded on LC–MS Triple Quad 6410 Agilent Technologies using series 1200 HPLC system (Tokoyo, Japan) column: C-18, 250 × 4.6 mm, 5 μm, mobile phase: A: H_2O + 0.1 % TFA, B: acetonitrile, flow rate: 1 mL/min, 20 μL, total run time: 40 min. A NaI well counter (Triathler multilabel tester, Hidex, Finland) and a dose calibrator (Atomlab 100, Biodex, NY) were used to measure low and high levels of radioactivity, respectively.

Peptide synthesis

Peptide LIKKPF (Leu-Ile-Lye–Lye-Pro-Phe) was manually synthesized on solid phase using standard Fmoc strategy, based on the Merrifield method [25]. Briefly, the peptide sequence Leu-Ile-Lye–Lye-Pro-Phe was assembled on Wang Resin with two equiv of N-α-Fmoc-protected amino acid and two equiv HOBt and DIC as a coupling reagent in six steps. A solution of two equiv of Succinimidyl-N-Boc-HYNIC and two equiv of DIPEA in dry DMF was added to the resin. After shaking for 45 min at room temperature (RT), the solution was removed and the resin was washed with DMF and CH_2Cl_2, respectively. The completeness of the coupling reaction was checked by a Kaiser Test. The cleavage of peptide from resin was checked using cocktail TFA/TIS/H_2O (95:2.5:2.5) for 45 min [26]. The solvents evaporated and peptide was precipitated with diethyl ether. The identity of peptide was confirmed by LC–MS.

Labelling studies

Labelling of HYNIC-LIKKPF with cold FDG (^{19}FDG)

The HYNIC functionalized peptide (2 mg) was reacted with 2 mg of ^{19}FDG in 96 % ethanol in saline (200 μL) pH = 2–3 for 30 min at 100 °C. The reaction mixture was immediately cooled diluted with water to a final volume of 1 mL. Purification was achieved by a Sep-Pak C18 cartridge. The cartridge was activated using 10 mL ethanol, followed by 10 mL of water and 20 mL of air. The reaction mixture was passed through a cartridge, followed by 5 mL of H_2O to remove un-reacted FDG and finally, 2 mL of 96 % ethanol to obtain peptide [24]. The identity of ^{19}FDG-HYNIC-LIKKPF was confirmed by LC-MS.

Radiolabeling of HYNIC-LIKKPF with ^{18}FDG

Different concentrations of peptide, (0.1–5) mg in 40–400 μL of 96 % ethanol, pH (2–2.5, 5–6, 8–8.5) were incubated with ^{18}FDG (1–5 mCi/200–250 μL) at 25, 80, 100 and 120 °C for 30 min. The pH was adjusted by TFA or NaOH. Reaction mixtures were purified by a Sep-Pak C18 cartridge. On the collected fractions, TLC chromatography was performed on TLC silica gel 60 F_{254}, acetonitrile:water (95:5) as a mobile phase, over a path of 8 cm [24, 27]. For HPLC radiochromatogram, 20 μL of purified mixture was applied to LC–MS. The outlet of LC–MS column was disconnected from mass and connected to a fraction collector. Forthy fractions (1 mL/min) were collected and activity was measured.

Radiolabelling of HYNIC-LIKKPF with ^{99m}Tc

HYNIC-peptide was labelled with ^{99m}Tc using $SnCl_2$ as a reducing reagent and EDDA and tricine as co-ligand. Briefly, (15–1000) μg peptide was mixed with 10 mg tricine, 5 mg EDDA, 7 μg $SnCl_2$ in a final volume 550 μL at pH 5–6. The labelling was initiated with the addition of 5–10 mCi ^{99m}Tc. The incubation was completed at 100 °C for 30 min. The radiochemical purity was determined by Radio-TLC, using TLC-SG as a stationary phase and MEK, sodium citrate 0.1 M pH 5, and methanol: ammonium acetate 1 M (1:1) as mobile phases, over a path of 8 cm. HPLC radiochromatogram was obtained as mentioned on "Radiolabeling of HYNIC-LIKKPF with ^{18}FDG" section.

Radiolabelling of HYNIC-LIKKPF with ^{18}FDG in presence of $SnCl_2$, tricine and EDDA

The radiolabelling was achieved as mentioned on "Radiolabeling of HYNIC-LIKKPF with ^{18}FDG" section.

Stability studies

The stability of ^{99m}Tc-HYNIC-peptide and ^{18}FDG-HYNIC-peptide were studied at room temperature for 12 and 4 h, respectively. At different time points, radiochemical purity of complexes was checked by a Radio-

TLC. In another study, approximately 10 μg of radiolabelled peptides were individually added to 0.5 mL of human serum plasma and incubated at 37 °C. At different time points (maximum 12 h for ^{99m}Tc and 4 h for ^{18}FDG), plasma proteins were precipitated out by reacting with 0.5 mL acetonitrile. The activity bound to the plasma protein was measured by counting the activity associated with the precipitate. The supernatant was analysed by Radio-TLC.

Results

LIKKPF was successfully synthesized via the standard Fmoc method, functionalized with HYNIC at the N-terminal and analysed by LC-MS. HYNIC-peptide: calculated for $C_{45}H_{69}N_{11}O_8$: 879.53; found m/z = 880 $[M + H]^+$. Analytical RP-HPLC: $t_R = 6.1$ min, 20 % A: 80 % B. At this MW (879.53 g/mol) 1 HYNIC molecule covalently attached to each molecule of peptide (Fig. 1).

The HYNIC-LIKKPF was conjugated with cold FDG (^{19}FDG). The yield of labelling was 51 %. The LC–MS analysis of purified labelled peptide (purification by C18 Sep Pack cartridge) showed a single mass peak in 1044 $[M + H]^+$ which corresponds to FDG-HYNIC-LIKKPF (Fig. 2) calculated for $C_{50}H_{78}FN_{11}O_{12}$: 1043.5. RP-HPLC: $t_R = 5.5$ min, 20 % A: 80 % B (Fig. 3).

The radiolabelling of HYNIC-LIKKPF with ^{99m}Tc was examined at temperatures (25, 80, 100, 120 °C), incubation times (5, 10, 15, 20, 25, 30 min), peptide (15, 100, 500, 1000 μg), and pH (2–2.5, 5–6, 8–8.5). Results are shown in Figs. 4, 5 and 6. HPLC radiochromatogram is shown in Fig. 7. The RCP was determined via Radio-TLC. *TLC-SG, MEK*: $^{99m}TcO_4^-$ ($R_f = 0.9$–1.0), $^{99m}TcO_2$, ^{99m}Tc-HYNIC-peptide, and co-ligand ($R_f = 0$). *TLC-SG*, *sodium citrate 0.1 M, pH 5:* $^{99m}TcO_4^-$, co-ligand ($R_f = 0.9$–1.0), $^{99m}TcO_2$, ^{99m}Tc-HYNIC-peptide ($R_f = 0$). *TLC-SG, methanol:ammonium acetate 1 M (1:1):* $^{99m}TcO_4^-$, ^{99m}Tc-HYNIC-peptide, and co-ligand ($R_f = 0.9$–1.0), $^{99m}TcO_2$ ($R_f = 0$). The RCP over 90 % was achieved with (15–1000) μg peptide, 5 mg EDDA, 10 mg tricine, 7 μg $SnCl_2$, pH 5–6, at 100 °C for 30 min.

The radiolabelling of HYNIC-LIKKPF with ^{18}FDG was examined at temperatures (25, 80, 100, 120 °C), incubation times (5, 10, 15, 20, 25, 30 min), and pH (2–2.5, 5–6, 8–8.5). Results are shown in Figs. 8 and 9. HPLC radiochromatogram is shown in Fig. 10. The RCP was determined via Radio-TLC. The fluorinated peptide remained at origin ($R_f = 0$), while ^{18}FDG moved up with solvent ($R_f = 0.45$). Preliminary studies showed that the optimal reaction temperature, incubation time and pH were 100 °C, 30 min, 2–2.5, respectively. By decreasing the volume of ^{18}FDG activity, the RCP increased. Considering the activity concentration of our daily synthesized ^{18}FDG, 50 mCi/mL, in all of the experiments, the final volume of reaction mixture was adjusted to 200–250 μL. ^{18}FDG (1–5 mCi) was used for the labelling of different amounts of peptide (100 °C, 30 min, pH 2–2.5). Since ^{18}FDG solution contains glucose, which competes with ^{18}FDG for hydrazone bond formation [24, 27], the amount of glucose was determined. This was achieved in a ^{18}FDG solution using HPLC (Agilent 1260, USA) equipped with a flow count Radio-HPLC detector system (B-FC-1000, FC-3300, Bioscan) and pulse amperometric detector (RID), column (anion exchange resin, 0.25 m, 4.0 mm, 10 μm), mobile phase (0.1 N NaOH), flow rate (1 mL/min), run time (30 min). The results showed a glucose concentration of 20–250 μg/mL of ^{18}FDG solution at different ^{18}FDG production runs. Tables 1 and 2 present the results of RCP with different glucose concentration. The highest RCP (>95 %) was obtained with glucose concentration <50 μg/mL (Table 2). The significant reduction of RCP was obtained by either a higher amount of activity or a lesser amount of peptide. The fluorinated peptide was purified by passing through C18 Sep Pack cartridge with 95 % efficiency. At the end of purification, the RCY (decay corrected) based on amount of ^{18}FDG activity was 40 ± 6.3 %. In vitro stability was checked via Radio-TLC at different time points. Results showed radiolabelled peptides were stable and no significant release of ^{99m}Tc or ^{18}FDG were detected for at least 12 and 4 h in aqueous and human serum solutions, respectively (Figs. 11, 12). The RCP was >95 % at all time points and less than 5 % of activity was transferred to serum proteins.

Discussion

The role and importance of peptide radiopharmaceuticals in imaging studies has encouraged vast research, with scholars working towards an easy, fast and efficient peptide radiolabelling method. ^{18}F and ^{99m}Tc are the best radionuclides for PET and SPECT studies. At present, a small number of peptides labelled with ^{99m}Tc are in clinical routine studies [28, 29]. Radiolabelling of peptides with ^{18}F is more complex than labelling with ^{99m}Tc. A small number of ^{18}Fluorinated prosthetic groups have been developed. There is not yet a clinically acceptable ^{18}fluorinated prosthetic group for the radiolabelling of peptides with ^{18}F. Synthesis of prosthetic groups is time consuming, containing multi-steps with a low to moderate yield. In addition, the peptide precursor should be protected before conjugation to prosthetic group. In vivo studies have also revealed a high hepatic and intestinal uptake, low target to non-target ratio, as well as a low stability of ^{18}Fluorinated peptide [12, 30, 31]. To overcome the

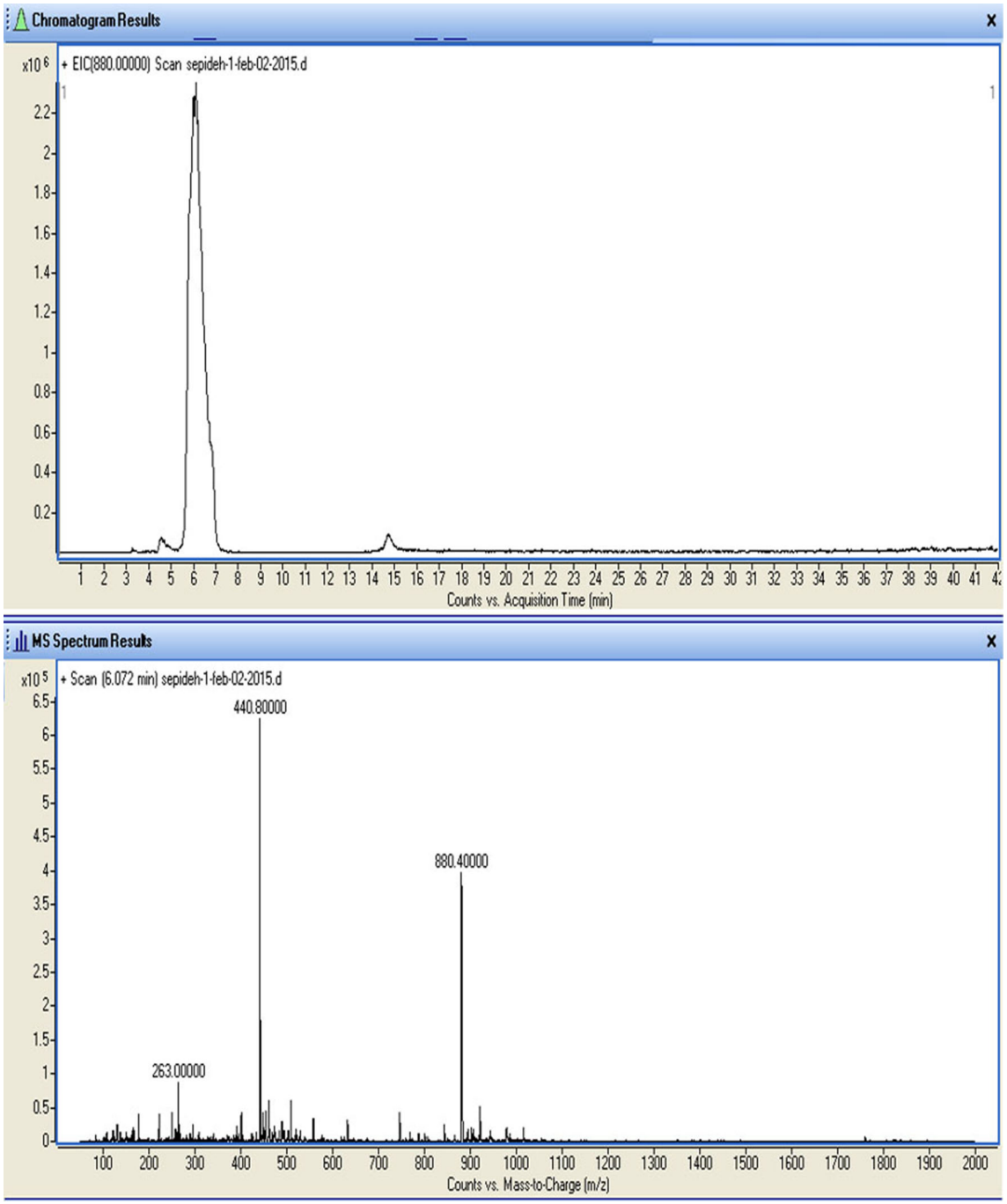

Fig. 1 LC–MS chromatogram of HYNIC-LIKKPF

mentioned problems, chemoselective oxime and hydrazone reactions have been introduced [16–18]. Mutarotation of sugars in aqueous solutions, from cyclic to acyclic forms, provides an aldehyde group for oxime or hydrazone bond formation. The acyclic form of ^{18}FDG, which increases at 100 °C, contains aldehyde group [18]. Due to the availability of ^{18}FDG in most PET centres, there is potential for ^{18}FDG as ^{18}F-fluorinated prosthetic group to be clinically accepted for the radiolabelling of peptides. Previous studies have revealed that the addition of carbohydrate into a peptide may improve the in vivo pharmacokinetics of peptide by decreasing lipophilicity [33, 34]. The hydrophilic nature of ^{18}FDG is expected to increase renal excretion, compared with hepatobiliary excretion [23].

^{18}FDG and ^{99m}Tc are the most available radiopharmaceuticals in nuclear medicine centres worldwide. Thus, the

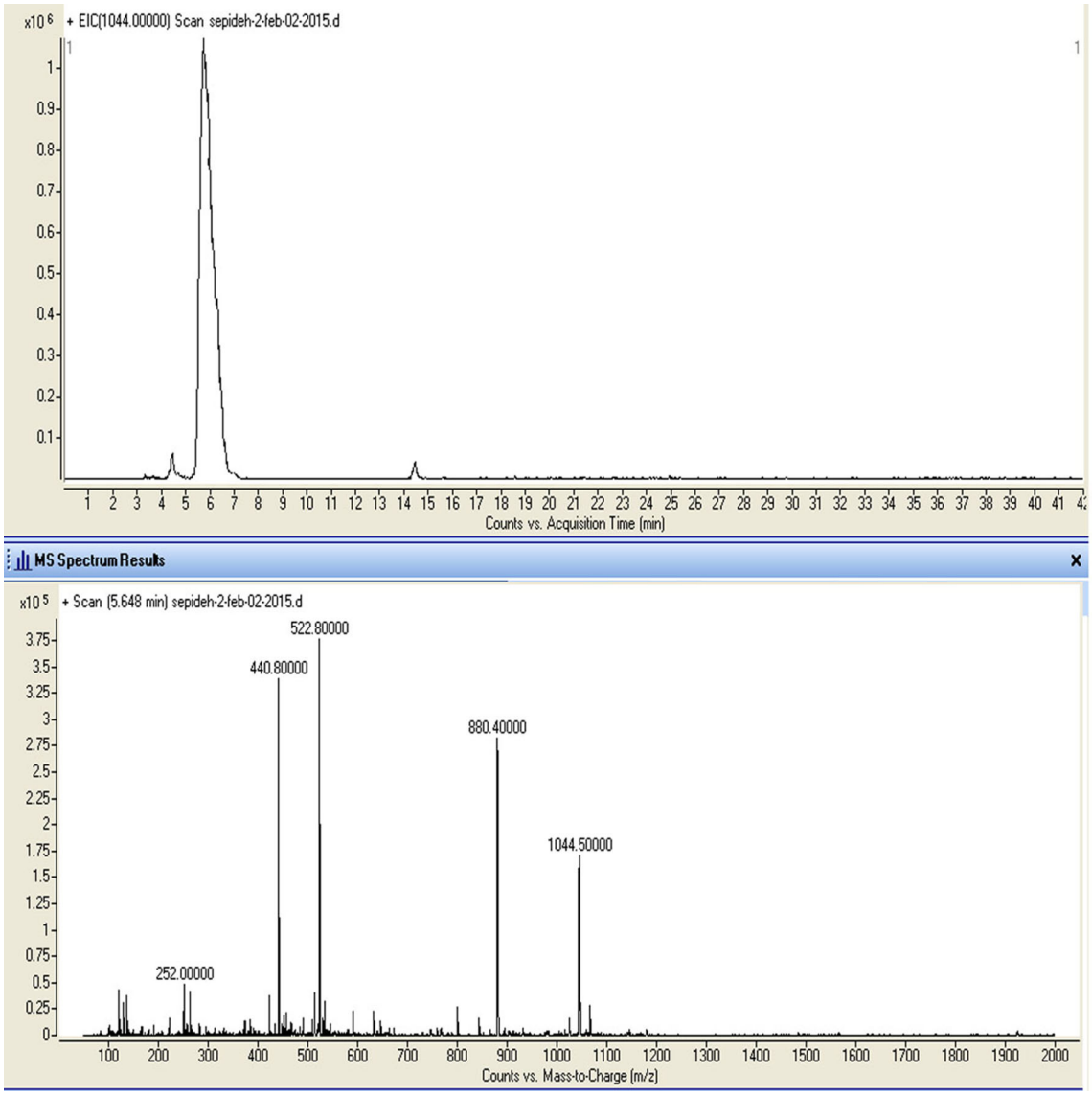

Fig. 2 LC–MS chromatogram of ^{19}FDG-HYNIC-LIKKPF

primary goal of this study was to prepare a peptide kit formulation to be easily radiolabelled with ^{99m}Tc or ^{18}FDG for imaging studies using SPECT or PET systems. The peptide LIKKPF was isolated by Burtea et al. [32] with high affinity and specificity for phosphatidyl serine. Since our group has recently been working on the design, synthesis and radiolabelling of peptides for apoptosis imaging, we selected the LIKKPF as the model peptide. The peptide was conjugated to HYNIC and characterized by LC–MS. The HYNIC-LIKKPF was labelled with ^{99m}Tc, using EDDA and tricine as co-ligand with RCP over 90 % (15–1000 μg peptide, 5 mg EDDA, 10 mg tricine, 7 μg $SnCl_2$, pH 5–6, at 100 °C for 30 min). In this study, we also evaluated the labelling of HYNIC-LIKKPF with ^{18}FDG as a prosthetic group. Our results showed that hydrazone bond formation between HYNIC-LIKKPF and ^{18}FDG is a fast, effective and chemoselective reaction, which performs in aqueous media in the presence of chemicals such as EDDA, tricine and $SnCl_2$. The optimal pH for hydrazone bond formation is 2–2.5. The amount of activity had a strong influence on RCP of oxime bond formation, as was mentioned by Hultsch et al. [27]. There is a competition between glucose and ^{18}FDG for hydrazone bond formation. In our experiments, the glucose was not removed from the ^{18}FDG solution. The radiolabelling of peptide was performed in the presence of glucose. The amount of glucose increases by using more ^{18}FDG activity, which results in low RCP. Meanwhile, a higher RCP is obtained by using a larger amount of peptide. The glucose concentration of our daily synthesized ^{18}FDG was usually

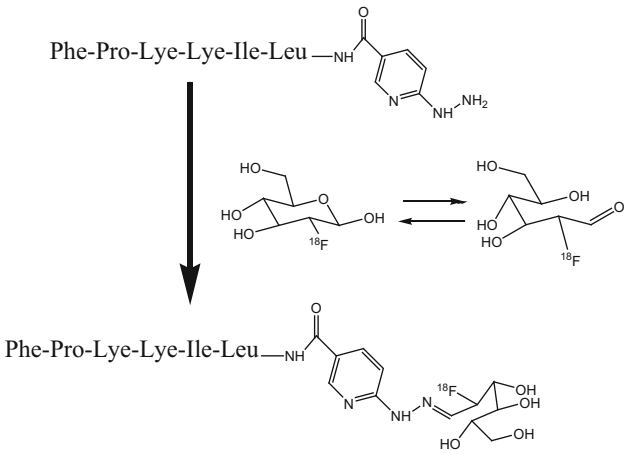

Fig. 3 Hydrazone bond formation between ^{18}FDG and HYNIC-LIKKPF

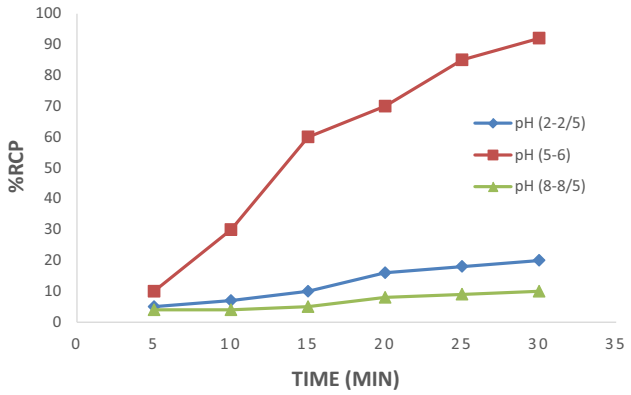

Fig. 4 RCP of ^{99m}Tc-HYNIC-LIKKPF as a function of time. The pH used 2–2.5, 5–6, and 8–8.5 with 100 μg of peptide, 5 mCi $Na^{99m}TcO_4$, at 100 °C

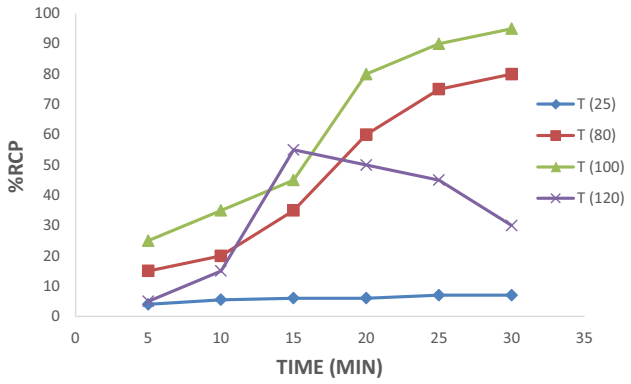

Fig. 5 RCP of ^{99m}Tc-HYNIC-LIKKPF as a function of time. The temperatures used 25, 80, 100, 120 °C with 100 μg of peptide, 5 mCi $Na^{99m}TcO_4$, pH 5–6

50–250 μg/mL. Some days, the glucose was <50 μg/mL. At this glucose concentration, RCP >95 % was achieved with peptide as low as 0.2 mg and 1 mCi ^{18}FDG. At

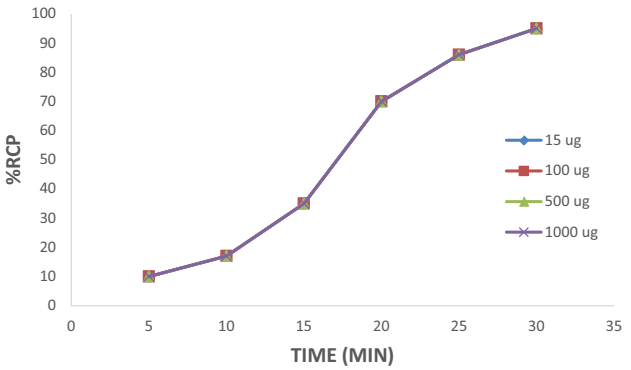

Fig. 6 RCP of ^{99m}Tc-HYNIC-LIKKPF as a function of time. The amounts of peptide used 15, 100, 500, 1000 μg at 100 °C, pH 5–6, 5 mCi $Na^{99m}TcO_4$

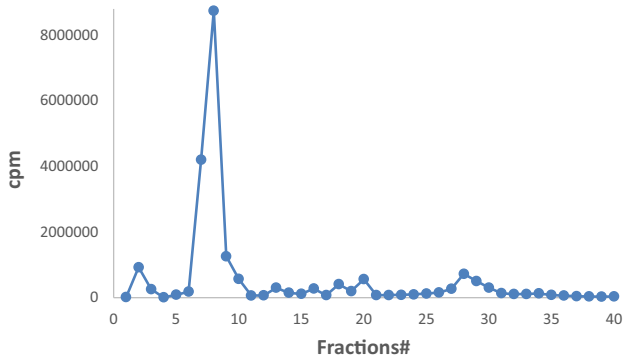

Fig. 7 HPLC radiochromatogram of ^{99m}Tc-HYNIC-LIKKPF

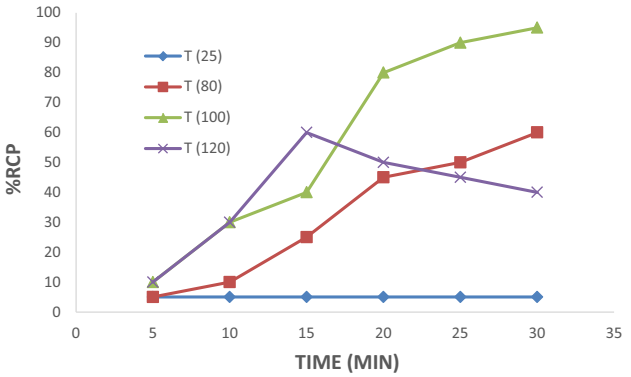

Fig. 8 RCP of ^{18}FDG-HYNIC-LIKKPF as a function of time. The temperatures used 25, 80, 100, 120 °C with 500 μg of peptide, 1 mCi ^{18}FDG, pH 5–6

glucose concentration of 50–250 μg/mL, RCP >90 % was achieved with peptide as low as 2 mg and 1 mCi ^{18}FDG. It decreased significantly by using less peptide or more ^{18}FDG activity. Since most of the days the glucose concentration was 50–250 μg/mL, with 1 mg HYNIC-

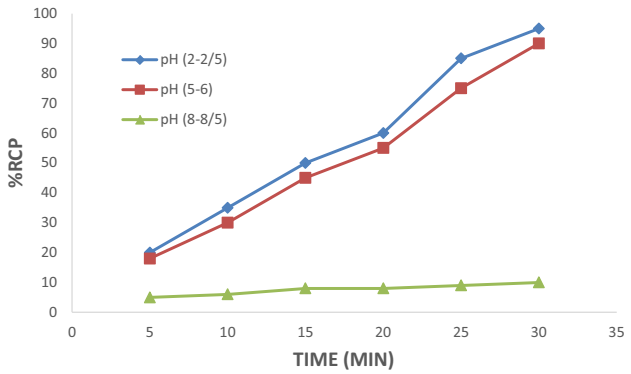

Fig. 9 RCP of ^{18}FDG-HYNIC-LIKKPF as a function of time. The pH used 2–2.5, 5–6, and 8–8.5 with 500 μg of peptide, 1 mCi ^{18}FDG at 100 °C

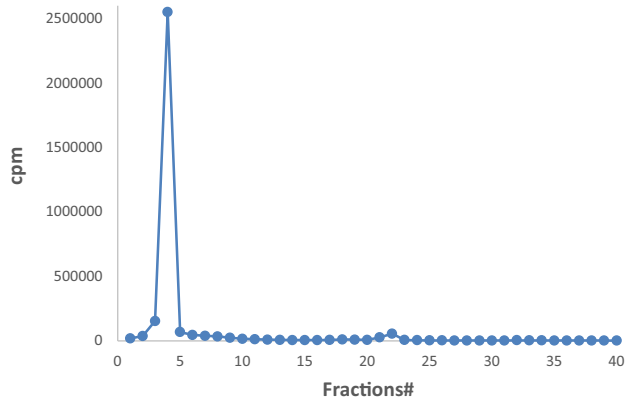

Fig. 10 HPLC radiochromatogram of ^{18}FDG-HYNIC-LIKKPF

Table 1 RCP of HYNIC-LIKKPF with ^{18}FDG (100 °C, 30 min, pH 2–2.5, glucose 50–250 μg/mL)

Peptide (mg)	0.1	0.2	0.5	1	2	3	5
^{18}FDG (1 mCi)	0	0	<10	50	>90	>95	>95
^{18}FDG (2 mCi)	0	0	0	30	50	>90	>95
^{18}FDG (3 mCi)	0	0	0	10	25	50	>90
^{18}FDG (5 mCi)	0	0	0	0	15	30	50

Table 2 RCP of HYNIC-LIKKPF with ^{18}FDG (100 °C, 30 min, pH 2–2.5, glucose <50 μg/mL)

Peptide (mg)	0.1	0.2	0.5	1	2	3	5
^{18}FDG (1 mCi)	50	>95	>95	>95	>95	>95	>95
^{18}FDG (2 mCi)	0	50	65	95	>95	>95	>95
^{18}FDG (3 mCi)	0	11	17	35	90	>95	>95
^{18}FDG (5 mCi)	0	0	0	5	60	90	>95

LIKKPF and 1 mCi ^{18}FDG, the RCP of at least 70 % was achieved. The labelled peptide was purified using C18 Sep Pack cartridge with 95 % efficiency for further studies.

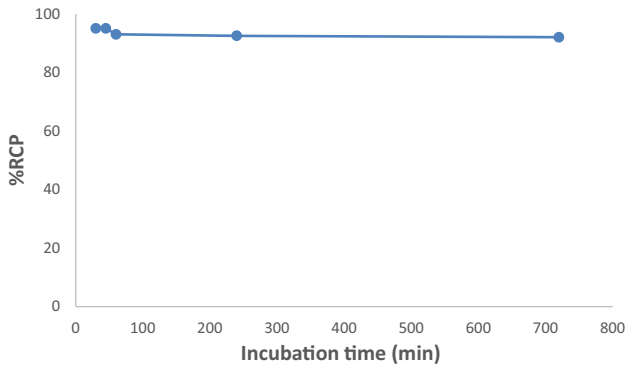

Fig. 11 Stability of ^{99m}Tc-HYNIC-LIKKPF in aqueous solution and human serum plasma

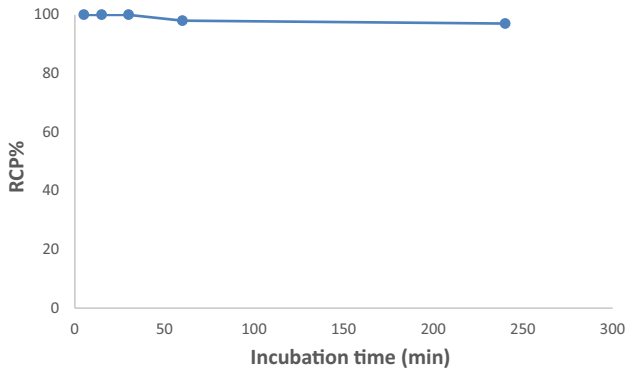

Fig. 12 Stability of ^{18}FDG-HYNIC-LIKKPF in aqueous solution and human serum plasma

In order to get RCP over 90 % without further purification, the glucose should be completely removed using HPLC, as suggested by Hultsch et al. [27]. Otherwise, ^{18}fluorinated peptide should be purified before in vivo experiments to remove un-reacted ^{18}FDG and other impurities. With ^{18}FDG solution free of glucose, it is expected to get RCP >90 % with peptide amount as low as 10–100 μg and ^{18}FDG activity >10 mCi. In these conditions, it is possible to have a bifunctional kit formulation for PET and SPECT imaging.

Conclusion

In this study, the HYNIC-LIKKPF was labelled for the first time with ^{18}FDG and ^{99m}Tc. In biological studies, ^{99m}Tc-HYNIC-LIKKPF with RCP >90 % is used without further purification, while ^{18}FDG-HYNIC-LIKKPF with RCP ≈ 70 % should be purified. It is an assumption that the higher RCP would be achieved with peptide as low as (10–100) μg and ^{18}FDG activity >10 mCi by removing glucose from ^{18}FDG solution. Since low amounts of peptide are usualy labeled with ^{99m}Tc, one peptide kit

formulation would be probably used for labeling with ^{18}FDG and ^{99m}Tc.

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