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Biodistribution of ^{99m}Tc Tricarbonyl Glycine Oligomers

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^{99m}Tc tricarbonyl glycine monomers, trimers, and pentamers were synthesized and evaluated for their radiolabeling and *in vivo* distribution characteristics. We synthesized a ^{99m}Tc-tricarbonyl precursor with a low oxidation state (I). $^{99m}Tc(CO)_3(H_2O)_3^+$ was then made to react with monomeric and oligomeric glycine for the development of bifunctional chelating sequences for biomolecules. Labeling yields of ^{99m}Tc-tricarbonyl glycine monomers and oligomers were checked by high-performance liquid chromatography. The labeling yields of ^{99m}Tc-tricarbonyl glycine and glycine oligomers were more than 95%. We evaluated the characteristics of ^{99m}Tc-tricarbonyl glycine oligomers by carrying out a lipophilicity test and an imaging study. The octanol-water partition coefficient of ^{99m}Tc tricarbonyl glycine oligomers indicated hydrophilic properties. Single-photon emission computed tomography imaging of ^{99m}Tc-tricarbonyl glycine oligomers showed rapid renal excretion through the kidneys with a low uptake in the liver, especially of ^{99m}Tc tricarbonyl triglycine. Furthermore, we verified that the addition of triglycine to prototype biomolecules (AGRGDS and RRPYIL) results in the improvement of radiolabeling yield. From these results, we conclude that triglycine has good characteristics for use as a bifunctional chelating sequence for a ^{99m}Tc-tricarbonyl-based biomolecular imaging probe.

Key words: ^{99m}Tc-tricarbonyl precursor, Glycine oligomer, Imaging moiety, Biomolecule tracing

INTRODUCTION

Molecular imaging techniques can provide biological information at the molecular level in living systems. Advances in molecular imaging techniques have been brought about by significant developments in instruments and specific imaging probes. Various imaging modalities are all successfully employed in the field of molecular imaging. Computerized tomography (CT) and magnetic resonance imaging (MRI) are still the main tools for providing structure-oriented information, while nuclear and optical imaging with the appropriate probes and reporters allow additional layers of molecular information. Despite the rapid progress of a number of imaging modalities, nuclear imaging is apremier clinical method and an advantageous approach for the *in vivo* tracking of biomolecules.

Technetium-99m is an ideal radionuclide for diagnostic organ imaging due to its optimum γ-energy (140 keV), short half-life (6 hr), low cost, and wide availability.

After the introduction of a ^{99m}Tc-tricarbonyl precursor with a low oxidation state (I) (Alberto *et al*., 1998), many approaches have been attempted to label a ^{99m}Tc-tricarbonyl precursor to a biomolecule, from glucose to an antibody (Schibli *et al*., 2000; Alberto *et al*., 1996; Chen *et al*., 2008; Taylor *et al*., 2010).

An ideal imaging probe would have high affinity and specificity for the target of interest. Peptides that target a number of disease-related receptors, as well as biomarker and the angiogenesis and apoptosis processes are in place. Small peptides have favorable pharmacokinetic and tissue distribution patterns as characterized by rapid clearance from blood and non-target tissues.

Many attempts have been made for the labeling of small peptides with ^{99m}Tc (Park *et al*., 2005; Reubi, 1995; Fischman *et al*., 1993; McAfee and Neumann, 1996; Lee *et al*., 2010).

The peptide labeling approach with ^{99m}Tc-tricarbonyl precursor can provide the highest possible specific activities with a minimal influence on the biologic properties of the peptide, including receptor affinity and metabolism (Egli *et al*., 1999). A small peptide sequence can be added to the key amino acid sequence as bifuctional chelating moiety of various molecular-targeting biomolecules for molecular imag-

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ing, such as RGD peptides, somatostatin, neurotensin, etc..

In this paper, we describe the evaluation of ^{99m}Tc-tricarbonyl labeled glycine monomer and oligomers as a bifunctionalpeptide moiety for nuclear imaging.

MATERINALS AND METHODS

Unless otherwise stated, all solvents, amino acids and their derivatives, and chemicals were of reagent grade. CO gas (99.5%) was obtained from the Daehan Gas Co. (Seoul, Korea) and prefiltered with an oxygen trap. ^{99m}Tc was obtained as pertechnatate by elution in an Unitech Tc-99m generator with 0.9% sodium chloride (Samyoung Unitech. Co. LTD., Korea). Gly(1), Gly-Gly-Gly(Gly(3)), Gly-Gly-Gly-Gly-Gly (Gly(5)) were obtained from Sigma Chemical Co. (St. Louis, USA).

Synthesis of ^{99m}Tc-tricarbonyl precursor. The $[^{99m}Tc(CO)_3(H_2O)_3]^+$(^{99m}Tc-tricarbonyl precursor) was prepared by adding 1 ml of $^{99m}TcO_4^-$ from a commercial generator (370 MBq) to a 5 ml vial containing potassium boranocarbonate (5.9 mg), sodium tetraboratedecahydrate (2.85 mg), sodium tartrate dihydrate (8.5 mg), and sodium carbonate (7.15 mg). The solution was heated for 30 min in boiling water under N_2. The labeling yield and stability of the ^{99m}Tc-tricarbonyl precursor were determined using reversed-phase high-performance liquid chromatography (HPLC). The ^{99m}Tc-tricarbonyl precursor was successfully prepared with a high radio-yields (> 95%).

HPLC conditions. Instrumentation consisted of an Agilent 1200 Series system (Agilent Technologies, Waldbronn, Germany) comprising a vacuum de-gasser, a dual pump, a temperature-controlled auto-sampler, a column oven compartment, a UV-Vis detector, and a radiometric detector. HPLC was carried out on a Nucleosil C18 (5 micron, 3.0 × 250 mm, Supelco Inc., PA, USA) maintained at ambient temperature. The mobile phase consisted of methanol (solvent B) and 0.05 M TEAP(solvent A). The HPLC gradient was made up of an isocratic elution (100% A) for the first 0~5 min; a linear gradient of 75% A/25% B to 100% A/0% B for 5~8 min; a linear gradient of 66% A/34% B to 75% A/25% B for 8~11 min; a linear gradient of 0% A/100% B to 66% A/34% B for 11~22 min; and an isocratic elution (100% B) for 22~25 min. The flow rate was 0.6 ml/min and a 10 μl aliquot was injected into the column.

Radiolabeling with ^{99m}Tc-tricarbonyl precursor. Labeling was performed by adding 1 ml of the prepared ^{99m}Tc-tricarbonyl precursor to the 50 μl of Gly(1), Gly(3), and Gly(5) (10 mg/ml in saline), at room temperature. Next, the reaction vial was heated at 75°C for 30 min. After cooling to room temperature, labeling yields were checked by radio-HPLC.

Lipophilicity of ^{99m}Tc-tricarbonyl Glys. The octanol-water partition coefficient of the complexes was measured in triplicate as shown below. Five-hundred μl of nitrogen-purged 0.05 M phosphate-buffered saline (PBS) (pH 7.4) was mixed together with 500 μl of n-octanol. After adding 10 μl of ^{99m}Tc-tricarbonyl glycine monomer, trimer, and pentomer (or ^{99m}Tc-tricarbonyl precursor), the samples were vortexed for 3 min, centrifuged at 3,000 g for 5 min using a SORVALL FRESCO centrifuge (Asheville, NC, USA), and allowed to separate the two phases. Each two-hundred μl of the PBS and octanol phases were measured in a well-type NaI(Tl) scintillation detector. The calculation was performed according to the following equation.

$$\text{Log p} = \log\left(\frac{\text{Octanol (cpm)}}{\text{Water (cpm)}}\right)$$

Animal studies. Female ICR mice (7 weeks old) were obtained from a specific pathogen-free colony at Orient, Inc. (Seoul, Korea). After quarantine and adaptation for three weeks, mice were used. The animals were housed in a room maintained at 23 ± 2°C with 50 ± 5% relative humidity, on a 12 h light/12 h dark cycle. The animals were fed a standard animal diet and water *ad libitum*. All animal studies were approved by the Institutional animal Care and Use Committee at Korea Atomic Energy Research Institute (KAERI).

Micro-SPECT/CT imaging studies. The mouse was scanned with an Inveon small-animal SPECT/CT system (Siemens Medical Solutions, Knoxville, TN, USA) equipped with a 1-pinhole mouse high sensitivity collimator. The mouse was anesthetized with 2% isoflurane (positioned prone in the cradle). The micro SPECT image was acquired at 30 min after intravenous injection of 37 MBq of ^{99m}Tc-tricarbonyl compounds. The CT scans were used for the anatomical reference. For the CT scans, the X-ray sources were used at 300 μA, and 60 kV for 15 min (one shot per projection). The CT resolution was 200 μm, and the number of acquired projections was 360.

Radiolabeling of Gly(3) added biomolecules with ^{99m}Tc-tricarbonyl precursor. For application of Gly(3) as an ending sequence for oligopeptides, Gly(3) added peptides were synthesized with their prototype peptides (AGTGDS, and GGGAGRGDS; RRPYIL and GGGRRPYIL). Labeling was performed using the ^{99m}Tc-tricarbonyl Gly(3) protocols prepared as described above.

RESULTS

Radiolabeling with ^{99m}Tc-tricarbonyl precursor. A ^{99m}Tc-tricarbonyl precursor was successfully prepared using

Table 1. ^{99m}Tc labeling yields and retention time of ^{99m}Tc-tricarbonyl glycine oligomers

Compound	Labeling yield[1]	Retention time (min)[2]
^{99m}Tc-tricarbonyl-Gly(1)	> 98%	11.3
^{99m}Tc-tricarbonyl-Gly(3)	> 90%	12.4
^{99m}Tc-tricarbonyl-Gly(5)	> 80%	12.4

1: Reaction conditions of ^{99m}Tc-tricarbonyl complex: 5 mg/0.2 ml of ligand solution was reacted with 1 ml ^{99m}Tc-tricarbonyl precursor, and then reaction vial was heated at 75°C for 30 min.
2: HPLC conditions:
Mobile phase - gradient system based on 0.05 M TEAP buffer and 100% MeOH.
Column - NucleosilC-18 column (3.0 × 250 mm).
Flow rate -0.6 ml/min.

a procedure described (Park *et al.*, 2005). The yield was higher than 98%. An additional purification step was not required. The radiolabeling results of glycine and glycine oligomers with a ^{99m}Tc-tricarbonyl precursor are summarized in Table 1. The labeling yields of three ^{99m}Tc-tricarbonyl complexes with Gly, Gly(3), and Gly(5) were greater than 80%. Analysis by RP HPLC reveals a single peak at 10.3 min for ^{99m}Tc-tricarbonyl-Gly, while ^{99m}Tc-tricarbonyl core elutes at 4 min. Typical chromatograms of a ^{99m}Tc-tricarbonyl precursor and ^{99m}Tc-tricarbonyl-Glys are shown in Fig. 1. Glycine and glycine oligomers were simply labeled with the ^{99m}Tc-tricarbonyl precursor with a high labeling yield.

Table 2. Lipophilicity of ^{99m}Tc-tricarbonyl glycine oligomers

Compound	Lipophilicity[1] (K_{ow} log P)
^{99m}Tc-tricarbonyl-Gly(1)	−0.48 ± 0.00
^{99m}Tc-tricarbonyl-Gly(3)	−1.53 ± 0.02
^{99m}Tc-tricarbonyl-Gly(5)	−1.50 ± 0.01

1: Octanol-water partition coefficient.

Lipophilicity of ^{99m}Tc-tricarbonyl glycinces. The octanol-water partition coefficient of ^{99m}Tc-tricarbonyl glycine and oligomersare summarized in Table 2. The values of their n-octanol/buffer partition coefficients were in the range of −0.5 to −1.6. This result indicates that these complexes are hydrophilic.

Animal studies. The SPECT images of ^{99m}Tc-tricarbonyl glycine and glycine oligomers in normal female ICR-miceacquired at 30 min post injection are shown in Fig. 2.

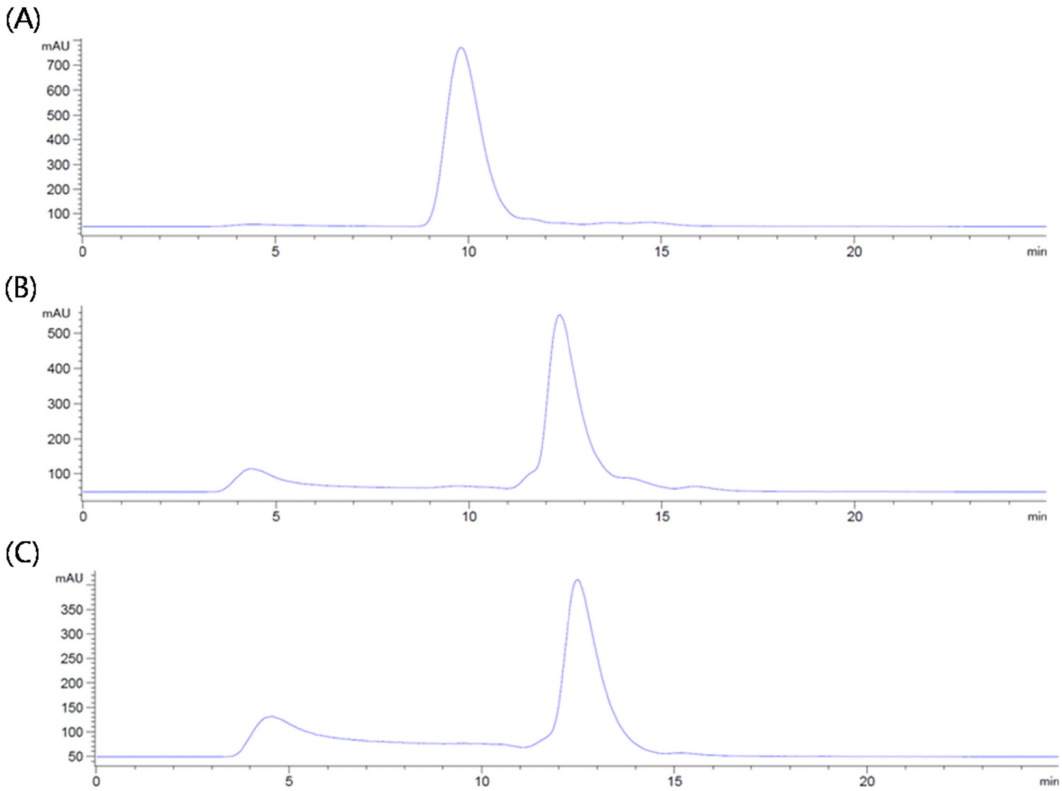

Fig. 1. The HPLC chromatogram of ^{99m}Tc-tricarbonyl Gly(1) (A), ^{99m}Tc-tricarbonyl Gly(3) (B), and ^{99m}Tc-tricarbonyl Gly(5) (C) on C-18 column. HPLC conditions: Mobile phase - gradient system based on 0.05 M TEAP buffer and 100% MeOH; Column -NucleosilC-18 column (3.0 × 250 mm); Flow rate −0.6 ml/min.

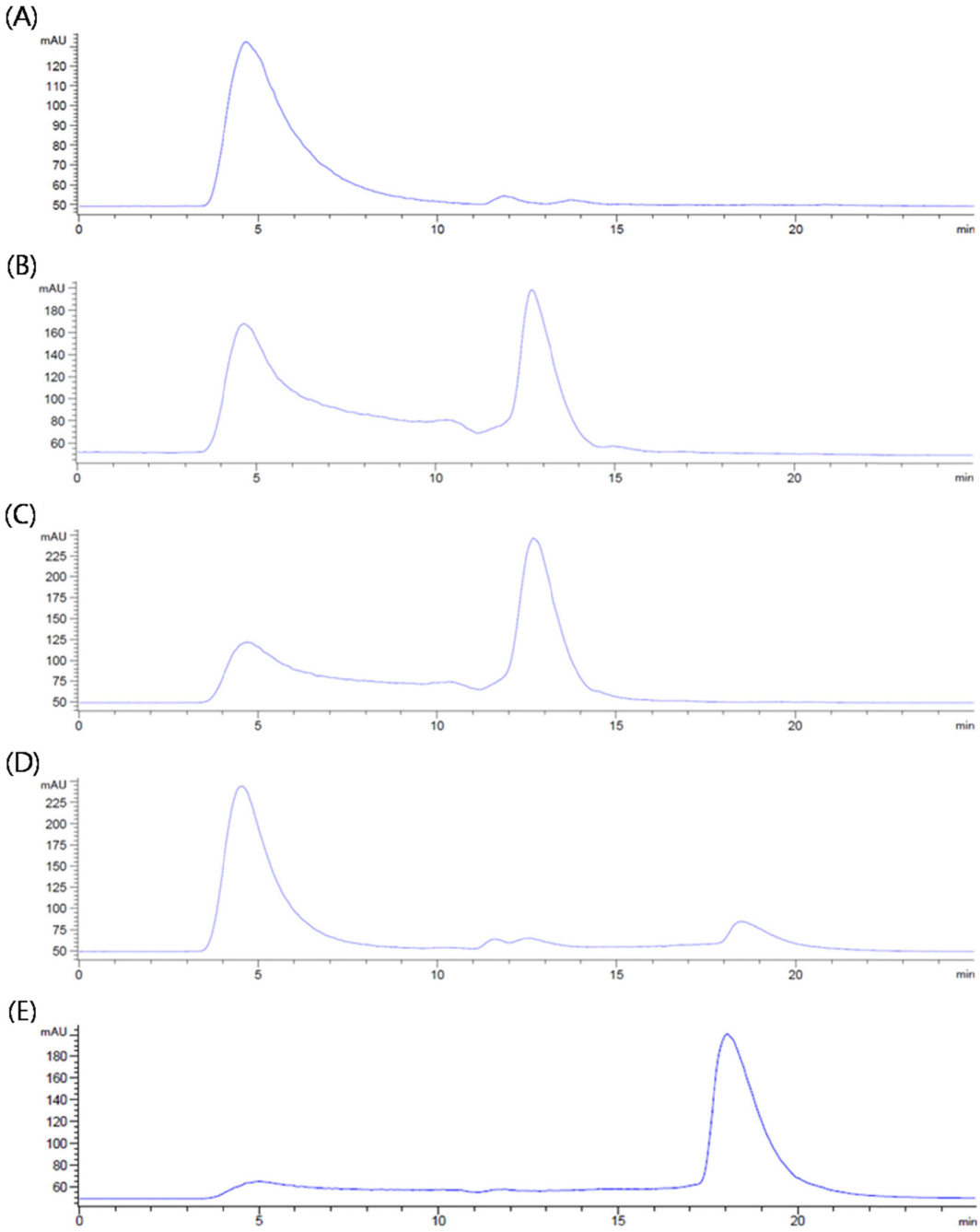

Fig. 2. The Reverse-Phase HPLC Profiles of ^{99m}Tc-tricarbonyl precursor (A), ^{99m}Tc-tricarbonyl AGRGDS (B), ^{99m}Tc-tricarbonyl GGGAGRGDS (C), ^{99m}Tc-tricarbonyl RRPYIL (D), and ^{99m}Tc tricarbonyl GGGRRPYIL (E). HPLC conditions: Mobile phase - gradient system based on 0.05 M TEAP buffer and 100% MeOH; Column -NucleosilC-18 column (3.0 × 250 mm); Flow rate −0.6 ml/min.

The ^{99m}Tc-tricarbonyl glycinetrimer had fast blood clearances and showed most activity in the bladder and some activity in the kidneys. Both ^{99m}Tc-tricarbonyl glycine and pentaglycine also showed fast blood clearance, but some radioactivity was retained in the liver. From these imaging studies, all three complexes of ^{99m}Tc-tricarbonyl glycines were cleared rapidly from the body by the renal excretion, particularly ^{99m}Tc tricarbonyl-triglycine.

Radiolabeling of Gly(3) added biomolecules with ^{99m}Tc-tricarbonyl precursor. The labeling yields of ^{99m}Tc-tricarbonyl GGGRRPYIL was higher than 95%, whereas that of ^{99m}Tc-tricarbonyl RRPYIL was less than 10% at 18 min. Also, ^{99m}Tc-tricarbonyl GGGAGRGDS showed a higher labeling yield than ^{99m}Tc-tricarbonyl AGRGDS at 13 min. Typical chromatogram of ^{99m}Tc-tricarbonyl precursor and ^{99m}Tc-tricarbonyl-Glys are shown in Fig. 1. Glycine

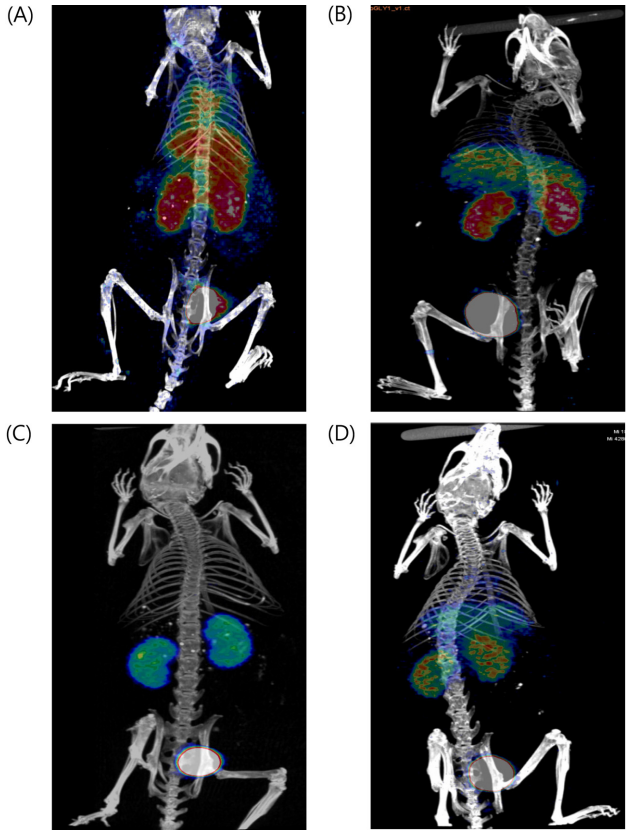

Fig. 3. The SPECT/CT images of ICR mice administered with of ^{99m}Tc-tricarbonyl precursor (A), ^{99m}Tc-tricarbonyl Gly(1) (B), ^{99m}Tc-tricarbonyl Gly(3) (C), and ^{99m}Tc-tricarbonyl Gly(5) (D). Imaging instrument: Inveon SPECT/CT system (Siemens Medical Solutions) equipped with 1MHS pinhole collimator. Images were obtained at 30 minutes after 37 MBq of radiolabeled compound injection intravenously. The condition of CT scan was set as 300 microampere at 60 kilovolts for X-ray source.

and glycine oligomers were simply labeled with a ^{99m}Tc-tricarbonyl precursor with a high labeling yield.

DISCUSSION

The synthesis of peptide metalloconjugation has an important role in providing radiolabelled probes for molecular imaging and therapy (Egli *et al.*, 1999; Psimadas *et al.*, 2012). Modification of native amino acid side chains with bifunctionalchelators was commonly used. This non-site-specific approach can lead to heterogeneously labeled products with a distribution of modified sites on the peptide, including the molecular recognition site. In attempt to overcome this undesirable conjugation, currently single amino acid chelate (SAAC) systems for the incorporation of the ^{99m}Tc-tricarbonyl-based radiopharmaceuticals have been successfully established into novel synthetic strategies (Maresca *et al.*, 2010). During the past decade, the field of technetium coordination chemistry has seen a rising interest in the ^{99m}Tc-tricarbonyl core (Schibli *et al.*, 1998; Schibli *et al.*, 2001). But the hydrophobicity of the metal complexes resulted in poor pharmacokinetic profile. From the classic renal functional radiopharmaceutical, ^{99m}Tc-mercaptoacetyl-Gly(3) (MAG_3), the strategy of conjugation of MAG_3 to the biomolecules has been used for biomolecules' imaging such as antibodies, affibodies, anti-sense DNA, and so on (Zhang *et al.*, 2000; Vanderheyden *et al.*, 2006; Liu *et al.*, 2006; Wang *et al.*, 2007).

In this study, the ^{99m}Tc-tricarbonyl labeled glycine monomer and oligomers was evaluated and addition of Gly(3) ending sequence to the biomolecules was investigated as a feasibility study. The preparation of ^{99m}Tc-tricarbonyl-glycine oligomers was well established. Gly(3) was showed that could be radiolabeled with a ^{99m}Tc-tricarbonyl precursorwith radiochemical analysis. Also ^{99m}Tc-tricarbonyl-Gly(3) showed a good clearance property *in vivo*. Furthermore, ^{99m}Tc-tricarbonyl-Gly(3) revealed hydrophilic property in the lipophilicity test that may contribute to a rapid excretion from the body when released from a biomolecule. From the nuclear image of ^{99m}Tc-tricarbonyl-Gly(3) in normal mice, this rapid excretion property was observed with our prediction based on the lipophilicity test.

Since Gly(3) can be added to the peptide sequence of biomoleculesit can be used in the same manner as MAG_3. Gly(3) added biomolecule (GGGAGRGDS and GGGRRPYIL) showed enhanced radiolabeling yield in comparison with unmodified one (AGTGDS and RRPYIL) as from 12 to 95% in case of GGGRRPYIL.

In conclusion, a facile preparation of ^{99m}Tc-tricarbonyl glycine oligomersand Gly(3) modification of biomolecule was successfully established. The ^{99m}Tc-tricarbonyl Gly(3) described here shows a rapid renal excretion without retention in other organs, furthermore the addition of Gly(3) to peptides showed an improved radiolabeling yield. The small animal nuclear imaging study could be useful to investigate the biodistribution of the biomolecules in living animal. Gly(3) modified peptides with the rapid whole-body clearance pharmacokinetics warrant further studies on peptide-based biomolecule imaging.

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