Labeling of Bifunctional Chelating Agent, MAG3, with Carrier-free ^{188}Re

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The labeling of MAG3 with carrier-free ^{188}Re from the $^{188}\text{W}/^{188}\text{Re}$ generator was investigated by the direct Sn reduction (solid-phase synthesis) and the indirect labeling method using citrate or gluconate as a transfer ligand. Optimum pH range were 12.1–12.6 for the direct Sn reduction, 2–5 for the use of citrate and 2.6–3 for the use of gluconate. Although the differences of the optimum conditions such as pH and reaction time at room temperature were observed by using a different transfer ligand, the labeling yield of ^{188}Re -MAG3 synthesized by the all methods was over 90% under the optimum conditions. The solid-phase synthesis requires the operation under a stream of nitrogen gas and the evaporation of solvent. On the other hand, the method using a transfer ligand is one-pot preparation by just heating a reaction mixture. Thus, judging from the ease of operations, the method using a transfer ligand is more convenient.

1. Introduction

The radioisotopes of rhenium (^{186}Re and ^{188}Re) are attractive radionuclides for radiotherapy because of their energetic beta particles and gamma rays suitable for imaging.^{1–3} Especially, rhenium-188 has an advantage of easy availability, because ^{188}Re can be obtained in a carrier-free level from a $^{188}\text{W}/^{188}\text{Re}$ generator. Oxorhenium(V) and oxotechnetium(V) complexes with quadridentate ligands such as N_3S and N_2S_2 ligands are often used as radiopharmaceuticals.^{4,5} Among them, mercaptoacetyltriglycine, MAG3 (N_3S ligand, Figure 1) labeled with $^{99\text{m}}\text{Tc}$ is widely used as a renal imaging agent. Furthermore, MAG3 is a useful bifunctional ligand in labeling biomolecules (such as monoclonal antibodies^{6,7} and peptides⁸) with $^{99\text{m}}\text{Tc}$ for radioimmunoscinigraphy of cancer and with $^{186,188}\text{Re}$ for radioimmunotherapy of cancer.

In this work, the labeling of MAG3 with carrier-free ^{188}Re from a $^{188}\text{W}/^{188}\text{Re}$ generator was investigated in detail. The ^{188}Re -MAG3 complex was synthesized by the direct Sn reduction (solid-phase synthesis)⁶ and by the indirect labeling method using a transfer ligand (citrate⁹ or gluconate). The dependence of the labeling yield upon the reaction conditions such as the concentrations of tin(II) chloride dihydrate as a reducing agent, S-benzoyl MAG3 and the transfer ligand, pH, temperature, reaction time and the addition of a carrier (final concentration: 20 μg Re/mL) was examined.

Figure 1. Chemical structure of MAG3.

2. Experimental

2.1. Production of $^{188}\text{W}/^{188}\text{Re}$ generator. Typically 25–35 mg of enriched ^{186}W as WO_3 (99.79% enrichment: Euriso-top, France) was irradiated for 26–52 days in JAERI JMTR (a thermal neutron flux of $2.7 \times 10^{14} \text{ n cm}^{-2} \text{ s}^{-1}$).⁹ The irradiated target was allowed to stand for more than 4 weeks for ^{187}W decay. The $^{188}\text{W}/^{188}\text{Re}$ generator was prepared by the alumina column system.¹⁰ The irradiated WO_3 was dissolved in 2 M NaOH. The pH of the ^{188}W solution was adjusted to about 2 using HCl. This solution was passed through the alumina column (10 $\times$ 60 mm; BIO-RAD, AG-4, 100–200 mesh), which was conditioned with 0.01 M HCl. The column was then washed with normal saline. Rhenium-188 was eluted with normal saline after the equilibrium between ^{188}W and ^{188}Re had almost reached. Rhenium-188 solution (0.2–3 MBq/mL) was obtained from the generator in a saline solution and was used for labeling purposes without further purification.

2.2. Synthesis of ^{188}Re -MAG3 complex. S-Benzoyl-MAG3 (S-Bz-MAG3) was supplied from Center for Development of Radioisotopes and Radiopharmaceuticals, BATAN (the National Nuclear Energy Agency, Indonesia). S-Triphenylmethyl-MAG3 (S-Tr-MAG3) was a gift from Prof. Arano, Chiba University. All other chemicals used were of guaranteed reagent grade.

(a) Direct Sn reduction (solid-phase synthesis)⁶

To 150 μL of 1 M Na_2CO_3 , 500 μL of a ^{188}Re solution from the generator, 150 μL of a freshly prepared Na_2SO_3 solution (100 mg/mL), 75 μL of HCl or NaOH solution for pH adjustment, 25 μL of S-Bz-MAG3 (2 mg/mL acetonitrile/ H_2O , 9:1) and 100 μL of a freshly prepared 2 mg/mL tin(II) chloride dihydrate aqueous solution were added. The reaction mixture in a closed vial was heated in boiling water for 10 min and evaporated to dryness under a stream of N_2 gas. The mixture was further heated for another 15 min. After cooling on ice, the mixture was reconstituted with 500 μL of water. The pH was brought in 5.7 to 6.3 with 490 μL of 1 N H_2SO_4 and 25–70 μL of 1 M Na_2CO_3 , and the solution was filtered through a 0.22 μm filter. Radiochemical yields of ^{188}Re -MAG3 were determined by HPLC. Free MAG3 was also used instead of S-Bz-MAG3. Free MAG3 was prepared from S-Tr-MAG3 by treating with trifluoroacetic acid and triethylsilane just before radiolabeling.

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(b) Citrate as a transfer ligand⁸

To 0.55 mg of S-Bz-MAG3, 450 μ L of solution for pH adjustment (0.1–0.6 M HCl, 0.2 M NaOH, 0.2 M acetic acid and/or 0.2 M sodium acetate) and 450 μ L of a freshly prepared $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 0.1 M citrate-buffer (pH = 5) were added. The reaction mixture was vigorously stirred by ultrasonic waves and 300 μ L of a ^{188}Re solution from the generator was added. The final concentration of Re varied from carrier-free levels to 20 $\mu\text{g Re/mL}$ (1.07×10^{-4} M) by adding NH_4ReO_4 to the ^{188}Re solution. After stirring the solution by vortex, the mixture in a closed vial was allowed to react in boiling water or at room temperature for 1 h. The mixture was cooled on ice for 5–10 min. After the solution was filtered through a 0.22 μm filter, radiochemical yields of $^{188}\text{Re-MAG3}$ were determined by HPLC.

The labeling yield of $^{188}\text{Re-citrate}$ was determined by silica gel TLC (Merck No. 5735/acetone) and silica gel ITLC (Gelman Sciences/0.9% NaCl) as well as $^{99\text{m}}\text{Tc-gluconate}$.¹¹ The distribution of ^{188}Re in TLC and ITLC was measured with a radioanalytic imaging system (AMBIS-100).

(c) Gluconate as a transfer ligand

To 0.55 mg of S-Bz-MAG3, 450 μ L of solution for pH adjustment (0.0–10.1 M HCl, 0.01–0.2 M NaOH and/or 0.2 M sodium acetate), 225 μ L of sodium gluconate aqueous solution and 225 μ L of a freshly prepared $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 0.1 M HCl were added. The reaction mixture was vigorously stirred by ultrasonic waves and 300 μ L of a ^{188}Re solution from the generator was added. The final concentration of Re varied from carrier-free levels to 20 $\mu\text{g Re/mL}$ by adding NH_4ReO_4 to the ^{188}Re solution. The mixture in a closed vial was allowed to react in boiling water or at room temperature for 1 h. The white precipitation was occurred at pH < 11. After the pH of the solution was brought to about 12 by adding NaOH, the precipitation was dissolved. Therefore, the same volume of 0.1 M NaOH was added to the reaction mixture just before the HPLC analysis when the precipitation had occurred. Radiochemical yields of $^{188}\text{Re-MAG3}$ were determined by HPLC.

2.3. HPLC analysis. The liquid chromatograph used was a Waters 2690 separations module equipped with a Waters 996 photodiode array detector and a radio-HPLC detector (Packard Radiomatic 515TR). The column was kept at 25 $^{\circ}\text{C}$. Radiochemical yield of $^{188}\text{Re-MAG3}$ was determined by reversed phase HPLC (Hypersil BDS-5C18, 4.6 $\times$ 150 mm, Chemco Science Co., Japan) using 4% EtOH - 0.01 M phosphate buffer (pH = 7). The flow rate was 1.0 mL/min. Typical chromatograms are shown in Figure 2. Retention times of $^{188}\text{ReO}_4^-$ and $^{188}\text{Re-MAG3}$ were 2.4 min and 3.7–3.9 min, respectively.

2.4. Stability studies of $^{188}\text{Re-MAG3}$ complex. The pH of $^{188}\text{Re-MAG3}$ solution (100 μ L) was changed to higher values (pH 2–14) by adding 400 μ L of HCl, NaOH and/or sodium

Figure 2. Typical chromatograms of carrier-free $^{188}\text{Re-MAG3}$ synthesized by using citrate at different pH.

acetate solution for pH adjustment. At regular intervals (1 hour to 70 hours), the radiochemical yield of $^{188}\text{Re-MAG3}$ was determined by HPLC as mentioned earlier.

3. Results and Discussion

3.1. Direct Sn reduction (solid-phase synthesis). By just heating the reaction mixture, no formation of $^{188}\text{Re-MAG3}$ was observed. When the solution was evaporated at 100 $^{\circ}\text{C}$ under a stream of N_2 until dry and heating continued, $^{188}\text{Re-MAG3}$ formation occurred. This phenomenon would be due to increase of concentration of the reagents (SnCl_2 , MAG3) during the evaporation process.

The yield of $^{188}\text{Re-MAG3}$ increased with the concentration of tin chloride and reached a constant value at 2 mg/mL of tin(II) chloride dehydrate in the initial solution. The concentration of tin chloride was fixed at 2 mg/mL in this work (0.2 mg/mL in the initial reaction mixture), however the concentration in the literature⁶ using ^{186}Re was 1 mg/mL of tin(II) chloride dehydrate solution.

The yields of $^{188}\text{Re-MAG3}$ were 83% and 89% at pH 12.5, when 1 mg/mL and 2 mg/mL of S-Bz-MAG3 solution were used respectively. The concentration of S-Bz-MAG3 was fixed at 2 mg/mL in this work (0.05 mg/mL in the initial reaction mixture), however the concentration in the literature⁶ was 1 mg/mL of S-Bz-MAG3 solution.

The dependence of the labeling yield of $^{188}\text{Re-MAG3}$ on pH in the initial reaction mixture was shown in Figure 3. The yield was more than 90% in the pH range 12.1–12.6. Our results are somewhat different from the results in the literature⁶ that the optimum pH was 11.7. The formation of $^{188}\text{Re-MAG3}$ by the direct Sn reduction consists of some reactions such as the deprotection of benzoyl-group from S-Bz-MAG3, the reduction of Re(VII) to Re(V) by tin chloride and the reaction of MAG3 and Re(V). The pH dependence of the yield of $^{188}\text{Re-MAG3}$ may reflect individual pH dependences in these reactions. The yield of $^{188}\text{Re-MAG3}$ has the maximum value in the alkaline pH region, as shown in Figure 3. Generally, the deprotection of benzoyl-group from S-Bz-MAG3 is carried out in NaOH solution. If the deprotection process is important for determination of the pH dependence of the $^{188}\text{Re-MAG3}$ yield, the pH dependence will be changed by using free MAG3 instead of S-Bz-MAG3. As shown in Figure 3, the pH dependence using free MAG3 produced from S-Tr-MAG3 was almost the same as that using S-Bz-MAG3. Thus, the deprotection of benzoyl group is less important for determination of the pH dependence of the $^{188}\text{Re-MAG3}$ yield.

Figure 3. Influence of pH on the labeling yield of carrier-free $^{188}\text{Re-MAG3}$ prepared by the solid-phase synthesis.

3.2. Indirect labeling method using a transfer ligand.

3.2.1. Effect of the concentration of S-Bz-MAG3. Citrate: The influence of S-Bz-MAG3 concentration (0.45–1.67 mg/mL) in the reaction mixture was studied at pH 1.7–1.8, $[\text{SnCl}_2 \cdot 2\text{H}_2\text{O}] = 2.25 \text{ mg/mL}$. The labeling yield of ^{188}Re -MAG3 was almost constant (about 90%) for the carrier-free ^{188}Re . However, the yield increased with the concentration of S-Bz-MAG3 and reached a constant value (about 90%) at 1.3 mg/mL for the carrier-added ^{188}Re (20 $\mu\text{g Re/mL}$). Higher concentration of S-Bz-MAG3 was required to obtain more than 90% of the yield for the carrier-added ^{188}Re .

Gluconate: The influence of S-Bz-MAG3 concentration (0.11–1.66 mg/mL) in the reaction mixture was studied at pH 3, $[\text{SnCl}_2 \cdot 2\text{H}_2\text{O}] = 2.25 \text{ mg/mL}$. The labeling yield of ^{188}Re -MAG3 was almost constant (about 90%) for the carrier-free ^{188}Re . However, the yield increased with the concentration of S-Bz-MAG3 and reached a constant value (about 90%) at 0.8 mg/mL for the carrier-added ^{188}Re .

The results indicated almost the same tendency for citrate and gluconate.

3.2.2. Effect of the concentration of tin chloride. Citrate: The influence of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ concentration (0.38–4.5 mg/mL) in the reaction mixture was studied at pH 1.6–1.9, $[\text{S-Bz-MAG3}] = 0.45 \text{ mg/mL}$. The labeling yield of ^{188}Re -MAG3 was about 90% for the carrier-free ^{188}Re and about 80% for the carrier-added ^{188}Re not less than 1.1 mg/mL ($4.9 \times 10^{-3} \text{ M}$). The yield decreased below 0.38 mg/mL ($1.7 \times 10^{-3} \text{ M}$).

Gluconate: The influence of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ concentration (0.59–9.0 mg/mL) in the reaction mixture was studied at pH 2.8–3.1, $[\text{S-Bz-MAG3}] = 0.46 \text{ mg/mL}$. The labeling yield of ^{188}Re -MAG3 was about 90% in the range 1–3 mg/mL for both the carrier-free and the carrier-added ^{188}Re . A precipitate was formed and the yield decreased more than 3 mg/mL. At pH 12, the yield increased (36–38% at 1.1 mg/mL of SnCl_2 , 70–72% at 9.0 mg/mL of SnCl_2) with the concentration of tin chloride for both the carrier-free and the carrier-added ^{188}Re .

3.2.3. Effect of pH. Citrate: The influence of pH on the labeling yield was investigated at $[\text{SnCl}_2 \cdot 2\text{H}_2\text{O}] = 2 \text{ mg/mL}$, $[\text{S-Bz-MAG3}] = 0.45 \text{ mg/mL}$ for the carrier-free ^{188}Re , 1.67 mg/mL for the carrier-added ^{188}Re , as shown in Figure 4. The maximum labeling yield (more than 90%) was obtained in the pH range 2–5 and the yield decreased sharply above pH 5.

Gluconate: The influence of pH on the labeling yield was investigated at $[\text{SnCl}_2 \cdot 2\text{H}_2\text{O}] = 2 \text{ mg/mL}$, $[\text{S-Bz-MAG3}] = 0.46 \text{ mg/mL}$, as shown in Figure 5. The maximum labeling yield (more than 90%) was obtained in the pH range 2.6–3 and the

Figure 4. Influence of pH on the labeling yield of ^{188}Re -MAG3 prepared by using citrate as a transfer ligand ($[\text{SnCl}_2 \cdot 2\text{H}_2\text{O}] = 2 \text{ mg/mL}$, $[\text{S-Bz-MAG3}] = 0.45 \text{ mg/mL}$ for the carrier-free ^{188}Re , 1.67 mg/mL for the carrier-added ^{188}Re).

yield decreased sharply above pH 3. In the alkaline region (pH 10–13), the labeling yield using gluconate was higher than that using citrate. Furthermore, the labeling yield using gluconate increased with the concentrations of tin chloride (as described above) and gluconate (as described later).

The pH dependence of the yield of ^{188}Re -MAG3 by the indirect labeling method may reflect some factors such as the deprotection of benzoyl-group from S-Bz-MAG3, the reduction of Re(VII) to Re(V) by tin chloride, the reaction of MAG3 and Re(V), the formation of Re(V)-X and the reaction of MAG3 and Re(V)-X (X = transfer ligand). The pH dependence of the ^{188}Re -MAG3 labeling yield was influenced by a transfer ligand as mentioned above. And, it was reported that the optimum pH was 5–6 for ^{188}Re -MAG3 prepared by using sodium potassium tartrate as a transfer ligand.¹² It was different from our results using citrate or gluconate. Thus, the reactions in which a transfer ligand participates such as the formation of Re(V)-X and the reaction of MAG3 and Re(V)-X would be important for determination of the pH dependence. Furthermore, it would support the above induction that the pH dependence (such as the optimum pH range) of the labeling yield of $^{186,188}\text{Re}$ -citrate¹³ as shown in Figure 6 was similar to that of ^{188}Re -MAG3 prepared by using citrate (Figure 4).

Figure 5. Influence of pH on the labeling yield of ^{188}Re -MAG3 prepared by using gluconate as a transfer ligand ($[\text{SnCl}_2 \cdot 2\text{H}_2\text{O}] = 2 \text{ mg/mL}$, $[\text{S-Bz-MAG3}] = 0.46 \text{ mg/mL}$).

Figure 6. Influence of pH on the labeling yield of $^{186,188}\text{Re}$ -citrate (○: This work (cf. ^{188}Re , $[\text{SnCl}_2 \cdot 2\text{H}_2\text{O}] = 2.25 \text{ mg/mL}$, $[\text{citrate}] = 0.038 \text{ M}$), ●: R. Konířová et al. (^{186}Re)¹³).

Figure 7. Influence of the concentration of gluconate on the labeling yield of ^{188}Re -MAG3 prepared by using gluconate as a transfer ligand ($[\text{SnCl}_2 \cdot 2\text{H}_2\text{O}] = 2.25 \text{ mg/mL}$, $[\text{S-Bz-MAG3}] = 0.46 \text{ mg/mL}$).

3.2.4. Effect of the concentration of a transfer ligand. Citrate: The influence of citrate concentration (7.9 and 39 mg/mL, 0.038 and 0.19 M) was studied at pH 5.6, 7.4, 13–14, $[\text{SnCl}_2 \cdot 2\text{H}_2\text{O}] = 2.25 \text{ mg/mL}$, $[\text{S-Bz-MAG3}] = 0.45 \text{ mg/mL}$. The labeling yield using 0.19 M citrate was a little higher (5–10 points) than that using 0.038 M citrate at all pH.

Gluconate: The influence of gluconate concentration (2.0–49.1 mg/mL, 0.0094–0.225 M) was studied at pH 3 and 12, $[\text{SnCl}_2 \cdot 2\text{H}_2\text{O}] = 2.25 \text{ mg/mL}$, $[\text{S-Bz-MAG3}] = 0.46 \text{ mg/mL}$, as shown in Figure 7. The labeling yield at pH 3 indicated a maximum at 8.2 mg/mL of gluconate. The labeling yield at pH 12 increased up to 85% with the concentration of gluconate for both the carrier-free and the carrier-added ^{188}Re .

3.2.5. Effect of reaction time and temperature. At the optimum pH (2.4–2.5 for citrate and 2.8–3.0 for gluconate), the labeling yield was more than 90% for 15 min in boiling water for the carrier-free ^{188}Re . For the carrier-added ^{188}Re , the reaction time in boiling water was required 60 min for citrate and 30 min for gluconate to obtain more than 90% of the yield. The labeling yield prepared at room temperature was lower than that in boiling water for both citrate and gluconate. The labeling yield using gluconate at pH 3 for 30 min at room temperature was more than 88% for the carrier-free ^{188}Re , however the labeling yield using citrate was lower than 15% under the same conditions.

3.3. Stability of ^{188}Re -MAG3. The influence of pH on the stability of the ^{188}Re -MAG3 prepared by using citrate or gluconate under the optimum conditions was investigated. The pH of ^{188}Re -MAG3 was changed to a higher value (pH 2–14). The decomposition of ^{188}Re -MAG3 prepared by using citrate was observed only when the pH was over 12, as shown in Figure 8. The survival $\{ = (\text{the amount after the pH change} / \text{the initial amount}) \times 100 \}$ of carrier-free ^{188}Re -MAG3 was over 97% at pH 6.7 and 94% at pH 11.3 even after 70 hours. The same results were obtained for ^{188}Re -MAG3 prepared by using gluconate. Furthermore, there was no difference in stability between carrier-free ^{188}Re -MAG3 and carrier-added one.

4. Conclusion

The labeling of MAG3 with carrier-free ^{188}Re from the $^{188}\text{W}/^{188}\text{Re}$ generator was investigated. The labeling yield of ^{188}Re -MAG3 synthesized by the direct Sn reduction (solid-phase synthesis) and the indirect labeling method using citrate or gluconate as a transfer ligand was over 90% under the optimum

Figure 8. Stability of carrier-free ^{188}Re -MAG3 prepared by using citrate.

conditions. The solid-phase synthesis requires the operation under a stream of nitrogen gas and the evaporation of solvent. On the other hand, the method using a transfer ligand is one-pot preparation by just heating a reaction mixture. Thus, judging from the ease of operations, the method using a transfer ligand is more convenient. However, the solid-phase synthesis required the smaller amounts of the reagents (S-Bz-MAG3: 0.05 mg per 1 mL of the reaction mixture, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$: 0.2 mg) than the method using a transfer ligand (S-Bz-MAG3: 0.5 mg per 1 mL of the reaction mixture, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$: 1–2 mg) because the concentrations of the reagents in the solid-phase synthesis increased gradually during the evaporation process. The effect of pH on the labeling yield of ^{188}Re -MAG3 was influenced by the difference of transfer ligands. Citrate works effectively in the acid pH region and gluconate dose in the alkaline pH region.

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