Stabilization of ^{90}Y-Labeled DOTA-Biomolecule Conjugates Using Gentisic Acid and Ascorbic Acid

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Radiolytic degradation of radiolabeled compounds is a major challenge for the development of new therapeutic radiopharmaceuticals. The goal of this study is to explore the factors influencing the solution stability of a ^{90}Y-labeled DOTA-peptide conjugate (RP697), including the amount of total activity, the activity concentration, the stabilizer concentration, and the storage temperature. In general, the rate of radiolytic decomposition of RP697 is much slower at the lower activity concentration (<4 mCi/mL) than that at the higher concentration (>10 mCi/mL). RP697 remains relatively stable at the 20 mCi level and room temperature while it decomposes rapidly at the 100 mCi level under the same storage conditions. Radical scavengers, such as gentisic acid (GA) and ascorbic acid (AA), were used in combination with the low temperature (−78 °C) to prevent the radiolytic decomposition of RP697. It was found that RP697 remains stable for at least 2 half-lives of ^{90}Y when GA or AA (10 mg for 20 mCi of ^{90}Y) is used as a stabilizer when the radiopharmaceutical composition is stored at −78 °C. The stabilizer (GA and AA) can be added into the formulation either before or after radiolabeling. The post-labeling approach is particularly useful when the use of a large amount of the stabilizer interferes with the radiolabeling. The radiopharmaceutical composition developed in this study can also apply to other ^{90}Y-labeled DOTA-biomolecule conjugates. The amount of the stabilizer used in the radiopharmaceutical composition and storage temperature should be adjusted according to the sensitivity of the radiolabeled DOTA-biomolecule conjugate toward radiolytic decomposition.

INTRODUCTION

We have been interested in the development of new diagnostic and therapeutic radiopharmaceuticals based on small-molecule receptor ligands (*1*−*18*). In our previous contribution (*19*), we reported the synthesis of a DOTA-conjugated vitronectin receptor antagonist (SU015: 2-(1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)-1-cyclododecyl)-acetyl-Glu(cyclo{Lys-Arg-Gly-Asp-D-Phe})-cyclo{Lys-Arg-Gly-Asp-D-Phe}) and its ^{90}Y complex (RP697 in Figure 1). Through a series of radiolabeling experiments, we developed a formulation for routine preparations of RP697 in high yield with the radiochemical purity (RCP) of > 95%. However, RP697 prepared using this formulation was not stable over time at room temperature, particularly at high activity levels.

^{90}Y is an β-emitter with a 2.27 MeV β-particle and a 2.67 day half-life. Due to the high energy of the β-particle, the radiolabeled cyclic peptide is very susceptible to radiolytic decomposition. Since the tumor uptake of RP697 is largely dependent on the receptor binding of the two cyclic RGD-containing peptide motifs, radiolytic decomposition may lead to the decreased therapeutic efficacy and unwanted radiation toxicity to other normal organs such as liver and bone marrow. The mechanism for radiolytic decomposition is thought to be mediated by the formation of free radicals, such as superoxide ($^{\bullet}O_2$) and hydroxyl radicals ($^{\bullet}$OH), in the presence of a large amount of high-energy β-particles (*20*). Radical scavengers such as human serum albumin (HAS), gentisic acid (GA), and ascorbic acid (AA) have been used as stabilizers for the radiolabeled antibodies (*21*−*23*). It was also found that freezing the antibody after radiolabeling up to the time of administration could considerably improve the immunoreactivity by decreasing the diffusion and interaction of free radicals with the radiolabeled biomolecule (*22*).

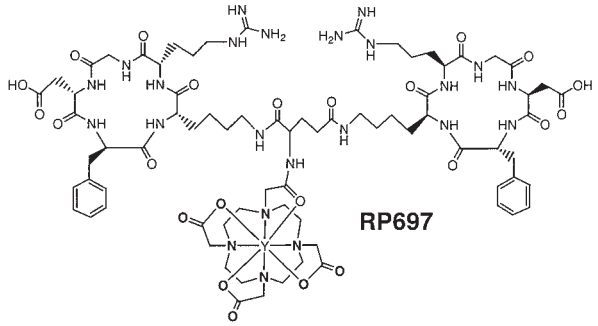

Figure 1. Structure of RP697.

The purpose of this study is to explore the factors influencing the solution stability of RP697, including the activity concentration, the total activity level, the stabilizer concentration, and the storage temperature. We have chosen AA and GA as stabilizers since they have been approved for pharmaceutical or radiopharmaceutical applications. The ultimate goal is to find the optimized radiopharmaceutical composition to maintain the solution stability of RP697. In principle, the radiopharmaceutical composition developed in this study should also apply to other ^{90}Y-labeled DOTA-biomolecule conjugates.

EXPERIMENTAL PROCEDURES

Materials. Acetic acid (ultrapure), ammonium hydroxide (ultrapure), ascorbic acid (sodium salt), diethylene-

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triaminepentaacetic acid (DTPA), and gentisic acid (sodium salt) were purchased from either Aldrich or Sigma Chemical Co., and were used as received. $^{90}YCl_3$ (in 0.05 N HCl) was purchased from New England Nuclear Life Sciences, North Billerica, MA. The cyclic pentapeptide cyclo(Arg-Gly-Asp-D-Phe-Lys), as its trifluoroacetic acid (TFA) salt, was prepared according to the literature method (*24*). Synthesis of SU015, 2-(1,4,7,10-tetraaza-4,7,10-tris(carboxymethyl)-1-cyclododecyl)-acetyl-Glu(cyclo-{Lys-Arg-Gly-Asp-D-Phe})-cyclo{Lys-Arg-Gly-Asp-D-Phe}), as its trifluoroacetic acid (TFA) salt will be described in a companion paper (*19*).

General Procedure for the Synthesis of RP697. To a shielded clean 5 mL vial containing 100 μg of SU015 and 0−10 mg of sodium gentisate (GA) or sodium ascorbate (AA) dissolved in 0.5 mL of 0.5 M ammonium acetate buffer (pH 8.0) was added 20−40 μL of $^{90}YCl_3$ stock solution (~20 mCi) in 0.05 N HCl. The reaction mixture was heated at 95−100 °C for 5 min. A sample of the resulting solution was diluted 20-fold with 2 mM DTPA solution (pH 5), and then analyzed by radio-HPLC and ITLC. Each condition was run twice, and the radiochemical purity (RCP) data are presented as an average of two independent measurements.

Analytical Methods. The HPLC method used a HP-1100 HPLC system with a UV/visible detector (λ = 220 nm), an IN-US radio-detector, and a Zorbax C_{18} column (4.6 mm × 250 mm, 80 Å pore size). The flow rate was 1 mL/min. The mobile phase was isocratic from 0 to 18 min using 87% solvent A (0.025 M ammonium acetate buffer, pH 6.8) and 13% solvent B (acetonitrile), followed by an isocratic wash using 40% solvent A and 60% solvent B from 19 to 25 min. The retention time of RP697 is 14−16 min. The ITLC method used Gelman Sciences silica gel ITLC paper strips and a 1:1 mixture of acetone and saline as eluant. By this method, RP697 migrates to the solvent front while [^{90}Y]colloid and [^{90}Y]acetate remain at the origin. The corrected RCP for RP697 was calculated by subtracting the percentage of [^{90}Y]colloid and [^{90}Y]acetate obtained by ITLC from that obtained by radio-HPLC.

RESULTS

Room Temperature Stability of RP697 at Low Concentration (4 mCi/mL). In the first experiment, we prepared three RP697 vials according to the standard procedure. The first vial contains only 100 μg of SU015, the second vial contains 100 μg of SU015 and 5 mg of GA, and the third vial contains 100 μg of SU015 and 5 mg of sodium ascorbate (AA). After addition of 20 mCi of $^{90}YCl_3$ in each vial, the reaction mixtures were heated at 100 °C for 5 min. After radiolabeling, the resulting solution was diluted to a concentration of 4 mCi/mL with saline, and the solution stability of RP697 at room temperature was monitored by radio-HPLC over 6 days. Figure 2 shows a typical radio-HPLC chromatogram for RP697. The peak at 14 min is from RP697. The peak at ~3.5 min is probably from a combination of several hydrophilic ^{90}Y-containing species while the peak at 24 min is from more lipophilic ^{90}Y-containing species. Since most of the radioimpurities are less than 1%, no efforts were made for further characterization. Figure 3 shows the RCP change over time for RP697. It is clear that RP697 remains relatively stable (RCP = 96.5% at 0 h and 94% at 144 h post-labeling) in the presence of GA and AA while it decomposes rapidly in the absence of GA and RCP is only 86% at 6 days post-labeling.

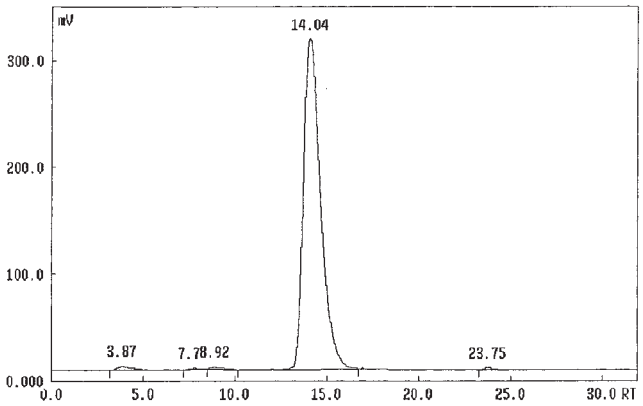

Figure 2. Typical radio-HPLC chromatogram of RP697.

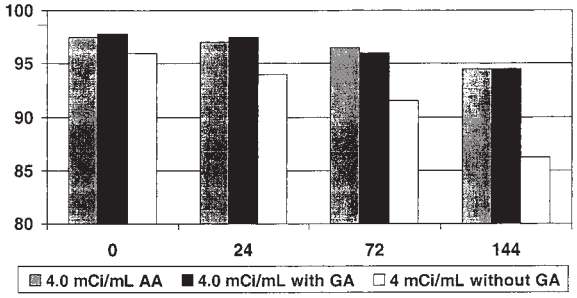

Figure 3. Effect of stabilizers on solution stability of RP697 at room temperature. Each condition was run twice, and the radiochemical purity (RCP) data are presented as an average of two independent measurements.

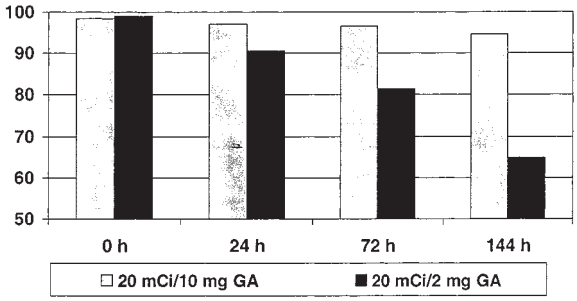

Figure 4. Effect of GA concentration on solution stability of RP697 at room temperature. Each condition was run twice, and the radiochemical purity (RCP) data are presented as an average of two independent measurements.

procedure using two levels (2 and 10 mg) of GA and 100 μg of SU015 for 20 mCi of $^{90}YCl_3$. The total volume was 0.5 mL, and the activity concentration was 40 mCi/mL. After radiolabeling, the resulting solution was kept at room temperature, and the solution stability of RP697 was monitored by radio-HPLC over 6 days. Figure 4 shows the RCP change over 6 days for RP697. The RCP of RP697 decreased from 96.5% at 0 h to 93% at 144 h post-labeling when 10 mg of GA was used for 20 mCi of activity. The RCP dropped much more rapidly from 97% at 0 h to 65% at 144 h when only 2 mg of GA was used to stabilize 20 mCi of RP697. This suggests that 2 mg of GA is not sufficient to maintain RCP above 90% and 10 mg of GA is needed to stabilize RP697 over 2 half-lives of ^{90}Y. Compared to the results from the previous experiment, it seems that the rate of decomposition of RP697 is also dependent on the activity concentration.

Activity Level and Solution Stability of RP697. In this experiment, we prepared two RP697 vials using

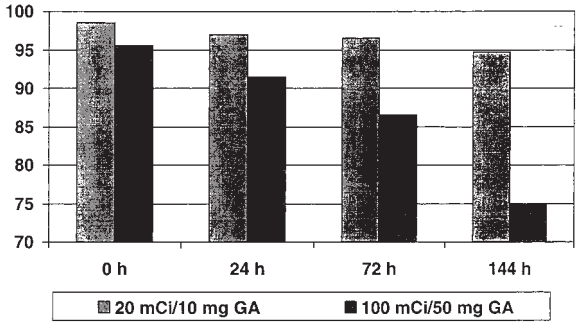

Figure 5. Effect of the activity concentration on solution stability of RP697 at room temperature. Each condition was run twice, and the radiochemical purity (RCP) data are presented as an average of two independent measurements.

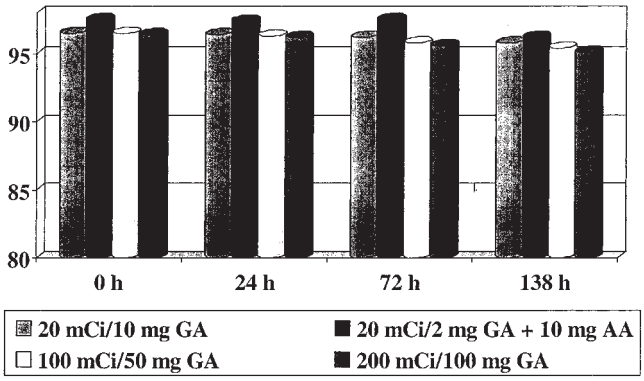

Figure 6. Solution stability of RP697 at −78 °C. Each condition was run twice, and the radiochemical purity (RCP) data are presented as an average of two independent measurements.

volume = 0.5 mL). In another vial, RP697 was prepared using 500 μg of SU015, 50 mg of GA, and 100 mCi of $^{90}YCl_3$ (total volume = 2.5 mL). After radiolabeling, the resulting solution was kept at room temperature, and the solution stability of RP697 was monitored by radio-HPLC over 6 days, as shown in Figure 5.

Solution Stability of RP697 at −78 °C. RP697 was prepared using the standard radiolabeling procedure (100 μg of SU015, 10 mg of GA for 20 mCi of $^{90}YCl_3$, and heating at 100 °C for 5 min). For the vials containing 100 and 200 mCi of activity, all the component levels were increased proportionally. For the vial using AA as the stabilizer, AA (200 mg/mL) was dissolved in 0.5 N ammonium acetate buffer (pH 7.4) and added to the reaction mixture (10 mg of AA for 20 mCi of ^{90}Y) after radiolabeling. After addition of AA, all the vials containing RP697 were kept in a dry ice box (−78 °C). Samples of the reaction mixture were analyzed by HPLC at 0, 24, 56, and 138 h. Figure 6 shows the RCP change over 6 days for RP697. Apparently, RP697 can be stabilized by the combination use of a stabilizer and low-temperature storage at −78 °C.

DISCUSSION

There has been great current interest in radiolabeled small peptides as therapeutic radiopharmaceuticals (*25*−*27*). In developing a new receptor-based therapeutic radiopharmaceutical, several factors need to be considered to satisfy the clinical need and to comply with FDA regulations. The therapeutic radiopharmaceutical must demonstrate therapeutic efficacy and favorable pharmacokinetics, including high and fast tumor uptake, high renal clearance are particularly important to improve the tumor-to-background ratio and to reduce the radiation burden to other major organs (kidney, liver, and bone marrow). The new radiopharmaceutical must have high radiochemical purity (RCP ≥90%). Unlike diagnostic radiopharmaceuticals, therapeutic radiopharmaceuticals have to be manufactured and released under GMP (Good Manufacturing Practice) conditions, and delivered for clinic applications. Therefore, the new therapeutic radiopharmaceutical must retain its chemical and biological integrity during release and transportation.

Therapeutic radiopharmaceuticals comprising β-emitting radionuclides may undergo autoradiolysis during preparation, release, transportation, and storage. During radiolysis, emissions from the radionuclide attack the metal chelate, targeting the biomolecule, and other compounds in proximity, which results in inter- and intramolecular decomposition or destruction of the radiometal chelate or the biomolecule. Radioactivity, which is not linked to the targeting biomolecule, will accumulate in nontargeting tissues and lead to the unwanted radiation toxicity to nontargeting tissues. Thus, it is important that the radionuclide remains linked to the targeting moiety, and the specificity of the targeting biomolecule is preserved. As a matter of fact, a major challenge for the development of a therapeutic radiopharmaceutical is to stabilize the radiolabeled compound during radiolabeling, release, and transportation.

Radiolysis is caused by the formation of free radicals such as hydroxyl and superoxide radicals. Free radicals are very reactive toward organic molecules such as peptides. To prevent radiolysis and stabilize the radiolabeled biomolecule, a radical scavenger or radiolytic stabilizer is often used either during or after the radiolabeling. A stabilizer is often an antioxidant, which readily reacts with hydroxyl and superoxide radicals. In general, the stabilizer for the therapeutic radiopharmaceutical should have the following characteristics: low or no toxicity or immunoreactivity when it is used for human administration, no interference with the receptor binding of the radiolabeled compound to the target cells or tissue(s), and the ability to stabilize the therapeutic radiopharmaceutical for a reasonable period of time (preferably 2 half-lives of ^{90}Y) for preparation, release, storage, and transportation.

There are several commercially available antioxidants. Human serum albumin (HAS) has been used as a stabilizer for the radiolabeled antibodies (*21*−*23*). Ascorbic acid is an antioxidant, and an FDA-approved pharmaceutical suitable for human injection. Gentisic acid has been used as a stabilizer in ^{99m}Tc radiopharmaceutical formulations (*28*−*30*). The stabilizer can be added into a therapeutic radiopharmaceutical formulation either before or after the radiolabeling. For pre-labeling addition, the stabilizer is added before addition of $^{90}YCl_3$ stock solution. During the radiolabeling process, the stabilizer is subjected to a certain degree of thermal decomposition, particularly at elevated temperatures. For the post-labeling addition, the stabilizer is not exposed to the heating process; thereby it may have longer stabilizing effect and higher stabilizing efficiency. The post-labeling approach is particularly useful when the addition of a large amount of stabilizer in the radiopharmaceutical formulation interferes with the radiolabeling.

There are several factors influencing the solution stability of RP697. These include the amount of activity in each vial, the activity concentration, the relative

is much slower at the activity concentration of 4 mCi/mL than that at a concentration of 40 mCi/mL (Figure 3). It needs 10 mg of GA or AA to maintain the RCP of RP697 (20 mCi) above 90% over 2 half-lives of ^{90}Y (Figure 4). RP697 remains relatively stable at room temperature if 10 mg of GA is used for 20 mCi of activity. It decomposes much faster when 50 mg of GA is used for 100 mCi of activity under the same storage conditions, even though the GA/activity ratio remains the same (Figure 5). Therefore, the solution stability data obtained from the low activity level (<20 mCi) cannot be simply extrapolated to high activity levels (>100 mCi).

In addition to the use of an antioxidant in the radiopharmaceutical formulation, storing the reaction mixture at low temperature (−78 °C/dry ice) also has a dramatic impact on the solution stability of RP697. For example, RP697 remains stable for at least 2 half-lives of ^{90}Y at −78 °C (Figure 6) while it decomposes rapidly at room temperature. Freezing the reaction mixture containing RP697 offers two advantages: stopping the diffusion of free radicals and slowing down the reaction kinetics between free radicals and radiolabeled cyclic peptide. Thus, it is recommended that the radiopharmaceutical be stored at low temperatures to avoid extensive radiolysis during release and transportation. The amount of antioxidant used in the radiopharmaceutical formulation and the storage temperature during release and transportation should be adjusted according to the sensitivity of a specific radiolabeled receptor ligand toward radiolytic decomposition.

CONCLUSIONS

In this study, we explored the factors influencing the solution stability of RP697, a ^{90}Y-labeled DOTA-peptide conjugate, and found that both the amount of total activity and the activity concentration have significant impact on the stability of the ^{90}Y-labeled cyclic peptide. The rate of radiolytic decomposition of RP697 is much slower at the lower activity concentration (<4 mCi/mL) than that at the higher concentration (>10 mCi/mL). RP697 remains relatively stable at room temperature when it is prepared at the 20 mCi level while it decomposes rapidly at the 100 mCi level under the same storage conditions. Therefore, the solution stability data obtained from the low activity level (<20 mCi) cannot be simply extrapolated to high activity levels (>100 mCi).

To prevent extensive radiolysis, we used both GA and AA as stabilizers. The stabilizer can be added into the formulation either before or after radiolabeling. For the post-labeling addition, the stabilizer is not exposed to the heating process; thereby it may have a longer stabilizing effect and higher stabilizing efficiency. The post-labeling approach is particularly useful when the addition of a large amount of stabilizer in the radiopharmaceutical formulation interferes with the radiolabeling of the DOTA-biomolecule conjugate.

ACKNOWLEDGMENT

Acknowledgment is made to Dr. M. Rajopadhye and Anothy R. Harris for the synthesis of DOTA-conjugated cyclic peptide (SU015).

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BC000145V