A Convenient Method of Preparing High Specific Activity Technetium Complexes Using Thiol-Containing Chelators Adsorbed on Gold

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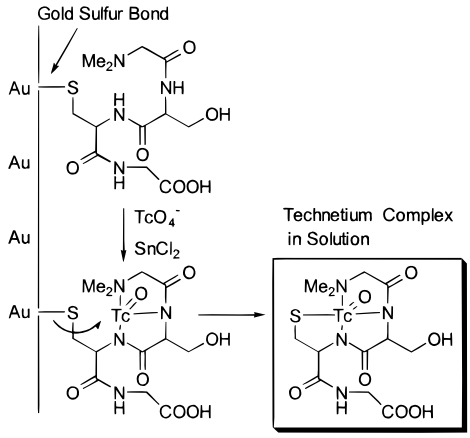
The physical and chemical properties of technetium-99m make it an ideal radioisotope for use in medical imaging agents.[1−5] The use of metal chelating moieties,[6] of which a large number are known, allows the technetium to be incorporated into the chemical structure of the targeting molecule. The most common contain nitrogen and/or sulfur, although complexes based on atoms such as phosphorus[7] and cyclopentadienyl ligands[8] are also known. Typically, technetium is incorporated into the chelator conjugate by treatment of an excess of the chelator molecule with a solution of pertechnetate and a suitable reducing agent. Although this procedure is often necessary to force the technetium complexation to completion, it results in a reaction mixture that contains a large excess of uncomplexed chelator (low specific activity formulations). With newer radiopharmaceuticals, such as bioactive peptides, the unlabeled molecule may be present in sufficient quantities to produce pharmacological or toxic effects and may saturate the receptor.[9] These problems can be reduced by purification of the radiopharmaceutical but this requires lengthy, labor intensive, and inefficient techniques such as HPLC. We recently described a method of avoiding these problems by the use of metal chelators attached to a solid support in such a way that the molecules are released only when complexed to the metal (generating high specific activity formulations).[10] However, the organic nature of the support means that a need still exists for a method of preparing radiopharmaceutical kits, capable of producing high specific activity formulations, which can be readily rendered sterile and manufactured in a facile manner.

Several reports have recently been published discussing the attachment of long chain alkane thiols to a gold surface and the structure of these adducts investigated.[11] This addition has been used in applications such as formation of monolayers of alkane thiols[12−14] and nanolithography.[15] More recently the process of thiol attachment has been extended to the attachment of peptides and protein fragments.[16] Utilization of this attachment provides an ideal opportunity to develop a procedure to generate high specific activity formulations of technetium radiopharmaceuticals according to the mechanism shown in Scheme 1. Thus a chelator would be attached to the gold surface using known chemistry.[11−14] Initial complexation of the surface bound chelator with the technetium (after reduction) weakens the gold−sulfur bond. This bond breaks and a sulfur−technetium bond forms causing the technetium complex to be released into solution. Uncomplexed chelator remains attached to the gold surface. The end result of this is the generation of technetium complexes having high specific activity in which the only receptor-targeting molecule in the solution is the radiopharmaceutical. In this paper we describe the preparation and labeling of the thiol-containing dimethyglycine-serine-cysteine-glycine (RP414), adsorbed onto a gold surface.

Scheme 1



The chelator having the cysteine protected by the acetamidomethyl (Acm) group was easily prepared by standard solid-phase peptide synthesis techniques employing FMOC chemistry.[17] Removal of the Acm group using mercury acetate in the presence of acetic acid,[18] followed by precipitation of the mercury with H_2S gas, gave the chelator as the free thiol. A solution of this chelator in phosphate buffered saline (PBS) and ethanol was then used to load the gold surface.[19] Unreacted chelator was removed by thorough washing of the gold and complete removal was confirmed by HPLC analysis of the supernatant.

Amino acid analysis techniques were employed to characterize the quantities of chelator adsorbed onto the gold. The analysis

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(19) A general procedure for loading the dimethylglycine-serine-cysteine-glycine onto solid phase is as follows: Dimethylglycine-serine-cysteine(Acm)-glycine (50 mg) was dissolved in 30% acetic acid (4 mL) and mercury(II) acetate (75 mg) added. The mixture was stirred at room temperature for 3 h. Hydrogen sulfide gas was bubbled through the solution with the formation of a black precipitate. This suspension was centrifuged and the supernatant decanted off and concentrated under reduced pressure to give a colorless solid. Gold powder (1−15 μm particle size) (80 mg) was heated over a flame and allowed to cool. It was then wetted with methanol in distilled water, stirred vigorously, and centrifuged and the solution was decanted off. A solution of deprotected dimethylglycine-serine-cysteine-glycine in 0.01 M phosphate buffered saline (2 mL) and ethanol (2 mL) was added and the mixture stirred at room temperature under argon for 20 h. The supernatant was removed and the gold powder washed with successive portions of water (5 times 4 mL).

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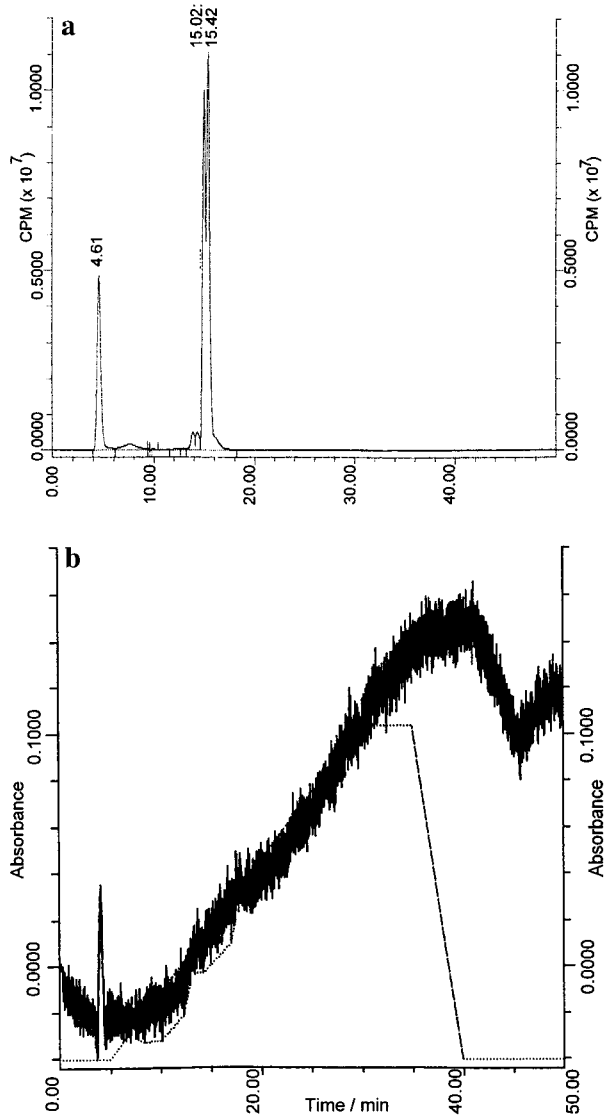

Figure 1. (a) Radiometric trace of solution after radiolabeling of gold-supported RP414 showing uncomplexed pertectnetate (retention time 4.61 min) and radiolabeled RP414 (two isomers with retention times of 15.02 and 15.42 min). (b) UV HPLC trace of solution after radiolabeling of gold-supported RP414 showing lack of unlabeled RP414 (retention time ca. 9 min).

showed 113 ng of attached peptide per mg of gold.[20] Radiolabeling of the gold-supported chelator was carried out using a procedure similar to that employed for solution-phase radiolabeling of the chelator. Hence, addition of stannous gluconate to a saline suspension of the gold was followed by addition of a solution of sodium pertechnetate. The resulting mixture was stirred at room temperature for 1 h and then heated at 60 °C for 30 min.[21] Analysis of the reaction was carried out using HPLC and the

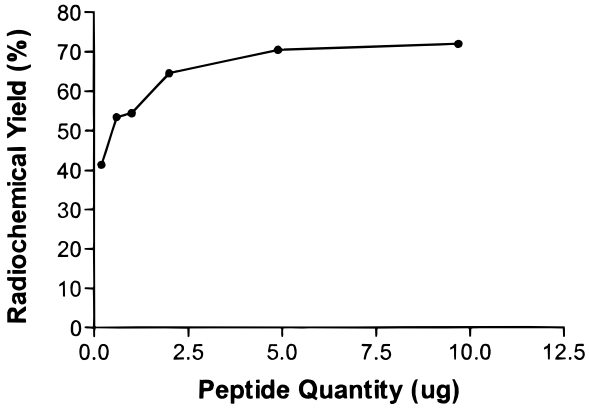

Figure 2. Effect of increasing peptide quantity, on a gold support, on radiolabeling yield.

results shown in Figures 1a and 1b. These show a clean labeling of the chelator (which exists as two isomers when complexed)[22] with a clear distinction between the radiolabeled molecules and the small amount of uncomplexed pertechnetate remaining (retention time 4.61 min). The structure of the radiolabeled compound was confirmed by co-injection of the reaction mixture with the corresponding rhenium complex, and the absence of unlabeled peptide was confirmed by examination of the UV absorbance trace from the HPLC. This did not contain any peaks corresponding to unlabeled peptide (retention time of ca. 9 min).

The effect of chelator quantity on the efficiency of radiolabeling was investigated by carrying out the labeling reaction on increasing amounts of gold-supported chelator. Since an increase in surface area corresponds to a greater amount of adsorbed chelator it was expected that the radiolabeling yield would increase with the increasing amounts of gold. This hypothesis was confirmed by the results obtained (Figure 2).

We have previously shown the ability to prepare technetium labeled agents in high specific activity formulations using a solid supported metal chelator. A problem associated with the commercial development of radiopharmaceutical kits is the need to produce sterile and pyrogen free formulations. The use of a gold surface for the attachment of the chelator moiety has several potential advantages over those described previously. The metal itself is easily sterilized and a gold surface is easily applied to a variety of supports. Clearly there are many potential applications for a technetium metal chelator attached to a gold surface in the field of radiopharmaceuticals.

Acknowledgment. This manuscript is dedicated to Professor George. M. Whitesides on the occasion of his 60th birthday. We would like to thank Rey Interior of the University of Toronto for carrying out amino acid analysis on the gold-supported samples.

Supporting Information Available: Synthetic procedures for dimethylglycylserinylcysteine(Acm)glycine and dimethylglycylserinylcysteine(Acm)glycine, amino acid analysis of gold supported peptide, and UV HPLC trace of dimethylglycylserinylcysteinylglycine (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Gold powder was purchased from Alfa Aesar as gold flake/sphere 1−15 μm, 99.95% and has a quoted surface area of 0.24−0.34 m^2/g.

(21) Radiolabeling of the gold-supported peptide is carried out as follows: 1.0 mL of saline was added to the gold-supported dimethylglycine-serine-cysteine-glycine. 100 μL of stannous gluconate (prepared by the addition of 20 μL of 20 mg/mL of stannous chloride to 1.0 mL of 13 mg/mL of sodium gluconate) was added followed by approximately 10 mCi of Tc-99m pertechnetate in 200 μL of saline. The reaction vessel was then shaken vigorously and the reaction allowed to proceed for 1 h at room temperature followed by 30 min at 60 °C. A 500 μL sample was analyzed using HPLC with a reverse phase C-18 column and a step gradient method of 0 to 70% acetonitrile w/0.1% TFA over 25 min. The sample was analyzed by both radiometric and UV detectors.

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