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Selective Release of Technetium Complexes from a Solid Phase due to C–N Bond Cleavage upon Metal Coordination

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Abstract: A new method for ^{99m}Tc-labeling using solid-phase technology is described. It allows the separation of unlabeled from labeled bioactive molecules by simple filtration. The procedure is based on a site-specific C–N bond cleavage reaction initiated by the formation of $[Tc^{I}(CO)_3L]$-complexes.

Keywords: Cleavage reactions · Radiopharmaceuticals · Solid-phase synthesis · Technetium

Radioactive substances are widely used for diagnostic purposes in medicine. The most frequently used radioactive isotope is ^{99m}technetium, a γ-emitter with a half-life of 6 h. ^{99m}Tc is produced by the beta decay of $Na^{99}MoO_4$ to $Na^{99m}TcO_4$ and separated from the molybdenum starting material by eluting an alumina column with saline. Ready-made generators are commercially available, enabling hospitals to produce $^{99m}TcO_4^-$ solutions just prior to use. Several ^{99m}Tc complexes have been developed that selectively accumulate in a specific tissue and permit the imaging of tissue function [1]. The last steps in the preparation of ^{99m}Tc-based radiopharmaceuticals are the reduction to a lower oxidation state of the Tc and complex formation (Scheme 1).

In our group, the method of converting $[TcO_4]^-$ to the general labeling precursor *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ is used routinely [2]. The three labile water ligands can be replaced easily by tridentate ligands containing amino and carboxylate groups as donors. Due to the short half-life of ^{99m}Tc, the synthesis of the Tc compounds must be completed at the hospital immediately prior to the administration to the patients. This limitation requires the development of a robust and easy to handle reaction procedure that avoids elaborate purification steps such as chromatography.

First-generation ^{99m}Tc-radiopharmaceuticals consisted of low molecular weight coordination compounds. They are clinically used for diverse applications, such as imaging of renal function, cerebral blood flow, myocardial perfusion and bone metastases or fractures. Second-generation ^{99m}Tc radiopharmaceuticals are in current development. They are functionalized by a highly bioactive receptor binding residue *i.e.* peptide, hormone or antibody, which directs the radiotracer selectively to the target, such as cancer cells. Generator eluates contain $Na^{99m}TcO_4$ in a concentration range of 10^{-8} to 10^{-6} M, depending on the age of the generator and the time span since the last eluation. To ensure complete binding of the ^{99m}Tc precursor in a reasonable time, high excess of the chelator has usually to be applied. With second-generation radiopharmaceuticals, the use of excess ligand entails the risk of toxic side effects and/or receptor saturation due to competition from unlabeled chelator-bioconjugate *in vivo*. Here we describe a new labeling method using solid-phase technology to separate unlabeled from labeled bioactive molecules by simple filtration.

We found that some molecules, bound to a chelator *via* a tertiary amino group, were cleaved from the chelator upon formation of $[^{99m}Tc(CO)_3L]$-complexes [3]. Cleavage is clearly mediated by the coordination of the $Tc^{I}(CO)_3$ fragment, but does not occur under the same reaction conditions in the absence of $[^{99m}Tc(H_2O)_3(CO)_3]$. This specific cleavage reaction can be utilized for the preparation of targeting radiotracers which are virtually free of uncomplexed chelator. The chelator is covalently bound to a solid support *via* formation of a tertiary amino group from the chelating unit. This C–N bond to the solid-phase anchoring group cleaves during formation of the technetium complex releasing only $[^{99m}Tc(CO)_3L]$ into solution, while uncomplexed ligand remains bound to the solid phase. The Tc complex can be isolated from the solid support by simple filtration (Scheme 2). Cleavage yields depend on temperature, pH and ligand type, and are up to 50% under optimized conditions. Analysis of the solution revealed concentrations of the free ligand in the 10^{-7} M range. This corresponds to an improvement of the complex to ligand ratio by at least one to two orders of magnitude in comparison to the best chelators in homogenous labeling.

The selectivity of the C–N bond cleavage prompted us to investigate the mechanism of the reaction. Experiments on the solid support as well as on soluble model

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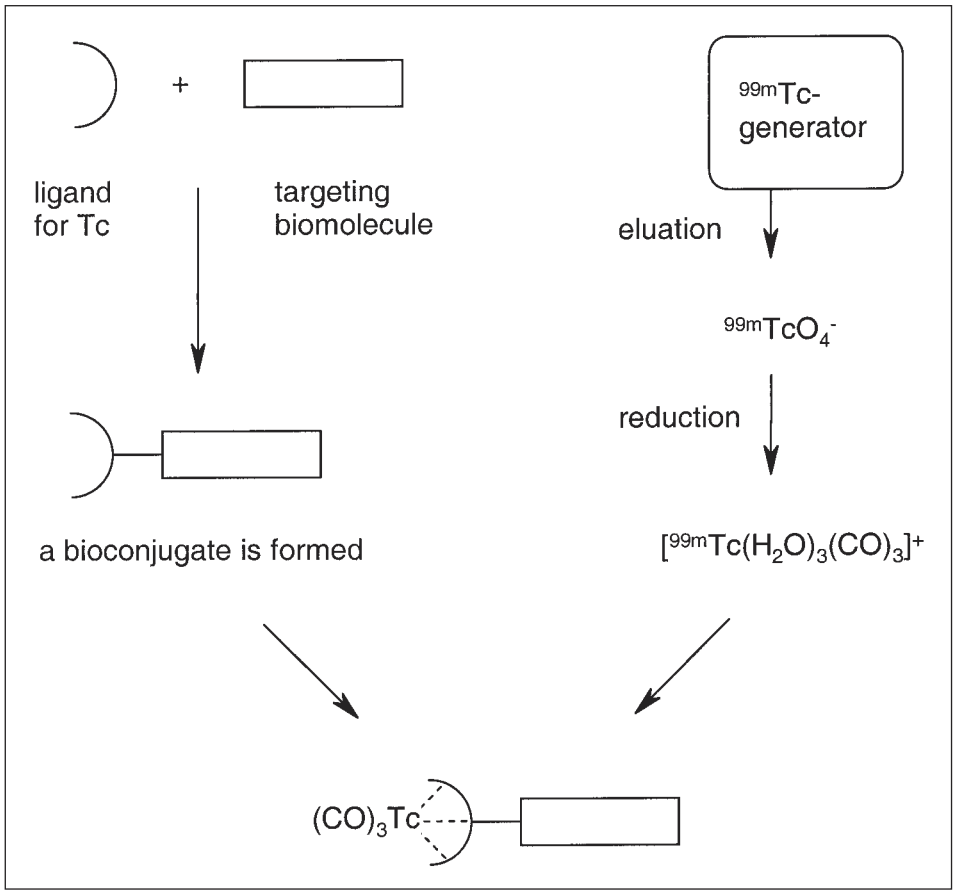
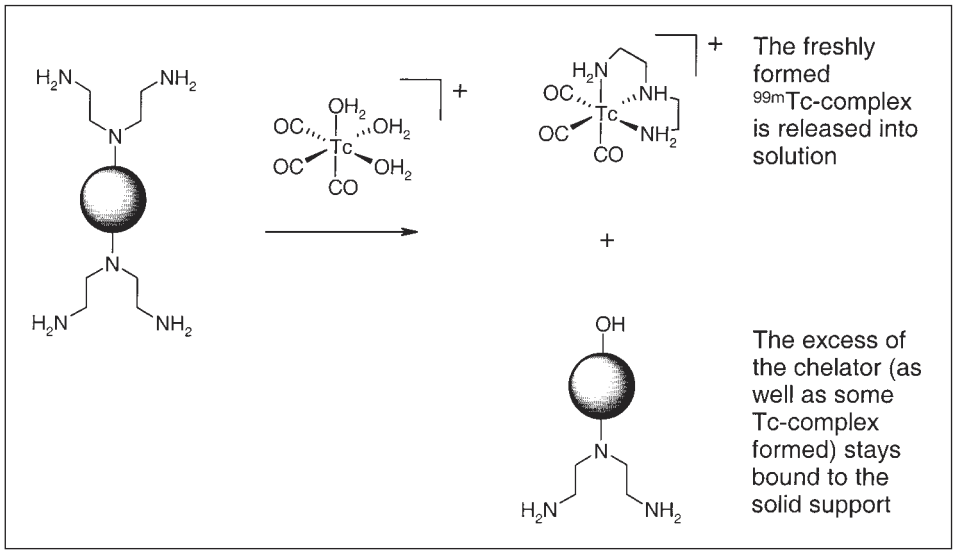
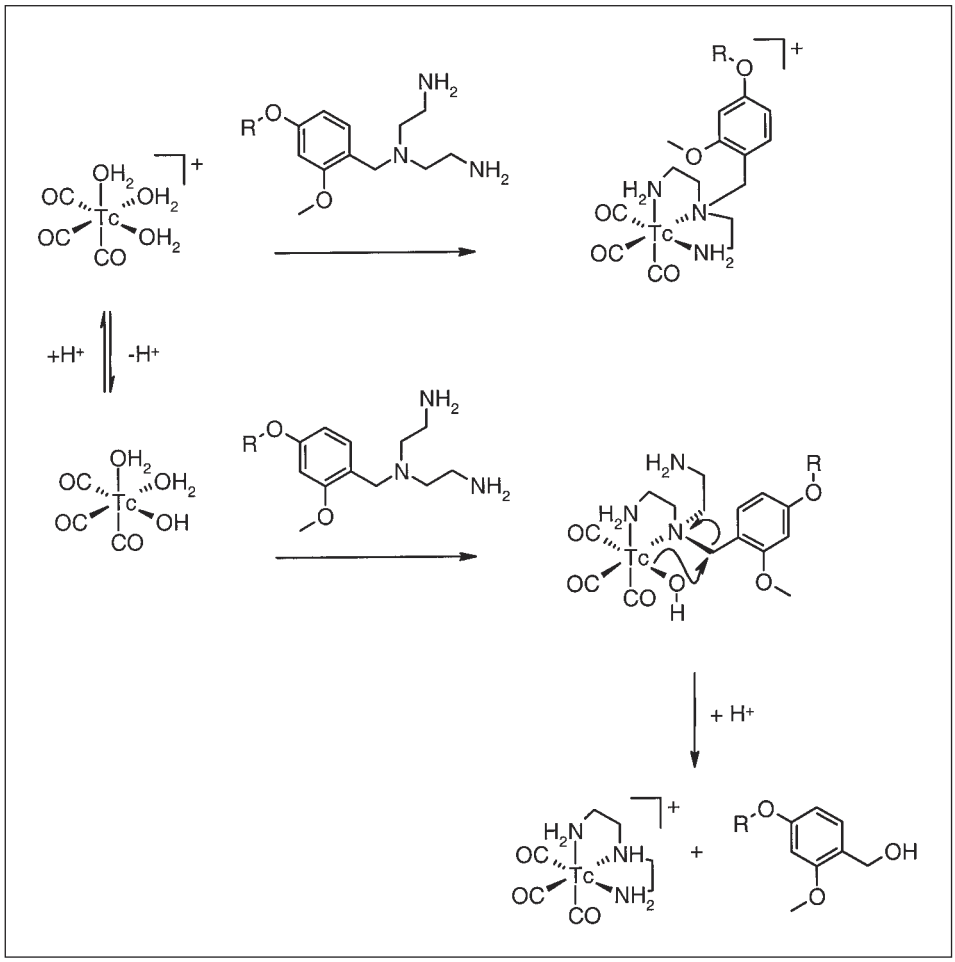


Scheme 1. Preparation of a second generation ^{99m}Tc imaging agent



Scheme 2. Preparation of a ^{99m}Tc-compound *via* metal assisted cleavage from a solid support

compounds were done to understand more about the reaction mechanism. As evident from these investigations, cleavage took place only during the complex formation step. The ligand itself as well as the formed complexes are stable under the conditions used. We also observed a strong temperature dependence. At room temperature, the complexes were formed without any cleavage. In fact, it was possible to load the resin with the maximal theoretical value of radioactivity under these conditions, and virtually no radioactivity could be released into solution afterwards, not even with heating. The optimal pH for cleavage was found to be between 8 and 9.5, which is in the range of the formation of the monodeprotonated hydroxy complex [^{99m}Tc(H$_2$O)$_2$(CO)$_3$(OH)]. It could also be shown that the methylene group has to carry an unsaturated carbon such as an aryl or vinyl group which activates nucleophilic substitution. Tertiary amines with an aliphatic chain are not prone to cleavage. Reaction of [^{99}Tc(H$_2$O)$_3$(CO)$_3$]$^+$ with a soluble model system (R = CH$_3$ in Scheme 3) allowed the detection of the organic cleavage product by analytical HPLC. It was assigned as 2,4-dimethoxybenzyl alcohol by co-injection of the corresponding reference material. GC-MS of the collected peak showed the mass of the methylated product. Methylation is believed to occur during work-up in methanolic solution, similar results were found by stirring commercially available 2,4-dimethoxybenzyl alcohol in ethanol. Based on these experimental re-



Scheme 3. Proposed cleavage mechanism

sults we suggest the following mechanism. Upon coordination of the tertiary amino group to the Tc-center, it becomes partially positively charged. The adjacent carbon is then activated for nucleophilic attack. In competition to complete the tridentate complex formation, the remaining hydroxy ligand on the Tc intramolecularly attacks the methylene group of the chelator inducing a C–N bond cleavage, thus releasing the complex into solution (Scheme 3).

Until now, only pure ligand systems bearing no additional functionalities were studied. Future work will be focused on attaching targeting vectors to the solid bound ligands. We have already shown that it is possible to functionalize the solid-phase bound chelator *via* reductive amination of an aldehyde with its primary amino groups [4]. This approach should allow the preparation of second-generation radiopharmaceuticals with improved complex to ligand ratio.

Acknowledgements

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