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S-bridged complex of ^{99m}Tc with *fac*(S)-[Rh(aet)$_3$]: Quality control, characterization and biodistribution studies in rats

N. Amir,[1]* S. Roohi,[1] S. Pervez,[1] A. Mushtaq,[1] M. Jehangir,[1] Y. Miyashita,[2] K. Okamoto[2]

[1] *Isotope Production Division, Pakistan Institute of Nuclear Science and Technology, P.O. Nilore, Islamabad, Pakistan*
[2] *Department of Chemistry, University of Tsukuba, Tsukuba, 305-8571, Japan*

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fac(S)-[Rh(aet)$_3$] (aet = 2-aminoethanethiolate) is N_3S_3 metalloligand which can coordinate to transition metal ions to form S-bridged polynuclear complexes. The reaction was carried out between $^{99m}TcO_4Na$ and *fac*(S)-[Rh(aet)$_3$] in the presence of $SnCl_2{\cdot}2H_2O$. A complex analogous to $[Re\{Rh(aet_3)\}_2]^{3+}$ is formed.[6] A simple method for radiolabeling of *fac*(S)-[Rh(aet)$_3$] with ^{99m}Tc has been developed and radiolabeling efficiency was higher than 99%. Effect of $SnCl_2{\cdot}2H_2O$ concentration, electrophoresis, HPLC, UV-Visible absorption spectra and biodistribution studies in rats were performed. Higher uptake by kidneys showed rapid distribution of the labeled *fac*(S)-[Rh(aet)$_3$]. Liver uptake was significant, stomach, lungs and intestine uptake was high at 4 hours post injection time.

Introduction

Nuclear medicine is a branch of medical imaging that uses radioactive tracers to examine the function of body systems. The radionuclide used in about 90% of all examinations is ^{99m}Tc, which is available from ^{99}Mo/^{99m}Tc generators. ^{99m}Tc in some chemical form is used in more than 85% of the diagnostic scans done each year in hospitals. To reach targets in the human body other than glandula thyreoidea, ^{99m}Tc needs a carrier molecule, usually a chelating agent. Many chelators that form stable complexes with ^{99m}Tc have affinities for certain tissues in the human body. Other chelators can be manipulated by pharmaceutical formulation to be retained in certain body systems. In order to form bonds with ^{99m}Tc, the chelator must contain electron donors like N, O and S. Today, chelators for the use with ^{99m}Tc exist for a number of highly sensitive scintigraphic studies of the brain, heart, skeleton, kidneys, hepatobiliary system and lungs. This includes chelator such as dimercaptosuccinic acid, 1,2-ethylenediylbis-L-cysteine diethyl ester, methylene-diphosphonate, hexamethylpropylene-amineoxime and hexakis(methoxy isobutylisonitrile).[1,2]

The estrogen, progesterone and androgen receptors may be useful for targeting breast and prostrate cancers. The ^{99m}Tc labeling of progesterone receptor has been studied utilizing conjugation to N_2S_2 ligands via phenyl spacer. An alternative approach is to integrate the receptor binding sites directly onto the outer periphery of the Tc ligands.[3,4] Bioconjugates of octreotide using the N_2S_2 ligand approach have been made for ^{99m}Tc and have been investigated as a potential method for the imaging of tumors. Steroids can be attached to rhenium oxo-complexes of N_2S_2 ligand systems in a directly analogous fashion. This therefore is a promising approach to the delivery of therapeutic radiation to appropriate tumors.[5]

The extensive investigations by numerous research groups on a variety of N_2S_2 and N_3S donor type ligands have revealed that the chemistries of these ligands with Tc and Re are rather complex, giving rise to considerable difficulties in the development of reliable procedures for the development of radiopharmaceutical reagents It is significant that thiolate based ligands appear to offer considerable advantages in purity of preparations and stability of the complexes. This led to the development of a class of tridentate ligands, such as bis(mercaptoethy1)methylamine (NS_2), which may constrain the possible coordination geometries and improve overall stability.[6]

Metalloligands, *fac*(S)-[M(aet)$_3$] (M = Rh^{III}, Ir^{III}, aet = 2-aminoethanethiolate) can react with transition metal ions to form S-bridged polynuclear complexes. These complexes indicated unique reactivity and electrochemistry. Incorporation of Re ion into S-bridged structure resulted in a fairly stable S-bridged complex $[Re\{Rh(aet)_3\}_2]^{3+}$. Investigations of redox properties and stability of Re ion is important as fundamentals for the application of Re compounds and development of radiopharmaceuticals.[7,8]

^{99m}Tc complexes are widely used for diagnostic imaging. The chemistry of S-bridged metal complexes has attracted much interest because of the importance of the thiolato groups in a variety of systems ranging from inorganic to organic and biological chemistry.[9,10] A number of complexes containing N and S donor ligands have been reported and play an important role in nuclear medicine. For example, ^{99m}Tc-*L,L*-ECD

* E-mail: areena_a@hotmail.com

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(ECD = ethylenecysteine dimer) is a potential cerebral perfusion imaging agent. It passes the blood brain barrier and is retained in the brain by ester hydrolysis. The hydrolysis is stereospecific that is only the complex with the *L,L* enantiomer of ECD is trapped while *D,D* enantiomer is not.[11–18]

Ga^{III} complexes with very high invitro and in vivo stability are formed with hexadentate ligands N_3X_3 (X = S, O).[19–21] Linker group technology has also been used in the development of ^{99m}Tc radioimmunoscintigraphic agents and ^{186}Re radio immunotherapeutic agents. One example is ^{99m}TcO-N_2S_2 derivatized with TFP (TFP = tetrafluorophenyl) active ester linked with a suitable linker and used for imaging melanoma and lung cancers.[22] Introduction of oxorhenium-N_2S_2 chelate system in diagnostic imaging agents for steroid positive tumors lowered the receptor binding affinity of the resultant molecule.[23,24]

Small molecule radiopharmaceuticals whose mode of localization involves biochemistry either specific receptor interactions or metabolism are the future of nuclear medicine.[25]

In this work S-bridged complex of ^{99m}Tc with *fac*(S)-[Rh(aet)$_3$] was formed in the presence of $SnCl_2{\cdot}2H_2O$ as a reducing agent. An analogous complex of Rhenium has been synthesized and characterized by spectroscopic techniques and X-ray crystallography as $[Re\{Rh(aet_3)\}_2]^{3+}$.[6] ^{99m}Tc is considered to show the same behavior towards *fac*(S)-[Rh(aet)$_3$] as Re. Quality control, effect of reducing agents concentration and time on labeling efficiency, electrophoresis, HPLC, UV-Visible absorption spectra and biodistribution studies in rats were performed and discussed.

Experimental

Materials

fac(S)-[Rh(aet)$_3$] was obtained from the Ken-ichi Okamoto Laboratory, University of Tsukuba, Japan. Rats (Sprague-Dawley) were obtained from the National Institute of Health (NIH) Islamabad. The Animal Ethics Committee of Institute gave an ethical approval for the animal experiments. ^{99m}Tc was obtained from locally produced fission based PAKGEN ^{99}Mo/^{99m}Tc generator system, PINSTECH, Islamabad. All the other chemicals used were analytical grade and purchased from Merck, Germany.

Method

1.0 mg of metalloligand, *fac*(S)-[Rh(aet)$_3$] was dissolved in 1.0 mL of 0.8N HCl. To this yellow solution was added 30 μL of $SnCl_2{\cdot}2H_2O$ followed by the addition of 1.0 mL of ^{99m}Tc as $Na^{99m}TcO_4$ (5–10 mCi). The pH of the solution was ~2. The solution was incubated for 20 minutes at room temperature. Reaction mixture volume used in all experiments was 2±0.1 mL. All the experiments were carried out at room temperature (22±2 °C).

Quality control

Radiochemical yield of ^{99m}Tc-*fac*(S)-[Rh(aet)$_3$] was assessed by thin layer chromatographic method using Whatman No. 3. Free $^{99m}TcO_4^-$ in the preparation was determined by using Whatman No. 3 as the stationary phase and acetone as the mobile phase. Reduced and hydrolyzed activity was determined by using Whatman No. 3 as the stationary phase and saline as mobile phase.

Paper electrophoresis

The charge of ^{99m}Tc-*fac*(S)-[Rh(aet)$_3$] was determined by paper electrophoresis using sodium phosphate buffer of pH 6.8 as electrolyte and Whatman No. 1 as a support. The sample was run at a constant voltage of 300 V for 1 hour. The strip was scanned by 2π scanner. For comparison, a sample of $Na^{99m}TcO_4$ was also run under identical condition.

HPLC

The sample was passed through a millipore filter carefully and injected into the HPLC column (peptide system). The Hitachi L-6200 intelligent pump and L-4200 UV-Vis detector systems were used for HPLC analysis. Analytical reverse phase HPLC analysis was performed with an analytical C18 column (RP-18 Lichrosorb, 25×0.45 cm) using a continuous gradient of methanol/water (20:80) to (80:20) in 30 minutes. Elutions were performed at a flow rate of 1.0 mL/min after an injection volume of 20 μL tracer. The eluted radioactivity was monitored on line using a NaI probe (Raytest-Steffi) collected fractions were also measured by well-type gamma-counter. A typical elution profile of ^{99m}Tc-*fac*(S)-[Rh(aet)$_3$] complex from an analytical HPLC at 20-minute post labeling was obtained. HPLC analysis revealed formation of single species with a retention time of 5.53 minutes while free pertechnetate was eluted at 7.27 minutes.

UV-Visible absorption spectroscopy

UV-Visible absorption spectrum was determined by 220S (Hitachi) UV/Visual-spectrophotometer with a scan speed of 240 nm/min and chart format 20 nm/cm.

Effect of time and reducing agent concentration on labeling efficiency

The effect of stannous chloride concentration (15, 30, 50, 100 μL) on labeling efficiency was observed.

Labeling efficiency was also studied by varying time interval (10, 20, 30, 60, 120, 240 minutes). pH of the mixture was in the range of ~2 in all cases. In quality control method Whatman No. 3 strips were used as stationary phase while acetone and saline as the mobile phases. All the experiments were performed at room temperature ~22 °C.

Biodistribution studies in rats

Male Dawley-Sprague rats weighing ~200 g were used in all animal experiments. 1 mg of *fac*(S)-[Rh(aet)$_3$] was dissolved in 1.0 mL of 0.8M HCl. To this solution was added 30 μL of stannous chloride solution. $^{99m}TcO^{4-}$ (1.0 mL) was then added. The mixture was incubated for 20 minutes. Quality control was done using Whatman No. 3/acetone and Whatman No. 3/saline. 0.5 mL of the labeled *fac*(S)-[Rh(aet)$_3$] was then injected to each rat. At 0.5, 4 and 24 hours post injection time, the rats were sacrificed after ether anesthesia and biodistribution was determined. The whole animals were then weighed and dissected. Liver, spleen, stomach, intestine, femur, bladder, lung, kidney and heart were weighed, and the activity was measured using a gamma-counter. The results were expressed as the percent uptake of injected dose per organ. The level of significance was set at 0.05.

Results and discussion

The reaction of *fac*(S)-[Rh(aet)$_3$] with Na$^{99m}TcO_4$ in acidic solution (HCl) using $SnCl_2{\cdot}2H_2O$ as a reducing agent at room temperature gave S-bridged complex expected to be $[^{99m}Tc\{Rh(aet)_3\}_2]^{3+}$ similar to the case of $[Re\{Rh(aet)_3\}_2]^{3+}$.[6] Tc and Re belong to the same group in periodic table, therefore, they are quite similar in their behavior. The complex formed in this case is expected to be trinuclear S-bridged complex consisting of two octahedral units of *fac*(S)-[Rh(aet)$_3$] and one ^{99m}Tc atom. Namely, the thiolato sulfur atoms in *fac*(S)-[Rh(aet)$_3$] unit coordinate to the central ^{99m}Tc atom forming an octahedral ^{99m}TcN6S6 chromophore.[6] Labeling efficiency, radiochemical purity and stability were assessed by a combination of ascending paper chromatography. In paper chromatography using acetone as the solvent, free $^{99m}TcO_4^-$ moved towards the solvent front (R_f = 1), while ^{99m}Tc- *fac*(S)-[Rh(aet)$_3$] and reduced/hydrolyzed ^{99m}Tc remained at the point of spotting. Using saline as the solvent, reduced/hydrolyzed ^{99m}Tc remained at the point of spotting, whereas ^{99m}Tc-*fac*(S)-[Rh(aet)$_3$] and free $^{99m}TcO_4^-$ moved towards the solvent front.

The positive charge of complex was confirmed by paper electrophoresis which showed that the ^{99m}Tc-*fac*(S)-[Rh(aet)$_3$] species move to cathode indicating that the compound exhibit cationic behavior. This result is similar to $[Re\{Rh(aet)_3\}_2]^{3+}$ where molar conductivity values showed that complex is positively charged.[6] The crystal structure of $[Re\{Rh(aet)_3\}_2]^{3+}$ has been reported earlier which was the first example of d^4 metal ion incorporated into S-bridged polynuclear structures.[6] In our work it is also believed that an S-bridged polynuclear complex with ^{99m}Tc is formed (Fig. 1).

In HPLC the UV chromatogram and radiochromatogram were similar. The UV chromatogram showed UV peak at 3.96 minutes. The same peak was found on the radiochromatogram at 5.53 minutes (86%). The activity peak at 7.27 minutes represents free TcO_4^- (14%). The UV-Visible absorption spectra showed a number of bands. Because the TcO_4^- counter anions indicate a few absorption bands in the whole region. The most intense band (220–280 nm) in the UV region corresponds well to sulfur-to-rhodium charge transfer band of terminal *fac*(S)-[Rh(aet)$_3$] units as observed in the previous complexes which have *fac*(S)-[Rh(aet)$_3$] terminal units.[6] Similarly the band around 320–360 nm is a d-d transition band of Tc(III) ion. This band in Re complex was found at ca. 357 nm.[6] As *fac*(S)-[Rh(aet)$_3$] has few energy bands in the energy region below 357 nm, bands from 450–800 nm are assigned to be arising from the central $Tc^{III}S_6$ chromophore. These bands were in the range of 430–830 nm in $[Re\{Rh(aet)_3\}_2]^{3+}$.[6] The sulfur-to-metal charge transfer band which is expected to be around 260 nm is not well defined in the present technetium complex because a broad band is formed in the region of 200–280 nm. This band is found at 263 nm in the case of corresponding rhenium complex.[6]

The amount of the reducing agent, $SnCl_2{\cdot}2H_2O$, which gave the highest labeling efficiency, was 30 μL (Fig. 2). The complexation of ^{99m}Tc with *fac*(S)-[Rh(aet)$_3$] was rapid and maximum labeling efficiency was achieved after 20 minutes.

Effect of time on labeling efficiency was studied at room temperature. It was noted that labeling reaches to its maximum value in 20 minutes where as it decreases down in 60 minutes reaching to 62% in 240 minutes (Fig. 3).

The tissue distribution of ^{99m}Tc-*fac*(S)-[Rh(aet)$_3$] expressed as percentage of injected dose per organ (%ID/organ) in rats studied at 0.5, 4 and 24 hours after intravenous administration is presented in Table 1 (Fig. 4). The ^{99m}Tc-*fac*(S)-[Rh(aet)$_3$] was rapidly distributed after intravenous injection as shown by the value of renal elimination (Table 1). This predominant renal clearance shows high hydrophilic character of ^{99m}Tc-*fac*(S)-[Rh(aet)$_3$].[26]

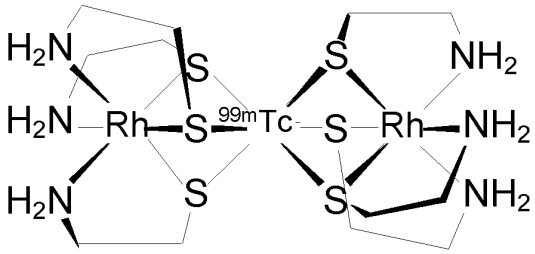

Fig. 1. Proposed structure of the complex formed between *fac*(S)-[Rh(aet)$_3$] and ^{99m}Tc

Liver uptake was significant while lungs, stomach and intestine uptake increased from 0.5 to 4 hours and then decreased at 24 hours. The activity in heart decreased with time and spleen showed an increase of activity with time (0.5–24 hrs). After intravenous injection the compound apparently becomes colloidal by chelating with serum calcium or any other element, forming insoluble compound, which is then trapped in liver and lungs.

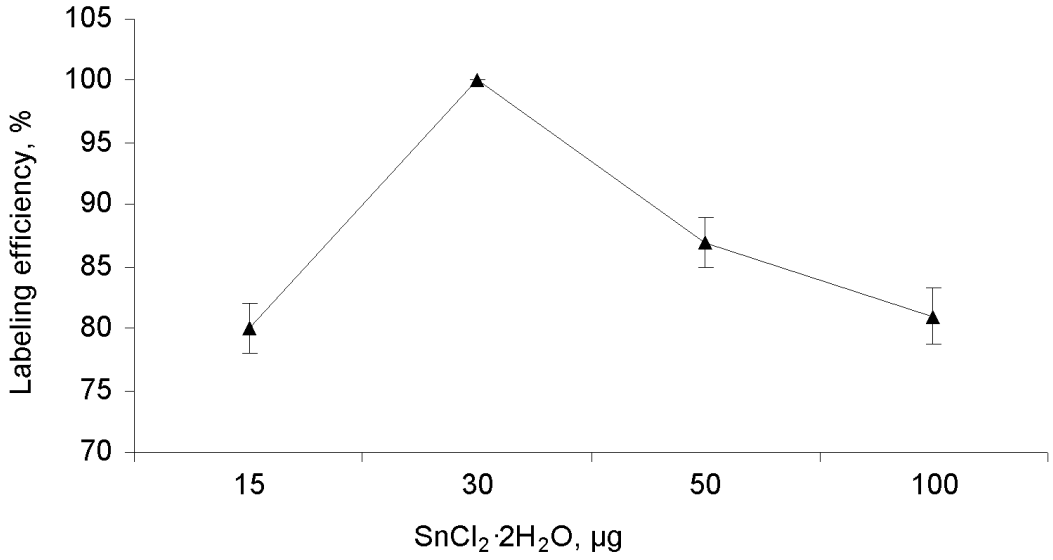

Fig. 2. Effect of reducing agent $SnCl_2 \cdot 2H_2O$ amount on the labeling efficiency of ^{99m}Tc labeled *fac*(S)-[Rh(aet)$_3$] ($n = 4$ per experiment)

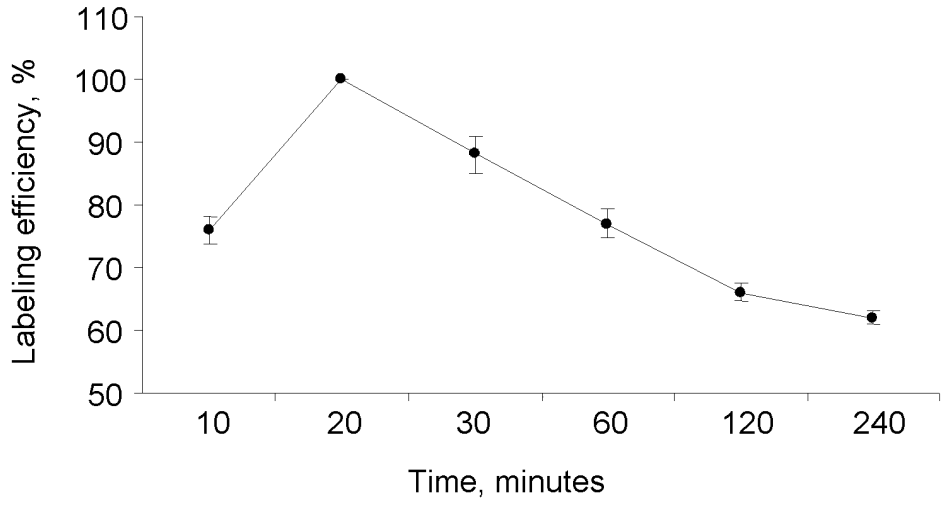

Fig. 3. Rate of complexion of ^{99m}Tc with *fac*(S)-[Rh(aet)$_3$] and stability of ^{99m}Tc labeled *fac*(S)-[Rh(aet)$_3$] ($n = 4$ per experiment)

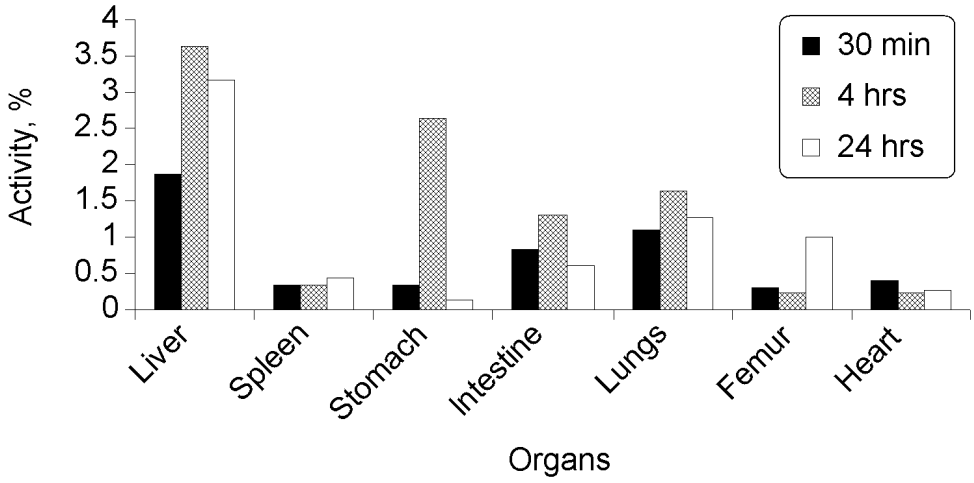

Fig. 4. Biodistribution of ^{99m}Tc labeled *fac*(S)-[Rh(aet)$_3$] in Dawley–Sprague rats. The rats were administered with 20–25 MBq of ^{99m}Tc labeled *fac*(S)-[Rh(aet)$_3$] intravenously and radioactivity was measured after 0.5, 4 and 24 hours (n = 4 per experiment)

Table 1. Biodistribution data in percent injected dose per organ for ^{99m}Tc labeled *fac*(S)-[Rh(aet)$_3$] and after 0.5, 4 and 24 hours of post injection in infected Dawley-Sprauge rats

Organ	0.5 hour	4 hours	24 hours
Liver	1.88 ± 0.05	1.88 ± 0.08	3.18 ± 0.09
Spleen	0.34 ± 0.04	0.34 ± 0.07	0.43 ± 0.075
Stomach	0.32 ± 0.1	0.32 ± 0.09	0.13 ± 0.02
Intestine	0.82 ± 0.2	0.82 ± 0.15	0.61 ± 0.19
Lungs	1.10 ± 0.09	1.10 ± 0.08	1.27 ± 0.14
Kidney	7.38 ± 1.1	7.38 ± 1.67	25.83 ± 2.89
Femur	0.30 ± 0.03	0.30 ± 0.06	1 ± 0.09
Heart	0.39 ± 0.08	0.39 ± 0.04	0.26 ± 0.06

Conclusions

A simple method for radiolabeling of *fac*(S)-[Rh(aet)$_3$] with ^{99m}Tc has been developed and standardized. Radiolabeling efficiency monitored by Whatman No. 3/acetone and Whatman No. 3/saline was higher than 99%. No post-labeling purification was required. Labeling efficiency decreased by increasing the concentration of stannous chloride and maximum labeling was observed at 30 μL. High uptake by kidneys showed rapid distribution of the labelled *fac*(S)-[Rh(aet)$_3$]. Liver uptake was significant, stomach, lungs and intestine uptake was high at 4 hours post injection time. Further studies are needed to understand the properties, applications and importance of the complex formed between ^{99m}Tc and *fac*(S)-[Rh(aet)$_3$].

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