PREPARATION AND BIODISTRIBUTION OF A TECHNETIUM CATIONIC COMPLEX - $[Tc^{III}(sacac)_2en(PPh_3)_2]^+$

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SUMMARY

The new,"mixed-ligand",Tc^{III}-N,N'-ethylene-bis-(acetylacetone-thioiminato)-bis-(triphenylphosphine)-hexafluorophosphate complex, $[Tc^{III}(sacac)_2en(PPh_3)_2]PF_6.2H_2O$, was prepared by reaction of the corresponding oxo-technetium (V) precursor with triphenylphoshine, in methanol.It was characterized by elemental analysis, IR, UV-Vis and ^1H NMR spectroscopy and HPLC. Biodistribution studies showed a considerable heart uptake, but rather slow blood clearance and hepatic elimination.

Keywords : Tc^{III}-N,N'-ethylene-bis-(acetylacetone-thioiminato) bis-(triphenylphosphine) complex, characterization, biodistribution.

INTRODUCTION

In the last decade much research has been focused on the development of cationic ^{99m}Technetium- complexes for myocardial perfusion studies(1-3). The design of these agents was based on the rational that monovalent cationic species are well-known heart-affine substances. The biological evaluation, however, of the various

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^{99m}Tc-compounds synthesized,indicated that human heart can be successfully imaged only with monocationic technetium complexes which cannot undergo in vivo reduction. These nonreducible agents do not exhibit detectable myocardial washout,even several hours after injection (4,5).

Considerable work has been performed on the chemistry and biology of Tc-complexes containing N_2O_2-type tetradentate ligands,the N,N'-bis-acetylacetone imines (6,7). A new class of cationic,"mixed ligand, Tc^{III}-agents, designated as $[Tc^{III}(L)(PR_3)_2]^+$, where L = the tetradentate Schiff base and PR_3 = tertiary phosphine,were synthesized via the corresponding Tc^V- precursors and proved to be electrochemically inert in the redox range accesible to biological systems (8).

Recently, we have studied the coordination of the N_2S_2-type Schiff base ligands N,N'-ethylene-bis-(acetylacetone-thioimines)to the $[Tc^VO]^{+3}$ core (9). Neutral complexes of the general formula $[Tc^VO(L)X]$(X=Cl, H_2O) have been synthesized and characterized.This paper describes the synthesis,characterization and biological evaluation of the "mixed-ligand" Tc^{III}-N,N'-ethylene-bis(acetylacetone-thioiminato)-bis-(triphenylphosphine) complex, $[Tc^{III}(sacac)_2en(PPh_3)_2]^+$, containing both the tetradentate thioimine,$(sacac)_2en$, and the monodentate triphenylphosphine,(PPh_3), ligands.

EXPERIMENTAL

General

All laboratory chemicals used, were of reagent grade. Solvents for High Performance Liquid Chromatography (HPLC), were specified as being of HPLC purity.

^{99m}Tc as $NaTcO_4$ was eluted from a commercial $^{99}Mo/^{99m}Tc$ generator with saline. ^{99}Tc as NH_4TcO_4 in 0.088 M NH_4OH (34 mg Tc/ml) was purchased from the Radio Chemical Centre, Amersham.

Elemental analyses were performed in the Centre National de la Recherche Scientifique,Service Central d' Analyse,Vernaison,France

Infrared spectra, as KBr pellets,were obtained on a Perkin-Elmer 1600 FT-IR spectrometer.UV-Vis spectra were recorded in methanol, on a Beckman DU-65 spectrophotometer. ^{1}H-NMR spectra were run on a Bruker spectrometer operating at 200 MHz.High performance liquid chromatography (HPLC) analysis was carried out on an LDC/Milton Roy Chromatography Gradient System equipped with UV-Vis Detector (LDC/ Milton Roy, MP 3000) and a Beckman 171 Radioisotope Detector for β- or low γ-detection.

Radioactivity of the biological samples was measured by a Packard Minaxi-5000 series γ-counter on line with a dedicated computer where the % dose/g of tissue was calculated.

Synthesis

Synthesis of $[Tc^{III}$-(sacac)$_2$en $(PPh_3)_2]$ PF_6 is outlined in Fig. 1. Tetrabutylammonium-tetrachloro-oxotechnetate, n-$Bu_4NTcOCl_4$ was synthesized by the method of Davison et al (10), and shown to exhibit the same UV -Vis and IR spectrophotometric parameters as reported. The synthesis of the ligand (sacac)$_2$en, proceeded in dichloromethane, by the reaction of Et_3OBF_4 with N,N'-ethylene-bis

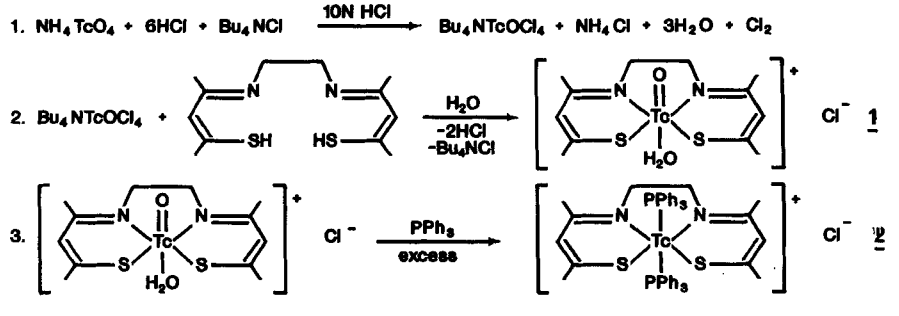

Fig. 1. Synthesis of Tc^{III}-N,N'-ethylene-bis-(acetylacetone-thioiminato)-bis-(triphenylphosphine) hexafluorophosphate.

(acetoimine) and the subsequent addition of NaHS in EtOH, according to previously reported methods (9,11). The oxotechnetium complex, $[Tc^{V}O(H_2O)(sacac)_2en]Cl$ 1, was prepared in ethanol, by ligand-exchange reaction of the Schiff base with $Tc^{V}OCl_4$, as described elsewhere (9,12).

$[Tc^{III}-(sacac)_2en\ (PPh_3)_2]\ PF_6$. 2.

53.60 mg (0.12 mmol) of trans-$[TcO(H_2O)(sacac)_2en]Cl$ were dissolved in ca. 10 mL of methanol. To the brown solution, 94.42 mg (0.36 mmol), of triphenylphosphine were added. The reaction solution became an intense blue-green and was stirred at room temperature for 15 min.Tetrabutylammmonium hexafluorophosphate, (100mg) was then added and immediately an olive-green solid product precipitated. It was filtered, washed twice with methanol and dried over P_2O_5 in vacuo: yield 90%.

Elemental analysis calculated for $C_{48}H_{48}N_2S_2P_3F_6Tc*2H_2O$:
C, 54.44; H, 4.95; N, 2.65; S, 5.16. Found: C, 53.94; H, 4.68; N, 2.92; S, 4.89.
IR spectrum (KBr): 1551 cm^{-1} (C=N), 1434 cm^{-1} (P-C), 745, 696 cm^{-1} (C-H, arom.), 840 cm^{-1} (P-F).
UV-Vis spectrum (methanol): 340 nm,456 nm, 580 nm.
Chemical shifts, δ ppm, and multiplicity of peaks in chloroform-d solution: $NCCH_3$ 2.725 (d, J~0.8 Hz), $SCCH_3$ 2.885 (s), NCH_2-CH_2N 4.398 (multiplet), CH 6.826 (q,J~0.8 Hz), aromatic area 7.450-7.724 (multiplet).

$^{99+99m}Tc^{III}-(sacac)_2en\ (PPh_3)$-chelate. 3.

Monocationic species of $Tc^{III}-(sacac)_2en\ (PPh_3)_2$ were prepared also in the presence of both ^{99m}Tc and ^{99}Tc, following a synthetic procedure similar to that described for 2. Tetrabutylammonium oxochlorotechnetate was formed by adding a small volume of $^{99m}TcO_4$ (ca. 65mCi) to 0.4 mL of $NH_4{}^{99}TcO_4$ solution. Equimolar quantities

(0.02 mmol) of $(sacac)_2en$ and the yielded $^{99+99m}TcOCl_4$ (5.2 and 10 mg respectively) reacted in methanol, to form the Tc^{V}-$O(sacac)_2en$ complex 1. To the brown solution a 3fold excess (0.06 mmol) of triphenylphosphine was added and the vial was sealed and heated at 95±2 ^{0}C for 15 min to yield the monocationic Tc^{III}-$(sacac)_2en$ $(PPh_3)_2]^{+}$,in more than 95% yield as was identified by HPLC analysis of the reaction mixture. This preparation was used for further comparative HPLC and biodistribution studies.

HPLC analysis

HPLC profiles of Tc-$(sacac)_2en$ complexes were worked out using a C-18 reversed phase (4.6x250mm) column, preceded by a pre-column and eluted with methanol: 0.01M CH_3COONH_4 ($95:5^{v}/_{v}$),as mobile phase. Flow rate was adjusted to 2.5 mL/min. Aliquots (100 µL) of methanolic solution of ^{99}Tc-complexes(1 and 2), were injected and peaks were detected at 254 and 470 nm.

$^{99m+99}$ Tc-complexes, (Tc^{V} and Tc^{III}-species), were identified under the same analytical conditions. Mass and activity peaks of the injected samples (100 µL, 100-200µCi) were detected by the radiometric and UV detectors in series.In all experiments to measure recovered yields, 100±5% of the injected radioactivity was recovered from the HPLC columns.

Biodistribution studies

Tissue distribution of $[Tc^{III}$-$(sacac)_2en$ $(PPh_3)_2]^{+}$ complex 3, was conducted in Swiss albino mice. HPLC purified 3 was evaporated to dryness under nitrogen, and redissolved in methanol : water 30:70 $^{v}/_{v}$). 0.1 mL (5 µCi) of this solution was injected intravenously in the tail-vein of the mice. Groups of five animals were sacrificed by cardiectomy, under ether anesthesia, at each of 10, 20, 45 and 90 min p.i.,time intervals. The various organs or samples of tissues were dissected, wipped by filter in order to re-

move the excess of blood, weighed and assayed for Tc-99m activity along with appropriate standards,for calculation of percent uptake uptake per gram of tissue.

RESULTS AND DISCUSSION

$[Tc^{III}\text{-}(sacac)_2en\ (PPh_3)_2]\ PF_6{*}2H_2O$ was prepared via the oxo-technetium^{V}-$(sacac)_2$en complex. Reacting $Tc^{V}O\text{-}(sacac)_2en$ 1 with triphenylphosphine, PPh_3,in methanol, resulted in the formation of $[Tc^{III}\text{-}(sacac)_2en\ (PPh_3)_2]PF_6$ complex 2. The reaction involved the two equivalent reduction of Tc^{V} to Tc^{III} by excess of phosphine, with the concomitant coordination of two monodentate phosphine ligands to the reduced technetium center. Fig. 1 illustrates schematically the synthetic procedure followed up.

Elemental analysis and spectral data confirmed the structure of the complex 2. IR spectrum showed no Tc=O band, while intense absorptions were observed at 840 cm^{-1} and 745 cm^{-1}, characteristic of the PF_6 anion and phenyl groups respectively. The absorbance at 1551 cm^{-1} was assigned as the C=N stretching of the imine group and the other one at 1434 cm^{-1} as the P-C stretching, typical for phosphines.

Further on, cationic species of $Tc^{III}\text{-}(sacac)_2en\ (PPh_3)_2$ were obtained in methanol, following the synthetic route outlined in Fig. 1. In this preparation, the $Tc^{V}O\text{-}(sacac)_2en$, 1, precursor was formed in the presence of both ^{99}Tc and ^{99m}Tc. The addition of phosphine to this solution, yielded $[^{99+99m}Tc\text{-}(sacac)_2en\ (PPh_3)_2]^{+}$ 3. At this step heating of the reaction mixture at $95^{0}C$ was found necessary in order to obtain high radiochemical yield of the final complex in solution. The latter was identified by UV-Vis and HPLC analysis comparatively to the ^{99}Tc-congener 2, as it is shown in Fig. 2.

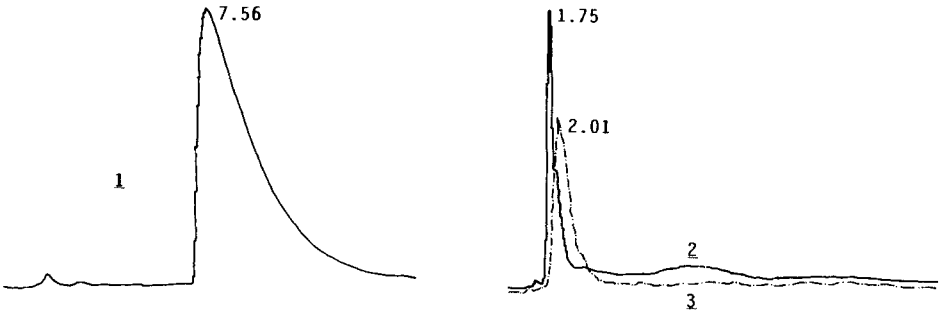

Fig. 2. HPLC profiles of technetium complexes : 1 $[Tc^{V}O(H_2O)(sacac)_2en]Cl$, 2 $[Tc^{III}-(sacac)_2en\ (PPh_3)_2]PF_6$, UV detection, 3 $^{99+99m}Tc^{III}-(sacac)_2en\ (PPh_3)_2$-chelate, radioactivity detection. Mobile phase: Methanol: 0.01M CH_3COONH_4(95:5 v/v). Flow rate: 2.5 mL/min.

Peaks traced by a UV detector that monitors Tc-99 and by a γ-radiometric monitoring Tc-99m were well correlated. $Tc^{V}O-(sacac)_2en$ complex 1, under the same HPLC conditions, was eluted at 7.6 min. after the injection of the intermediate preparation sample. This peak was not anymore observable after the addition of PPh_3 and the formation of $[Tc^{III}-(sacac)_2en\ (PPh_3)_2]$,3. The radioactive peak of complex 3 was collected, diluted with water and administered in mice as 30% methanolic solution.

Biodistribution studies, as presented in Table 1, showed that $[Tc^{III}-(sacac)_2en\ (PPh_3)_2]^+$ exhibits a considerable heart uptake which gave the highest radioactivity value 45 min p.i.(8,6 + 2.6 % dose/g).Though blood clearance is not fast and lung uptake is rela tively high, heart to blood as well as heart to lung ratios at 45 min p.i., raised up to 0.87 and 0.93 respectively. The complex cleared to some extent through the hepatobiliary system, leading to liver uptake values that decreased during the course of the study. Excretion via the kidneys was also observed, the renal clearance being relatively slow. The stomach activity appears to

Table 1. Tissue distribution of $[^{99m+99}Tc\text{-}(sacac)_2en\ (PPh_3)_2]^+$, complex 3, in mice.

Organ	% Dose /g 10 min	20 min	45 min	90 min
Blood	13.12 ± 2.01	12.15 ± 1.80	9.91 ± 2.09	9.47 ± 2.37
Heart	4.58 ± 0.39	7.81 ± 0.79	8.61 ± 2.62	4.61 ± 0.77
Lung	28.27 ± 2.19	10.27 ± 3.77	9.30 ± 2.40	6.44 ± 2.30
Liver	8.62 ± 1.31	16.05 ± 1.37	13.30 ± 1.98	12.81 ± 2.60
Spleen	4.28 ± 1.87	5.52 ± 1.62	4.49 ± 0.38	3.59 ± 1.96
Int.tract	3.74 ± 0.23	3.83 ± 1.23	5.52 ± 0.58	5.43 ± 2.54
Kidneys	4.50 ± 2.65	13.76 ± 1.93	11.26 ± 2.91	9.66 ± 2.61
Stomach	2.10 ± 1.09	5.42 ± 1.85	5.75 ± 1.54	6.09 ± 1.09
Muscles	2.56 ± 0.72	1.84 ± 1.14	2.62 ± 0.47	1.90 ± 0.57

*Data are the mean ± std.dev. for five animals.

increase with time maybe due to a possible instability of the complex in vivo.

In conclusion, a new "mixed-ligand", Tc^{III}-N,N'-ethylene-bis (acetylatone thioiminato)-bis-(triphenylphosphine)complex was prepared directly via the oxotechnetium (V)- Schiff base precursor. Synthesis proceeded in high yield, under the conditions tested. Moreover, $[Tc^{III}\text{-}(sacac)_2en\ (PPh_3)_2]^+$, formed in the presence of both Tc-99 and Tc-99m, was administered in animals.Biodistribution studies demonstrated a considerable heart uptake of the complex. However,the rather slow blood clearance and high lung accumulation observed, may consist relative drawbacks. Further investigation of this type of ligands with other phosphines would be interesting and may improve the biological characteristics of these complexes.

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