Characterization of Tris(N-substituted-2-methyl-3-hydroxy-4-pyridinonato)technetium(IV) Cations

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Introduction

A burgeoning interest in technetium coordination chemistry has been spurred by its widespread use in nuclear medicine.[2–7] Recent focus has been on the design and preparation of new technetium complexes, the lipophilicity, polarity, and overall charge of which can be readily altered by simple substitutions in the molecular framework of the ligand in order to optimize the biodistribution and target specific organs.[2–5,7–11] Examples are cationic (Cardiolite) and neutral (Ceretec) technetium complexes,[7] used as myocardial and brain imaging agents, respectively.

We have been pursuing a continuing study of tris(3-hydroxy-4-pyridinonato)gallium and -indium complexes that are water soluble, are hydrolytically stable, and are of varying lipophilicity and neutral charge.[12–17] The combination of these properties, and the great mobility of these complexes *in vivo*, has suggested their potential application as heart imaging agents.[17] In an effort to extend these properties to technetium, we have investigated the chemistry of technetium complexes with these same ligands. We found that cationic tris(ligand) complexes [Tc(L)₃]⁺ could be easily synthesized from the reaction of TcO₄⁻ (using ⁹⁹ᵐTc and ⁹⁹Tc) with an excess of a N-substituted-3-hydroxy-4-pyridinone in the presence of a reducing agent such as Na₂S₂O₅

Scheme 1

Hmepp, R = C₂H₅
Hpap, R = p-C₆H₄OCH₃

[Tc(mepp)₃]⁺, R = C₂H₅
[Tc(pap)₃]⁺, R = p-C₆H₄OCH₃
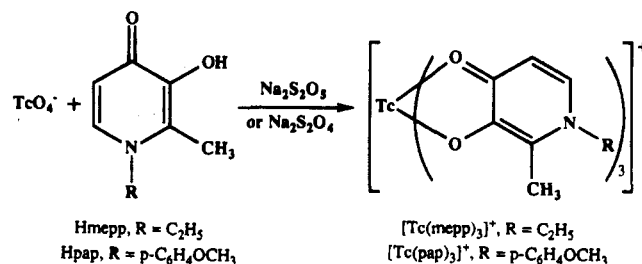
or Na₂S₂O₄ (as shown in Scheme 1). This simple, rapid radiolabeling procedure, which gives high radiochemical purity and yield, provides a kit amenable to clinical application.

Studies *in vivo* in rabbits and mice have revealed that several of these cationic complexes may be useful as morphologic kidney imaging agents, with [⁹⁹ᵐTc(pap)₃]⁺ showing elevated levels in the kidneys even after 24 h.[18] In this report, we present the synthesis (using ⁹⁹Tc) and characterization of two of these cationic tris(ligand) Tc(IV) complexes, [Tc(mepp)₃]PF₆ and [Tc-(pap)₃]Cl, in order to demonstrate conclusively that [Tc(L)₃]⁺ cations are formed at both the no-carrier-added (⁹⁹ᵐTc) level and the carrier-added (⁹⁹Tc) level.

Experimental Section

Materials. The ligands 1-ethyl-3-hydroxy-2-methyl-4-pyridinone (Hmepp)[14] and 3-hydroxy-1-(p-methoxyphenyl)-2-methyl-4-pyridinone (Hpap)[16] were synthesized as described previously. Sodium bisulfite, ammonium hexafluorophosphate, and maltol (3-hydroxy-2-methyl-4-pyrone) were purchased from Aldrich and used as received. NH₄[⁹⁹-TcO₄] was obtained from Oak Ridge National Laboratory and was recrystallized from hot 5 N HNO₃ prior to use. Deionized water of >18 MΩ quality was obtained from a Millipore MilliQ system or from a Corning MP-1 Megapure still with Barnstead D8902 and D8904 cartridges. All other chemicals and solvents were obtained from commercial sources and were used as received.

Instrumentation. Mass spectra were obtained with either a Kratos MS 50 (electron-impact ionization, EI) or an AEI MS-9 (fast-atom-bombardment ionization, FAB) instrument. Infrared spectra were recorded as KBr disks in the range 4000–400 cm⁻¹ either on a Nicolet 5DXB instrument or on a Perkin-Elmer PE 783 spectrophotometer and were referenced to polystyrene. Analyses for C, H, and N were obtained from either Galbraith Laboratories, Inc. (Knoxville, TN), or Canadian Microanalytical Laboratory (Delta, BC, Canada).

Chromatography. Chemical purity for each complex was determined by TLC (Whatman reversed-phase MK C₁₈ plates, CH₃CN:MeOH:0.5 M NH₄OAc:THF = 4:3:2:1) and HPLC (Hamilton PRP-1 column, flow rate 1.5 mL min⁻¹, gradient method, 40:60 to 80:20 CH₃-CN (0.1% TFA):H₂O (0.1% TFA) over 20 min, isocratic at 80:20 for 10 min).

Electrochemistry. Cyclic voltammetry data were obtained using a PARC (Princeton Applied Research Co.) Model 264 polarographic analyzer/stripping voltammeter and a PARC Model RE0089 X-Y recorder. Electrochemical measurements were carried out under an argon atmosphere at room temperature. Solution concentrations were 10⁻³ M in complex and 0.1 M in the supporting electrolyte TEAP (tetraethylammonium perchlorate). Voltammograms were recorded using a platinum working electrode, a platinum wire counter electrode, and an Ag/AgCl reference electrode checked periodically relative to a 1.0 × 10⁻³ M solution of ferrocene in acetonitrile containing 0.1 M TEAP for which the ferrocenium/ferrocene reduction potential was 0.40 V and ΔE_p = 72 mV at a scan rate of 100 mV s⁻¹.

Caution! ⁹⁹Tc is a low-energy (0.292 MeV) β⁻ emitter with a half-life of 2.12 × 10⁵ years. All manipulations of solutions and solids were performed in laboratories approved for the handling of low-level

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radioisotopes, and normal safety procedures were used at all times to prevent contamination.

Tris(1-ethyl-2-methyl-3-hydroxy-4-pyridinonato)technetium-(IV) Hexafluorophosphate (Tc(mepp)₃PF₆). NH₄TcO₄ (87 mg, 0.48 mmol) and Hmepp (390 mg, 2.5 mmol) were dissolved in 40 mL of a 1:3 mixture of ethanol and saline. Solid Na₂S₂O₅ (290 mg, 1.53 mmol) was added, and the solution was refluxed for 16 h. The volatiles were removed under reduced pressure. The residue was dissolved in 25 mL of CH₂Cl₂, and the solution was extracted with 25 mL of water. The orange-red CH₂Cl₂ layer was collected and evaporated to dryness. The residue was dissolved in a mixture of 5 mL of acetone and 20 mL of water. Addition of [NH₄][PF₆] (160 mg, 1.0 mmol), dissolved in 3 mL of water, gave an orange-red precipitate, which was isolated, washed twice with 10 mL of water, and dried *in vacuo*. The yield was 65 mg, 20% based on Tc. Anal. Calcd (found) for C₂₄H₃₀F₆N₃O₆PTc ([Tc-(mepp)₃]PF₆): C, 41.15 (40.70); H, 4.32 (4.53); N, 6.00 (5.92). IR (cm⁻¹): 1605, 1555, 1489 and 1470 (vs, $\nu_{C=O}$ and ν_{C-C}); 847 (PF₆⁻). FABMS: m/z = 555 ([Tc(L)₃]⁺), 403 ([Tc(L)₂]⁺). R_f was 0.86 and R_t was 3.30 min.

Tris(1-(*p*-methoxyphenyl)-2-methyl-3-hydroxy-4-pyridinonato)-technetium(IV) Chloride ([Tc(pap)₃]Cl). To a mixture of Hpap (200 mg, 0.8 mmol), NH₄TcO₄ (36 mg, 0.2 mmol), and NH₄Cl (100 mg) in a 25:10:2 CH₃OH:CHCl₃:H₂O mixture was added a freshly prepared solution of Na₂SO₄ (90 mg, 0.5 mmol) in 3 mL of water. The reaction solution became purple-red immediately. After 3 min of refluxing, it was checked by TLC (R_f = 0.78 (Hpap) and 0.59 ([Tc(pap)₃]⁺). The reaction solution was refluxed for another 10 min and was then left in the fumehood to evaporate solvents slowly at room temperature. A purple-red solid formed on the surface of the water in the flask; this solid was isolated by filtration and was recrystallized from a 1:1 CHCl₃:Et₂O mixture by vapor diffusion to give purple-red microcrystals. [Tc(pap)₃]Cl is very soluble in chloroform and methanol but not soluble in water. The yield was 73 mg, 50% based on Tc. Anal. Calcd (found) for C₄₀H₃₇Cl₄N₃O₉Tc ([Tc(pap)₃]Cl·CHCl₃): C, 50.92 (51.08); H, 3.95 (4.25); N, 4.45 (4.71). IR (cm⁻¹): 1605, 1538, 1505, 1495 and 1465 (vs, $\nu_{C=O}$ and ν_{C-C}). FABMS: m/z = 789 ([Tc(L)₃]⁺), 575 ([TcO-(L)₂]⁺), 559 ([Tc(L)₂]⁺), 361 ([TcO₂L]⁺). R_f was 0.59 and R_t was 21 min.

Results and Discussion

Since ⁹⁹ᵐTc is a short-lived γ-emitting isotope ($t_{1/2}$ = 6 h), it is impractical and not feasible to synthesize enough of a ⁹⁹ᵐTc complex to perform chemical analysis. In order to confirm the structural and chemical properties of ⁹⁹ᵐTc complexes which have potential as morphologic kidney imaging agents,[18] we prepared two representative examples, the cationic tris(ligand) Tc(IV) complexes [Tc(mepp)₃]PF₆ and [Tc(pap)₃]Cl, from the reaction of TcO₄⁻ with an excess (4–5 equiv) of Hmepp and Hpap, respectively, in the presence of a reducing agent such as Na₂S₂O₄ or Na₂S₂O₅ (Scheme 1). The complexes were isolated as either hexafluorophosphate or chloride salts and were stable both in the solid state and in solution. Both salts were soluble in a variety of polar organic solvents (chloroform, methanol, acetonitrile, acetone) and sparingly insoluble in diethyl ether and water. They were characterized by elemental analysis, spectroscopic (IR and FABMS), chromatographic (HPLC, TLC), and electrochemical (cyclic voltammetry) methods. Unfortunately, single crystals for an X-ray diffraction study were never isolated, despite hundreds of attempts to grow crystals with a variety of different anions and N-substituted-2-methyl-3-hydroxy-4-pyridinonato ligands.

The IR spectra of the [Tc(L)₃]⁺ cations displayed four strong bands, which are characteristic of coordinated pyridinonate ligands,[12–16] in the region 1605–1400 cm⁻¹; for [Tc(mepp)₃]-PF₆, the spectrum also showed a band at 847 cm⁻¹ from the hexafluorophosphate counterion. The FAB mass spectra were obtained in a 3-nitrobenzyl alcohol matrix in the positive ion detection mode and showed the expected predominant [M(L)₃]⁺ peak as well as [M(L)₂]⁺ and [M(L)]⁺ fragmentations. In [Tc-

Figure 1. HPLC concordance for [⁹⁹ᵐTc(pap)₃]⁺ and [⁹⁹Tc(pap)₃]⁺.
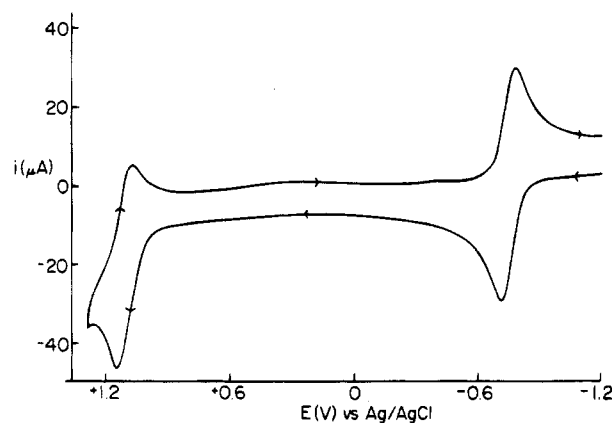
Figure 2. Cyclic voltammogram for [Tc(mepp)₃]PF₆.

(pap)₃]Cl, the FAB mass spectrum also showed peaks at m/z = 575, 361, and 345 corresponding to TcO species [TcO(pap)₂]⁺, [TcO₂(pap)]⁺, and [TcO(pap)]⁺, respectively. The ¹H NMR spectrum of [Tc(pap)₃]Cl in CDCl₃ displayed the broadened, shifted resonance signals characteristic of a paramagnetic complex, consistent with a d³ Tc(IV) center.

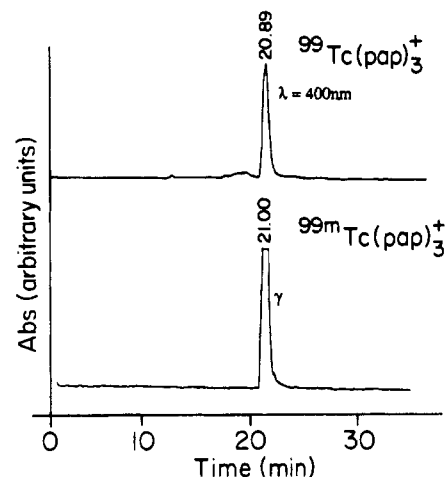**Chromatography.** Comparison of the TLC and HPLC chromatographic data for [⁹⁹Tc(mepp)₃]⁺ and [⁹⁹Tc(pap)₃]⁺ with those for their ⁹⁹ᵐTc analogs[18] shows clearly that the same complexes are prepared on both the no-carrier-added (⁹⁹ᵐTc) level and the carrier-added (⁹⁹Tc) levels. For comparison, the complex [⁹⁹ᵐTc(pap)₃]Cl was prepared in high yield (>95% radiochemical purity as determined by TLC) by reacting ⁹⁹ᵐTcO₄⁻ (3–5 mCi) in an ethanolic saline solution with a large excess of Hpap (4–5 mg) in the presence of sodium bisulfite (15–30 mg). Co-injection of the carrier-added and non-carrier-added complexes showed identical HPLC chromatographic profiles (Figure 1), demonstrating again that the same complex was formed on both macroscale and radiotracer levels.

Electrochemistry. The cyclic voltammogram of [Tc(mepp)₃]-PF₆ was recorded in the potential range −1.2 to +1.25 V vs Ag/AgCl with TEAP as supporting electrolyte in acetonitrile (Figure 2). Two well-defined quasi-reversible one-electron-transfer waves were detected at −0.74 and 1.07 V vs Ag/AgCl at a scan speed of 100 mV/s with ΔE_p = 68 and 82 mV, respectively. Since no redox activity was detected under the same conditions for Hmepp, these two redox processes arise from oxidation state changes associated with the Tc complex. These two redox processes are tentatively assigned to the two one-electron transfers [Tc^IV(mepp)₃]⁺/[Tc^III(mepp)₃] and [Tc^V-(mepp)₃]²⁺/[Tc^IV(mepp)₃]⁺ according the following formal

reductions:

$$[Tc^{IV}(mepp)_3]^+ + e^- \rightarrow Tc^{III}(mepp)_3$$

$$E_1 = -0.74 \text{ V vs AgCl}$$

$$[Tc^{V}(mepp)_3]^{2+} + e^- \rightarrow [Tc^{IV}(mepp)_3]^+$$

$$E_2 = 1.07 \text{ V vs AgCl}$$
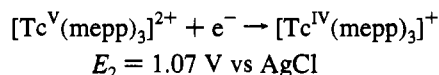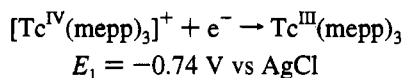
The very large separation (>1.8 V) between the two redox waves for the Tc(V)/Tc(IV) and Tc(IV)/Tc(III) couples clearly indicates that the intermediate Tc(IV) oxidation state is preferentially stabilized by six O donors from three bidentate N-substituted-3-hydroxy-4-pyridinonate ligands. The conproportionation constant (K_{con}) was calculated to be 5.4×10^{30} for the equilibrium

$$[Tc^{V}(mepp)_3]^{2+} + [Tc^{III}(mepp)_3] \xrightleftharpoons{K_{con}} 2[Tc^{IV}(mepp)_3]^+$$

$$\log K_{con} = (E_2 - E_1)/0.059$$
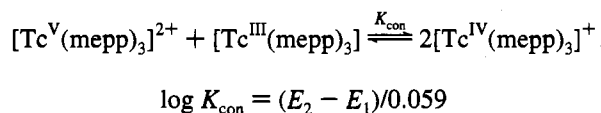
These data strongly corroborate the assignments of these redox waves. In addition, the relatively high reversibility ($\Delta E_p < 82$ mV) for both electron transfers suggests that there are neither significant geometric changes in the inner coordination sphere nor chemical complications when Tc(IV) (inert d^3) is reduced to Tc(III) (low spin d^4, also expected to be inert[19]) or oxidized to Tc(V) (d^2).

In acetonitrile, [Tc(pap)$_3$]Cl also showed two redox waves at ~-0.8 V ($\Delta E_p = 95$ mV) and $+1.03$ V vs Ag/AgCl under

similar conditions; however, the redox wave at the higher potential for the Tc(V)/Tc(IV) couple is irreversible. This is probably caused by the presence of a Cl$^-$ anion, which might undergo a irreversible oxidation at this potential. Since the cathodic and anodic separation for the lower potential wave is 95 mV and increases with higher scan speed, the one-electron transfer at -0.8 V vs Ag/AgCl is not truly reversible. This conclusion is also evidenced by the asymmetric cathodic and anodic waves and the deviation of i_c/i_a from unity.

It is interesting to note that Davison and co-workers have shown[20] that a series of Tc(L)$_3$ complexes with acetylacetonate ligands comprise the cationic Tc(IV), neutral Tc(III), and anionic Tc(II) complexes with a preference for oxidation states 1 unit lower than those of the 3-hydroxy-4-pyridinone complexes describe herein. Clearly the latter are very easy to oxidize.

In conclusion, the [Tc(L)$_3$]$^+$ complexes formed with N-substituted-3-hydroxy-2-methyl-4-pyridinonate ligands with both ^{99m}Tc and ^{99}Tc have been unambiguously demonstrated to be chemically identical.

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