

TECHNICAL NOTES

The Investigation of Radiopharmaceutical Components by Fast Atom Bombardment Mass Spectrometry: The Identification of Tc-HIDA and the Epimers of Tc-CO₂DADS

Catherine E. Costello, James W. Brodack, Alun G. Jones, Alan Davison, Denis Lee Johnson, Sudhakar Kasina, and Alan R. Fritzberg

Massachusetts Institute of Technology, Cambridge, Massachusetts, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts, and University of Colorado Health Sciences Center, Denver, Colorado

The nature of two technetium-labeled radiopharmaceutical components has been established by means of fast-atom-bombardment mass spectrometry (FABMS) in combination with carrier-added (CA) and no-carrier-added (NCA) reversed-phase high-pressure liquid chromatography (HPLC). Negative-ion FABMS was used to determine that the epimers of Tc-CO₂DADS are the oxo[N,N'-(1-carboxyethylene)-bis-(2-mercaptoacetimido)]technetate(V) ions; positive-ion FABMS showed that Tc-HIDA is bis[N-(2,6-dimethylphenyl-carbamoylmethylimino)diaceto]technetate(III).

J Nucl Med 24: 353-355, 1983

The combination of high-pressure liquid chromatography (HPLC) and mass spectral analysis has provided an extremely powerful method for the analysis and characterization of radiopharmaceutical components. We have already demonstrated the utility of field desorption (FD) as an ionization mode using both positive-ion (1) and negative-ion detection (2). This in turn has enabled us (a) to characterize new technetium complexes; (b) to study the nature of reactions on coordinated ligands; and (c) to determine the fate of technetium complexes after administration into animals. In this communication we show that the relatively new technique of fast-atom-bombardment mass spectrometry (FABMS) (3) can also be used to establish the nature of water-soluble technetium complexes separated from radiopharmaceutical preparations.

MATERIALS AND METHODS

Long-lived technetium-99 as ammonium pertechnetate was obtained as a gift and Tc-99m as sodium pertechnetate from a commercial Mo-99→Tc-99m generator. In order to establish the equivalence of the chemistry occurring at carrier-added (CA: Tc = 10⁻⁵ M) and at no-carrier-added (NCA: Tc ≤ 10⁻⁸ M) concentrations, Tc-99m as pertechnetate was added as a tracer so that the HPLC separations could be monitored radiometrically as required.

Received April 15, 1982; revision accepted Dec. 3, 1982.

For reprints contact: Professor Alan Davison, Dept. of Chemistry, Rm. 6-435, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA 02139.

Racemic N,N'-(1-carboethoxyethylene)-bis-(S-benzoyl-2-mercaptoacetamide) (CO₂DADS precursor) was synthesized from DL-ethyl-2,3-diaminopropanoate as described elsewhere (4). The stannous HIDA complex was obtained from a commercial radiopharmaceutical kit.*

All of the reversed-phase ion-pair HPLC analyses and separations were performed as reported previously (2) using a μ-Bondapak[†] C₁₈ column (30 × 0.39 cm). Solvent A was 0.05 M ammonium sulfate in water, and solvent B HPLC-grade methanol. Initial conditions were set at A = 100, B = 0, with a flow rate of 2.0 ml/min. Upon injection, a 10-min linear gradient to A = 0, B = 100 was applied, followed by a 5-min hold at these conditions.

The mass-spectrometry studies were done on a MAT 731 double-focusing mass spectrometer[‡] equipped with a combination EI/FI/FD ion source that can be operated in either positive- or negative-ion mode. The ion source was modified for FABMS by fitting it with an B11 Fine Beam neutral-atom gun.[§] Xenon was used as the reagent gas. An aqueous solution of the sample was mixed 1:1 with glycerol and 1 μl of this was placed on the probe tip.

Preparation of Tc-HIDA. No-carrier-added preparations were made by reconstituting Lidofenin kits according to the manufacturer's instructions, with 4.0 ml saline containing 1 mCi of Tc-99m. Analysis of these showed a single radioactive component to be present, with a retention time of 11.1 min.

Experiments showed that this component could be reproduced at CA levels by the following procedure. To a Lidofenin kit containing 10 mg of the ligand and SnCl₂ (manufacturer's specification: SnCl₂ = 0.80 mg minimum, maximum tin content 0.64 mg)

was added a further 5.8 mg of freshly ground $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, and the kit was reconstituted by adding 5 ml of saline containing 1 mCi of Tc-99m and 4.26 μmole of Tc-99 as ammonium pertechnetate. The product was then used to calibrate both the ultraviolet (UV) (254 nm) and radiometric outputs of the HPLC.

For the sample analyzed by fast-atom-bombardment mass spectroscopy, the Tc-99m was omitted as follows. Freshly ground $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, 6.5 mg, were added to a Lidofenin kit, followed by 2.0 ml H_2O . An aliquot (25 μl) of this was taken for HPLC analysis. To the remaining mixture, 1.0 ml H_2O containing 10.5 μl of 0.406 M Tc-99 as ammonium pertechnetate (4.26 μmole) was added and another 25 μl withdrawn for analysis. The entire preparation was then placed onto a C_{18} SEP-PAK[†] (that had been prewashed, first with methanol and then water) and then washed with 10 ml 0.05 M $(\text{NH}_4)_2\text{SO}_4$. The complex was removed by eluting with 5 ml MeOH, the solution then being passed through a 0.5- μm filter and evaporated to dryness *in vacuo*. The residue was dissolved in 1 ml MeOH, and 25 μl was taken for chromatographic analysis. The remainder was again evaporated to dryness and sealed under argon before mass-spectral analysis.

Preparation of the epimeric mixture of Tc-CO₂-DADS. The protected ligand N,N'-(1-carboethoxyethylene)-bis-(S-benzoyl-2-mercaptoacetamide) (36.16 mg) was dissolved in a mixture of 1 ml ethanol, 1 ml H_2O , and 0.5 ml 1 M NaOH, and heated to 80° for 5 min. After cooling to room temperature, 167 μl 0.443 M (74 μmole) Tc-99 as ammonium pertechnetate and 3 mCi of Tc-99m in 2 ml saline were added, followed by 30 mg of sodium dithionite. The clear yellow solution was adjusted to pH 6.9 by the dropwise addition of 1 M HCl, and passed through a 0.22- μm filter prior to chromatography. Two peaks were observed in HPLC analysis, with retention times of 7.8 and 8.25 min respectively and in a ratio of approximately 1:2.

The first component was collected from 40 repetitive 25- μl injections (fraction collected 7.7–8.1 min) and the second from 42 such injections (fraction collected 8.1–8.6 min). Each of the pooled fractions were then treated to remove the excess ion-pairing agent using a C_{18} SEP-PAK as follows. Each was separately evaporated to dryness *in vacuo*, the yellow residue dissolved in 5 ml H_2O and placed onto a C_{18} SEP-PAK (prepared as above) in order to remove excess ion-pairing agent. The flask was rinsed with 5 ml H_2O and the wash passed through the SEP-PAK. The complexes were each eluted with 5 ml MeOH and kept for mass-spectral analysis.

RESULTS

Tc-HIDA. The retention time of the sole radioactive component in a standard NCA kit preparation of Tc-Lidofenin was 11.1 min under the conditions of analysis. The spiked CA preparation also showed a single component in both the UV and radiometric traces, with the same retention time. A number of prior experiments, using different CA syntheses and analyzed chromatographically under a variety of conditions, had shown it was possible to produce a number of complexes simultaneously, none of which was ever detected in standard NCA kit preparations. Furthermore, degradation over hours was noted even in sealed vials with the CA species having the same retention times as the kit product. For this reason all stages of the manipulations were monitored by HPLC.

Additionally, solutions of the CA Tc-HIDA species degraded more rapidly in air over a period of hours after synthesis. The samples prepared for mass-spectrometric analysis were therefore prepared using Tc-99 only, in the manner described, the preparation being immediately evaporated to dryness and kept under argon. A sample of each was analyzed by HPLC using the UV (254 nm) output to confirm the retention time of the technetium-HIDA species. Figure 1 shows the positive-ion FAB mass spectrum of this preparation. Ions are seen at $m/z = 685$ and a

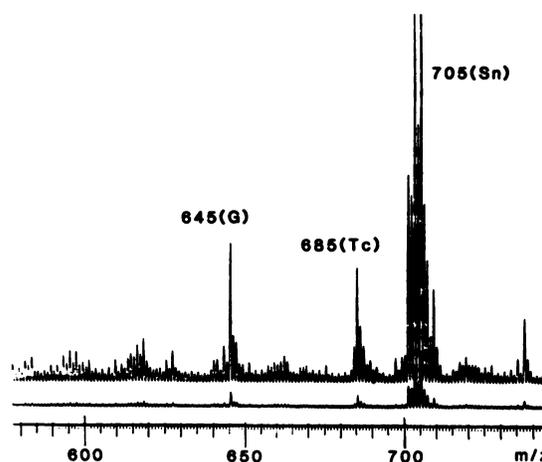


FIG. 1. Positive-ion fast-atom-bombardment mass spectrum of the Tc-HIDA (Tc) and Sn-HIDA (Sn) complexes recovered from a Lidofenin kit. For details of sample preparation see text [reagent gas xenon, matrix glycerol (G)].

cluster at $m/z = 701$ – 709 , demonstrating the presence of two distinct species, a technetium-HIDA and a tin-HIDA complex. The peak at 685 ($\text{A}^- + 2\text{H}^+$) is attributed due to the species $\{[\text{H}]_2[\text{Tc}(\text{III})(\text{HIDA})_2]\}^+$ (calculated for $\{[\text{H}]_2[\text{Tc}(\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_5)_2]\}^+ = 685$).

The cluster at $m/z = 701$ – 709 has the intensity distribution expected for the species $\{[\text{H}][\text{Sn}(\text{IV})(\text{HIDA})_2]\}^+$. Thus the most intense peak at $m/z = 705$ is due to the Sn-120 isotope, which has a natural abundance of 32.4% (calculated for $\{[\text{H}][^{120}\text{Sn}(\text{IV})(\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_5)_2]\}^+ = 705$). The cluster at $m/z = 645$ is due to the glycerol matrix; it consists of seven glycerol molecules and one proton.

Using negative-ion FAB, a more complex spectrum resulted. However, a peak was observed at $m/z = 683$, corresponding to the anion $[\text{Tc}(\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_5)_2]^-$.

Tc-CO₂-DADS. The reaction of N,N'-(1-carboethoxyethylene)-bis-(S-benzoyl-2-mercaptoacetamide) with pertechnetate and dithionite in aqueous ethanolic base leads to the detection of two distinct peaks under HPLC analysis at both NCA and CA levels. The negative-ion FAB mass spectrum of each fraction showed a peak at $m/z = 363$ (A^-) corresponding to the oxotechnetium(V) anion $[\text{TcC}_7\text{H}_8\text{N}_2\text{O}_5\text{S}_2]^-$. Attempts to record negative-ion field-desorption mass spectra with these materials provided only very weak signals and transient ion currents.

DISCUSSION

The experiments of Loberg and Fields (5–7) showed that Tc-99m HIDA could be produced in greater than 95% yield by reduction of pertechnetate ion with stannous tin in the presence of excess ligand. They also demonstrated that the ligand-to-metal ratio in the resulting technetium complex was 2:1 and that it did not contain tin. Electrophoretic studies (8) confirmed the ligand-to-metal ratio and suggested a net charge of -1 . The complex was assumed to be monomeric, with the two dianionic ligands functioning as tridentate iminodiacetates bonding in the triangular faces of an octahedron. With a bis-ligand structure, their assumptions led to a formal charge of $+3$ on the technetium center. In the light of the then existing body of knowledge, the most straightforward choices were Tc^{3+} (oxidation state III) and TcO^{3+} (oxidation state V).

The mass-spectral results here clearly show that the form proposed by Loberg is correct: a bis-dianionic ligand complex of technetium(III), presumably octahedral in structure. Note that

the result also provides an independent verification of the electrophoretic techniques developed by Burns et al. (8).

The reactions of the fully protected (carboxy and both thiols) ligand N,N'-(1-carboethoxyethylene)-bis-(S-benzoyl-2-mercaptoacetamide) evidently lead to the production of an epimeric mixture of the oxo[N,N'-(1-carboxyethylene)-bis-(2-mercaptoacetimido)]technetate(V) anions. The mass spectra of the two fractions seen in the HPLC analysis both showed a single peak at $m/z = 363$, corresponding to the anions $[\text{TcC}_7\text{H}_8\text{N}_2\text{O}_5\text{S}_2]^-$. Thus not only are the benzoyl protecting groups lost during hydrolysis, but also the ester functionality is hydrolyzed completely under the conditions of synthesis. The epimers result from the syn and anti disposition of the carboxylate group on the ethylene bridge with respect to the technetium-oxo bond (4).

These results show clearly that it is possible, using a combination of HPLC and mass spectrometry, to characterize discrete radiolabeled species present in radiopharmaceutical preparations.

ACKNOWLEDGMENTS

This work was supported by the Department of Energy under contract number DE-AC02-81EV10649. The Mass Spectrometry Facility is supported by the NIH Division of Research Resources Grant Number RR00317 (to K. Biemann).

FOOTNOTES

* Merck Frosst Laboratories, Kirkland (Montreal), Canada.

† Waters Associates, Inc., Milford, MA 01757.

‡ Finnegan MAT, Sunnyvale, CA 94086.

§ Ion Tech, Ltd., Teddington, U.K.

REFERENCES

1. WILSON BW, COSTELLO CA, CARR SA, et al: Field desorption mass spectrometry of complex anions containing rhenium and technetium. *Anal Lett* 12(A3):303-311, 1979
2. JONES AG, DAVISON A, LATEGOLA MR, et al: Chemical and in vivo studies of the anion oxo[(N,N'-ethylenebis(2-mercaptoacetimido)]technetate(V), *J Nucl Med* 23:801-809, 1982
3. BARBER M, BARDOLI RS, SEDGWICK RD, et al: Fast atom bombardment of solids as an ion source in mass spectrometry, *Nature* 293:270-275, 1981
4. FRITZBERG AR, KUNI CC, KLINGENSMITH WC III, et al: Synthesis and biological evaluation of Tc-99m N,N'-bis-(mercaptoacetyl)-2,3-diaminopropionate: A potential replacement for I-131 hippuran. *J Nucl Med* 23:592-598, 1982
5. LOBERG MD: Radiolabeled drug analogs based on N-substituted iminodiacetic acid. In *The Chemistry of Radiopharmaceuticals*. Heindel ND, Burns HD, Honda T, Brady LW, Eds. New York, Masson, U.S.A., Inc. 1978, pp 191-204
6. LOBERG MD, COOPER M, HARVEY E, et al: Development of new radiopharmaceuticals based on N-substitution of iminodiacetic acid. *J Nucl Med* 17:633-638, 1976
7. LOBERG MD, FIELDS AT: Chemical structure of technetium-99m-labeled N-(2,6-dimethylphenylcarbamoylmethyl)-iminodiacetic acid (Tc-HIDA). *Int J Appl Radiat Isotopes* 29:167-173, 1978
8. BURNS HD, SOWA DT, WORLEY P, et al: Electrophoretic determination of charge on carrier-free 99m-Tc-labeled complexes. *J Pharm Sci* 70:436-439, 1981

Society of Nuclear Medicine 8th Annual Western Regional Meeting

October 6-9, 1983

Westin Plaza Hotel

Seattle, Washington

Announcement and Call for Abstracts

The Scientific Program Committee welcomes the submission of abstracts of original contributions in Nuclear Medicine from members and nonmembers of the Society of Nuclear Medicine for the 8th Annual Western Regional Meeting. Physicians, scientists, and technologists—members and nonmembers—are invited to participate. The program will be structured to permit the presentations of papers from all areas of interest in the specialty of Nuclear Medicine. Abstracts submitted by technologists are encouraged and will be presented at the Scientific Program. Abstracts for the Scientific Program will be published as a Journal Supplement and will be available to all registrants at the meeting.

The Western Regional Scholarship and Award Fund will make one award in the name of Norman D. Poe for the most outstanding paper in the field of pulmonary or cardiac nuclear medicine and a second award for an outstanding Technologist paper.

The abstracts will be printed from camera-ready copy provided by the authors. Therefore, only abstracts prepared on the official abstract form will be considered. These abstract forms will be available from the Western Regional Chapter office (listed below) after April 1, 1983. Abstract forms will be sent to members of the Pacific Northwest, Northern California, Southern California and Hawaii Chapters in a regular mailing in early May, 1983. All other requests will be sent on an individual basis.

All participants will be required to register and pay the appropriate fee. Please send the original abstract form, supporting data, and seven copies to:

Justine J. Parker, Administrator
8th Western Regional Meeting, SNM
P.O. Box 40279
San Francisco, CA 94140

The 8th Annual Western Regional Meeting will have commercial exhibits and all interested companies are invited.

For information contact Becci Lynch at the Western Regional SNM office (address above). Tel: (415)647-0722 or 647-1668.

Deadline for abstract submission: Postmark by midnight, June 24, 1983