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COMPUTATIONAL CHEMISTRY AND METAL-BASED RADIOPHRMACEUTICALS

M. NEVES
Instituto Tecnológico e Nuclear, Sacavém

R. FAUSTO
Departamento de Química, Faculdade de Ciências e Tecnologia,
Universidade de Coimbra, Coimbra

Portugal

Abstract

Computer-assisted techniques have found extensive use in the design of organic pharmaceuticals but have not been widely applied on metal complexes, particularly on radiopharmaceuticals. Some examples of computer generated structures of complexes of In, Ga and Tc with N,S,O and P donor ligands are referred. Besides parameters directly related with molecular geometries, molecular properties of the predicted structures, as ionic charges or dipole moments, are considered to be related with biodistribution studies. The structure of a series of oxo neutral Tc-biguanide complexes are predicted by molecular mechanics calculations, and their interactions with water molecules or peptide chains correlated with experimental data of partition coefficients and percentage of human protein binding. The results stress the interest of using molecular modelling to predict molecular properties of metal-based radiopharmaceuticals, which can be sucessfully correlated with results of *in vitro* studies .

1. INTRODUCTION

Molecular computer modeling describes the generation, manipulation and representation of 3D structures of molecules and their associated physicochemical properties. In addition, the simulation of intermolecular interactions between the optimized molecular structures and specific macromolecular targets, could be used to recognize biological potential binding sites, and leads to the understanding of biological phenomena.

This research area has been developed for largely on the search for new drugs in the pharmaceutical industry. However, its application is still relatively uncommon in the field of metal-based radiopharmaceuticals, mainly due to the difficulty in the determination of reliable force field parameters for transition metal ions. Reliable parameters were obtained by modeling metal complexes and varying the parameters involving in Me-L bonds until the agreement between the molecular generated geometry and the crystallographically observed structures was as close as possible. According to R.D.Hancock [1], optimized parameters

for octahedral complexes of In(III) and Ga(III) with N, S and O donors were applied to predict several structures which demonstrated good agreement with those crystallographically determined. In the case of Tc(V) oxo complexes, the apex of the Tc=O group is readily reproduced by defining appropriate ideal O-Tc-L angles. Some structures predicted by molecular mechanics are:

- N,N'-ethylene bis(3-ethyl-3-mercaptobutyl) oxo Tc(V), with root mean deviations relatively to the crystallographic structure of 0.196 Å, for the heavy atoms, and 0.347Å , for all atoms [2].
- $[TcO(DME)_2]^-$ (DME= ), [TcO(DMEA)SPh] (DMEA= ) and $[TcO(TCC)_2]$ (TCC=tetrachlorocatechol). For these compounds, the overlap of the sulfur p_z orbital with the d_{xz} orbital of Tc (π-bond) retrains the rotation about the Tc-S bond and the calculations were not able to reproduce exactly the crystallographic structures [1,3].

On the other hand, the structures of five oxo Tc(V) complexes bearing several ligands with Tc-N, Tc-S and Tc-P bonds were predicted by density-functional methods and the results compared with the corresponding crystal structures. The authors claim the advantages of this approach, in spite of the computational resources needed be considerable [4] .

When reliable force field parameters are achieved and confidently tested, the use of molecular mechanics to predict molecular structures of Tc-complexes, that cannot be readily determined experimentally (such as ^{99m}Tc-complexes at carrier-free level, 10^{-6} - 10^{-8} M, in where inorganic classic analytical techniques are not easy to apply and the corresponding ^{99}Tc chemistry is complex), appears as a very powerful method of study. Indeed, when supported by both the available informations on the specific peculiarities of the ^{99}Tc and/or Re and the previously determined molecular structures of the ligands, molecular mechanics has been proved its great power in furthering our understanding of the chemical reactivity and fundamental structural features of Tc-radiopharmaceuticals [5,6]. Besides the bond lenghts and valence or dihedral angles, a series of other relevant molecular properties can be evaluated by molecular mechanics, including dipole moments, surface areas, atomic charges, Van der Waals (VDW) energies, dipole-dipole or charge-charge interaction energies (DD/QQ) etc., which can then be correlated with experimental data. Hansen et al. [7], developed a series of a second-generation ^{99m}Tc renal radiopharmaceuticals based on the prototype agent $TcO(MAG_3)]^{2-}$ (MAG=mercaptoacetyltriglicine) on the basis of results provided by molecular mechanics studies. These authors tried to find a correlation between both the position of the carboxyl group and the oxo-carboxyl interatomic distances with biologic activity, and understand the relevance of the above molecular parameters to renal excretion. In a different molecular mechanics study, Reichert and Welch [8] established

correlations between dipole moments and ionic charges of several copper complexes of macrocyclic ligands (cyclen, cyclam, Etcyclam, DOTA, TETA), and their liver uptake by rats.

Another interesting problem, is the evaluation of specific molecular interactions between the predicted metal-based radiopharmaceuticals and biological molecules. Each complex has its own specific properties, such as electron donor/acceptor character, lipophilicity-hydrophilicity balance, redox potential, pK, chirality and isomerism. These specific properties are the key-base to molecular recognition by biological molecules. So, it is expected that simulations of intermolecular interactions can shed light, at a molecular level, to the specific binding involving the radiopharmaceuticals and the biological molecules.

In this paper, the computer generated optimized geometries of the oxo neutral complexes $[TcOL_2(OH)]$ are reported, where L is the monoanionic form of biguanide $[H_2NC(=NH)NHC(=NH)NH_2]$ (Big) or the N1 substituted dimethyl (DMBig), phenyl (PBig) and phenethyl (PEBig) biguanides. The calculations were undertaken by *molecular mechanics,* being the reliability of the approach used first checked by detailed comparison between theoretically predicted geometrical parameters (atomic distances, valence and dihedral angles) and the correspondent experimental values obtained by X-ray crystallography for the cationic complex $[TcO(DMBig)_2]^+$. In addition to the structural predictions, the computational method used also enabled a detailed evaluation of the main intermolecular interactions between the Tc-complexes and simple molecules, such as water or small peptide chains. Correlations were found between interaction energies and surface areas of the low energy aggregate species and the results of *in vitro* studies, as partition coefficients and percentage of human protein binding of the ^{99m}Tc-biguanide complexes.

2. MOLECULAR MECHANICS CALCULATIONS

The calculations were undertaken with the PCMODEL program (version 3.0) [9], in a 486DX4-100 PC. PCMODEL is a simple interactive molecular modeling program that can handle up to 296 total atoms. The MMX force field is an extension of Allinger' s MM2 force field which, besides performing conventional *molecular mechanics* calculations on the molecular σ-system, undertakes a simultaneous π-system semi-empirical valence electron self consistent field calculation. This approach improves considerably both the qualitative and quantitative description of systems having delocalized π-electrons, such as the ligands studied in this work. General parameters for technetium are available in this force field, where the metal covalent radius is taken to be 135.5 pm [10]. To check the reliability of the PCMODEL/MMX calculations for the systems considered, a minimized structure of the analogous complex $[TcO(DMBig)_2]^+$ was first generated and compared with available

experimental structural data[13]. Satisfactory agreement is observed, even when relevant dihedral angles, which are in general difficult to reproduce by *molecular mechanics*, are considered [11,12]. In particular, the calculations agree with the experimental data showing that the Tc(V) ion close a pseudoaromatic ring, due to extensive π electron delocalization along the N-C-N skeleton and that the Tc=O core lies slightly above the plane formed by the two ligands

The structures of the neutral oxo Tc-complexes, $[TcO(Big)_2(OH)]$, $[TcO(DMBig)_2(OH)]$, $[TcO(PBig)_2(OH)]$ and $[TcO(PEBig)_2(OH)]$, were analyzed by similar calculations. Finally, the intermolecular interactions between each one of the complexes and water (or peptide chain) molecules were evaluated by:

i) optimizing the structures for each Tc-complex/water molecule or Tc-complex/peptide chain aggregate resulting from a systematic search in their configurational space, the whole process being systematically checked by inspection of relative energies of the final structures,

ii) calculating a series of relevant structure-related properties (such as polar, apolar and total surface areas, VDW and DD/QQ interaction energies) that, together with the same properties obtained for the individual components of the aggregates, can be correlated with the degree of interaction between these species within the aggregates.

3. RESULTS AND DISCUSSION

3.1. Geometries

The values of the theoretically predicted parameters (bond distances, valence and dihedral angles) of the proposed structures of Tc-biguanide complexes: $[TcO(Big)_2(OH)]$, $[TcO(DMBig)_2(OH)]$, $[TcO(PBig)_2(OH)]$ and $[TcO(PEBig)_2(OH)]$ are shown in Table 1. In all complexes studied the ligand molecules were found to be quasiplanar, with the C-N-C-N(R) torsion angles, (R = H_2 , $(CH_3)_2$, HPh, HCH_2CH_2Ph) varying from 169.7 to 177.5°. The ligand substitution effect is more relevant in the case of the phenyl and phenethyl derivatives, where the torsion angle O=Tc-O-H are 143.5° and 148.2°, respectively, while in the case of non-substituted and dimethyl substituted complexes this angle is 178.3° and 177.3°. The deviation from planarity observed in these complexes having ligands bearing a phenyl group, can be explained considering that the presence of the aromatic rings considerably increase the overall steric hindrance, thus forcing the axial ligands to assume the observed non-planar geometry. In the case of the phenethylene derivative this effect is less pronounced, since the presence of the ethylenic chains leads to reduce steric contacts.

Table 1. Relevant calculated geometrical parameters for the oxo ^{99m}Tc -biguanide complexes*

	TcOBig	TcODMBig	TcOPBig	TcOPEBig
bond distance(Å)				
Tc=O	1.72	1.71	1.73	1.73
Tc-OH	1.90	1.89	1.91	1.92
Tc - N	1.92-1.96	1.92-1.97	1.91-1.97	1.92-2.04
C - N	1.38-1.41	1.35-1.46	1.31-1.42	1.30-1.46
C - C	-	-	1.39-1.42	1.40-1.54
N - H	0.96-0.97	0.96-0.97	0.96-0.98	0.96-1.00
C - H	-	1.11	1.10	1.10-1.12
valence angle (°)				
O =Tc - O	156.5	154.6	171.6	157.0
O = Tc - N	91.6-111.4	90.1-106.4	82.0-102.8	77.6-124.1
N - Tc - OH	65.9-111.7	66.0-112.5	83.6-103.9	78.7-108.8
N - Tc -N	85.9-176.8	87.2-179.0	83.5-167.0	85.1-160.8
C - N - Tc	125.8-127.4	125.8-128.2	118.1-127.1	120.7-126.1
N - C - N	117.4-124.0	118.1-124.7	111.4-127.1	114.1-128.7
C - N - C	124.7;125.0	119.2-126.2	125.6-131.1	124.2-126.5
C - C - N	-	-	116.6-126.6	109.8;112.4
C - C - C	-	-	118.4-121.7	118.9-130.2
N - C - H	-	109.7-111.8	-	105.9-109.3
C - N - H	115.6-120.8	114.8-120.8	114.4-121.3	105.7-118.1
H - N - H	119.0-119.7	119.0;119.1	119.0;119.4	118.1;119.7
torsion angle (°)				
O = Tc - O - H	178.3	177.3	143.5	148.2
C - N - C - NH_2	171.2-176.6	169.7;176.3	174.2;179.7	173.3;173.7
C - N - C - $N(CH_3)_3$	-	169.9;177.5	-	-
C - N - C - NH(Ph)	-	-	169.7;174.5	-
C - N - C - NH(EtPh)	-	-	-	171.6;176.9

*TcOBig, TcODMBig, TcOPBig and TcOPEBig are abbreviated notation of $[TcO(Big)_2(OH)]$, $[TcO(DMBig)_2(OH)]$, $[TcO(PBig)_2(OH)]$ and $[TcO(PEBig)_2(OH)]$

3.2. Molecular interactions

Simulation of molecular interactions between water molecules and $[TcO(Big)_2(OH)]$, $[TcO(DMBig)_2(OH)]$, $[TcO(PBig)_2(OH)]$ or $[TcO(PEBig)_2(OH)]$ suggest the attachement of two water molecules to the complexes, by means of hydrogen bond formation between the nitrogen atoms of the guanidine imino groups and hydrogen atoms of the water molecules, as shown in Figure 1, for the specific case of $[TcO(Big)_2(OH)]$. Additional water molecules stay close to the Tc aggregate environment, but no more hydrogen bond formation was predicted.

The relative changes in polar surface areas (ΔSA), for each molecular aggregate (Tc-complex and two water molecules), relative to the sum of the polar surface areas of the individual components may be used as a measure of the relative stability of the various aggregates (or the relative water affinity to the complex). A greater water affinity would correspond to a greater surface area reduction and, consequently, to a greater ΔSA value.

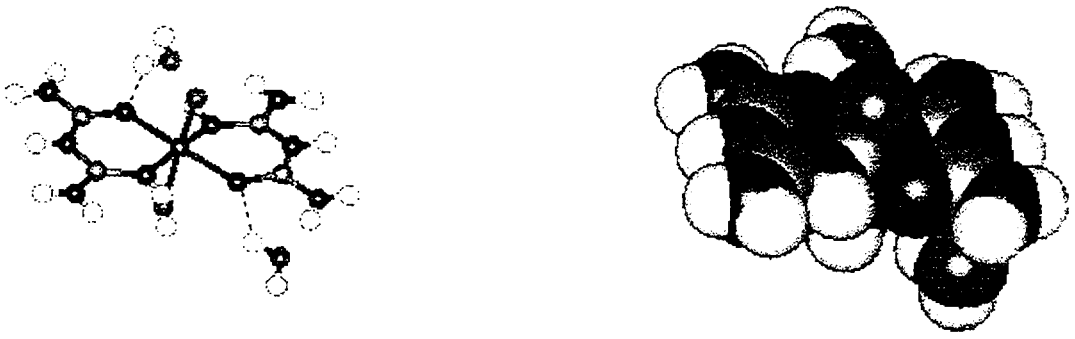

FIG. 1. Minimum energy aggregate resulting from interaction of $[TcO(Big)_2(OH)]$ with water (ball & stick and CPK space filling models; H bonds represented as dash lines)

In addition, the relative values of ΔDD/QQ=DD/QQ (aggregate) - Σ DD/QQ (individual compo nents) may be correlated with the partition coefficients experimentally evaluated for each complex. In fact, the minimum ΔDD/QQ value (-33.15 kcal.mol^{-1}) corresponds to the more hydrophilic Tc-complex, and the maximum ΔDD/QQ value (+0.81 kcal.mol^{-1}) to the less hydrophilic (or more lipophilic), which means that the electrostatic interaction energies could also be related to water affinity along the same series of Tc-complexes. The observed correlations of relative water affinity with both, ΔSA and ΔDD/QQ, are consistent with the dipolar character of water molecules and consequently with the prevalence of Coulomb electrostatic interactions in these systems. Other structure-related parameters, such as relative changes in molecular apolar surface areas or VDW interactions, are not expected to play an important role in stabilizing the aggregates, and were not considered. The graphical representation of partition coefficients (PC), ΔSA and ΔDD/QQ along the series of studied Tc-complexes, gives a clear indication that calculated ΔSA and ΔDD/QQ follow the same pattern of variation of the experimentally determined partition coefficients (Figure 2).

Using the same methodology, the molecular interactions between the complexes, $[TcO(Big)_2(OH)]$, $[TcO(DMBig)_2(OH)]$, $[TcO(PBig)_2(OH)]$, $[TcO(PEBig)_2(OH)]$, and the simple dipeptide chain $^+H_3N\text{-}CH_2\text{-}C(=O)\text{-}NH\text{-}CH_2\text{-}COO^-$ (whose structure was previously optimized as described for all other systems here considered) were also evaluated. The optimized geometry of the dipeptide has the relevant bond distances and valence angles similar to those described in literature (in parentheses), *i.e.*, a C=O distance of 1.21Å (1.24Å), a C(=O)-N distance of 1.34Å (1.32Å), a N-C distance of 1.46Å (1.47Å) and a O=C-N angle of 125.3° (125°).

The observed multiple interactions between the complex $[TcO(Big)_2(OH)]$ and the dipeptide could be explained in terms of electrostatic interactions involving the different atoms of the system. Then, the hydrogen atoms of the NH_3^+ group of the peptide chain (charge +0.22e)

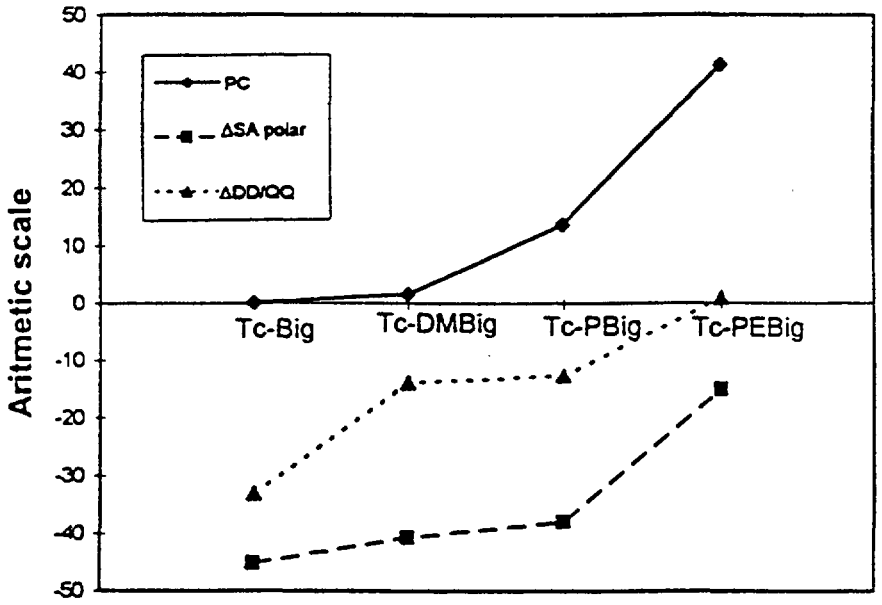

FIG. 2. Partition coefficients (PC), ΔSA polar and ΔDD/QQ for Tc-biguanide complexes.

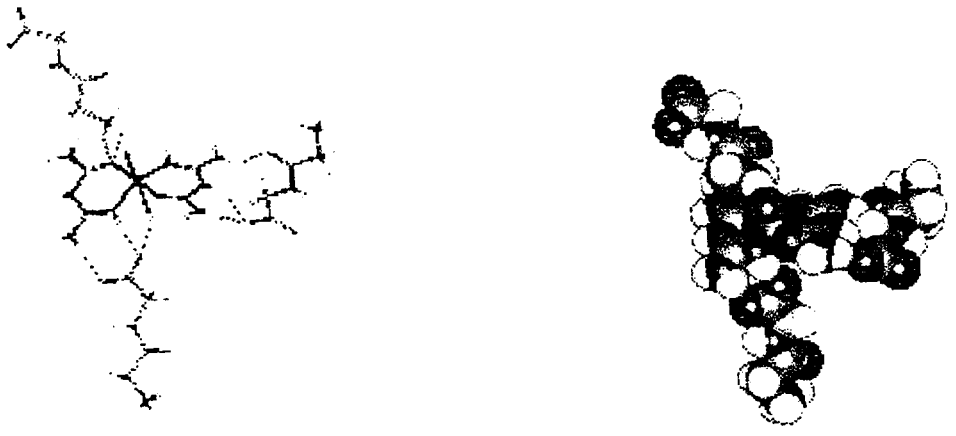

FIG. 3. Minimum energy aggregate resulting from interaction of $[TcO(Big)_2(OH)]$ with three peptide chains (ball & stick and CPK space filling models; H bonds represented as dash lines)

form hydrogen bonds with the electronegative nitrogen atoms of the Tc-complex (charges ranging from -0.19 to -0.46e), while the oxygen atoms of both the C=O and COO^- groups of the dipeptide (charges: C=**O**, -0.29e; C(=**O**)O^-, -0.41e; C(=O)**O**$^-$, -0.79e) form hydrogen bonds with the electropositive hydrogen atom of the Tc-OH group (charge +0.29e) and with the hydrogen atoms of the imino (+0.22e) and amino (+0.15e) groups of the biguanide ligands. The cyclization of the dipeptide can occur as shown in Figure 3, through hydrogen bond formation between the COO^- and C=O groups with imino and amino groups. The COO^- group of a second dipeptide, is also hydrogen bonded to the Tc-OH, imino and amino

groups. A third dipeptide is hydrogen bonded by its NH_3^+ group to an imino group of the complex.

The Tc-complexes of the remaining ligands studied do not have as many amino groups acessible as [TcO(Big)$_2$(OH)]. In addition, the effect of substitution of hydrogen atoms by the dimethyl, phenyl and phenethyl groups on the N1 atom leads also to a reduction of the charges on all nitrogen atoms, specially those that stay closer to the substitution site. This decreasing effect can be explained considering that coordination favors electronic delocalization involving the N atoms. On the other hand, the presence of non-polar groups leads to the appearance of essentially hydrophobic surfaces that limit the extension of the attractive interactions involving polar surfaces of the complex and the peptide chains. In the case of the N1 dimethyl derivative only one peptide chain interacts significantly with the complex (two hydrogen bonds, between the COO^- and Tc-OH groups and an imino group are predicted). Additional peptide chains are pushed out by the essentially non-polar dimethyl groups. In the [TcO(PBig)$_2$(OH)] and [TcO(PEBig)$_2$ (OH)] complexes a similar effect is promoted by the phenyl and phenethyl groups.

The interaction of [TcO(Big)$_2$(OH)] with the peptide chains is clearly distinct from the processes involving the other complexes studied. It is interesting to note that the percentage ges of human protein binding for the various complexes evaluated by gel filtration, are: [TcO(Big)$_2$(OH)]=44.9±2.3; [TcO(DMBig)$_2$(OH)]=13.4±1.1; [TcO(PBig)$_2$ (OH)]=10.6±0.9 and [TcO(PEBig)$_2$(OH)]=7.1±0.7. Thus the percentage of human protein binding for [TcO(Big)$_2$ (OH)] is considerably higher than for the other complexes. This findings agrees with the theoretical results, which predict that [TcO(Big)$_2$(OH)] is considerably more efficient in establishing energetically favorable hydrogen bonds with the peptide chains.

4. CONCLUSION

The elucidation, at a molecular level, of the way of functioning of pharmacologically relevant systems is one of the most important challenges in biomedical research. Molecular modeling has been playing an ever growing role in this field, and it appears nowadays as a very powerful method of study, whose relevance in the specific field of radiopharmaceuticals is very promising. The present study adopts this methodology to shed light on the molecular structures of pharmacologically relevant Tc-complexes of biguanides, and enabled us to establish fundamental relationships between some structure-related molecular properties and important physicochemical properties of the studied systems, such as partition coefficients and water affinity, as well as protein binding and peptide interactions. The success of this approach to study the kind of systems here considered opens good perspectives to start a series of systematic studies by this method directed both to the computer assisted design of

new Tc-radiopharmaceuticals and to the establishment of important correlations between their relevant molecular properties and results obtained from experimental *in vitro* studies.

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