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Molecular Mechanics of Oxaliplatin and an Oxaliplatin Derivative with Their Relevant Biological Targets

Daniel C. Jackson
Western Kentucky University, Daniel.Jackson429@topper.wku.edu

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MOLECULAR MECHANICS OF OXALIPLATIN AND AN OXALIPLATIN
DERIVATIVE WITH THEIR RELEVANT BIOLOGICAL TARGETS

A Capstone Experience/Thesis Project

Presented in Partial Fulfillment of the Requirements for

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Honors College Graduate Distinction at Western Kentucky University

By

Daniel C. Jackson

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Western Kentucky University

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CE/T Committee: Approved by

Professor Kevin M. Williams, Advisor

Professor Rajalingam Dakshinamurthy

Professor Philip Lienesch   _____

                                                                              Advisor
                                                                   Department of Chemistry

ABSTRACT

Molecular mechanics and dynamics calculations were used to simulate reactions of oxaliplatin and Pt(Me$_2$dach)(oxalate) with methionine and guanine, where Me$_2$dach is N,N-dimethyl-1,2-diaminocyclohexane. The results were consistent with steric effects that resulted in chelation when Pt(Me$_2$dach)(oxalate) reacted with N-acetylmethionine experimentally (Williams et al., 2013). The energy difference due to ligand bulk that was predicted using molecular mechanics was also consistent with experimental results: oxaliplatin's ligand bulk did not prevent the formation of bis products with 9-ethylguanine and N-acetylmethionine, but the ligand bulk of Pt(Me$_2$dach)(oxalate) did prevent bis product formation with N-acetylmethionine, resulting in chelation with the sulfur and oxygen atoms of the methionine residue (Williams et al., 2013). Pt(Me$_2$dach)(oxalate) did not prevent bis products with 9-ethylguanine (Williams et al., 2013).

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VITA

September 26, 1990…………………………..Born – Bowling Green, Kentucky

2005...Greenwood High School, Bowling Green, Kentucky

2009...Western Kentucky University

PUBLICATIONS

1. Williams, K. M., Poynter, A. D., Hendrie J. D., Jackson D. C., & Martin, V. K. (2013). Comparison of N-acetylmethionine reactivity between oxaliplatin and an oxaliplatin derivative with chiral (S,S) amine nitrogen atoms. *Inorganica Chimica Acta, 401,* 64-69. doi:10.1016/j.ica.2013.03.008

FIELDS OF STUDY

Major Field: Biology

Minor Field: Psychology

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Molecular mechanics is a form of software that models organic and inorganic compounds through parameters that determine the conformation and energy of a compound. Molecular mechanics software utilizes force fields, which are sets of parameters used to calculate energy. An AMBER (Assisted Model Building with Energy Refinement) force field was used in the molecular mechanics software because of its parameters for amino acids and DNA bases, molecules which were of focus.[1] The AMBER force field has been modified several times from the force field developed by Weiner and his colleagues in the 1980's.[2] How parameters are obtained varies from each modification, but in general, these force fields rely heavily upon *ab initio* calculations of simple molecules, X-rays of crystal structures, IR (Infrared spectroscopy), and NMR (Nuclear Magnetic Resonance spectroscopy) spectra to obtain the data necessary to create parameters for factors such as bond lengths, bond angles, and electrostatic interactions.[2-4]

This software was used to model oxaliplatin, a platinum-containing anticancer compound used in the treatment of colorectal cancer (Figure 1.1).[5-7] It was developed and utilized because it is not cross-resistant with its predecessors, cisplatin and carboplatin.[8] Oxaliplatin and other platinum-containing anticancer compounds function by having their leaving groups (the portion of the compound that is removed by another molecule, for example, oxalate for oxaliplatin) replaced with two water molecules inside

a cell. Upon reaching the nucleus, the water molecules are replaced by DNA bases as

platinum binds to DNA. It is the binding to DNA that can result in replication errors that

lead to apoptosis. Though platinum is thermodynamically favored to bind to DNA bases

over amino acids, platinum is kinetically favored to bind to amino acids.[9,10] It is

therefore relevant in anticancer research to determine what extent of bulk will shift

platinum's kinetic preference to DNA bases.
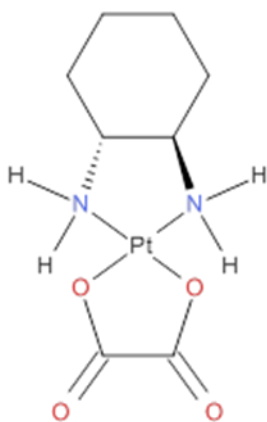
Figure 1.1: Oxaliplatin

The carrier ligand is the top portion of this molecule, including Pt. The leaving ligand is everything below in this two-dimensional representation.

In addition to oxaliplatin, molecular mechanics was also used to model

Pt(Me$_2$dach)(oxalate), a derivative of oxaliplatin (Figure 1.2). The difference between

these two compounds is due to their carrier ligands (Figure 1.1). Oxaliplatin contains the

dach (diaminocyclohexane) carrier ligand, while Pt(Me$_2$dach)(ox) contains the Me$_2$dach

(1,2-dimethyl diaminocyclohexane) carrier ligand. Both compounds contain the oxalate

leaving group. Me$_2$dach differs from dach in that both of its chiral nitrogen atoms have a

single bond with a methyl group, creating a larger bulk than dach. Since amino acids are

larger than DNA bases, our goal was to see if the bulk difference between oxaliplatin and

Pt(Me$_2$dach)(ox) created a difference in reactivity between these two compounds with

guanine and methionine.

2

Figure 1.2: Pt(Me$_2$dach)(oxalate)

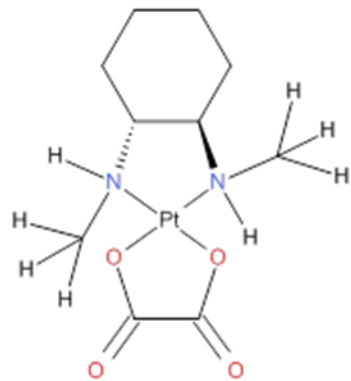Contains the oxalate leaving ligand like oxaliplatin, but the carrier ligand contains two methyl groups that replace two of the hydrogens in oxaliplatin's dach carrier ligand.

CHAPTER 2

EXPERIMENT

Molecular mechanics data were obtained using HyperChem 7 (Hypercube, Inc.)

on a Dell Optiplex GX260 computer with Windows XP. An AMBER89 force field was

used with modifications to contain parameters developed in past research for platinum

atoms bound to guanine and methionine.[3,11] To analyze the bulk difference between

Pt(Me$_2$dach)(oxalate) and oxaliplatin, molecular mechanics was used to construct models

with varying conformations, including various chiralities of the amine nitrogens, carbons

1 and 2 of the cyclohexane chair of the carrier ligand, and the sulfur of the methionine

residue. Once structures were made, energy minimizations were done on the models.

Molecular dynamics calculations were then done on the models analogously with

previous research: models were simulated at 300 Kelvin for 250 picoseconds, with a

conformation saved at each picosecond and the lowest total energy of each conformation

was recorded.[9]

CHAPTER 3

RESULTS

Oxaliplatin with bis-methionine: [Pt(dach)(N-AcMet-S)$_2$]

To distinguish rotamers, the first two chiralities are those of the carbons of the

cyclohexane ring bound to the amine nitrogens. The next two chiralities are those of the

two sulfur atoms of the two methionine residues. Head-to-head (HH), tails-to-tails (TT),

head-to-tails (HT), or tails-to-head (TH) refer to the positioning of the methyl group

attached to each methionine's sulfur atom. If it is bound in such a way that it points

above the platinum plane, it is in the heads (H) configuration, and below the platinum

plane is tails (T). The methyl groups' orientations are listed in order from the methionine
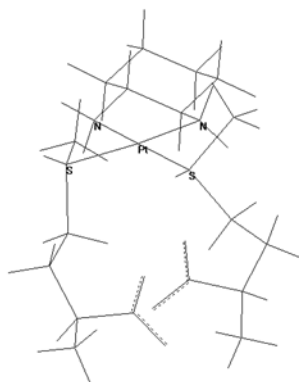
residue on the left first.

Figure 3.1: RR-RR-HH, the lowest total energy conformation for oxaliplatin with bis-methionine, with the methionine residues oriented closest to viewer. The methyl groups attached to the sulfur atom of each methionine residue are pointing above the platinum plane, while the rest of each residue is positioned below the platinum plane.

In the graph below (table 3.1), it is shown that RR-RR-HH (Figure 3.1) was the conformation that provided the lowest total energy. The reason why it was the lowest total energy conformation can in part be explained by running single point calculations, which show electrostatic interactions, hydrogen bond strain, and total bond strain. Compared to the relatively highest total energy structures, the relatively lower total energy structures had lower total bond strain and lower electrostatic interactions and hydrogen bond strain.

Rotamer	Lowest Total Energy (kcal/mol)	Time (ps)
RR-RR-HH	-11.4834	85
RR-RR-TT	-6.49225	59
RR-RR-HT	-10.7318	91
RR-SS-HH	9.671332	2
RR-SS-TT	11.00478	158
RR-SS-HT	16.87738	1
RR-SR-HH	9.457934	10
RR-SR-TT	-10.4101	21
RR-SR-HT	19.58132	151
RR-SR-TH	-1.19333	32

Table 3.1

Oxaliplatin with bis-guanine: [Pt(dach)(9-EtG)$_2$]

To distinguish rotamers, the first two chiralities listed are those of the two cyclohexane ring carbons bound to the amine nitrogens. Since each guanine residue

6

contains no chiral atoms, the direction and placement of the hydrogen bonded to the C8 carbons (the carbons double-bonded to the N7 nitrogens bound to platinum) were used to label conformations as HH (head-to-head), and lambda HT and delta HT.[12] Lambda HT refers to the C8 hydrogen bond pointing below and above the platinum plane from the left and right guanine residues, respectively.[12] Delta HT (Figure 3.2) refers to the C8 hydrogen pointing above and below the platinum plane from the left and right guanine residues, respectively.[12]

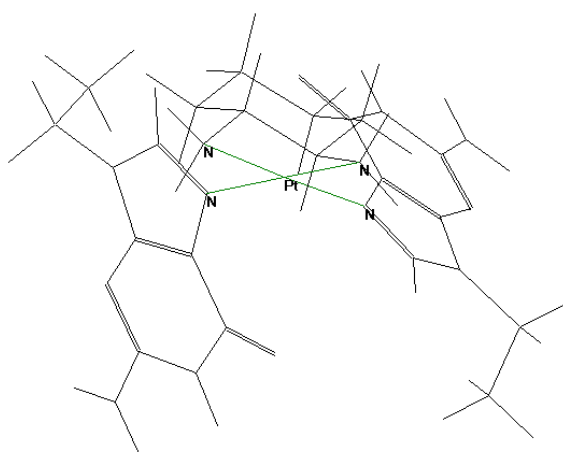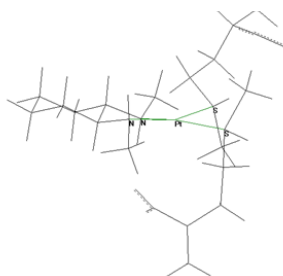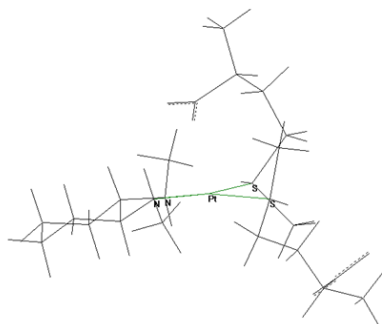| Rotamer	Lowest Total Energy (kcal/mol)	Time (ps)
RR-HH	-16.7662	242
RR-Lambda HT	-16.097	142
RR-Delta HT	-16.998	164

Table 3.2

Figure 3.2: RR-Delta HT

From the left to right direction, the carbon-hydrogen bond of the carbon double-bonded to the nitrogen in each guanine residue points above and below the platinum plane.

Pt(Me$_2$dach) with bis-methionine: [Pt(Me$_2$dach)(N-AcMet-S)$_2$]

Rotamers were distinguished by the chiralities of the amine nitrogens and cyclohexane ring carbons (ex. SRRS), and the chiralities of the sulfur atoms and the orientation of the methyl groups bound to the sulfurs were distinguished by the same method as the oxaliplatin with bis-methionine rotamers.

The energy differences seen in the structures below are mostly due to total bond strain; SRRS-SR-HT (Figure 3.3, the lowest total energy structure) had a relatively low total bond strain (~21 kcal/mole) compared to SRRS-SS-HT (Figure 3.4, the highest total energy structure), which had a total bond strain of ~38 kcal/mol. The bond strain difference is illustrated by how the square planar geometry of the four bonds to platinum is distorted to a larger degree in SRRS-SS-HT (Figure 3.4).

Figures 3.3 & 3.4: SRRS-SR-HT(above) and SRRS-SS-HT (below). Note that in SRRS-SS-HT, the platinum plane is to a larger degree distorted from an ideal square planar geometry due to bond strain.

Rotamer	Lowest Total Energy (kcal/mol)	Time (ps)
SRRS-RR-HH	6.95555	242
SRRS-RR-TT	9.414564	249
SRRS-RR-HT	15.15593	115
SRRS-SR-HH	9.524042	159
SRRS-SR-TT	18.42109	49
SRRS-SR-HT	5.4734	73
SRRS-SR-TH	12.08102	21
SRRS-SS-HH	7.578558	177
SRRS-SS-TT	6.665445	218
SRRS-SS-HT	21.69378	68

Table 3.3

Pt(Me$_2$dach) with bis-guanine: [Pt(Me$_2$dach)(9-EtG)$_2$]

Rotamers were distinguished like those of dach with bis-guanine, except the amine nitrogens' chiralities are included. The three conformations had similar electrostatic interactions and hydrogen bond strains combined at about -23 kilocalories per mole. SRRS-HH and SRRS-Delta HT had higher total bond strains because their cyclohexane rings were out of the stable "chair" conformation.

Rotamer	Lowest Total Energy (kcal/mol)	Time (ps)
SRRS-HH	-5.50239	102
SRRS-Lambda HT	-11.7471	249
SRRS- Delta HT	-6.87903	126

Table 3.4

SRRS S,O-Chelates: $[(S,R,R,S)\text{-Pt}(Me_2dach)(N\text{-AcMet-S,O})]^+$

Chelates within the SRRS and RSSR chirality were separated by the chirality of

the sulfur atom and whether the carbonyl oxygen (OXT) pointed above or below the

platinum plane (up or down) and then whether it pointed toward or away from the

platinum atom (in or out). Electrostatic interactions, hydrogen bond strain, and total bond

strain differences were seen by comparing relatively low total energy structures to

relatively high total energy structures. In high energy structures, the total bond strain

makes the cyclohexane chair out of plane with the square planar geometry of the four

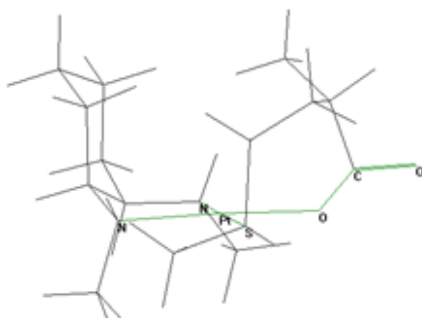platinum bonds (Figure 3.5).

Figure 3.5: SRRS-R-COOH-up and out

The bond strain is high enough that the cyclohexane chair on the left side of the molecule is nearly perpendicular to the platinum plane though it should lie within the plane.

Rotamer	Lowest Total Energy (kcal/mol)	Time (ps)
R-COOH up and in	22.19559	32 & 58
R-COOH up and out	33.47956	129
R-COOH down and in	21.1443	250
R-COOH down and out	13.86348	197
S-COOH up and in	14.2397	129
S-COOH up and out	31.66563	39
S-COOH down and in	19.78293	55
S-COOH down and out	16.3332	186 & 232

Table 3.5

RSSR S,O-Chelates: $[(R,S,S,R)\text{-}Pt(Me_2dach)(N\text{-}AcMet\text{-}S,O)]^+$

A similar relationship to the SRRS chelates was seen in that the low total energy structures had both lower electrostatic interactions, hydrogen bond strain, and total bond strain than the high total energy structures. The structures with the relatively lowest total energies were the ones that had their cyclohexane chairs more in line with the platinum plane.

Rotamer	Lowest Total Energy (kcal/mol)	Time (ps)
R-COOH up and in	19.23563	130
R-COOH up and out	36.70739	218
R-COOH down and in	17.73952	219
R-COOH down and out	21.28113	60
S-COOH up and in	19.00508	152
S-COOH up and out	33.68429	64
S-COOH down and in	21.64079	48
S-COOH down and out	28.57723	175

Table 3.6

In summary, all of the lowest total energy structures are listed:

Rotamer	Lowest Total Energy (kcal/mol)	Time (ps)
[Pt(dach)(N-AcMet-S)$_2$] RR-RR-HH	-11.4834	85
[Pt(dach)(9-EtG)$_2$] RR-Delta HT	-16.998	164
[Pt(Me$_2$dach)(N-AcMet-S)$_2$] SRRS-SR-HT	5.4734	73
[Pt(Me$_2$dach)(9-EtG)$_2$] SRRS-Lambda HT	-11.7471	249
[Pt(Me$_2$dach)(N-AcMet-S,O)]$^+$ R-COOH down and out	13.86348	197
[Pt(Me$_2$dach)(N-AcMet-S,O)]$^+$ R-COOH down and in	17.73952	219

Table 3.7

12

CHAPTER 4

DISCUSSION

The lowest total energy conformation of oxaliplatin with bis-methionine was about 6 kilocalories per mole higher than the lowest total energy conformation of oxaliplatin with bis-guanine. With $Pt(Me_2dach)$ with bis-methionine, the lowest total energy conformation was about 17 kilocalories per mole greater than the lowest total energy conformation with bis-guanine. This 11 kilocalories per mole difference between oxaliplatin and $Pt(Me_2dach)(ox)$ is the result of the bulk difference between dach and Me_2dach being used as carrier ligands. This is comparable to past research with the Me_4en ligand (N,N,N$^{'}$,N$^{'}$-tetramethylethylenediamine) in that bis products with methionine "would have severe interligand clashes," while this effect would be lessened with bis-guanine products.[11]

NMR data revealed that the reaction of $Pt(Me_2dach)(ox)$ with N-acetylmethionine resulted in chelation with the sulfur and oxygen atoms of a single methionine residue.[1] Oxaliplatin could form bis products with methionine at high concentrations of N-acetylmethionine; at low concentrations a sulfur-nitrogen chelate could form.[1] Both $Pt(Me_2dach)(oxalate)$ and oxaliplatin could form bis products with 9-ethylguanine.[1] This is consistent with molecular mechanics and dynamics calculations in that the lowest total energy structures of $Pt(Me_2dach)(oxalate)$ and oxaliplatin with bis products with guanine

had comparably low total energy values. These values, as well as the energy of the lowest total energy structure of oxaliplatin with bis-methionine, are significantly lower than the energy of the lowest total energy conformation of Pt(Me$_2$dach)(oxalate) with bis-methionine (table 3.7), showing the thermodynamic disparity.

It was originally determined that the sulfur-oxygen chelates formed by the reaction of Pt(Me$_2$dach)(oxalate) with N-acetylmethionine were of SRRS (N,C,C,N) chirality.[1] It was later shown that when Pt(Me$_2$dach)(oxalate) reacted with N-acetylmethionine, chelates were also made of RSSR chirality. Of the SRRS and RSSR chelates, NMR and other spectra data were unable to determine any further the conformations of the chelates, including the chiralities of the sulfur atoms. Molecular mechanics and dynamics data showed three conformations of the SRRS chelates having relatively low energies compared to the other conformations. The data predicts that the SRRS chelates made have two conformations with the sulfur atoms in the S chirality and one conformation with the sulfur atom in the R chirality. Of the RSSR chelates, both spectra data and molecular mechanics data were insufficient to determine the conformation of the RSSR chelates formed. Molecular mechanics and dynamics data were unable to provide a prediction of the conformation because there were multiple conformations of relatively low energy.

In regard to practical applications, the formation of sulfur-oxygen chelates by the reaction of Pt(Me$_2$dach)(ox) and N-acetylmethionine does not shift reactivity with platinum in guanine's favor because the chelate product is stable enough that it cannot be knocked off by even a more thermodynamically favored reactant.[1] Therefore, the ability of Pt(Me$_2$dach)(ox) to bind to DNA is limited by the formation of a product with an

amino acid that could possibly be stable for years. It is also significant that

Pt(Me$_2$dach)(ox) and oxaliplatin reacted with N-acetylmethionine at equal rates, showing

that the extra bulk of the Me$_2$dach ligand did not prevent the bonding of N-

acetylmethionine before chelation.[1] Therefore, it can be concluded that the bulk

difference between oxaliplatin and Pt(Me$_2$dach)(ox) does not create a difference in

reactivity between methionine and guanine, thus not giving an advantage to either of

these compounds in this aspect of anticancer activity.

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