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Bioorganic & Medicinal Chemistry Letters 15 (2005) 1471–1474
Bioorganic & Medicinal Chemistry Letters

Electronic structure calculations on the thiazole-containing antibiotic thiostrepton: molecular mechanics, semi-empirical and ab initio analyses

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Received 28 October 2004; revised 22 December 2004; accepted 29 December 2004
Available online 22 January 2005

Abstract—Thiostrepton is a highly complex cyclic thiazoyl peptide antibiotic and is active against Gram-positive bacteria. Molecular mechanics, semi-empirical and ab initio studies were utilized to further understand the structural and electronic properties of this antibiotic.

Thiostrepton (TS) (Fig. 1) ($C_{72}H_{85}N_{19}O_{18}S_5$, mw = 1665) is a paradigm for the class of multicyclic thiazole-containing peptide antibiotics. This family of antibiotics has been reported to exhibit potent antimicrobial activity against Gram-positive bacteria through a common mechanism: inhibition of bacterial protein synthesis.[1] The best studied member, TS, interacts with a region of 23S rRNA and ribosomal protein L11 of the large 50S ribosomal unit termed the guanosine triphosphatase (GTPase) centre.[2] Tight binding of this drug within this vicinity imposes conformational constraints on protein L11 resulting in an abolishment of GTP hydrolysis reactions involved in the protein elongation cycle.[3] TS also exhibits antimalarial activity, displaying activity against *Plasmodium falciparum*, the major causative agent of malaria.[4] Additionally, it has been shown that TS suppresses T-cell independent antibody production in murine models.[5] Much interest has been focused on the biochemistry and chemistry of TS culminating in the recent total synthesis of the antibiotic, allowing for the preparation of analogues to probe structure–activity relationships.[6] Multidrug resistance is a critical problem and the development of either new targets or increasing the efficiency of known antibiotics is essential.[7]

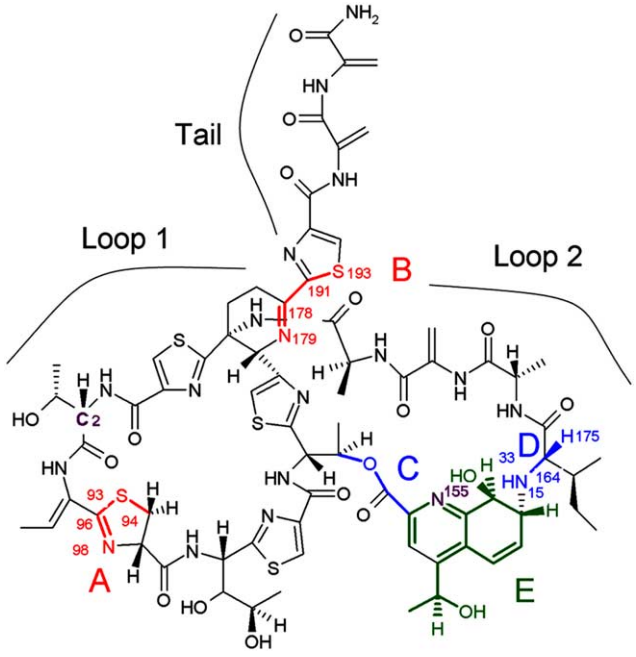

Figure 1. Thiostrepton. (A) The dihydrothiazole moiety; (B) linkage between tail and body of thiostrepton; (C) ester linkage; (D) secondary amine (N^{15}); (E) quinaldic acid moiety. Red: two low quality torsion angles found using the OPLS-AA force field. Blue: dihedral angles used to monitor the stochastic dynamics run. Purple: distance monitored during the stochastic dynamics run between N^{155} and C^2.

Our current repertoire of knowledge on TS provides limited understanding of its antimicrobial activity. TS is an

Keywords: Thiostrepton; Molecular modelling; Semi-empirical; Ab initio; Conformation; Antibiotic.
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doi:10.1016/j.bmcl.2004.12.076

extraordinarily complex macrocyclic peptide antibiotic containing thiazoline and quinaldic acid moieties along with a didehydroalanine tail. The complexity of its molecular architecture underlines the intense need to characterize its electronic and conformational properties, which should provide the opportunity to extend our insight into its possible modes of binding to ribosomal RNA.[8]

The crystal structure of TS was first reported in 1970, although the structural coordinates have never been released.[9] However, the recent release into the public domain of an X-ray structure based on sulfur anomalous dispersion techniques has provided the coordinates for the heavy atoms in TS.[10] This information has since been utilized in docking experiments against the TS RNA-L11 target complex.[11] The crystal structures of other cyclic thiazole peptides such as nosiheptide[12] and GE2270A[13] have also been determined. Nevertheless it is well known that crystal packing forces can play important roles in the overall structures determined by X-ray diffraction methods.[14] In addition, the ability of a molecule to sample different conformations makes it imperative to study the structure of an antibiotic by a variety of techniques. Computational chemistry is a technique that can lead to insight into the conformational profile of a molecule.

This report presents a detailed study on the structure of TS. The TS crystal structure was geometry optimized using molecular mechanics and semi-empirical methods. Information acquired from these computations were then utilized for detailed calculations including conformational searches and electron density calculations at the AM1 and ab initio levels to obtain partial charges of atoms that will be important in future modelling efforts.

The recently reported crystal structure of TS (PDB: 1E9W) was imported into WebLab ViewerPro 3.7 (MSI) where bond types and hydrogen atoms were added to produce the completed structure. One of the proton additions was a secondary amine (N^{15}) linked to the quinaldic acid moiety of TS (E in Fig. 1). Secondary amines are known to pyramidalize and the heavy atom data for the crystal structure does not indicate the orientation of the proton. A number of steric interactions were visually observed if the proton was pointed inwards to the centre of TS, hence the proton was added such that it pointed outwards with a dihedral angle (H^{175}–C^{164}–N^{15}–H^{33}) of 65.1°. As well, the water of hydration bound to TS was removed. This TS structure, with all required hydrogens, was utilized as the starting structure for the series of calculations outlined below.

The first approach utilized molecular mechanics to scan the various available force fields in MacroModel 8.0[15] such that an appropriate force field might be found without the need for the development of new force field parameters. The force fields available and the number of high, medium and low bond stretch/bend/torsional qualities were evaluated (Supplementary table). The OPLS-AA force was determined to be the best of the available force fields. Two low quality torsional angles however were noted: N^{98}–C^{96}–S^{93}–C^{94} and N^{179}–C^{178}–C^{191}–S^{193} (Fig. 1, red). The localization of these torsion angles are situated in the ring of the dihydrothiazole group and the bond attaching the thiazole of the tail to the piperidine group. Due to ring constraints of the dihydrothiazole, these low quality torsional angles would not be expected to greatly affect the conformational studies, as for the tail, it was expected to freely rotate in solution, which was taken into account when evaluating the generated conformers for relatedness (see below). It is important to note that although we utilized OPLS-AA as the molecular mechanics force field of choice for this particular problem, more detailed future studies may require the development of force field parameters for these particular torsional rotations.

OPLS-AA energy minimizations[16] were performed in vacuo and in water, utilizing the implicit GB/SA methodology.[17] The minimized structures obtained had energies of: −827.83 kcal/mol (vacuum) and −1014.75 kcal/mol (water). Superimposition of non-hydrogen atoms of the TS crystal structure with these two energy minimized structures revealed low RMS values of 0.4380 and 0.5431, respectively, suggesting that the two low quality torsional angle parameters discussed earlier do not appear to be major obstacles in modelling TS. The didehydroalanine tail showed the largest difference with RMS values 1.787 and 1.820 for the gas and liquid phase, respectively. Figure 2 shows the overlay of these structures.

A second approach was undertaken to determine the minimized structure obtained by utilizing semi-empirical AM1 calculations.[18] The AM1 structure (vacuum) obtained from the starting crystal structure minimized to a low energy structure had no negative frequencies based on frequency calculations. When superimposed on the starting crystal structure, the RMS difference obtained was 0.9623. Additional comparisons of the AM1 structure to the OPLS-AA structures determined in vacuo and in water resulted in RMS differences of 1.0433 and 0.9904, respectively (Supplementary material). The two torsion angles N^{98}–C^{96}–S^{93}–C^{94} and N^{179}–C^{178}–C^{191}–S^{193} in the AM1 structure were determined to be 0.8° and 12.0°, respectively. The values for these dihe-

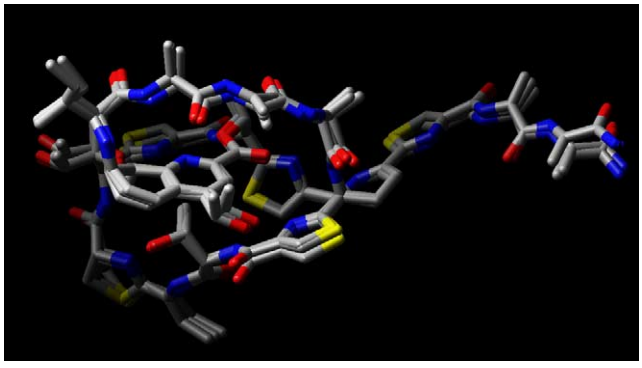

Figure 2. The superimposed images of the thiostrepton starting crystal structure with OPLS-AA energy-minimized structures in vacuo and water. Hydrogens have been eliminated for clarity.

dral angles in the OPLS-AA gas phase calculated structure were −11.7° and 0.4°, respectively. The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) at the AM1 level of calculation were determined to be localized on the dihydrothiazole and quinaldic acid moieties, respectively (Fig. 3). The HOMO and LUMO are expected to be important in contributing to the interaction of TS with ribosomal RNA. It has been suggested that the quinaldic acid and the thiazole(s) moieties of TS engage in pi-stacking interactions with adenosine 1067 and 1095, respectively.[11] Additionally, the AM1 minimized structure was utilized as the geometry for a single point energy calculation at the B3LYP/631-G(d)//AM1 level. This calculation led to a detailed analysis of the electronic structure of TS. The mulliken charges determined by the AM1 and B3LYP/6-31G(d) calculations, as well as CHelpG (B3LYP/6-31G(d)) electrostatically fit charges on the AM1 geometry-optimized structure are available as Supplementary material.[19]

Although the semi-empirical and ab initio calculations provide partial charges for the atoms on this large drug molecule, it is also important to realize that the crystal structure and the minimized structures directly obtained from it, are each but one structure on the energy potential surface. Such a large molecule such as TS should exhibit a multiplicity of conformations in spite of the fact that it is restricted by two internal loops. In order to explore this in a computationally efficient manner, we utilized the energy-minimized OPLS-AA (vacuum and water) structures for a series of conformational searches. There are 39 rotatable bonds in the TS structure, not including methyl group rotations. This number precludes approaches that utilize dihedral angle drive-based search protocols. We therefore applied the highly efficient low mode (LMOD)[20] conformational search protocol implemented in MacroModel 8.0. This method explores the low frequency eigenvectors of the molecular system and is expected to follow 'soft' degrees of freedom, such as those found in torsional rotations. Due to the aggressive search protocol utilized in this approach, the chirality of the 17 chiral centres were held fixed during the conformational search. Only the secondary amine (N^{15}) was not fixed, as it was believed that pyramidalization of this nitrogen in addition to its inherent flexibility might be a contributing factor to the conformational flexibility of TS. Both vacuum and water phase OPLS-AA minimized structures were used in separate calculations using LMOD.[21]

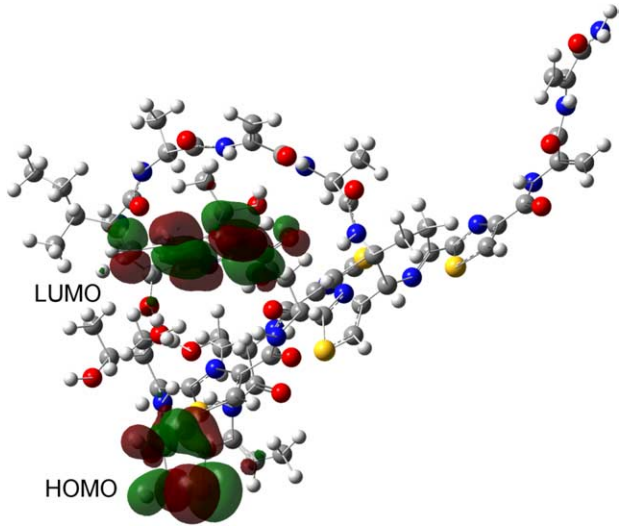

Figure 3. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) diagram at the AM1 level were overlapped, and were calculated to be localized on the dihydrothiazole and quinaldic acid moieties of thiostrepton, respectively.

LMOD calculations performed in vacuo found a total of 158 conformations within ~10.0 kcal/mol of the global minimum (−908.06 kcal/mol), 31 of which are within 3 kcal/mol. In the case of water (GB/SA), 293 unique conformations were found, 76 of which were within 3 kcal/mol of the global minimum (−1133.81 kcal/mol). Overlaying the two global minimum structures (vacuum and water) with the starting TS crystal structure (Fig. 4) revealed that Loop 2 and the tail region are more open and exposed in vacuo, whereas the structure obtained in implicit water forms a more tightly packed architecture. The secondary amine (N^{15}) was observed to have pyramidalized and faces inwards towards the TS core in the LMOD structure in vacuo. This suggests that the binding interactions of TS could potentially involve significant conformational changes of Loop 2 that maybe be facilitated by the pyramidalization and torsional flexibility of N^{15}.

Although by no means exhaustive, the above approaches did find a number of conformations lower in energy than the OPLS-AA minimized crystal structure (Supplementary material). A number of the conformations found in the LMOD searches showed the N^{15} pyramidalization, where approximately 16% and 13% of the LMOD-generated vacuum and water conformations (1000) had the amine proton pointed inward. Hence this process may play an important role in defining the conformation ensemble of TS. In order to gain some insight into the frequency of this possible pyramidalization process, as well as to explore further the conformational mobility of the loop regions in TS, stochastic dynamics studies[22] were undertaken in vacuo and in implicit water (GB/SA). One nanosecond dynamics experiments were undertaken at 300 K. The dihedral angles around the internal lactone (C^{142}–O^{144}–C^{153}–C^{154}),

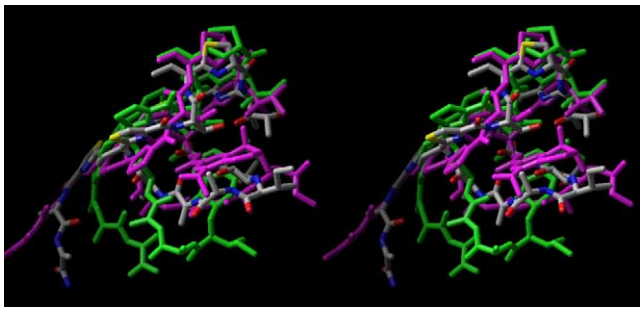

Figure 4. Superimposed global minimum energy structures calculated from LMOD in vacuum (green) and water (CPK) and the starting thiostrepton structure (magenta). For details, refer to text.

the secondary amine (H^{175}–C^{164}–N^{15}–H^{33}), and the atomic distance between the two of the loops (C^{2}–N^{155}) were also monitored to gain additional information as to conformational changes over the one nanosecond time frame.

Results from the stochastic dynamics studies performed in vacuo indicate that early in the run, the N^{15} proton orientates itself inwards (steps 17–74, 130–167) periodically, while no such behaviour was observed in the water run. The region of the internal lactone ring does not appear to change significantly in both cases, although conformations of the opposite orientation were sampled frequently throughout the duration of the analysis (see Supplement material). The distance between the two loops was found to increase over the dynamics run (vacuum and water) experiment thereby expanding the core of TS. Data from these stochastic dynamics suggest that TS exhibits conformational flexibility, a 'breathing' dynamic for binding interactions. Stochastic dynamics plots can be found in the Supplementary material.

In conclusion, a detailed molecular mechanics-based conformational search, stochastic dynamics, semi-empirical and ab initio studies on TS, a representative of a major class of the thiazole peptide antibiotics, have yielded a number of insights into its structure including the potential pyramidalization of the secondary amine and aspects of the mobility of the TS loops. In addition, the focus has provided detailed electronic structure parameters on TS, including providing knowledge of the position of the HOMO and LUMO orbitals and the electrostatic charges on TS. This information should serve as an important basis for future studies on TS and other thiazole peptide antibiotics and their interactions with biological systems.

Acknowledgements

The authors wish to thank NSERC, OGSST and the University of Waterloo for research support and access to the UW High Performance Computing Centre facilities. Additional thanks goes to Robyn Landers for excellent technical assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.12.076.

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