Investigating effects (steric, electronic) and the thermal stability of disulfide bridges in peptides by use of semi-empirical, DFT and MP2 level methods.

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Introduction:

The possibility to predict the characteristics or behavior of a small part of a bio-molecules would give scientists a powerful insight into the exact role played by this small part into the whole function of the bio-molecule. Furthermore, it would lead to more rational designing of new molecules. In this context we considered somatostatin[1-2], a hypothalamic peptidic hormone, whose functions include the inhibition of secretion of growth hormone, glucagon, insulin, gastrin and secretin. This peptide has wide potential as a clinical agent. However, this idea of using somatostatin as a drug is hampered due to its very short half-life (<3 minutes) in circulation and also due to lack of selectivity. To overcome these problems, several peptide analogs of somatostatin have been synthesized. One of the resulting octa-peptide is octreotide[4]. Octreotide conjugated to a bifunctional chelator such as DTPA (diethylenetriamine pentaacetic acid) have been labeled with a variety of radioactive isotopes and these are being used in imaging of somatostatin receptor –positive tumors[5].

Both somatostatin and octreotide contain a disulfide bridge. Presence of disulfide bridges creates conformation constraints and provides thermostability to the peptide[6]. Such conformational constraint is often necessary for biological activity.

Substituting the amino acid residue that contains the sulfhydryl group (for example substituting cysteine with penicillamine) could have considerable changes in the stability of the disulfide bridge. This is due to the fact that penicillamine analogues have bond lengths similar to the normal disulfide but are sterically hindered due to the two-methyl groups present on the β carbon[7].

The general problem we would like to address here is to investigate the steric and electronic effect induced by the neighbor atoms on the stability of the disulfide bridge. To solve this problem, we would need to consider the thermo-dynamical profile of the reaction of the bond forming and bond breaking of the disulfide bridge. Linear substrates

of the peptide (free sulfhydryl) are converted to the disulfide form by chemical methods such as air oxidation[8]. These substrates need to be sufficiently flexible to access thermodynamically favored conformations in which to-be-paired cysteine residues come into reasonable spatial proximity. Environment (steric, neighboring charges, buried versus surface positions of the cysteine residues) affects their pKa values and consequently ease of oxidation[9].

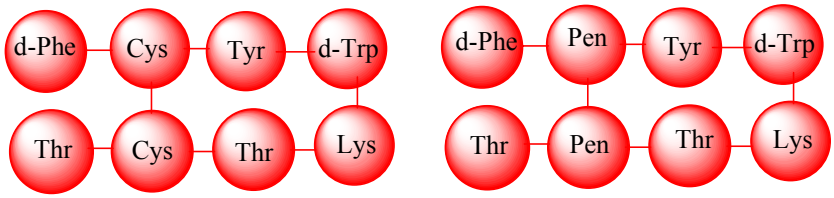
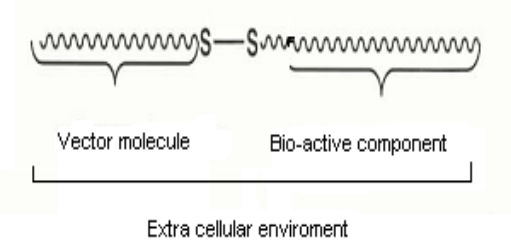


Figure 1. Peptide sequences of a) cys-octreotide b) pen-octreotide.

Goals and objectives:

This project would address the effect of geminal methyl groups present in the penicillamine residue on the disulfide bond in octreotide. The approach of this project is to use theoretical calculations to investigate the comparative stability of the disulfide bridge formed between two cysteine residues or two penicillamine residues in octreotide. Consequently, the obtained data might lead to a better understanding of the role of the geminal methyl groups on the stability of the disulfide bridge. The knowledge comprehended from the theoretical calculations would then help in a more rational design of synthetic molecules containing disulfide bridges. If this theoretical project turns out successful in yielding meaningful data, then this approach can be amplified to encompass other peptides. One project that my lab group is interested is to synthesize a compound that has a cleavable covalent bond (such as a disulfide bond) between a vector moiety (such as octreotide) and a bioactive component. The disulfide bridge will be cleaved inside the reducing environment of the cell and consequently the bio-active component will detached from the vector as soon as it reaches the interior of the cell.



Theoretical level calculations can give insights into what kind of amino acid would be more appropriate to be used to form such a cleavable covalent bond.

Reaction Mechanism:

Reduction cleavage of the S-S bonds[10] takes places electrochemically. In absence of any side reactions, the process of the overall 2-electron reduction is shown below. There is evidence that that $RSSR^{\bullet -}$ (a radical anion species) is formed, but with a very short lifetime.

$$RSSR + e \rightleftharpoons RSSR^{\bullet -} \quad (1)$$

$$RSSR^{\bullet -} \longrightarrow RS^{\bullet} + RS^{-} \quad (2)$$

$$RS^{\bullet} + e \longrightarrow RS^{-} \quad (3)$$

$$RSSR + 2\,e \longrightarrow 2\,RS^{-} \quad (4)$$

Proposed research:

A) Choice of methodology:

Semi-empirical methods of computation will be used in the initial part of the project to optimize the structure of all the molecules. This method is the method of choice due to the huge size of the molecules of interest[11]. The molecular formulas of the molecules give an indication of their size. Cys-octreotide- $C_{49}H_{66}N_{10}O_{10}S_2$; Pen-octreotide – $C_{53}H_{74}N_{10}O_{10}S_2$. Indeed, semi-empirical methods have been already used for a variety of jobs concerning modeling of large molecules. The optimized structure obtained from the semi-empirical

method will then be utilized in the future calculations that will be done by use of ab-initio (DFT and MP*n*) methods.

B) Scope of project:

As described earlier, the method of calculations to be done for the preliminary part of the project is semi-empirical and subsequently by use of ab initio methods.

Peptide chain effect: Computing the structure of the peptide by AM1 and PM3[12] methods would yield the optimal structure of the peptide (bond lengths, bond angles, dihedral angles etc) and Hartree-Fock energies of each molecule. These optimized structures will be used for further computations, using DFT methods.

DFT[12] methods are less expensive in terms of CPU time compared to the MP*n*[12] method for mid-sized and large systems and produce significantly greater accurate results[13].

Further calculations would need to be done on step 2 of the reaction scheme shown above by use of ROHF/UHF levels with various basis sets. This is because of the fact this is the step where disulfide bond is actually breaking up. Data obtained from all these calculations would allow for calculation of the energetics of the reaction.

Neighborhood effect: Computations done by isolating only a small part of the whole peptide chain would give some insight on the effect (steric energies, electronic etc) of the neighboring atoms on the disulfide bridges. Here we have more scope to use higher-level calculations such as MP2 level etc as the size of the molecule is much more manageable. All the steps of the reaction scheme will be computated for this section.

C) Interpretation:

The results obtained from the optimization of the peptide chain by use of AM1 and PM3 methods are given in the preliminary results section. This optimization reduces the CPU time required to the DFT level calculations. Calculations done by DFT methods will give more accurate Hartree-Fock energies of the full molecules. From the data obtained, an energy profile of the

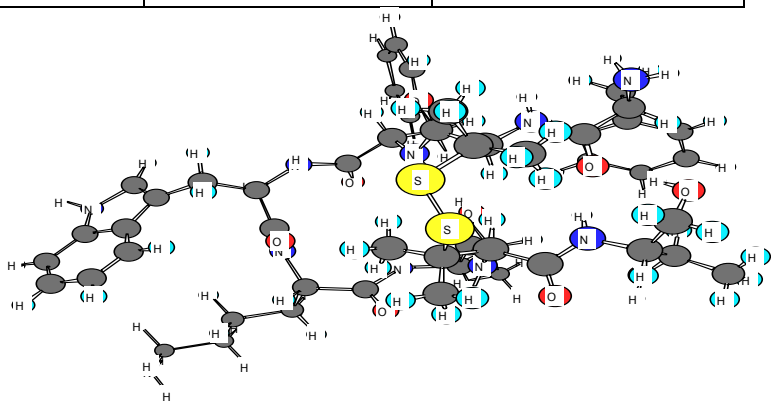
reaction bond cleavage of the neutral compound and the radical anion etc can be drawn. Energetics of the fragmentation reaction can be calculated.

It is assumed that the data obtained from the computations done by isolating only a small part of the peptide should give a more accurate picture about the free energy change and the energetics of the fragmentation reaction. These computations done on the isolated part of the molecule also should contribute towards greater understanding of the effect (steric, electronic etc) that the neighboring atoms have on the disulfide bridge.

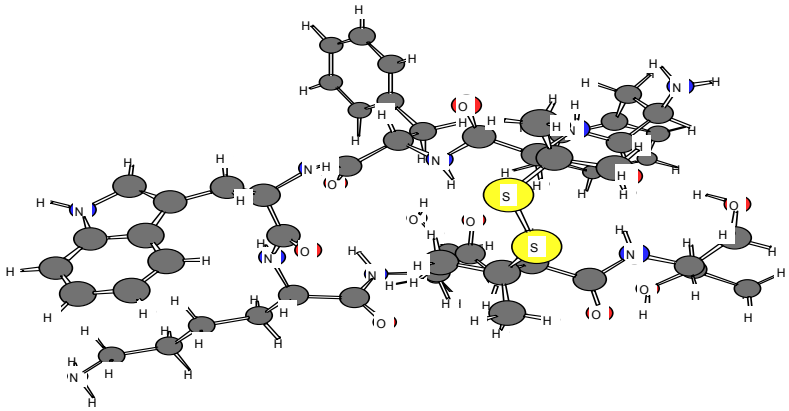
Preliminary results: Optimization of both the di-sulfide form of pen-octreotide and cys-octreotide with AM1 and PM3[12] methods has been done and the data obtained is tabulated in the following table.

Optimized energies of the molecules from AM1 and PM3 calculations:

Molecule	AM1 in A.U	PM3 in A.U
Cys-Octreotide	-0.5463036	-0.537041
Pen-Octreotide	-0.5479494	-0.5427258



(Above)Cys-octreotide (Below) Pen-Octreotide



D) Facilities and feasibility:

In order to compute such calculations, we would definitely need hardware at least similar to the ones available to us at MU for this class, 'Shiva' which is a Silicon Graphics Power Challenge Computer with 8 R12000 processors or better. A PC would be rather insufficient for this kind of project. Use of a mini-super computer would be rather time consuming.

E) Timeline:

This project is time-consuming. The molecule of interest being so large in size, semi-empirical calculations each took at least 24 hours to reach optimized structures. Use of DFT and MP2 methods to compute energies would require considerably longer CPU time. Additional interpretations would extend the time required to finish the project. A reasonable time frame required to finish this project should be about 4-5 months.

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