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COMPUTATIONAL INSIGHTS INTO THE SELF-ASSEMBLY OF PHENYLALANINE-BASED MOLECULES

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Abstract: In a recent paper "Self-Assembly of Phenylalanine-Based Molecules", we have studied the formation and stability of phenylalanine and diphenylalanine constructs. In the case of diphenylalanine we observe nanotubes, however, phenylalanine molecules aggregate in layers of four, not six, molecules. In the preset paper, we extend this previous work and compare the energetics of all experimentally observed structures, simulated structures, and designed structures, by way of single point Density Functional Theory (DFT) calculations. We take a detailed look at water content, pore size and dipole moments inside our phenylalanine-containing tubes and analyze stabilizing factors in the nanostructures.

Keywords: phenylalanine, diphenylalanine, self-assembly, molecular dynamics

1. Introduction

Many biological processes require self-assembly of biomolecules into ordered structures. The common characteristic in these processes is the hierarchical assembly of monomers into new, compact, and complex structures that are stabilized by non-covalent interactions such as hydrogen bonding, π-π stacking, or electrostatic interactions between the monomers. While each of those interactions is comparatively weak, together they sum up to a strong driving force [1] that is controlled by the composition of the monomers. A well explored example is diphenylalanine which is known to form nanotubes [2, 3], however, recently it was found that phenylalanine molecules can also form nano fibrils [4]. It was found that both synthesized fibrils and those isolated from mice with the illness phenylketonuria, exhibited amyloid-like characteristics [4]. This potential connection to amyloidosis has motivated this and a previous study which aim to

determine the structure and energetics of phenylalanine aggregates, comparing them with diphenylalanine aggregates.

2. Methods

In a previous study ("Self-Assembly of Phenylalanine-Based Molecules") [5] we have generated idealized structures of phenylalanine (F) and diphenylalanine (FF) built out of rings with either four or six members (F4, FF4, F6, FF6) Figure 1. Here, we took these idealized structures along with the crystal structure of phenylalanine Figure 2 (b), and estimated their single point energies using Density Functional Theory (DFT). The calculations were performed with B3LYP [6, 7] and the 6–31G** basis set using Q-Chem 4.2 [8]. In order to simplify the calculations, the middle two layers of each structure were considered and the non-polar hydrogen atoms were deleted. Single point energies for both gas-phase and polarized continuum solvent (PCM) at 78.39 debye were calculated. For our

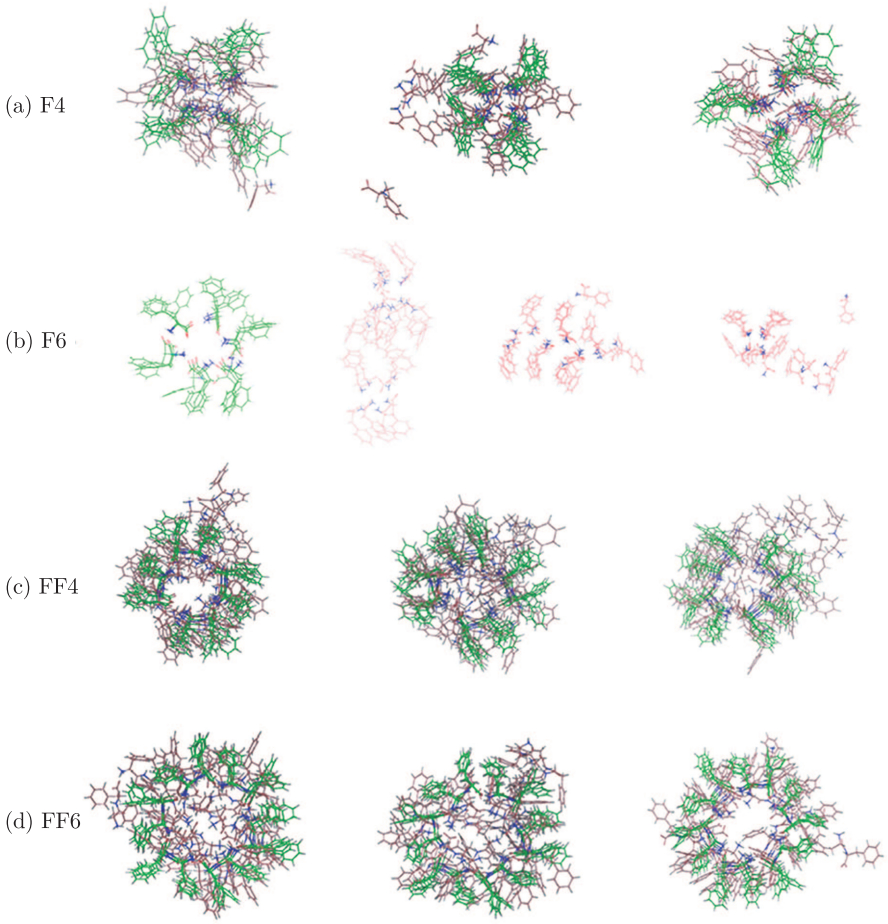

Figure 1. Stabilty Test Results [5]

analysis we used Discovery Studios Visualizer 4.0 [9] Quantities include number of water molecules per layer and dipole moments per layer. For each system the inner two layers (see Figure 3) were isolated for analysis in order to minimize finite-size effects and to be consistent with the previous study and the DFT analysis.

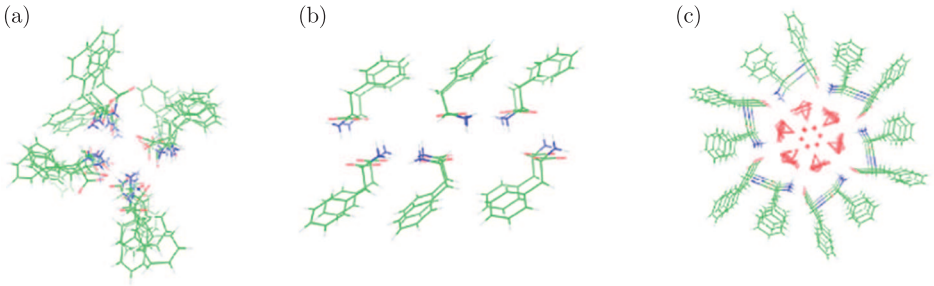

Figure 2. Proposed lowest-energy structure of phenylalanine (a) together with the experimentally determined crystal structure of phenylalanine (b) and the crystal structure of diphenylalanine (b); green atoms are carbon, blue are nitrogen, red are oxygens and white are hydrogens

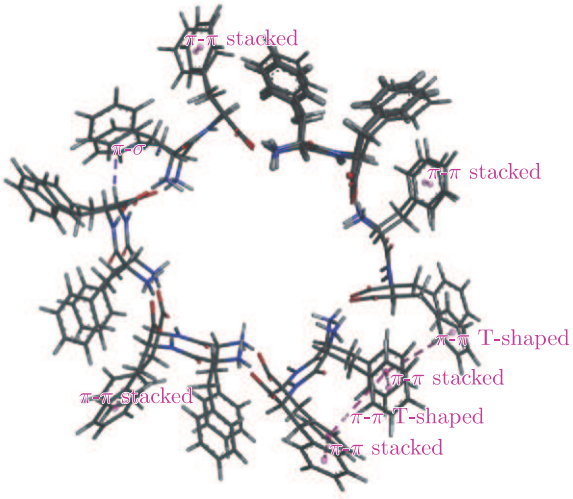

Figure 3. Method of Discovery Studio Anaysis

3. Results and discussion

In our previous molecular dynamics study [5], we observed that the phenylalanine oligomer with 4-fold symmetry (F4), the designed diphenylalanine oligomer with forced 4-fold symmetry (FF4) and the diphenylalanine oligomer with 6-fold symmetry (FF6) have retained their original symmetries and general structures. However, the designed phenylalanine oligomer with 6-fold symmetry (F6) fell apart quickly, and reassembled as either a F4 or phenylalanine crystal structure. The lack of stability for the F6 structure is likely due to a pore-size of

8.0[2.4] Å, which is 2 Å larger than that of the idealized F4 structure. The later measures 6.0[0.4] Å. Values in brackets are standard deviations over multiple measurements. As a consequence of the larger pore size, the distance between the end groups is larger for the F6 structure than for the F4 structure, and therefore the stabilizing interaction between them is smaller. Hence, the difference in stability between the F4 and F6 structures demonstrates the importance of end group interactions and suggests strong pH dependence towards aggregation.

While experimentally only the FF6 structure, but not the FF4 structure has been found for diphenylalanine, the later is interesting because it is an intermediate between nanotubes and nano wires. Comparing our results to that of the study of Jangbae Kim [10] it seems that our FF6 structure resembles a the nanotube, while our FF4 follows the same qualitative trends from nanotube (NT) to nanowire (NW) observed by these authors. We see a larger contraction in FF4 in both directions, aligned and perpendicular to the tube axis. In fact, if the grinding process necessary ended up disturbing the atomic structure to where both collapsed to the regular crystal structure [11, 12], our designed FF4 structure may be a more accurate description of the experimentally observed nanowire. Separate from the PXRD experiment, thermogravimetric analysis was performed on NW vs NT and found that their NW (possibly our FF4) is thermodynamically less stable than their NT (suggesting our FF6). This is in direct agreement with our DFT calculations showing that FF6 has a lower energy per layer than FF4.

Another study investigated phenylalanine by DFT and looked at the geometries that optimize the energetics of phenylalanine assemblies have also been studied by density-functional calculations. Three dominant structures were found for a system of four interacting phenylalanines [13]. One interacts only through the backbone with no indication of π stabilization. Another shows all four aromatic rings oriented and spaced in a manner typical for π stacking. The third structure, the global minimum, contains one T-shaped interaction similar to the crystal structure of benzene. Interestingly, from our Discovery Studio analysis, both our FF4 and FF6 (possibly a NW and NT) have the same number of this more energetically favorable T-shaped interactions. The FF6 has more π-stacking (not the T-shaped) and more hydrogen bonding than FF4. These both support our DFT results of FF6 being more energetically stable. Kim *et al.* compared the electron density of hydrogen bonding from the Powder X-Ray Diffraction results of their nanotube vs nanowire and discovered that there was roughly 3 times the amount of electron density found for the nanotube than the nanowire [10]. In our comparison of the six-fold diphenylalanine to the four-fold, we also see an increase (roughly 4 times) in the counted number of hydrogen bonds as defined by discovery studio.

The absolute value of ΔH for FF6 is larger in magnitude per molecule than FF4 which is consistent with the partial bond formation that would occur from more hydrogen bonding and pi stacking of the FF6. This is shown in Figure 4.

The results of the DFT study can be seen in Figure 5. The gas phase calculations showed the same trends as that of solvated molecules, but at a higher

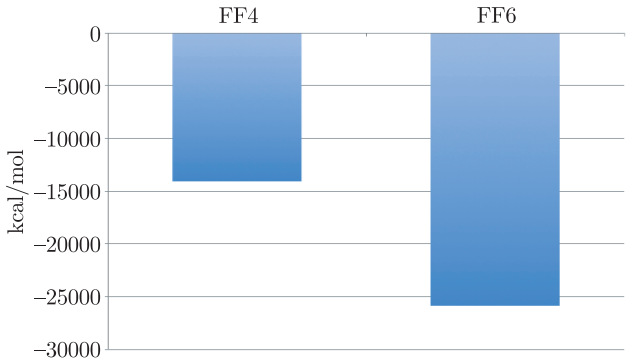

Figure 4. Difference in energy (per layer) between free monomers and formed structure

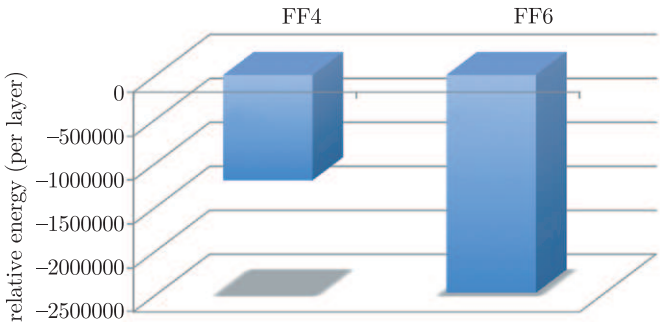

Figure 5. DFT results (kcal/mol, in idealized configuration)

energy. They are computationally less expansive than solvated one, and at the same time describe an environment similar to lipid layers. The results given in Figure 5 for the solvated layers make sense as FF6 is essentially the crystal structure observed in experiment. It stands to reason that the FF6 would be more energetically favorable and while FF4 has not been observed experimentally with this level of atomic detail, it is likely that FF4 is on the spectrum toward becoming a nanowire.

The water content and dipole moments were investigated since it is known that an increase in water content increases leads to higher conductivity in FF nanotubes [14]. While a previous investigation by Andrade-Filho *et al.* just models different numbers of water molecules in the same, symmetry of 6 FF tube, our FF4 results are also in line with the trends seen for FF6. In this study, with 15, 17 and 24 water molecules inside their FF6 layer correspond to 11.79 Å, 12.60 Å, and 13.10 Å, respectively. Our three FF6 stability runs (200 ns each with Molecular Dynamics) gave an average of 15.3 water molecules per layer with the inner channel distance of 12.38 Å following their method of measuring and averaging N-N distances across the pore. This lies right in the trend seen in the Andrade-Filho paper for FF6. FF4 also follows this trend with 9.42 Å pore size containing an average 4.8 water molecules. The shrinking of the pore size corresponding to decreased water content is not only a logical trend but also agrees with the

previously discussed observation comparing nanotubes to nano wires [10]. The 15, 17 and 24 water molecules resulted in 89.39, 91.67, 90.97 debye per layer, respectively. Our idealized structures had 15.9 (FF6-NT) and 9.6 (FF4-NW) water molecules resulting in 42.4 and 19.8 debye per layer respectively (see Figure 6). While the values themselves are quite different, it is worth noting that entirely different software programs were used in the different studies but that the trend is clearly in agreement.

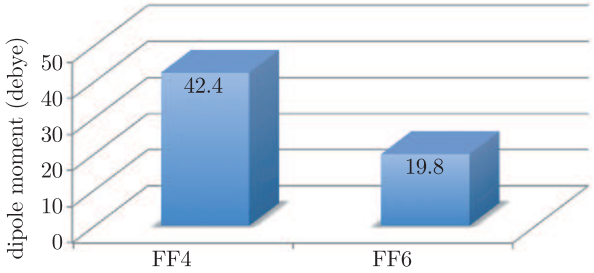

Figure 6. Dipole moment results (in idealized configuration)

Duan Li *et al.* [15] recently looked at the link between phenylalanine neurotoxicity and imbalances of cerebral energy metabolism and discussed the relationships between phenylalanine exposure and subsequently regulated factors. They reported that 0.9mM phenylalanine was neurotoxic to cultured fetal cortical cells and significantly decreased the cell viability after their 30 hours of treatment. This concentration is similar to the one in the study by Abramovich *et al.* [4] who showed that at 0.94 mM of the phenylalanine fibrils decreased cell viability decreased by approximately 10% for a PC12 cell line compared to its alanine control. At higher concentrations cell viability was more significantly reduced to 65%. Since we do not observe water molecules in the simulated F4 structure, this experimentally observed toxicity of phenylalanine fibrils is likely not due to tubular phenylalanine oligomers acting as pores that allow water leakage through the cell membrane. The form of our F4 aggregates rather suggest that the non polar interaction of the rings with the aliphatic chains of the lipids will separate the polar head groups of the lipids leading to membrane disruption as a cause of cell-toxicity.

4. Conclusion

The analysis in this brief report support our previous work [5] and is in agreement with other studies, both experimental and theoretical. The mechanism of toxicity in phenylketonuria is still not resolved and the possibility (supported by our results) that it is fibrils, and not simply the higher concentration of phenylalanine in patients, that is responsible for this toxicity gives new insight and may open new treatment options, The pore size, water content, dipole moments and relative stability of F4 and related structures not only give insight to this self-assembly process from a medical standpoint, but also from a materials perspective.

Comparing our results to that of the study of Jangbae Kim [10] it seems that our FF6 structure resembles a nanotube, while our FF4 follows the same qualitative trends from nanotube (NT) to nanowire (NW) observed by these authors. Our results also follow the trend of increased water content correlating with an increased dipole moment [14] which also suggests increased conductivity. From the study of four interacting phenylalanines [13] we know the relative energies of different ring orientations. Comparing both this π-stacking and the hydrogen bonding, we confirm that these factors agree with the FF6 nanotube being more energetically stable then the FF4 structure. In future work we will focus on the material science applications of our work and study the structures formed by mixtures of phenylalanine and diphenylalanine.

Acknowledgements

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