Book Chapter

Conceptual DFT as a Novel Chemoinformatics Tool for Studying the Chemical Reactivity Properties of the Amatoxin Family of Fungal Peptides

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Abstract

The chemical structures and molecular reactivities of the Amatoxin group of fungi-derived peptides have been determined by means of the consideration of a model chemistry that has been previously validated as well-behaved for our purposes. The reactivity descriptors were calculated on the basis of a methodological framework built around the concepts that are the outcome of the so called Conceptual Density Functional Theory (CDFT). This procedure in connection with the different Fukui functions allowed to identify the chemically active regions within the molecules. By considering a simple protocol designed by our research group for the estimation of the pKa of peptides through the information coming from the chemical hardness, these property has been established for the different molecular

systems explored in this research. The information reported through this work could be of interest for medicinal chemistry researchers in using this knowledge for the design of new medicines based on the studied peptides or as a help for the understanding of the toxicity mechanisms exerted by them.

Keywords

Amatoxins; Computational Chemistry; CDFT Descriptors; Chemoinformatics; pKa

Introduction

Amatoxins are a group of deadly toxins found in Amanita fungi consisting of nine octapeptides with a bicycle structure which are named as α-amanitin, β-amanitin, γ-amanitin, ϵ-amanitin, amanullinic acid, amanin, amaninamide, amanullin and proamanullin. These toxins are often associated with another group of bicyclic peptides, the phallotoxins, which only contain seven amino acids in the ring [1].

Amatoxins are found in mushrooms of the genera Amanita, Galerina, Lepiota, and Conocybe from the fungal phylum Basidiomycetes. α-amanitin poisoning causes irreversible liver damage and is responsible for 90% of fatal incidents with mushrooms. Amatoxins are synthesized as a 35 amino acid precursor, which is then cleaved, macrocyclized and further modified including the introduction of the covalent tryptathionine bond between a cysteine and a tryptophan, the defining feature of amatoxins. The identity of the enzymes and the order in which these reactions take place remain experimentally undetermined. Precursor peptides containing the N-terminal signature sequence MSDIN have been identified, as well as the protease that catalyzes the cleavage of the leader sequence (POPA) and the macrocyclase enzyme (POPB). These are the only enzymes involved in amanitin biosynthesis characterized to date, and both belong to the prolyl oligopeptidase super-family. An extensive kinetic characterization of the macrocyclase from Galerina marginata

revealed similarities with other prolyl oligopeptidases, as well as pronounced product inhibition caused by the long recognition sequence [2,3].

Chemoinformatics is an important field of research which involves several procedures for the management of chemical information and can be considered a wonderful instrument for the design of new medicines in the pharmaceutical industry. The majority of the applications dependent on Chemoinformatics look to make forecasts about the natural properties of molecular systems starting from a background built over their associated chemical structures, and the computational displaying of them by considering a tight coupling of biological and organic data. For this reason, the connections among biology and chemistry portrayed by the techniques assoaciated to Computational Chemistry are very important. For a given molecular system, the structure and its assoaciated chemical properties may be estimated and visualized on the basis of its related electron density, and it is in this way that molecular descriptors will without a doubt be identified with molecular properties; nonetheless, the degree of this link will rely upon the particular descriptors, properties and group of molecules considered in the analysis [4].

This research seeks to obtain the chemical reactivity information of the fungal peptides under study by means of the consideration of the Density Functional Theory (DFT) derived concepts. There is a lot of research where the Conceptual DFT is used to relate the reactivity of several compounds with biological activity [5-11]. The understanding of the chemical reactivity properties of the Amatoxin molecules will be crucially achieved by means of the consideration of the Fukui functions to extract the information about the reactivity of the peptides which can be of potential utility in the process of designin new pharmaceutical drugs [12-16]. The information reported through this work could be of interest for medicinal chemistry researchers in using this knowledge for the developing of new medicines based on the studied peptides or as a help for the understanding of the toxicity mechanisms exerted by them.

Computational Methodology

The determination of the conformers of the nine fungal molecules belonging to the Amatoxin group was performed by using the ChemAxon Calculator plugins included in MarvinView 17.15 available from ChemAxon (Budapest,Hungary), a graphical display software considered of utility for the study of chemical structures and reactions. The procedure stated by choosing the most stable conformer for each peptide by doing Molecular Mechanicscalculations through the overall MMFF94 force field [17-21]. The resulting lowest energy conformers for each peptide obtained during this process were then reoptimized through the Density Functional Tight Binding (DFTBA) functionality accesible within the Gaussian 09 software [22]. By considering the experience acquired in the previous research of our group [11, 23-30], the model chemistry based on the association of the MN12SX functional with the Def2TZVP basis set using water as the solvent was considered for the final optimization of the resulting molecular structures because it has been shown that it allows the verification of the 'Koopmans in DFT' (KID) procedure [11, 23-30]. In the same way, the process for the calculation of the electronic properties and the chemical reactivity descriptors of the fungal peptides involved the use of MN12SX/Def2TZVP/H2O model chemistry through the consideration of the previously optimized molecular structures.

Results and Discussion

As mentioned in the Computational Methodology section, the MN12SX/DefTZVP/H2O model chemistry combined with the SMD (Solvent Model Based on the Density) solvent model [31] was used for the calculation of the electronic properties of each peptide after using calculation analysis procedures to determine whether all the structures agree with the minimum energy requirements. All the calculations were performed in the presence of water as the solvent because the potential bioactivity and toxicity of this fungal peptides is intimately related with the absorption, distribution, metabolism and excretion that take place within the organisms. The graphical sketches of the

molecular structures of the Amatoxins are shown in Figure 1 below.

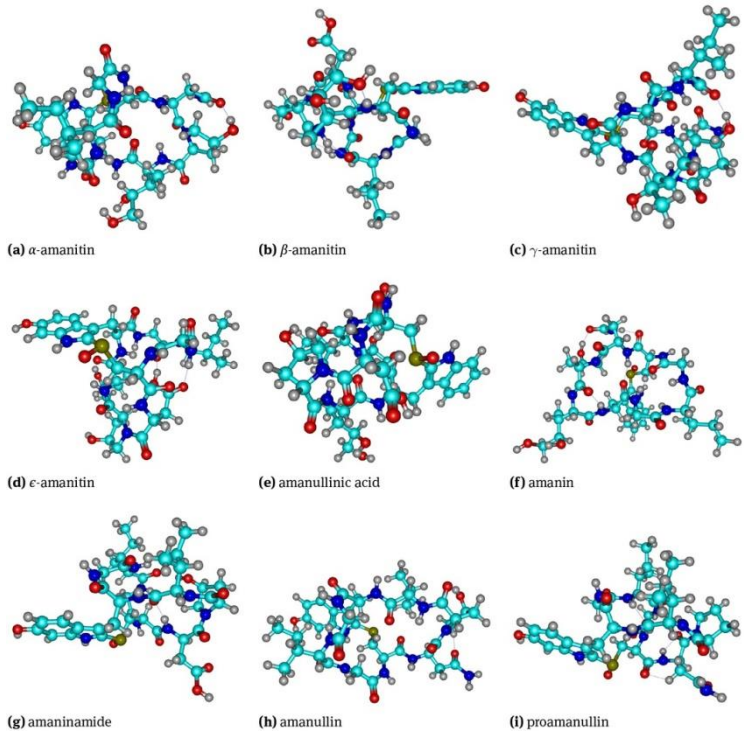

Figure 1: Graphical sketches of the molecular structures of a) α-amanitin; b) β-amanitin; c) γ-amanitin; d) ϵ-amanitin; e) amanin, f) amaninamide, g) amanullinic acid, h) amanullin and i) proamanullin

Following Becke's ideas [32] and the studies by Baerends et al concluding that the HOMO-LUMOgap of the Kohn-Sham (KS) system can be used as an effective measure of the molecular optical gap [33,34], ground state calculationswere used for the determination of the maximum absorption wavelength that belongs to the fungal peptides of the Amatoxin family to find the respective λ_{max} values through the application of chosen model chemistry to determine the HOMO-LUMO gaps. As we have shown in our previous research [23-29], the KID procedure is also valid in the presence of water as the solvent and represents

an advantage over the use of the vertical I and A for the calculation of the global descriptors because it avoids the separate calculation of the radical cation and anion which could be difficult for molecules of the size considered here. Therefore, the results for the calculation of the electronic properties of the Amatoxins fungal peptides are displayed Table 1.

Table 1: Electronic energies (in au) of the Amatoxins, HOMO, LUMO and HOMO-LUMO gap (in eV), and the maximum absorption wavelengths λ_{max} (in nm) calculated with the MN12SX/Def2TZVP/H2O model chemistry.

Molecule	**Total Electronic Energy**	**HOMO**	**LUMO**	**HOMO-LUMO Gap**	λ_{max}
α-amanitin	−3512.5105	−5.4760	−1.1657	4.3103	288
β-amanitin	−3532.3745	−5.5987	−1.3543	4.2444	292
γ-amanitin	−3437.4086	−5.6172	−1.3856	4.2316	293
ϵ-amanitin	−3457.2548	−5.5495	−1.3110	4.2384	293
amanullinic acid	−3382.1470	−5.5792	−1.3129	4.2662	291
amanin	−3457.2226	−5.8959	−1.4944	4.4014	282
amaninamide	−3437.3836	−5.8730	−1.4588	4.4142	281
amanullin	−3362.2831	−5.5615	−1.2972	4.2643	291
proamanullin	−3287.1706	−5.5547	−1.3184	4.2363	293

Calculation of the Global Reactivity Descriptors of the Amatoxins

It has been shown by Frau and Glossman-Mitnik [11, 23-30] that the HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) energies obtained with the MN12SX/Def2TZVP/H2O model chemistry allows the verification of the KID procedure, that is, rendering an approximate Koopmans behavior. With the aid of the KID technique and the finite difference approximation [11, 23-30], the following expressions can be used to define the global reactivity descriptors [14-16,35,36]:

Electronegativity

$$\chi = -\frac{1}{2}(I + A) \approx \frac{1}{2}(\varepsilon_L + \varepsilon_H)$$

Global Hardness

$$\eta = (I - A) \approx (\varepsilon_L - \varepsilon_H)$$

Electrophilicity

$$\omega = \frac{\mu^2}{2\eta} = \frac{(I + A)^2}{5(I - A)} \approx \frac{(\varepsilon_L + \varepsilon_H)^2}{4(\varepsilon_L - \varepsilon_H)}$$

Electrodonating Power

$$\omega^- = \frac{(3I + A)^2}{16(I - A)} \approx \frac{(3\varepsilon_H + \varepsilon_L)^2}{16\eta}$$

Electroaccepting Power

$$\omega^+ = \frac{(I + 3A)^2}{16(I - A)} \approx \frac{(\varepsilon_H + 3\varepsilon_L)^2}{16\eta}$$

Net Electrophilicity

$$\Delta\omega^{\pm} = \omega^+ - (-\omega^-) = \omega^+ + \omega^-$$

being ϵ_H and ϵ_L the HOMO and LUMO orbital energies.

The calculated values for these global reactivity descriptors using the MN12SX/Def2TZVP/H2O model chemistry and the associated HOMO and LUMO energies are displayed in Table 2.

Table 2: Global reactivity descriptors of the Amatoxins calculated with the MN12SX density functional with the Def2TZVP basis set and the SMD solvation model using water as the solvent.

Molecule	Electronegativity	Global Hardness	Electrophilicity
α-amanitin	3.3209	4.3103	1.2793
β-amanitin	3.4765	4.2444	1.4238
γ-amanitin	3.5014	4.2316	1.4486
ϵ-amanitin	3.4303	4.2384	1.3881
amanullinic acid	3.4461	4.2662	1.3918
amanin	3.6952	4.4014	1.5511
amaninamide	3.6659	4.4142	1.5222
amanullin	3.4293	4.2643	1.3789
proamanullin	3.4365	4.2363	1.3939
α-amanitin	4.4884	1.1675	5.6560
β-amanitin	4.8511	1.3746	6.2257
γ-amanitin	4.9124	1.4110	6.3234
ϵ-amanitin	4.7562	1.3260	6.0822
amanullinic acid	4.7732	1.3272	6.1004
amanin	5.2249	1.5297	6.7546
amaninamide	5.1533	1.4874	6.6407
amanullin	4.7390	1.3097	6.0487
proamanullin	4.7708	1.3343	6.1051

As expected from the analysis of the molecular and electronic structure of these peptides, their electrodonating powers are larger than their accepting powers. However, the differences in the chemical reactivity between them are not to large. The global hardness may be regarded approximately as the inverse of the polarizability and then is related to the deformability of the global electronic density. This means that a small hardness implies great reactivity and viceversa. Although the small differences, our methodology allowed to classify amanin and amaninamide as the lowest reacting peptides while γ-amanitin, ϵ-amanitin and proamanullin are the greatest reacting peptides of the group considered here. The same conclusions can be obtained from the analysis of the values of the global electrophilicity which follows from the relation between the electronegativity and the global hardness, although in this case, the differences are more marked.

Calculation of the pKas of the Amatoxin Family of Fungal Peptides

During a previous study of amino acids and peptides [37], a relationship between the pKa and the global hardness η has been developed in the form of pKa = 16.3088 - 0.8268 × η which is expected to be useful for the prediction of the pKa of larger peptides. The computation of the pKa values for all the peptides has been based on the η values presented in Table 2 and the results for the Amatoxin molecules are shown in Table 3. As to the best of our knowledge, the experimental pKas of the peptides considered in this work have not been reported and our results represent an approximate prediction of what those values could be.

Table 3: pKas of the Amatoxin family of fungal peptides.

Molecule	pKa
α-amanitin	12.75
β-amanitin	12.80
γ-amanitin	12.81
ϵ-amanitin	12.80
amanullinic acid	12.78
amanin	12.67
amaninamide	12.66
amanullin	12.78
proamanullin	12.81

The estimated pKa values displayed in Table 3 validate that the QSAR relationship utilized as successful in the differentiation of the particular pKa values for every peptide independent of the significance of the difference. The pKa estimations of these peptides could be of interest in the development of pharmaceutical medications by clarifying the drug delivery procedures and their respective action mechanisms.

Local Reactivity Descriptors Calculation

Applying the same ideas as before, the definitions for the local reactivity descriptors will be [14-16]: Nucleophilic Fukui Function

$$f^{+}(r) = \rho_{N+1}(r) - \rho_{N}(r)$$

Electrophilic Fukui Function

$$f^{-}(r) = \rho_{N}(r) - \rho_{N-1}(r)$$

which are relationships between the electronic densities of the neutral, positive and negative species.

The Electrophilic Fukui functions $f^{-}(\mathbf{r})$ and Nucleophilic Fukui functions $f^{+}(\mathbf{r})$ for the Amatoxin peptides are shown in Figure 2, where the colored regions allow to distinguish the electrophilic and nucleophilic regions within each of the studied peptides which could be of importance for the designing of new pharmaceutical drugs based on these moieties and also for getting and understanding of their toxicological properties.

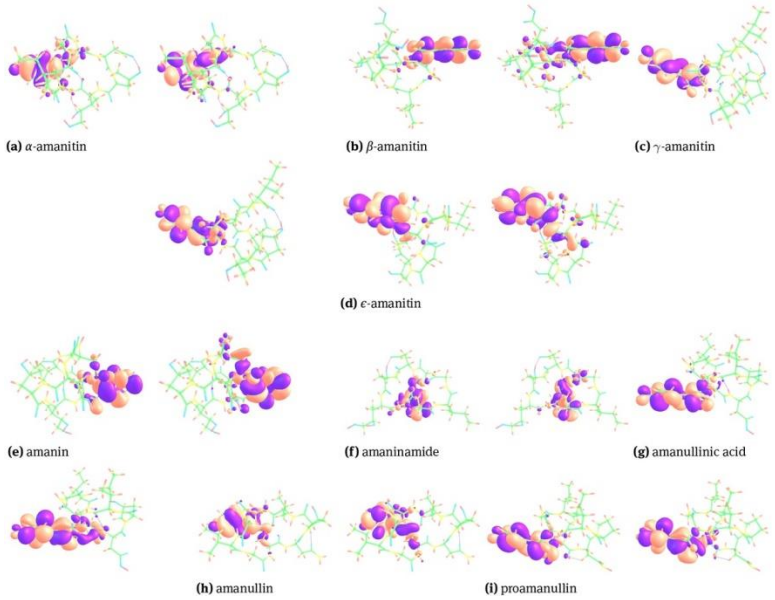

Figure 2: Graphical representation of the Electrophilic Fukui function $f^{-}(\mathbf{r})$ (left column) and Nucleophilic Fukui function $f^{+}(\mathbf{r})$ (right column) of the Amatoxins.

Conclusions

Throughout this study, the reactivity properties of nine molecules belonging to the Amatoxin group of fungal peptides was considered by making use of the Conceptual DFT model as an instrument to understand the electrophilic and nucleophilic interactions.

The data about the global and local chemical reactivity descriptors of the fungal peptides gained in this work could be useful to aid the plan of new pharmaceutical drugs relying on these information. The analysis of the molecular and electronic structure of these peptides revealed that their electrodonating powers are larger than their accepting powers. Moreover, although the differences in the chemical reactivity between them are not to large, a chemical reactivity order could be deduced based in our methodology that allowed to classify amanin and amaninamide as the lowest reacting peptides while γ-amanitin, ϵ-amanitin and proamanullin are the greatest reacting peptides of the group considered in this work. And the same conclusions were obtained from the analysis of the values of the global electrophilicity which follows from the relation between the electronegativity and the global hardness, although in this case, the differences were more marked.

The results related to the pKa could be of fundamental importance because it could give new information involving the drugs solubility. In this way, if the experimental values of the pKa the considered molecular systems are not available, the approximate previously developed QSAR equation employed in this study could be considered a nice predictive tool for the estimation of the pKas of small and large peptides.

As mentioned before, the information reported through this work could be of interest for medicinal chemistry researchers in using this knowledge for the developing of new therapeutic drugs based on the studied peptides or as a help for the understanding of the toxicity mechanisms exerted by them.

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