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PRELIMINARY STRUCTURAL OPTIMIZATION OF SOME FUMONISIN METABOLITES BY DENSITY FUNCTIONAL THEORY CALCULATION

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SUMMARY

Maize (Zea mays L.) is often contaminated with Fusarium verticillioides. This harmful fungus produces fumonisins as secondary metabolites. These fumonisins can appear both free and hidden form in planta. The hidden form is usually bound covalently to cereal starch. From the hidden fumonisins, during enzymatic degradation, glycosides are formed, and the fumonisin is further decomposed during a de-esterification step. In this short communication some preliminary DFT calculated structural results which could be useful in the future to help to understand the van der Waals force controlled molecular interactions between these kinds of mycotoxin molecules and enzymes are demonstrated.

Key-words: Fumonisin, fumonisin metabolites, DFT, electron density surfaces, structural optimization

INTRODUCTION

The term "mycotoxin" is usually reserved for the secondary metabolites produced by *fungi* that readily colonize crops. The sphingosine-like fumonisins are produced by several species of *Fusarium* molds, such as *Fusarium verticillioides*. The most commonly contaminated crop is maize (*Zea mays*). Fumonisins are common mycotoxins in maize, although these toxins can occur in a few other crops as well (Placinta et al., 1999). The primary health concerns associated with fumonisins are carcinogenic properties and acute toxic effects (Voss et al., 2007). The disruption of sphingolipid metabolism by inhibition of ceramide synthase has been proposed to be responsible for the carcinogenicity and toxicity (Wang et al, 1991). Four groups of fumonisins (FA, FB, FC and FP) were classified based on different structure of the carbon backbone and the location of the of nitrogen functional group (Musser and Plattner, 1997). Fumonisin (FB_1) is the most common and economically important form, followed by B_2 and B_3, where the index numbers refer to the different location of the hydroxyl groups on the carbon chain. Therefore, the metabolic investigations are focused on FB_1 *in vivo* (Fodor et al., 2008) and *in vitro* (Cirlini et al. 2015; Falavigna et al. 2012).

Mycotoxins undetectable by conventional, extraction-based analytical methods are termed as masked or hidden mycotoxins (Berthiller et al., 2013). Extractable mycotoxins can be easily detected but bound and/or hidden mycotoxins cannot be directly analysed. They have to be liberated from the matrix by chemical or enzymatic pre-treatment prior to chemical analysis. Dall'Asta et al. (2010) reported that with an *in vitro* digestion model method - after an enzymatic pre-treatment -, significantly more (30-40%) fumonisin was detected as compared to the measured moiety with conventional extraction method. In case of naturally contaminated (field derived) crop samples the hidden proportion of fumonisin is identical (35.6±22.3%, Dall' Asta et al., 2010). In case of inoculated crop cultures produced at laboratory-scale this value is 38.6±18.5% (Szabó-Fodor et al., 2015). Chemically modified mycotoxins are currently the largest group of modified mycotoxins and can be classified as "thermally formed" and "non-thermally formed" (Rychlik et al., 2014). Thermal degradation products have been described for several mycotoxins. A prominent example is fumonisin FB_1 which can react

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in a Maillard-type reaction with reducing sugars leading to *N*-(1-deoxy-D-fructos- 1-yl) fumonisin B_1 (NDF). The formation of hydrolyzed fumonisins (HFB_1), on one hand, are biologically modified and formed by the intestinal microbiota (Fodor et al., 2008) and, on the other, are formed under alkaline conditions during food processing (Humpf and Voss, 2004).

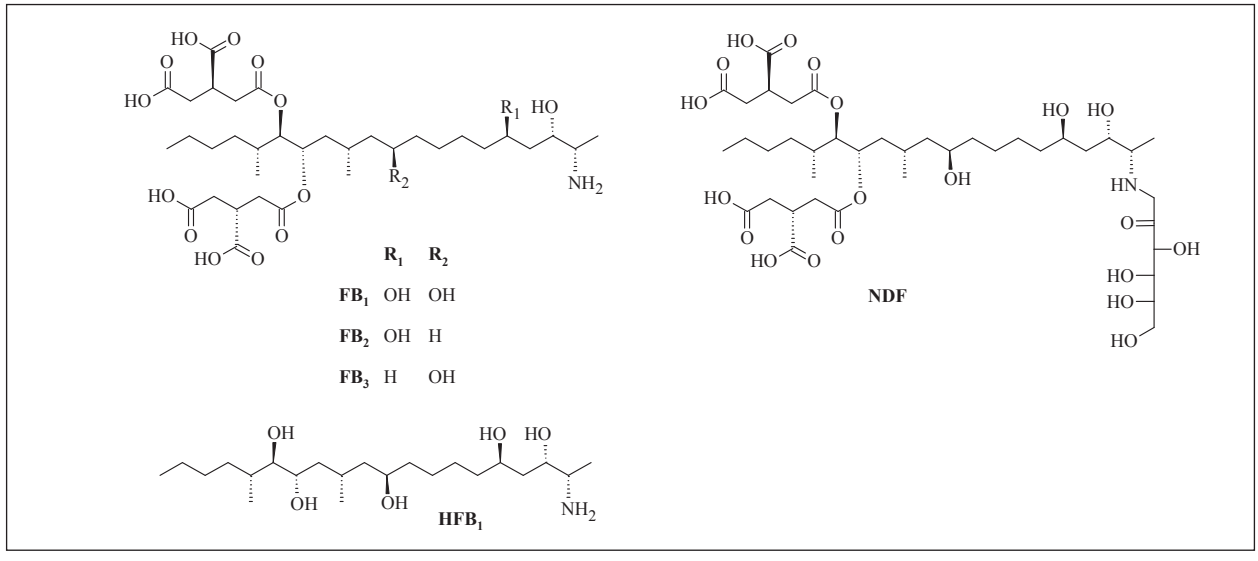

Figure 1. Structures of mentioned fumonisins

FB_1 is an inhibitor of sphinganine *N*-acyltransferase and increases the ratio of sphinganine/ sphingosine (Sa/So). Thus, elevation in the Sa/So ratio in different tissues (e.g. serum, urine, liver, kidney) is the exclusive biomarker of fumonisin exposure in exposed animals or human. However, best to our knowledge, no additional computation study was made to clarify the type of connection between sphinganine *N*-acyltransferase and fumonisin's monogastic metabolites. Density Functional Theory (DFT) calculation is an efficient method to determine the hydrophilic and hydrophobic site of molecules and it provides feasible input structures for the profound enzyme-substrate docking studies, in order to support the toxicological investigations.

From structural point of view, fumonisins provide a special nature: the long hydrophobic carbon chain bears electronegative (hydroxyl, amino- and carboxyl) functional groups. Due to free rotation around the carbon chain's single bonds, there could be several conformations – with different spatial location of functional groups –, being convenient for the above mentioned enzymes as "key", according to the lock and key enzyme model.

Several toxin molecules were investigated by theoretical DFT studies (e.g. Song et al., 2011; Rahmani brothers, 2014) including also mycotoxins (e.g. Türker and Gümüş, 2009). The first step of these computional studies is always the structure optimization followed by enzyme docking studies (e.g. Kumar and Garg, 2014). Docking studies needs huge computing power, usually executed in a computer cluster, which was not available in this case. The aim of this work was to highlight the lack amidst fumonisin metabolites and computational chemistry, as well as to provide some preliminary results for further, enzyme docking studies.

MATERIAL AND METHODS

All calculations were performed by Gaussian 03W. The structures were built in Advanced Chemistry Development, Inc. (ACD/Labs-ACD/3D) as .mol files and then converted to GaussView 3.09. The geometry of fumonisin metabolites were first pre-optimized with semi-empirical PM3 method and these structures were re-optimized in terms of DFT Gaussian 03 calculations for structural parameters using B3LYP (Becke's three-parameter functional with exact HF exchange and Lee-Yang-Parr exchange-correlation)/6-311G basis set. The FMO analysis (HOMO - highest occupied molecular orbital, LUMO - lowest unoccupied molecular orbital), are acronyms for and, involving hybridizations of selected bonds are also calculated at B3LYP methods and 6-311G level of the theory. Although most of all biologically relevant DFT studies set water as model solvent to investigate the solvation effect, in this case solvent interactions were neglected because of the long computation time; therefore all model calculations were executed in vacuum. GaussView 3.09's cube generator was used to calculate and visualize the electrostatic potential maps from the DFT results.

RESULTS AND DISCUSSION

Although the calculations are performed in vacuum – which is obviously not the medium of a living cell –, some ascertainment should be noted. The optimized structure of FB_1 is more spheroidal than expected which

is be explained with the zwitterion-like force between the Lewis base amine and acidic carboxyl group. It is in agreement with the more linear structure of NDF, where amine form *N*-glycosidic bond which is unable to show the above mentioned intermolecular interaction. HFB_1 became also a semicircle instead of a line, but with a smaller curve than FB_1, representing the ground state torsional angle between bonds (Figure 2.). Computed energy gap values (Δε) are shown in Table 1. The results are in agreement with the awaited stability (the first and second hydrolyzation step of NDF): $HFB_1 > FB_1 >$ NDF.

Table 1. B3LYP/6-311G calculated LUMO, HOMO and Δε (energies, are in eV) ($\Delta\varepsilon = \varepsilon_{LUMO} - \varepsilon_{HOMO}$)

Energy – (eV)	FB_1	HFB_1	NDF
LUMO	-4.08	-2.48	-5.01
HOMO	-9.82	-9.88	-8.98
Δε	5.74	7.40	3.97

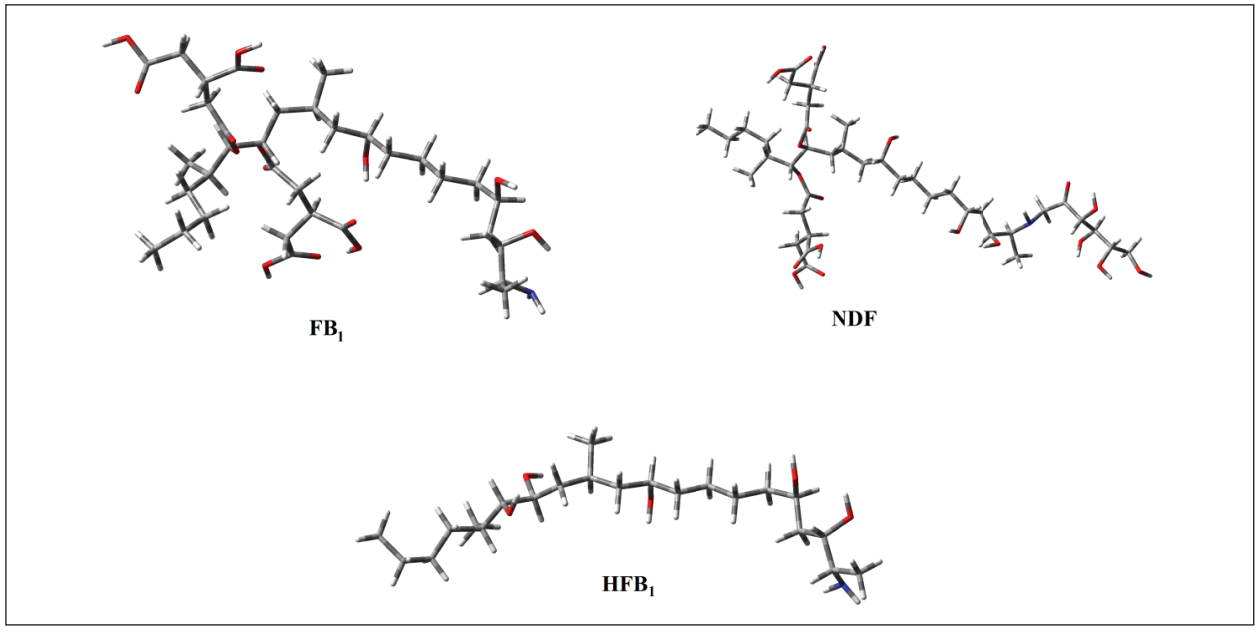

Figure 2. Optimized geometry of the investigated fumonisin molecules at 37°C *in vacuo* – tube model

CONCLUSION

The structures of FB_1, NDF and HFB_1 have been optimized by the Gaussian 03W at B3LYP/6-311G level. With the help of the results – and within the frame-work of the lock and key model –, researchers can better picture how the active site of the sphinganine *N*-acyltransferase approaches and joins the investigated fumonisin metabolites inhibiting the function of the enzyme in question.

By all means, these calculations should be handled as preliminary results, since solvation effects haven't been taken into consideration. Moreover, pH has momentous influence on the structure, especially in the case of FB_1. It should be calculated at isoelectric point as a zwitterion, with $-NH_3^+$ and $-COO^-$ functional groups. Due to the limited computational sources, we have to decide in the future to use smaller basis set instead of B3LYP/6311G and recomputing with solvent effect or to use a computer cluster to assess exact calculations.

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