In Silico Toxicology: Inhibiting Acrylamide Action in Food Products and Coffee.
A Project Suggested by Ernesto Illy

FINAL REPORT

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Research Framework and Research Activities

The E. Illy Foundation and the International School Superior of Advanced Studies of Trieste (SISSA) have started a research project in the field of Computational Toxicology. This project aims at combining chemical toxicology and computer simulations to generate a novel identification procedure of molecules acting as scavengers of toxic chemicals in food and coffee.

My participation as a researcher in this project led by Prof. P. Carloni (Lab of Comput. Biophysics, German Research School for Simulation Sciences GmbH & Lab of Comput. Biomedicine, Forschungszentrum Jülich (IAS-5/INM-9) and Prof. Micheletti (Sector of Statistical and Biological Physics, SISSA) and funded by E. Illy Foundation has resulted in a completed project (i) and one more (ii) that is ongoing and carried out in collaboration with Prof. M. L. Bolognesi (Dept. of Pharmacy and Biotechnology, University of Bologna). In addition, benefiting from the lively cultural environment in SISSA and in Juelich, I had the opportunity to be involved in two more projects (iii-iv, both completed projects).

In project (i), our aim was to study scavengers of toxic acrylamide (ACR). Transition state theory and density functional theory (DFT) were employed to reveal the relative free energy reaction profile of ACR with vitamin B3 (niacin).[1]

In project (ii) we use software tools of computational toxicology (e.g. QSAR Toolbox) to assess the toxicity of the ACR-nucleophile adducts.

In projects (iii-iv) I was involved in theoretical and experimental ^{1}H NMR studies of caffeine hetero-association with 3,4-, 4,5- and 3,5-di-O-caffeoylquinic acid.[2] I was then involved in the study of experimental and computed Vis-CD spectra of Cu^{2+}-loaded model peptides in square planar complexes, which resulted in a set of empirical rules useful for the elucidation of the complex conformation.[3]

A summary of the deliverables that came out and a brief description of the ongoing project follow.

Deliverables of the project "Computational Toxicology" funded by E. Illy Foundation

Project (i): Sequestering Acrylamide in coffee

Abstract

Acrylamide (ACR) is present in many food products cooked in high temperatures.[4] On average, more than 1/3 of the ACR content consumed by adult humans is due to coffee drinking.[5] ACR is neurotoxic in animals as well as in humans. It is also carcinogenic in rodents. Hence, reactions, which lead to ACR removal from food products and in particular from coffee, are of great importance for food research. In fried potato strips, the compound niacin sequesters ACR by forming a Niacin-acrylamide (NACR) adducts.[6] The same reaction also occurs in water. We have studied the mechanism and energetics of this reaction in aqueous solution by density functional theory. The CAM-B3LYP[7] and M06-2X[8,9] functionals with the 6-31+G(d,p)[10,11] basis set were employed. Single point calculations at the MP2 level with the same basis set were performed on optimized structures obtained at the M06-2X level, as well. Solvent effects comprehended both PCM[12,13] and SMD[14] solvation models. The calculated NMR chemical shifts of 1-propanamide-3-carboxy pyridinium are in agreement with experimental results.[3] The theoretical study showed that this reaction is favored thermodynamically. The relative electronic energy and free energy reaction profiles were calculated.

Paper published

1. G. Papamokos, J. Dreyer, L. Navarini, P. Carloni, Trapping acrylamide by a Michael addition: A computational study of the reaction between acrylamide and niacin. *Int. J. Quant. Chem.* 2014, 114, 553-559.

Project (ii) - ongoing: Employing computational tools to assess the toxicity of adducts formed by reactions between acrylamide and its potential scavengers.

Abstract

We are using available software tools to assess the toxicity of the acrylamide-nucleophile formed adducts so as to trap ACR in coffee and food products by non-toxic adducts. We are assessing the validity of the methods using ACR and similar substances that are known to be toxic following the protocols suggested by the developers. Then we will apply those methods to the acrylamide-nucleophile formed adducts reported in the literature. Freely available software tools used for this aim are: QSAR Toolbox[15], VEGA[16], Toxtree[17] and EPI Suite.[18] I plan to finish this research after I move to my new position.

Project (iii): An experimental and computational ^{1}H NMR study of hetero-association of caffeine with di-O-caffeoylquinic acid isomers in aqueous solution

Abstract

Caffeine hetero-association with 3,5-di-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid and 4,5-di-O-caffeoylquinic acid in aqueous solution has been investigated by one-dimensional (1D) and two-dimensional (2D) high resolution 1H-NMR spectroscopy. Self-association of the di-O-caffeoylquinic acid isomers has been studied as well. Caffeine-di-O-caffeoylquinic acid isomers association constants were measured. The value of the association constant of the caffeine-di-O-caffeoylquinic acid complexes is compatible with previous studies and within the typical range of reported association constants for other caffeine-polyphenols complexes. Structural features of the three different complexes have also been investigated, by NMR spectroscopy combined with quantum chemical calculations, and the complex conformation is discussed.

Paper under review

1. Nicola D'Amelio G. Papamokos, J. Dreyer, P. Carloni, L. Navarini. ^{1}H NMR studies of hetero-association of caffeine with di-O-caffeoylquinic acid isomers in aqueous solution. (*Submitted to Food Biophysics*)

Project (iv): Comparison of experimental and computed vis-CD spectra of Cu^{2+}-loaded model peptides in square planar complexes and development of empirical rules.

Abstract

Circular Dichroism (CD) spectroscopy in the visible region (Vis-CD) is a powerful technique to study metal-protein interactions. It can resolve individual *d–d* electronic transitions as separate bands and is particularly sensitive to the chiral environment of the transition metals. Modern quantum chemical methods enable CD spectra calculations and direct comparison with the experimental CD data, by which the conformations and the stereochemistry of the metal-protein complexes can be assigned. However, a clear understanding of the observed spectra and the molecular configuration is largely lacking. In this study, we compare the experimental and computed Vis-CD spectra of Cu^{2+}-loaded model peptides in square planar complexes. We find that the spectra can readily discriminate the coordination pattern of Cu^{2+} bound exclusively to main-chain amides from that also involving a side chain (i.e. histidine side chain). Based on the results, we developed a set of empirical rules that relates the appearance of particular Vis-CD spectral features to the conformation of the complex. These rules can be used to gain insight into coordination geometries of other Cu^{2+} or Ni^{2+}-protein complexes.

Paper in press

1. H. F. Stanyon X. Cong, Y. Chen, N. Shahidullah, Giulia Rossetti J. Dreyer, G. Papamokos, P. Carloni and J. H. Viles. Visible circular dichroism of Cu^{2+} and Ni^{2+} ions in histidine and amide main-chain coordinating complexes; developing predictive rules for coordination geometry (*FEBS Journal, in press*)

Concluding Remarks

The work in the project "Computational Toxicology" funded by E. Illy Foundation revealed the relative reaction profile and the mechanism of the reaction between ACR and niacin, a potential scavenger of ACR in coffee. Our results are consistent with experimental NMR data. We predict that similar natural chemicals to niacin could react with ACR following a similar mechanism. Such chemicals might perhaps be provided as coffee additives (e.g. milk, cream, flavor substances). From this work we have published one paper. Additionally, we have proposed and we are currently applying a relatively new methodology for the assessment of the toxicity of adducts formed by ACR and chemicals similar to niacin.

Moreover, the theoretical and experimental ^{1}H NMR studies of caffeine hetero-association with 3,4-, 4,5- and 3,5-di-O-caffeoylquinic acid and the study of experimental and computed Vis-CD spectra of Cu^{2+}-loaded model peptides in square planar complexes were completed. (One paper in press and another one under review)

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