Identification of CID fragmented oxidation products of tryptophan with chlorine dioxide solutions as marker of potential cell degradation

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Introduction

Chlorine dioxide is a widely used chemical for disinfection purposes applied mostly in water. The mechanisms of how it interacts with organisms are plentiful and seem to be barely understood. To get a hold of that the interaction with proteins is studied, as they could be used as a marker for oxidative stress induced by chlorine dioxide. The most reactive amino acids seem to be the aromatic ones. This leads to the conclusion that tryptophane can be used as a marker due to its specific UV-Vis-Absorption-spectrum and its fluorescent properties by an excitation with 280 nm. In case of oxidation this leads to either a change or loss of fluorescence. To understand the underlying mechanisms better the reaction products and kinetics have to be known.

So far the products were only analyzed with atmospheric pressure ionization methods after the use of not purified chlorine dioxide. This leads to the question if pure chlorine dioxide solution behaves fundamentally different.

Chemicals

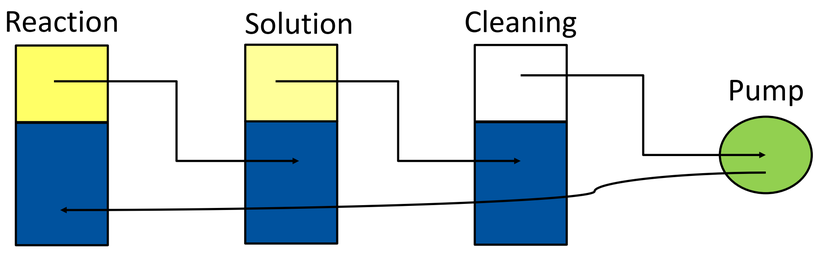
Chlorine dioxide is synthesized with a badge system that produces chlorine dioxide and continuously transfers it to the next vessels where the cleaned chlorine dioxide is concentrated.

In the reaction vessel chlorine dioxide is synthesized with natriumthiosulfate and natriumchlorite. This chemicals are supplied by Brenntag AG and are the same as used in the in situ plants. This reaction emits a lot of heat in the start, as such the vessel is cooled with an ice bath. After the ice is melted, the water bath gets heated to 40 °C for a higher yield and faster reaction. The gas phase of this vessel gets directed in a gas washing bottle and streams trough Millipore water. The gas phase of this bottle is then directed into the next identically built device and the process repeats. The gas phase then gets pumped by a peristaltic pump in the reaction vessel. With this setup, concentrations up to 5 w% of clean chlorine dioxide solution can be made and stored safely at 6 °C for up to 6 months. There are no other ions than the Chloride and Chlorate ions produced by the very slow decay of chlorine dioxide.

The tryptophane is commercially bought by Merck and graded for biochemical use.

Chlorine dioxide preparation

Figure 1: Chlorine dioxide reaction and purification setup.

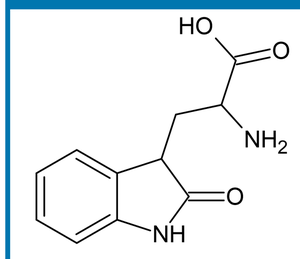


Reactions

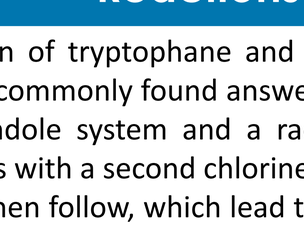
The mechanism of the reaction of tryptophane and chlorine dioxide is, as for all organic substances, barely known. The commonly found answer to that question is that in a first step chlorine dioxide attacks the indole system and a radical cation and chlorite ion are the products. Then the cation reacts with a second chlorine dioxide molecule.

Many different reaction steps then follow, which lead to a wide variety of products [1]. There it is important to differ between the ionization methods. GC-MS systems with an EI-source produce many small highly oxidated products [2]. However as described in literature, with atmospheric pressure ionization of solutions, bigger oxidated products. These let assume that reacted only with two chlorine dioxide molecules.

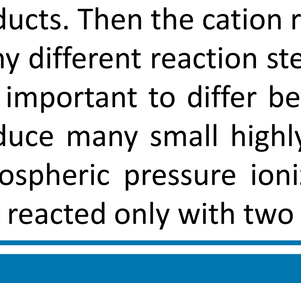
Expected Products



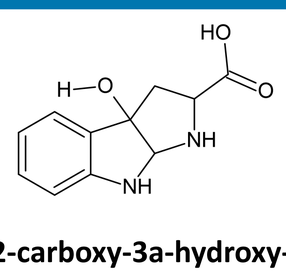
Oxindolylalanin (Oia)
MW = 220.22



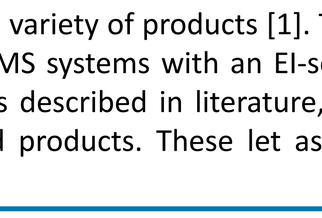
Dioxindolylalanine (Dia)
MW=236.22



N-formylkynurenine (NFK)
MW = 236.22



2-carboxy-3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrollol[2,3-b]indole (CPI)
MW = 220.22



2-carboxy-3a,8a-dihydroxy-1,2,3,3a,8,8a-hexahydropyrollo[2,3b]indole(CDPI)
MW = 236.22

MS-measurements and data analysis

The measurements were done with a SCIEX 6500 Triple-Quad-Massspectrometer with an SelexION-source.

The aqueous solution in which the reaction took place was mixed with an equal amount of acetonitrile solution with 0.2 % formic acid. The solution was injected with a syringe pump with a flow of 20 µl/min. The collision gas in q2 is nitrogen from the in house supply.

The fragment spectra were obtained with CFM-ID [3].

Macromolecular context

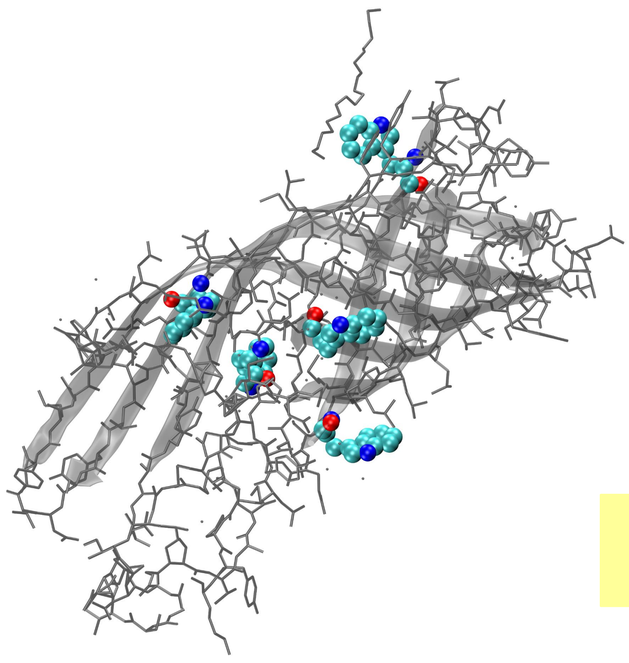
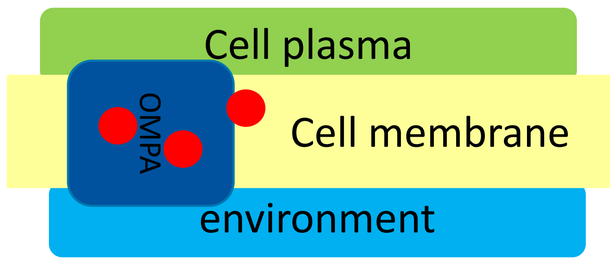


Figure 2, left: Structure of the membrane transporter protein OmpA of E. coli, tryptophane as van der Waals radii, protein structure as cartoon; Bottom left outside of the cell membrane; top right inside

Figure 3, bottom: scheme of cell membrane with OmpA; as red dots tryptophane



Results

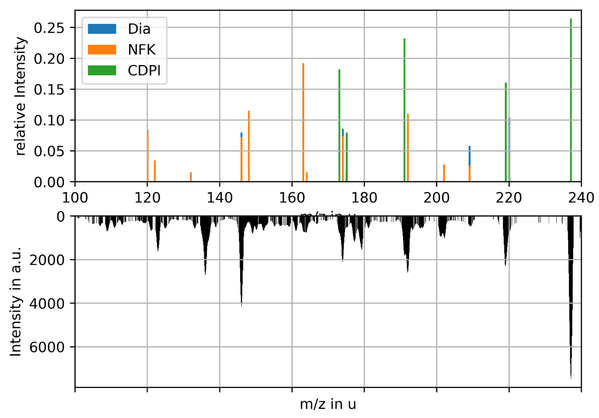
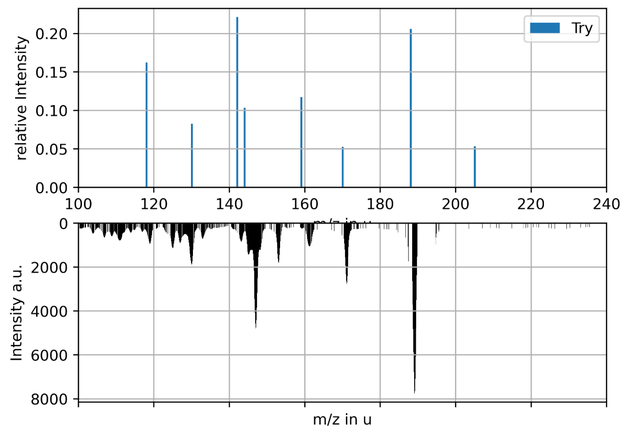


Figure 4, left: Spectrum of the extracted ion at 237 m/z with an CE of 20 eV in black; in the other half the calculated spectras of Dia, NFK, CDPI

Figure 5, right: Spectrum of the extracted ion with 205 m/z with an CE of 20 eV in black; in the other half the calculated spectras of Dia, NFK, CDPI



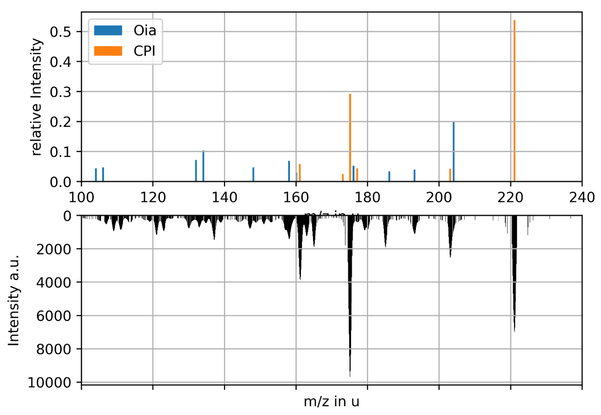


Figure 6, left: Spectrum of the extracted ion with 221 m/z with an CE of 20 eV in black; in the other half the calculated spectras of Dia, NFK, CDPI

Conclusion

As seen in Figure 4 to 6 it is clear that the m/z ratios of the expected products were found in the mass spectra. Furthermore, the fragment patterns match the expected scheme as calculated with CFM-ID.

This is also supported by DFT calculations that were performed with xtb [4]. All described species seem to be valid and also the protonated species were evaluated with crest and optimized with xtb [5].

That leads to the conclusion that all the postulated structures seem valid for the found m/z ratios. This enables further assumptions about toxicologic features and pathways of chlorine dioxide.

Outlook

In future there is the plan to determine the ratios of the products with the same mass should be determined by the fragmentation pattern. To get these ratios further theoretical data is needed. Additionally, some of the products that are commercially available will be acquired and measured.

There is also the plan to measure small peptides for the influence of the peptide chain and surrounding amino acids.

References

[1] Stewart DJ, Napolitano MJ, Bakhmutova-Albert EV, Margerum DW. Kinetics and mechanisms of chlorine dioxide oxidation of tryptophan. Inorg Chem. 2008 Mar 3;47(5):1639-47.

[2] Navalon S, Alvaro M, Garcia H. Chlorine dioxide reaction with selected amino acids in water. J Hazard Mater. 2009 May 30;164(2-3):1089-97.

[3] Wang F, Allen D, Tian S, Oler E, Gautam V, Greiner R, Metz TO, and Wishart DS. (2022) CFM-ID 4.0–a web server for accurate MS-based metabolite identification. Nucleic Acids Research 50 (W1), W165-W174.

[4] C. Bannwarth, E. Caldeweyher, S. Ehlert, A. Hansen, P. Pracht, J. Seibert, S. Spicher, S. Grimme WIREs Comput. Mol. Sci., 2020, 11

[5] Pracht, P.; Bohle, F.; Grimme, S.; Automated exploration of the low-energy chemical space with fast quantum chemical methods, Phys. Chem. Chem. Phys., 2020, 22, 7169-7192.