The response of lysozyme to oxidation by hypochlorite

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The oxidation of lysozyme with hypochlorite is a process of great interest, being one of the consequences of the uncontrolled activation of leukocytes of the immune system in various chronic pathological and inflammatory conditions. (Figure 1)[1]

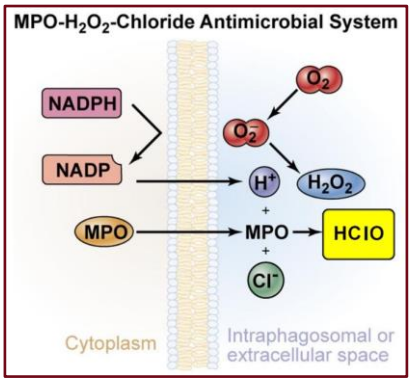

Figure 1. During the process termed "the respiratory burst", activated leukocytes produce highly oxidizing species, including hypochlorite.

The first phenomenon that was observed during this work, by mixing an aqueous solution of lysozyme in phosphate buffer at physiological pH with a solution of hypochlorite, is the almost instantaneous formation of a precipitate. The nature and formation mechanism of this precipitate are not known. To clarify these aspects, several experiments have been carried out, including many spectroscopic analysis of UV radiation absorption, Circular Dichroism (CD), Dynamic Light Scattering (DLS), and emission spectra with the thioflavin-T fluorescent probe (ThT). Some X-ray diffraction spectra and some microscopy investigations were also made using the atomic force microscope (AFM).

From this series of investigations it emerged that the percentage of lysozyme that precipitates strictly depends on the molar ratio between hypochlorite and protein. Once the precipitate was removed, it emerged through the DLS that the lysozyme that remains in solution aggregates over time more quickly and to a greater extent than the non-oxidized native protein. Furthermore, the UV absorption spectra showed a chemical modification of the lysozyme due to the reaction with hypochlorite, but also the occurrence of secondary reactions in the following hours on the oxidized protein.

The aggregates, that are present in solution even immediately after the reaction, have a weakly positive response to the ThT fluorescence assay, which is considered a specific assay for amyloid fibrils. (Figure 2)[2]

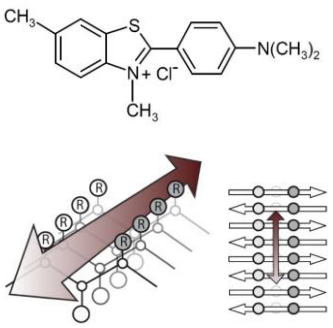

Figure 2. Interaction between thioflavin-T and the cross-β structure that is typical of amyloid fibers.

However, the characterization of the aggregates remains uncertain and the positive response of the ThT assay, in fact, may be compatible with the presence of zones with β-sheet interface that are initially formed due to the chemical modifications caused by oxidation on the lysozyme molecules, and then evolve into globular or amorphous clusters or aggregates, and not into amyloid fibrils.

CD measurements, on the other hand, clearly showed a change in the secondary structure of the protein after oxidation with hypochlorite, showing an increase in β-sheet structure and a decrease in α-helix structure with increasing oxidant/protein molar ratio.

The results of this work suggest that there is a value of the hypochlorite/lysozyme molar ratio beyond which the chemical damage to the lysozyme can trigger a series of processes, starting from a conformational transition or a modification of the secondary and tertiary structure of the protein, and this can lead to processes of aggregation and precipitation. (Figure 3)[3]

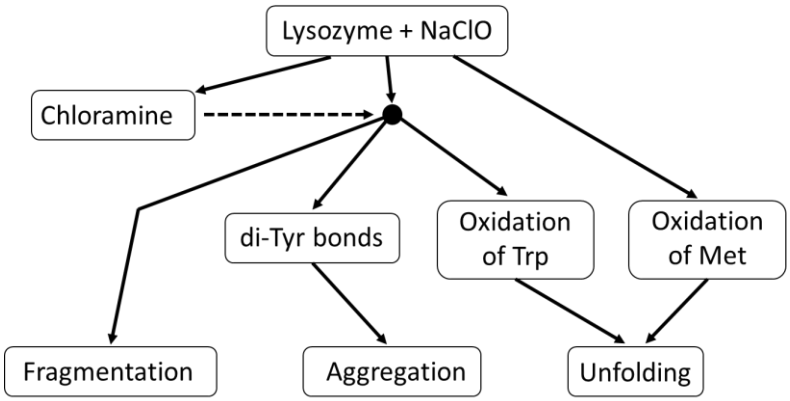

Figure 3. Possible consequences of the oxidation of lysozyme by hypochlorite.

A primary objective of further investigations is therefore the search for the best conditions to better identify these critical conditions and deepen the knowledge of the mechanism of this process.

References

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[3] Hawkins CL, Davies MJ. Inactivation of protease inhibitors and lysozyme by hypochlorous acid: role of side-chain oxidation and protein unfolding in loss of biological function. *Chem. Res. Toxicol*. 2005 18: 1600-1610.