Ewa Gapys, M.Sc.
Lodz University of Technology
Faculty of Chemistry

Supervisor: Andrzej Marcinek, Ph.D., D.Sc.
Additional Supervisor: Radosław Michalski, Ph.D.

Mechanistic aspects of the reactivity of amino acid, peptide, and protein hydroperoxides

Proteins are major non-water components of living organisms. Due to their abundance they are major target for different types of oxidants, continuously produced in biological systems, including reactive oxygen species (ROS). One of undesirable products of these reactions are hydroperoxides of amino acids, peptides and proteins.

Formation of hydroperoxides leads to changes in the structure and properties of proteins. Furthermore, hydroperoxides are unstable intermediates that can further propagate the oxidative damage (e.g. inducing enzymes inactivation or DNA damage). In view of their proposed involvement in multiple oxidative reactions it is important to develop easy to use, sensitive and reliable assay for measuring amino acid, peptide and protein hydroperoxides in real time. Existing methods, including FOX assay and the iodometric assay possess substantial limitations, and cannot be used in real time measurements.

In PhD thesis titled "Mechanistic aspects of the reactivity of amino acid, peptide, and protein hydroperoxides" the reactivity of hydroperoxides of amino acids, peptides and proteins towards various scavengers, including boron-based profluorescent probes, salen-manganese(III) complexes and seleno-L-methionine, is described. The amino acid, peptide, and protein hydroperoxides used in the reactivity studies were generated in enzymatic system based on horseradish peroxidase and xanthine oxidase, in the reaction with singlet oxygen, and in the reaction with radiolytically formed hydroxyl radicals. The rate constants of the reactions between boronate-based probes and selected hydroperoxides have been determined. On the basis of those studies the easy to use and real-time assay based on 7-coumarin boronic acid (CBA) probe was developed for the detection and quantification of hydroperoxides of amino acids, peptides and proteins.

In the second part of this work, the reactivity of amino acid, peptide and protein hydroperoxides towards their potential scavengers, salen-manganese complexes and organo-selenium compound, seleno-L-methionine, is presented.

Salen-manganese complexes are synthetic, low molecular weight scavengers that possess catalase- and SOD-like activity. These compounds show protective effect in various models of ROS-associated tissue damage that can be related to their catalytic activity. It is probable that the observed positive effect is not only related to the catalytic inactivation of superoxide radical anion and hydrogen peroxide by these complexes but can be also related to their ability to scavenge the other hydroperoxides, like amino acid, peptide, and protein hydroperoxides. In order to determine rate constants of the reactions between selected salen manganese complexes and L-tyrosine, L-tryptophan and L-histidine hydroperoxides,

the competition kinetic approach was applied. In this method CBA probe was used as a reference compound. The activity of salen-manganese complexes toward L-tryptophan hydroperoxides and hydroperoxides generated in more complex biological system such as lysozyme, bovine serum albumin or cell lysates from macrophages RAW 264.7 was confirmed.

The second order rate constants of the reaction between amino acid and peptide hydroperoxides with naturally occurring amino acid, seleno-L-methionine, were determined.

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Ewa Gapys