Abstract

Redox biology is one of most important interdisciplinary field of research that has been emerging dynamically in the recent years. Processes of oxidation and reduction of the cell components lay in the area of interest of this field of chemistry. Reactive metabolites of oxygen, called reactive oxygen and nitrogen species are engaged in those processes. According to current knowledge, those species are involved in transmission of biochemical signals, regulation of metabolism, and they are a crucial part of defense mechanisms. During normal operation of the organism, the concentration of those reactive species is kept on low, steady-state levels, due to the presence of enzymatic catalysts. However, this balance, called redox homeostasis, may be impaired during the course of many diseases, such as atherosclerosis, or neurodegenerative disorders such as Alzheimer's disease or Parkinson's disease. Overproduction of reactive oxygen and nitrogen species may lead to the oxidation of cell components, which lead to their impairment damaging the cell and causing the exacerbation of pathological condition. This phenomenon is known as an oxidative stress.

Hydrogen peroxide and peroxynitrite are very important representatives of the reactive oxygen and nitrogen species. Hydrogen peroxide is a powerful oxidant generated in spontaneous or catalytic dismutation of superoxide radical anion. Although its reactivity towards cell components is quite low, in the presence of ferrous ions, hydrogen peroxide is transformed into hydroxyl radical, which is the most reactive species generated *in vivo*. Therefore hydrogen peroxide is considered as highly toxic. Peroxynitrite is strong one- or two-electron oxidant, that is generated *in vivo* in the reaction between nitric oxide and superoxide radical anion. Peroxynitrite reacts with many cell components, i.a. with some amino acids, glutathione or oxyhemoglobin. Moreover, peroxynitrite is a precursor of highly oxidative and nitrosative radicals, such as nitrogen dioxide or carbonate radical anion, and thus it is also considered as highly toxic.

Determination of the role of reactive oxygen and nitrogen species, including hydrogen peroxide and peroxynitrite, in the functioning of living organisms requires reliable methods of their detection. Due to the high reactivity and poor spectroscopic properties, their direct detection is very difficult or impossible. Therefore, spectroscopic probes are used as a tool to detect reactive species. These probes during the reaction with reactive species change their spectroscopic properties, and give strong signal, detectable with common

analytical techniques. One of such probes is Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine), which is widely accepted as a probe for selective detection of hydrogen peroxide. This probe is used frequently in research, although mechanistic aspects of its oxidative conversion in the horseradish peroxidase-catalyzed (HRP) reaction are not known.

In PhD thesis “Mechanistic aspects of oxidative conversion of probes designed for the detection of hydrogen peroxide and peroxynitrite” the complex characteristic of the products of one-electron oxidation od Amplex Red, performed with the use of pulse radiolysis, is presented. It was shown, that not only enzymatic system H_2O_2/HRP can oxidize the probe, but strong one-electron oxidants, such as azide radical, nitrogen dioxide and carbonate radical anion, can oxidize the probe as well. The rates of the reaction between Amplex Red and azide radical and carbonate radical anion were determined. Based on the performed characteristics, the mechanism of this oxidative conversion of Amplex Red to colorful resorufin was proposed. Probably, Amplex Red is oxidized to its radical, which undergoes dismutation creating a transient species that decomposes to resorufin.

It was also shown, that Amplex Red incubation with peroxynitrite also leads to its oxidative conversion to resorufin. Based on the kinetic analysis it was ascertained that Amplex Red is not oxidized directly by peroxynitrite, but through the radicals of which peroxynitrite is a precursor. Horseradish peroxidase, used as an enzymatic catalyst in the reaction between hydrogen peroxide and Amplex Red, can also catalyze its oxidation in the presence of peroxynitrite. Catalytic rate constants for this two oxidants were determined, the values are similar, which leads to the conclusion that Amplex Red may detect hydrogen peroxide and peroxynitrite simultaneously. With the use of 7-coumarin boronic acid, that shows high reactivity towards peroxynitrite, and low towards hydrogen peroxide, the method of differentiation of the signals originating from those two oxidants generated in one system was proposed.

Some aspects of the boronate compounds, as probes for the detection of peroxynitrite, were shown. The reactivity of boronate probe Peroxy Crimson 1 towards peroxynitrite in different pH’s was determined, what allowed for the calculation of elementary rate constant for this reaction. The stability of the probe was determined in the presence of glutathione – Peroxy Crimson probe undergoes fast reaction with one or two molecules of glutathione, what discriminates the use of this probe in biological applications.

However, this probe was used to determine the rate constant of the reaction between three isomeric boronic acids, substituted with the -$CH_2P^+(C_6H_5)_3$ group, towards peroxynitrite, with the use of pulse radiolysis. The product profile was also determined. One of the products, substituted in the *ortho*- position, named *o*-MitoPhB$(OH)_2$ has shown different product reaction profile. Product of internal cyclization, produced in a large quantity, was the specific product for the reaction of the probe with peroxynitrite.

With the use of *o*-MitoPhB$(OH)_2$ and 7-coumarin boronic acid, based on the thorough analysis of the products profile in several experimental systems, it was shown that the product of the reaction between azanone (HNO) and oxygen, is unambiguously peroxynitrite, which has been disputed between researchers over the last years.