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Reactions of catalase with selected exo- and endogenous compounds

Catalase is one of the most important antioxidant enzymes. The main function of catalase is decomposition of hydrogen peroxide to water and molecular oxygen. Even a slight decrease of catalase activity, *in vivo*, may lead to hydrogen peroxide (and other reactive oxygen species) overproduction, and thus to the so-called oxidative stress. Therefore, it is extremely important to know the mechanisms of the reactions of catalase with exo- and endogenous compounds that may affect the catalytic cycle of the enzyme. From a pool of compounds potentially reactive to catalase I have selected flavonoids (plant polyphenols which are delivered to the body with foods), hypochlorous acid (highly chlorinating and oxidizing species, formed in the body in the immune response to invading pathogens), and nitrite (popular preservative, which is both delivered to the body with food, as well as produced *in vivo*). All investigations have been performed in the model systems.

In the first part of my dissertation, the influence of 19 flavonoids (from flavones, flavonols, and flavanones subclass) and model polyphenols on the activity of catalase was analyzed. I have found that all polyphenolic compounds used in these studies, especially myricetin, epicatechin gallate, epigallocatechin gallate, and quercetin, inhibit catalase. Based on the relationship between the structure and the inhibitive activity of flavonoids, the most important structural elements of flavonoids in catalase inhibition have been proposed. According to the obtained results it has been concluded that flavonoids, under low fluxes of hydrogen peroxide, reduce catalase Compound I to the inactive Compound II. In the absence of an external source of hydrogen peroxide, the rate-determining step of flavonoid-induced Compound II formation is the rate of hydrogen peroxide generation during the flavonoid autoxidation. I have concluded that, prior to the reduction of Compound I, flavonoid binds to the catalase molecule at the NADPH binding pocket. Competition between flavonoid and NADPH toward the same binding site may be the reason for partial protection of catalase against flavonoid-induced inhibition observed in the presence of NADPH.

The next part of my research concerned the reaction of catalase with hypochlorous acid. It has been shown that hypochlorous acid inhibits catalase and the degree of hypochlorous acid-induced catalase inhibition does not depend on pH in the range of 6.0-7.4. I have demonstrated that the reaction of catalase with hypochlorous acid proceeds in a different way, depending on the molar excess of hypochlorous acid over the enzyme. Hypochlorous acid reacts preferentially with amino acid residues on the enzyme surface. When the molar excess of hypochlorous acid exceeds 50 small fraction of the acid reacts directly with the heme center. Irreversible heme damage is observed at 1000-fold or higher molar excess of hypochlorous acid over catalase. The influence of flavonoids on the activity and mechanism of the reaction of catalase with hypochlorous acid, as well as antioxidant activity of flavonols modified by hypochlorous acid have also been investigated.

It has been confirmed that nitrite inhibits catalase, especially in the presence of chloride. Possible mechanisms of nitrite-induced catalase inhibition have been discussed. The kinetics of the reaction of nitrite with catalase and with its oxidized derivatives at various pH has been investigated and the rate constants of these reactions have been determined. Finally, it has been experimentally demonstrated that nitrite reduce both, Compound I and Compound II, directly to the native enzyme.

In the last part of dissertation, I have discussed possible biological consequences of catalase inhibition in physiological and pathological conditions. Having in mind, that in certain specific conditions, such as cancer or bacterial infection, catalase inhibition may be beneficial, knowledge of the mechanisms of the reaction of catalase with flavonoids, hypochlorous acid or nitrite may be helpful, e.g. in the designing of new anticancer therapy.

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