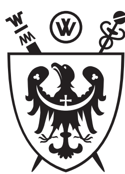

Lactoperoxidase as a new molecular target in infectious oral diseases

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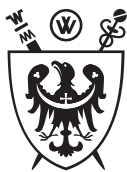
Lactoperoxidase (LPO) is a heme glikoprotein secreted, among others, into saliva, tears, respiratory tract and gastrointestinal mucus etc. This enzyme together with its (pseudo)halogenation cycle substrates—H_2O_2 and thiocyanate ions oxidized to hypothiocyanite ions, forms one of the main systems involved in antimicrobial defence within the oral cavity (Thomas & Aune, 1978; Magacz et al., 2019). Its antimicrobial action is based on oxidation of microorganisms thiol moieties of proteins leading to loss of their biological function (Bafort et al., 2014).

In bacterial and inflammatory diseases such as dental caries or periodontitis, as a result of extensive H_2O_2 production (oxidative stress) by host and microbial oxidases, lactoperoxidase active center is oxidized to a less reactive intermediate known as Compound II (viz. the oxo-iron(IV) intermediate of LPO) resulting in decreased generation of antimicrobial products. Compound II is a relatively stable form without the ability of hypothiocyanite synthesis (Gau et al., 2016). Moreover, this form when not reduced to native form could be oxidated to Compound III ultimately irreversibly losing its biological activity. Compound II could be reduced to the active native form by means of the reaction with broad spectrum of phenolic compounds of endogenous (proteins) and exogenous (xenobiontics, food, drugs) origin (Gau et al., 2018).

In presented research *Reynoutria* sp. rhizome extracts, due to their high polyphenol content, have been tested as potential source of compounds able to reactivate the antimicrobial activity of lactoperoxidase through converting the Compound II intermediate to native LPO state (FeIII(P)LPO). In our study, 70% acetone extracts of *Reynoutria japonica*, *Reynoutria sachalinensis* and *Reynoutria × bohemica* together with their five fractions (butanol, ethyl acetate, ethyl ether, dichloromethane and water residue) and four selected polyphenols dominating



in the studied extracts, were tested towards lactoperoxidase reactivating potential.

For this purpose, IC50, EC50 and activation percentage of hypothiocyanite synthesis was determined spectrophotometrically by Ellman's method. Furthermore, to evaluate the mechanism of reactivation, the rate constants for the conversion of Compound I (*viz.* the oxo-iron(IV) porphyrin π–cation radical intermediate of LPO) to Compound II and Compound II to FeIII(P)LPO in the presence of extracts, extracts fractions and selected polyphenols have been determined using direct stopped-flow spectroscopy. Finally, the ability to enhance the antimicrobial properties of the lactoperoxidase system was tested against *Streptococcus mutans* using direct optical density measurements of bacterial cultures.

We proved that *Reynoutria* sp. rhizome is the source of lactoperoxidase peroxidation cycle substrates which can act as both activators and inhibitors of the antimicrobial properties of that system. Concentration of tested extract/ fraction or compound was the deciding factor of observed effect. In lower concentrations activation of hypothiocyanite synthesis was observed nevertheless increase of concentration led to complete inhibition of lactoperoxidase (pseudo)halogenation cycle reaction. Presented study shows that the reactivation of lactoperoxidase by some phenolic compounds could become a potential therapeutic target in prevention and treatment support in some infectious inflammatory oral diseases.

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