Understanding the Mechanism of Polyphenolic Inhibition on Human Vascular NADPH Oxidase

by

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The goal of this thesis work is to employ a chemical biology approach to derive structural information on the binding of polyphenolic metabolites of apocynin-like compounds to vascular nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) subunits, which has been shown the main source of reactive oxygen species (ROS) generation in the vascular wall.

Using principles of biocatalysis, we have synthesized a library of phenolics using soybean peroxidase (SBP) catalysis in solution phase. The apocynin oxidation products (AOP) obtained mainly ranged from dimers to pentamers, and included phenolic coupled products as well as oxidized analogs (quinones), hydroxylated compounds, and *o*-demethylated variants. Resveratrol metabolites (ROP) obtained were two main dimers with different couplings.

The *in vitro* study showed that the apocynin metabolites highly inhibited NADPH oxidase activity in a dose – response way. Some of the components obtained from the precipitate fraction, demethylated tetramer quinone, hydoxylated tetramer phenol, tetramer phenol, and dimer from the soluble extraction might be the potential effective compounds.

We have set up a moderate – throughput *in vitro* screening platform for identifying active metabolites of phenols that can disrupt critical interactions between $p22^{phox}$ and $p47^{phox}$ subunit, which are important in NADPH oxidase assembly and activation. Interestingly, instead of inhibition of the interaction between $p22^{phox}$ peptide and $p47^{phox}$ protein, the phenol metabolites increased their interaction. The tetramer and tetramer quinone metabolites of apocynin might be functional in this process. Although the oligophenols can bind to the proline residues on $p22^{phox}$ peptide, their binding to $p47^{phox}$ seems more essential to induce $p47^{phox}$ further binding to $p22^{phox}$ proline - rich peptide. Since proper conformation of $p47^{phox}$ is required for the activation of NADPH oxidase complex, it is likely that $p47^{phox}$ was stimulated by oligophenols through conformational change, leading to the interaction with proline residues on $p22^{phox}$ peptide. However, which compounds of oligophenols actually work, where and how these oligophenols interact with $p47^{phox}$ will need more work to determine.

In addition, we confirmed that the quinone compounds of the apocynin metabolites inhibited the binding of $p47^{phox}$ to $p22^{phox}$ peptide. This result contrasted the mechanism proposed in the above paragraph, in which we speculate that the tetramers and tetramer quinones might stimulate the interaction between $p22^{phox}$ peptide and $p47^{phox}$ protein. Decisive results will require MALDI – TOF analysis of AOP and $p47^{phox}$ interaction.

The overall studies of this thesis further enhance our understanding of the molecular processes by which peroxidase – generated polyphenols inhibit the assembly and activation of the NADPH oxidase complex, and perhaps other multi-component signal transduction proteins. Such information will provide additional insight into how phenols, such as apocynin and resveratrol, found in natural products (e.g. green tea and red wine) and their bio-inspired counterparts, may act therapeutically to inhibit the development of vascular diseases.