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I E C B

Mechanistic Investigations of Four Enzymes Involved in the Transformation of Polyphenolic Substrates.

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Flavonoids are ubiquitous in plants where they constitute a very large family of polyphenolic structures. Among them, anthocyanidins and their glycosylated derivatives are largely responsible for the pigmentation patterns of flowers and fruits, whereas one of the best documented functions of flavan-3-ols and condensed tannins is to protect plants from infection by bacteria and fungi, as well as from destruction by insects or indiscriminate consumption by herbivorous animals. Flavonoids are increasingly recognized as important components of our diet, and many of them have been claimed to exhibit pharmacological properties which may be beneficial to human health.

Yet, understanding the catalytic and inhibition mechanisms of enzymes which transform polyphenolic substrates remains a challenging task. This is largely due to substrate or product properties such as limited solubility in water, instability and multiple protonation states, but also to marked substrate inhibition often observed at modest concentrations of polyphenols, and to frequent problems of aggregation of recombinant enzymes out of their usual environment.

This talk will focus on the main techniques which we used in recent years to understand the behavior of polyphenolic substrates with four distinct enzymes. We will discuss studies of three NADPH-dependent reductases of the flavonoid biosynthetic pathway, namely dihydroflavonol reductase (DFR), leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR). We will emphasize the use of deuterium labeling and tandem-mass spectrometry to shed light on the mechanisms of stereo- and regio-specificity of double-hydride transfers, and that of isothermal titration calorimetry as well as the chromatographic method of Hummel & Dreyer in equilibrium-binding studies. We will show from steady-state kinetics and binding studies that the unusually strong substrate inhibition observed with DFR and dihydroquercetin mostly results from the formation of a non-productive binary enzyme-polyphenol substrate in an ordered mechanism

where NADPH is the first substrate, and that this could be rationalized in terms of head-to-tail binding of the polyphenolic substrate when NADPH is not yet present at the active site.
Finally, we will show how mass spectrometry can be used in real time to monitor a complex sequence of one-electron transfers and dimerization initiated by a recombinant laccase on a tetracyclic polyphenol, namely brazilin.

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