REINVESTIGATING SUBSTRATE SPECIFICTY OF DIHYDROFLAVONOL 4-REDUCTASE OF *PETUNIA* × *HYBRIDA*

Daria Nitarska, Christian Haselmair-Gosch, Silvija Miosic, Christian Molitor, Karl Stich and Heidi Halbwirth*

Institute of Chemical, Environmental and Bioscience Engineering, Technische Universität Wien, Vienna, Austria

Dihydroflavonol 4-reductase (DFR) is an oxidoreductase, catalyzing the stereospecific reduction of the keto group of dihydroflavonols in position 4, in the presence of NADPH, to provide leucoanthocyanidins. This is a key step, influencing the formation of at least three flavonoid classes: flavonols, flavanols and anthocyanidins. As a rule, DFRs convert dihydroflavonols irrespective of their hydroxylation pattern, but some plant species, such as petunia and tobacco, possess specific DFRs that do not convert dihydrokaempferol (DHK) into the corresponding leucopelargonidin. This has a strong effect on flower color. Such species lack orange and bright-red pelargonidin accumulating phenotypes (Johnson et al. 1999).

The underlying molecular mechanism of DFR substrate specificity is not completely understood. In pioneering work, Johnson et al. (2001) tested the relevance of 6 putatively important positions by exchanging hydrophilic with hydrophobic residues of the same size. This identified amino acid (aa) 133 as being significant which is an asparagine (N) in the unspecific gerbera DFR and an aspartic acid (D) in the specific petunia DFR. Replacement of the N in the gerbera DFR with an apolar leucine (L) created a DHK preferring DFR, which has been patented for breeding towards orange flower color. Interestingly, Johnson et al never simply exchanged the N in the gerbera DFR with D to demonstrate the relevance for the observed substrate specificity. Later the crystal structure of DFR revealed that aa 133 indeed contributes to the coordination of the dihydroflavonol substrate in the active site (Petit et al. 2007).

We used purified recombinant *Petunia* × *hybrida* DFR (*Ph*DFR) and mutants thereof to analyze the influence of several amino acids on the substrate specificity. Most notably, *Ph*DFR is highly specific for dihydromyricetin (DHM), which is a generally overseen aspect, as most studies fundamentally focus on the inability of *Ph*DFR to convert DHK. The low specificity for DHQ was already demonstrated in early works with enzyme preparations from *P.* × *hybrida* (Forkmann and Ruhnau 1987). We show that the N/D model of Johnson indicates DHM preference rather than inability to convert DHK, and demonstrate how *Ph*DFR substrate specificity can be modulated into different types of substrate preference.

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