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**Abstract**

Malus × domestica (apple) accumulates particularly high amounts of dihydrochalcones in various tissues, with phloridzin (phloretin 20 -O-glucoside) being prevalent, although small amounts of 3-hydroxyphloretin and 3-hydroxyphloridzin are also constitutively present. The latter was shown to correlate with increased disease resistance of transgenic M. × domestica plants. Two types of enzymes could be involved in 3-hydroxylation of dihydrochalcones: polyphenol oxidases or the flavonoid 30 -hydroxylase (F30H), which catalyzes B-ring hydroxylation of flavonoids. We isolated two F30H cDNA clones from apple leaves and tested recombinant Malus F30Hs for their substrate specificity. From the two isolated cDNA clones, only F30HII encoded a functionally active enzyme. In the F30HI sequence, we identified two putatively relevant amino acids that were exchanged in comparison to that of a previously published F30HI. Site directed mutagenesis, which exchanged an isoleucine into methionine in position 211 restored the functional activity, which is probably because it is located in an area involved in interaction with the substrate. In contrast to high activity with various flavonoid substrates, the recombinant enzymes did not accept phloretin under assay conditions, making an involvement in the dihydrochalcone biosynthesis unlikely.

**Keywords: malus × domestica (apple); flavonoids; dihydrochalcones; chalcones; flavonoid 3-hydroxylation**