ISOLATION OF AN ACYLTRANSFERASE SEQUENCE PUTATIVELY INVOLVED IN CYNARIN BIOSYNTHESIS IN CYNARA CARDUNCULUS L.

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cynarin, Cynara cardunculus, acyltransferase isolation

Cynara cardunculus is a diploid \( (2n=2x=34) \) species which includes globe artichoke (var. scolymus L.), cultivated cardoon (var. altilis DC) and their progenitor wild cardoon [var. sylvestris (Lamk) Fiori].

Cynara cardunculus is source of biopharmaceuticals and its leaf extracts have been widely used in herbal medicine as hepatoprotectors and choleretics since ancient times. The chemical components of the leaves have been found rich in compounds originating from the metabolism of phenylpropanoids which (i) protect proteins, lipids and DNA from oxidative damage caused by free radicals, (ii) inhibit cholesterol biosynthesis and contribute to the prevention of arteriosclerosis and other vascular disorders, (iii) inhibit HIV integrase, a key player in HIV replication and its insertion into host DNA, and (iv) possess antibacterial activity.

The major phenolic compounds in artichoke extracts are di-caffeoylquinic acids (e.g. cynarin) which are present mainly in Cynara species, and its precursor chlorogenic acid, one of the most widespread soluble phenolic compound in the plant kingdom. While chlorogenic acid has practically no market value, cynarin is a molecule of large pharmaceutical interest because it exhibits a strong antioxidant activity.

In extracts from tobacco stem a protein: hydroxycinnamoyl-CoA:shikimate/quinate hydroxycinnamoyltransferase (HCT) has been purified, which controls the biosynthesis of chlorogenic acid. The HCT: (i) catalyses the esterification of caffeic acid with the 3-hydroxyl group of quinic acid in order to originate chlorogenic acid, (ii) catalyzes the esterification of \( p \)-coumaroyl-CoA with quinic acid in order to generate \( p \)-coumaroyl quinate. Furthermore, in tobacco, the cDNA responsible for HCT production has been isolated and sequenced.

Artichoke presumably possesses an extra HCT enzyme that converts chlorogenic acid into cynarin and the aim of this research was to isolate the corresponding cDNA. mRNAs were extracted from globe artichoke leaves and the cDNAs generated by reverse transcription. Degenerate CODEHOP primers were designed on conserved region of acyltransferase protein in order to amplify the HCT cDNA by PCR. The resulting DNA fragments were resolved by agarose gel electrophoresis and a band of 700 bp isolated, cloned into a plasmid and sequenced.

A translated database search (Blast x) revealed high similarity (79% identity and 86% homology) with
the tobacco *HCT* (cDNA: AJ507825) and global alignment (CLUSTAL W) revealed the artichoke *HCT*-like sequence clustering with one of the four main acyltransferase groups (i.e. anthranilate N-hydroxycinnamoyl/benzoyltransferase). Further work will try to isolate full length HCT-like cDNAs by using the isolated sequence as a probe to screen a cDNA library. After successful isolation, the cDNA candidates will be heterologously expressed in yeast or in bacteria, with the objective to characterize the cDNA coding for the enzyme converting chlorogenic acid into cynarin.