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REGIOSELECTIVE GLUCOSYLATION OF (+)-CATECHIN USING A NEW VARIANT OF THE SUCROSE PHOSPHORYLASE OF BIFIDOBACTERIUM ADOLESCENTIS

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PURPOSE OF THE ABSTRACT

Catechins belong to the flavan-3-ols class, a sub-family of flavonoids which have a role in preventing chronic inflammation responsible to neurodegenerative, cardiovascular or viral diseases.[1] (+)-Catechin (3,3',4',5,7-pentahydroxyflavan) is distributed in a wide variety of plants and has been reported to have a stronger antioxidant activity compared to other flavonoids thanks to its catechol moiety (C3'-OH and C4'-OH). It can scavenge free radicals and inhibit production of reactive oxygen species by transfer of a single electron to electron-accepting radicals.[2] Nevertheless, its low aqueous solubility prevents (+)-catechin from being well-absorbed by the human body. During the last steps of their biosynthesis, flavonoids are functionalized and, in most cases, they are beta-glucosylated to increase their biodisponibility in living organisms. While these glucosylated forms of (+)-catechin present interest, their extraction from plants as well as their chemical synthesis is not viable as they both lead to low yields of purified products. Thus, enzymatic synthesis is a solution as it is known to be efficient, specific and eco-friendly. Controlling the regioselectivity of the enzymatic glucosylation of flavonoids remains nevertheless a methodological challenge for scientists.

Sucrose phosphorylases (SPs) are enzymes from the Glycoside Hydrolase family GH13 subfamily 18 according to CAZY database (EC 2.4.1.7). They catalyze in vivo reversible phosphorolysis of sucrose into alpha-D-glucose-1-phosphate and D-fructose via a glucosyl-enzyme intermediate. Their natural substrate, sucrose, is a cheap and efficient glucose donor. It has been shown that SP from Bifidobacterium adolescentis (BaSP) could synthesize alpha-glucosylated phenolic compounds using other acceptors than phosphate.[3] The mutation of native BaSP Gln345 by a phenylalanyl residue (mutant Q345F) increased the size of the catalytic site entry, allowing the glucosylation of flavonoids. Three alpha-glucosylated products of (+)-catechin were synthesized, but with no control on the reaction regioselectivity.[4] In order to control the regioselectivity of the transglucosylation reaction of (+)-catechin, we engineered a new mutant of Q345F containing a second mutation and obtained the variant P134D/Q345F. Using this new mutant as catalyst, we observed a modification of the products proportions favoring the formation of (+)-catechin-3'-O-alpha-D-glucoside compare to (+)-catechin-5-O-alpha-D-glucoside. Indeed, we showed, using standard molecular modelling and docking protocols, that P134D mutation impacted the local geometry of the acceptor site leading to the formation of the majority product by destabilizing one possible orientation of (+)-catechin acceptor.

This original combination of theorical and experimental complementary approaches can be used to predict preferred orientations of flavonoids in acceptor sites, hence providing a rational basis for building variants towards

a better control of regioselective glucosylation using SPs.

FIGURES

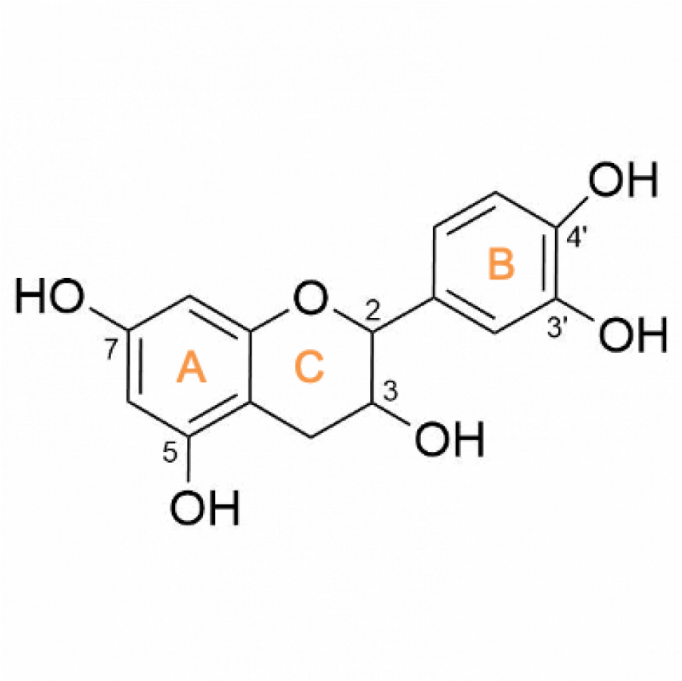

FIGURE 1

Molecular structure of (+)-catechin

(+)-catechin is composed of three rings designated by letters A, B and C. Ring B is the one with the catechol moiety.

FIGURE 2

KEYWORDS

Flavonoids | Regioselectivity | Sucrose phosphorylase | Modelling

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