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Laccase (EC 1.10.3.2) catalyses the conversion of procyanidin B-2 (epicatechin dimer) to type A-2

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Abstract—We report here the conversion of procyanidin B-2 (epicatechin dimer) to the procyanidin A-2 dimer by laccase (EC 1.10.3.2). The identity of the A-2 dimer was determined by its mass spectrum ($m/z = 577$), as well as by comparison with a product formed with the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. This latter system was previously shown to transform procyanidin type-B to type-A. Other quinonoid-type products, including an oxidation product ($m/z = 574.6$) of A-2, were also observed. We propose that in plants the conversion of natural procyanidins type-B to type-A might occur by enzymatic means rather than via a radical process as was previously suggested.

Procyanidins also known as condensed tannins are widely distributed in the plant kingdom.[1]

They are believed to protect plants against predators. Because of their potential beneficial effects to human health, there is a growing interest in these natural products. These condensed tannins possess a wide range of biological properties, including antioxidant activity,[2] antibacterial,[3] anticancer[4] and antiallergy effects.[5] For example, procyanidin B-2 was reported to have a hair growing effect,[6] whereas procyanidins of type-A linkage were shown to prevent urinary tract infections.[7]

Although the transformation of natural procyanidin type-B to type-A in an alkaline/H_2O_2 system[8] and in neutral conditions with DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals[9] have been previously reported, no direct evidence for enzymatic mediation of this conversion has been presented. The only data that suggested a possible involvement of enzymatic activity in this transformation was a report by Tanaka et al.,[10] in which phenazine derivatives of quinonoid structures were isolated after epigallocatechin was incubated in banana extracts. However, the isolated reaction products might have been formed via radical chemistry.

Recently, we reported that, in the presence and absence of the mediator (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), laccase converts (+)-catechin to oligomeric products, including hydrophilic type-B dimers as well as hydrophobic type-A dimers.[11] We have presented evidence that laccase could convert an enzymatically generated type-B dimer via an intermediate quinone methide to the type-A dimers. These dimers were shown to be formed by other enzymatic systems, such as peroxidase[12] and grape polyphenol oxidase.[13] However, the natural procyanidin type-B dimers are structurally different from the enzymatically generated type-B dimers.[13] They differ in the position of the inter-flavan bond, which in the former mainly occurs between C-4 of a flavan-3-ol unit and C-8 of another flavan-3-ol unit (Fig. 1). The type-A dimer has an additional ether bond between C-2 and O-7. In contrast to natural procyanidin type-B, the coupling of enzymatically produced type-B dimers occurs between the A-ring of a flavan-3-ol unit and the B-ring of another unit (head to tail polymerization). Because of this structural difference it is of interest to investigate whether or not laccase is capable of also converting natural procyanidin type-B dimer to type-A.

The reaction of procyanidin B-2 and DPPH radicals was performed in methanol and in aqueous ethanol (9:1). A previous report showed that DPPH radicals could transform procyanidin type-B to type-A under neutral conditions.[9] The reaction between laccase and PB-2 was

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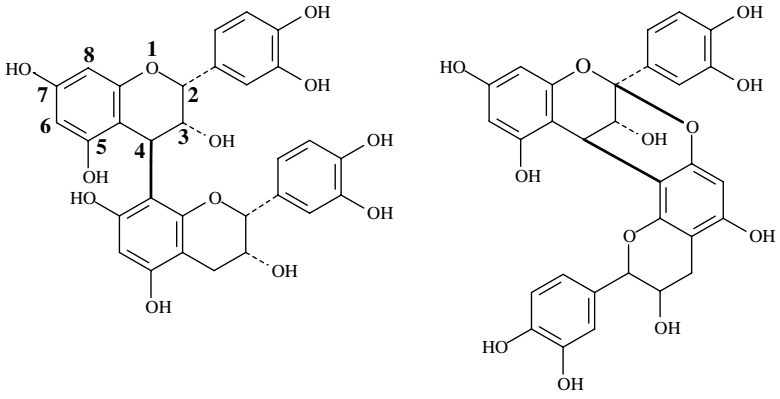

Procyanidin B-2 Procyanidin A-2

Figure 1. Structures of procyanidin type B-2 and A-2.

carried out in water and in buffers 100 mM acetate (pH 5) and in 125 mM phosphate (pH 7). Figure 2 shows a typical mass chromatogram of procyanidin B-2 obtained by HPLC–ESI-MS analysis, whereas Figure 3 compares the results of the incubation mixture of laccase with procyanidin B-2 (in water) (Fig. 3a) with that of DPPH radicals with PB-2 (aqueous ethanol) (Fig. 3b). The products formed in both systems were similar. The peak with the retention time (ca. 23 min) was identified as procyanidin A-2 as judged from its mass ($m/z = 577$) and mass spectrum (Fig. 3c). The characteristic ion peak with the m/z value 425 for type-A dimers[9,14] was observed. In addition, the results with the DPPH radical confirmed the formation of PA-2 (Fig. 3b). This latter system was previously shown to produce both A-1 and A-2 dimers from the corresponding PB-type dimers.[9] This PA-2 product had absorbance maxima at 230, 275 and around 420 nm (Fig. 3d), whereas the parent substrate had absorbance maxima at 230 and 275 nm (data not shown). In both enzymatic and radical systems, a product with a retention time 24.7 min was also detected, with an m/z value of 577 (Fig. 3a and b). At incubation times longer than 30 min the peak at 23 min decreased while the peak at 24.7 min increased (data not shown). This suggests a conversion of the former to the latter. This conversion is most likely an intramolecular rearrangement. A similar transformation of an enzymatically-produced type-A dimer to an isomer of the same type has been previously observed.[11,13]

At least three major peaks (t_R, ca. 8, 12.7, and 19.5 min.) with the m/z value of 575 were also observed under neutral conditions (Fig. 3a and b). These appear to be anthocyanidin-type products with the typical molecular ion $[M+H]^+$ 575.[15] The minor peak with the m/z value of 574.6 seems to be an oxidation product of the PA-2 dimer. At pH 7, in the enzymatic system, this product with the m/z value of 574.6 was the principal peak detected (Fig. 4). The peak with the m/z value of 576 (t_R, ca. 30.6 min) could be the semiquinone of the former product (m/z 574.6, Fig. 4). The formation of additional products in the DPPH-driven transformation of procyanidin B dimers to type-A has been described.[9] In contrast to the results of Kondo et al.,[9] we found that this DPPH-driven conversion of PB to type-A could also occur in alcohol (methanol) (data not shown).

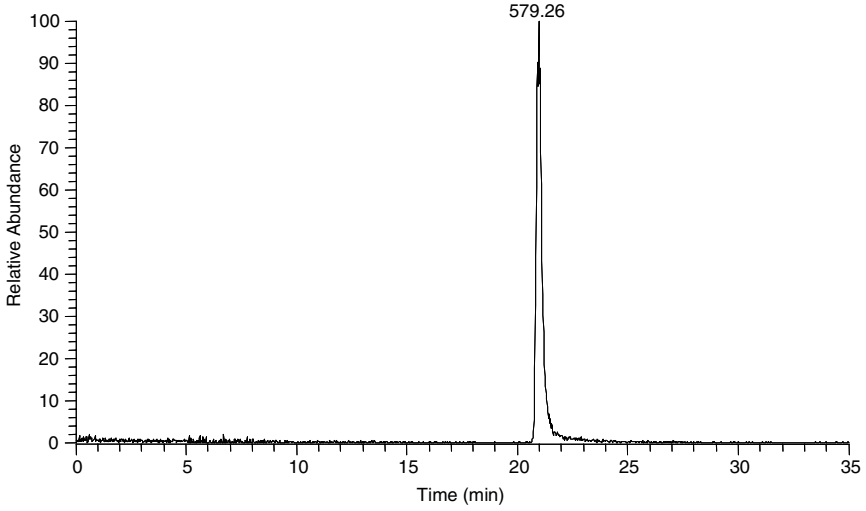

Figure 2. Mass chromatogram of procyanidin B-2.

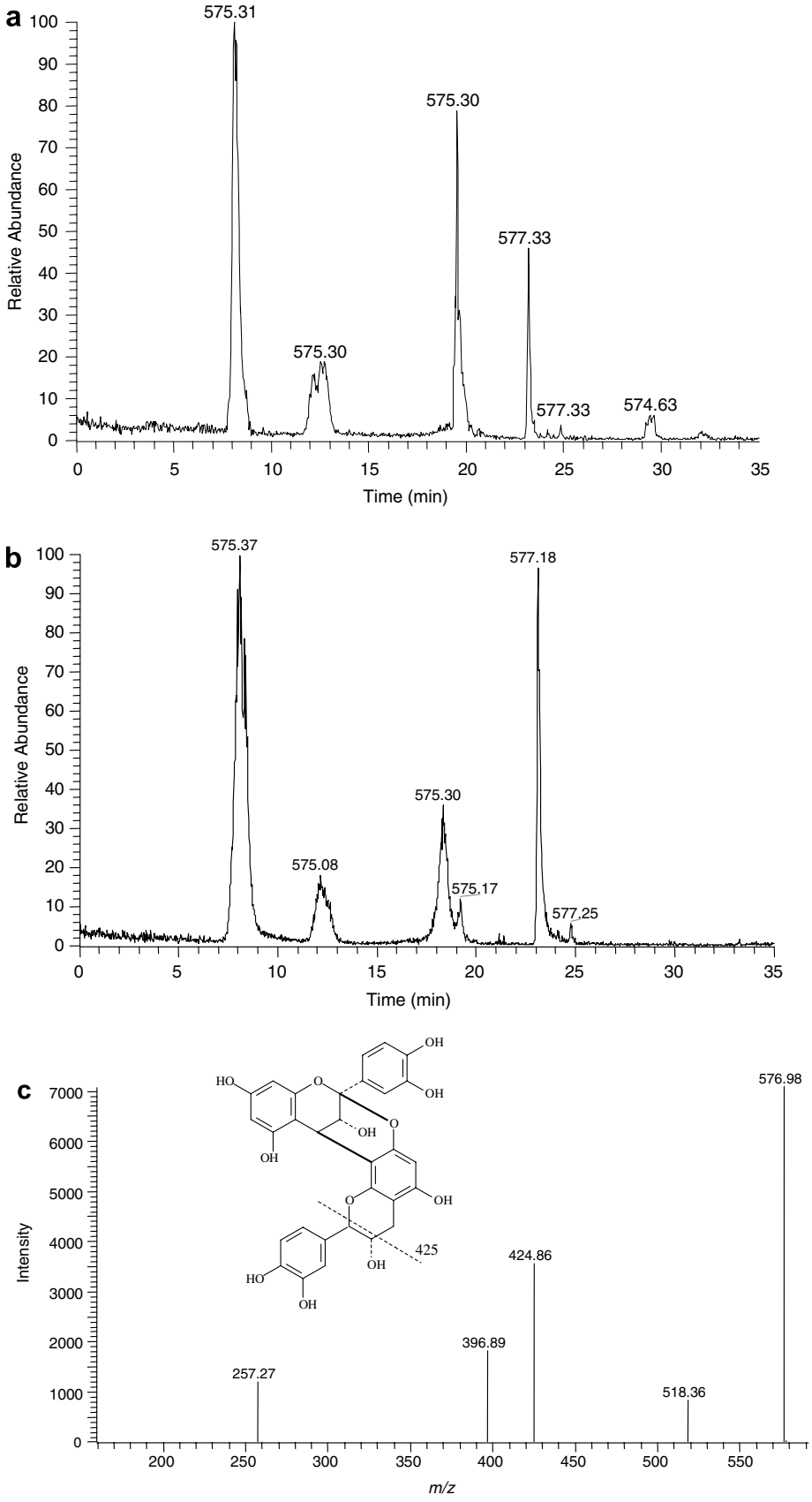

Figure 3. Mass chromatogram of the reaction products of procyanidin B-2 with (a) laccase; (b) DPPH radical; (c) mass spectrum of procyanidin A-2 and (d) UV–visible absorption spectrum of PA-2.

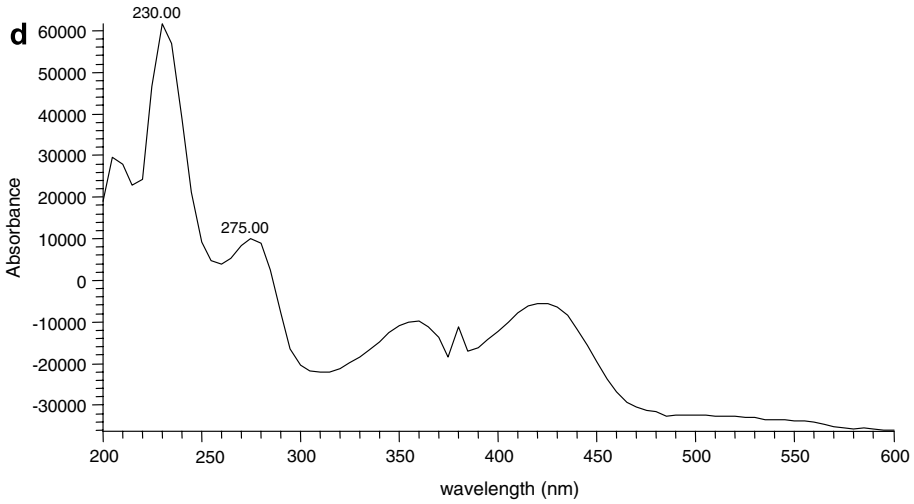

Figure 3 (*continued*)

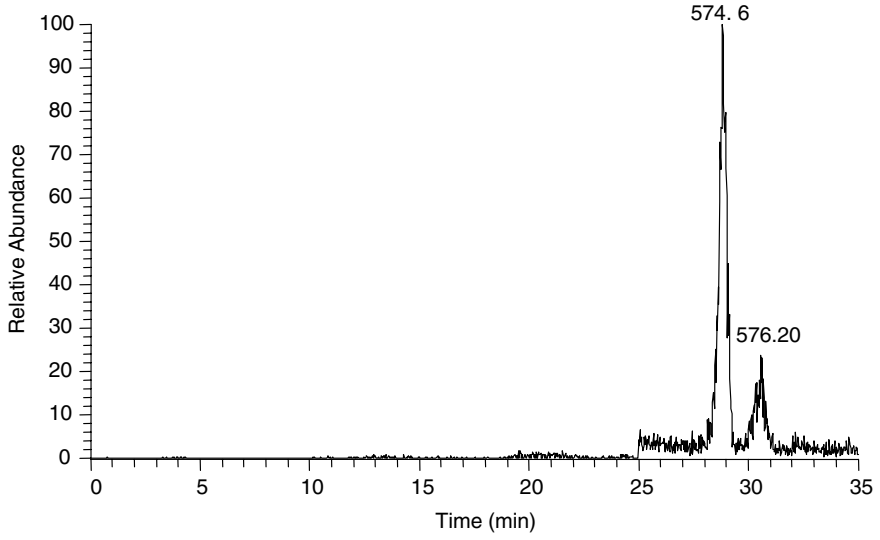

Figure 4. Mass chromatogram of the reaction products of procyanidin B-2 with laccase at pH 7. The reaction mixture contained 10 μL laccase, 200 μM PB-2 and 125 mM phosphate buffer, pH 7.

The observed laccase-catalysed transformation of PB-2 to A-2 is an oxidative intramolecular reaction, which might involve the same mechanism proposed for the DPPH radical mediated conversion.[9] Kondo et al.[9] noted that the C–H bond dissociation enthalpy at C-2 of the upper flavan-3-ol unit was low enough (68.7 Kcal/mol) to be abstracted by radicals. Presumably, laccase first oxidises a phenolic group and then abstracts an electron from C-2, followed by a deprotonation to form an intermediate quinone methide as was suggested for the DPPH radical reaction.[9] Nucleophilic attack of the hydroxyl group at C-7 of the lower flavan-3-ol unit at C-2 of the upper unit would lead to the formation of PA-2, whereas if the electron shift originated from the oxygen at position one of the upper flavan-3-ol unit, then anthocyanidin-type products would be formed. Probably, at pH 7 nucleophilic attack of the hydroxyl group at C-7 prevails, displacing the reaction towards the formation of PA-2, which is then oxidised by laccase to the product with the m/z value of 574.6.

In conclusion, our results suggest that in plants, the transformation of procyanidin type-B to type-A might involve an enzyme catalysed oxidation reaction rather than a radical driven process as was previously suggested by Kondo et al.[9] Polyphenol oxidases such as laccase could be responsible in vivo for this reaction. The presence of laccase in higher plants has been well documented[16] though its physiological role remains yet to be defined.

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References and notes

1. Xie, D-Y.; Dixon, R. A. *Phytochemistry* **2005**, *66*, 2127–2144.
2. Sang, S.; Tian, S. T.; Wang, H.; Stark, R. E.; Rosen, R. T. *Bioorg. Med. Chem.* **2003**, *11*, 3371–3378.
3. Scalbert, A. *Phytochemistry* **1991**, *30*, 3875–3883.
4. Katiyar, S. K.; Mukhtar, H. J. *Cell. Biochem.* **1997**, *27*, 59–67.
5. Sano, M.; Suzuki, M.; Miyase, T.; Yoshino, K.; Maeda-Yamamoto, M. *J. Agric. Food Chem.* **1999**, *47*.
6. Takahashi, T.; Yokoo, Y.; Inoue, T.; Ishii, A. *Food Chem. Toxicol.* **1999**, *37*, 545–552.
7. Foo, L. Y.; Lu, Y.; Howell, A. B.; Vorsa, N. *Phytochemistry* **2000**, *54*, 173–181.
8. Satoshi, M.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.* **1987**, *35*, 4717–4729.
9. Kondo, K.; Kurihara, M.; Fukuhara, K.; Tanaka, T.; Suzuki, T.; Miyata, N. *Tetrahedron Lett.* **2000**, *41*, 485–488.
10. Tanaka, T.; Kondou, K.; Kouno, I. *Phytochemistry* **2000**, *53*, 311–316.
11. Osman, A. M.; Wong, K. K. Y.; Fernyhough, A. *Enzyme Microb. Technol.* **2006**, Doi: 10.1016/j.enzmictec. **2006**. 09.018.
12. Weings, K.; Huthwelker, D. *Liebigs Ann. Chem.* **1970**, *731*, 161–170.
13. Guyot, S.; Vercauteren, J.; Cheynier, V. *Phytochemistry* **1996**, *42*, 1279–1288.
14. Karchesy, J. J.; Hemingway, R. W.; Foo, Y. L.; Barofsky, E.; Barofsky, D. F. *Anal. Chem.* **1986**, *58*, 2567–2568.
15. Wellmann, F.; Griesser, M.; Schwab, W.; Martens, S.; Eisenreich, W.; Matern, U.; Lukačin, R. *FEBS Lett.* **2006**, *580*, 1642–1648.
16. Mayer, A. M.; Staples, R. C. *Phytochemistry* **2002**, *60*, 551–565.