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Structural analysis of A-type or B-type highly polymeric proanthocyanidins by thiolytic degradation and the implication in their inhibitory effects on pancreatic lipase

Hideto Kimura [a,*], Satoshi Ogawa [a], Takashi Akihiro [b], Kazushige Yokota [c,d,*]

[a] The Department of Research and Development, Kotobuki Seika Co., Ltd, 2028 Hatagasaki, Yonago, Tottori 683-0845, Japan
[b] Department of Biological Science, Faculty of Life and Environmental Science, Shimane University, 1060 Nishikawatsu-cho, Matsue, Shimane 690-8504, Japan
[c] Department of Life Science and Biotechnology, Faculty of Life and Environmental Science, Shimane University, 1060 Nishikawatsu-cho, Matsue, Shimane 690-8504, Japan
[d] The United Graduate School of Agricultural Sciences, Tottori University, 4-101 Koyama-Minami, Tottori-shi, Tottori 680-8553, Japan

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ABSTRACT

Compared with oligomeric proanthocyanidins, highly polymeric proanthocyanidins have more difficulty to analyze the molecular sizes and the mode of interflavan linkages of flavan-3-ol units through doubly linked A-type bonds and single B-type bonds. Recently, we have shown that seed shells of the Japanese horse chestnut contain highly polymeric proanthocyanidins as dominant polyphenolics that can be separated into two fractions according to the difference in the molecular sizes. Here, we tried to perform the structural characterization of them in terms of the molecular sizes and the proportions of A-type linkages relative to B-type linkages. The results were compared with those of the corresponding preparations with variations in the sizes from fruits of blueberry and cranberry. Gel permeation chromatography revealed that the molecular sizes of them were higher in the order of blueberry > cranberry > seed shells of the Japanese horse chestnut when they are compared between the respective fractions. For the analysis of terminal and extension units of those proanthocyanidins, the isolated fractions were subjected to the thiolytic cleavage of the B-type linkages using 1-dodecanethiol, and the resulting degradation products were identified by ultra-performance liquid chromatography electrospray-ionization mass spectrometry (UPLC-ESI/MS). These analyses provided fast and good resolution of the degradation products and revealed higher proportions of A-type linkages compared with B-type linkages in the both isolated fractions in the order of the seed shells > cranberry > blueberry. Moreover, the isolated fractions with higher molecular sizes and those more abundant in the proportions of A-type linkages were found to be more effective in the inhibition of pancreatic lipase activity. The results suggest that higher molecular sizes and more abundance of A-type bonds in polymeric proanthocyanidins are promising key factors for the attenuation of lipid digestion as dietary supplements.

1. Introduction

Proanthocyanidins, generally known as subclasses of polyphenolic polymers, are found in a wide variety of cereals, fruits, vegetables, and other plant sources [1]. These compounds have a series of heteropolyflavan-3-ols, (+)-catechin/(−)-epicatechin units, which are linked through a single B-type linkage of C4 → C6 or C4 → C8, and a doubly linked A-type linkage including a C4 → C8 bond and an additional ether bond between O7 → C2 [1]. Generally, the polymeric proanthocyanidins can be classified as B-type proanthocyanidins linked mainly with B-type linkages and A-type proanthocyanidins having more abundant A-type bonds as well as B-type linkages. Recently, proanthocyanidins have been reported to exhibit various biological activities, such as antioxidant [1–3], anti-cancer [1,4], antimicrobial [5], and anti-allergy [6] activities as well as the inhibitory effects on digestive enzymes of carbohydrates and lipids [7–9]. The combined results raised the potential usefulness of proanthocyanidins as sources of dietary supplements for developing novel nutraceuticals.

Recently, our laboratory has reported the high levels of highly polymeric A-type proanthocyanidins in the seed shells of the Japanese horse chestnut (*Aesculus turbinata* BLUME) [10] (Fig. 1). These highly polymeric proanthocyanidins have been shown to possess healthy biological effects including antioxidant [10], antidiabetic [11,12], and anti-obesity effects [12,13].

Abbreviations: UPLC, ultra-performance liquid chromatography; ESI/MS, electrospray-ionization mass spectrometry; DP, degree of polymerization; HPLC, high-performance liquid chromatography; Mp, peak-top molecular weight.

* Corresponding authors. Tel.: +81 859 22 7459; fax: +81 859 22 7886.
E-mail address: h-kimura@chukai.ne.jp (H. Kimura).

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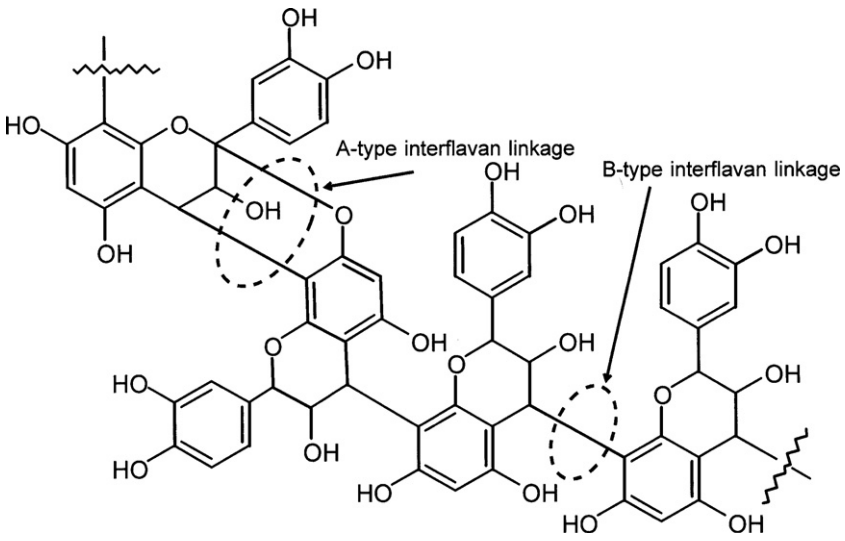

Fig. 1. Partial structural formulas of polymeric proanthocyanidins having a series of heteropolyflavan-3-ols, (+)-catechin/(−)-epicatechins, with doubly linked A-type interflavan linkages and single B-type bonds.

Nevertheless, the structural information on A-type proanthocyanidins exerting on their effects is still limited because these compounds are highly polymerized forms. Alternatively, A-type proanthocyanidins are found at high levels in common foods such as cranberry [4,14], litchi [15], and peanut [16]. As a characteristic feature of A-type cranberry pronanthocyanidins, these compounds have been described to exhibit bacterial anti-adhesion activity [14] and anti-cancer effect [4]. The findings implicate the importance of the doubly linked A-type interflavan linkage in conferring those unique biological activities [1,4,14]. Therefore, it is important to analyze the proportions of the A-type linkage in various types of polymerized proanthocyanidins and the binding mode of how those flavan-3-ol units are linked through the A-type and B-type interflavan bonds using different food sources. Furthermore, the degree of polymerization (DP) is another critical factor influencing the biological activities of proanthocyanidins. For example, these related polyphenolic compounds with higher DP have been shown to have more potent effects on antioxidative activity [17], inhibition of digestive lipase [9], and lipolysis in cultured 3T3-L1 cells [18].

Earlier, apple oligomeric procyanidins, one class of proanthocyanidins, with DP of less than 10 have been studied by combined instrumental analyses by normal-phase chromatography, high-performance liquid chromatography (HPLC)-ESI/MS, matrix-assisted laser-desorption ionization time-of-flight mass spectrometry [19]. On the other hand, higher molecular-weight proanthocyanidins with DP of higher than 10 have been difficult to separate effectively by conventional HPLC methods, so that efficient analytical procedures for highly polymerized compounds as intact forms have not been established until now [20–22].

In this study, we attempted to fractionate highly polymeric proanthocyanidins from the seed shells of the Japanese horse chestnut and cranberry fruit as rich sources of A-type proanthocyanidins, and those from blueberry fruit as a typical source of B-type proanthocyanidins. The isolated fractions were subjected to the structural characterization including the molecular sizes as estimated by gel permeation chromatography and the proportions of terminal and extension units with A-type linkages as determined by UPLC-ESI/MS after the thiolytic reaction using 1-dodecanethiol, which can only cleave the single B-type bond. Moreover, we assessed the effects of the isolated fractions from different sources on pancreatic lipase in vitro. Based on the comparisons between the results of separate preparations, we discuss the relationship between the structural features and lipase-inhibitory activity of highly polymeric proanthocyanidins with the variation in the molecular sizes and the proportion of the A-type linkage compared with the B-type linkage.

2. Experimental

2.1. Materials

Frozen fruits of blueberry (*Vaccinium angustifolium* Aiton) and cranberry (*Vaccinium macrocarpon* Aiton) were purchased from Shoei Food Industry (Tokyo, Japan). Seeds of the Japanese horse chestnut (*Aesculus turbinata* BLUME) were collected from the forest of northern Hyogo Prefecture in Japan and identified as described earlier [10–13,23]. Diaion HP-20 and Chromatorex ODS 1024T for column chromatography were obtained from Nippon Rensui (Tokyo, Japan) and Fuji Silysia (Kasugai, Japan), respectively. Folin-Ciocalteu reagent, procyanidin A2, and procyanidin B2, porcine pancreatic lipase (Type II), and 4-methylumbelliferyl oleate were supplied by Sigma (St. Louis, MO, USA). Sephadex LH-20 for column chromatography was purchased from GE Healthcare (Buckinghamshire, UK). The LUNA C18 (2) column for reverse-phase HPLC was the product of Phenomenex (Torrance, CA, USA). The Cosmosil 2.5C18-MS-II for UPLC was purchased from Nacalai Tesque (Kyoto, Japan). The TSK-gel Super AW 3000 column for gel permeation HPLC and standard polystyrene F4 were provided by Tosoh (Tokyo, Japan). The Shodex standard polystyrene kits, SL-105 and SM-105, for size exclusion chromatography were purchased from Showa Denko (Tokyo, Japan). All other reagents and chemicals including (+)-catechin and (−)-epicatechin were of analytical grade and purchased from Wako (Osaka, Japan), unless otherwise stated.

2.2. Extraction, fractionation, and isolation of highly polymeric proanthocyanidins

Extraction, fractionation, and isolation of highly polymeric proanthocyanidins were carried out essentially according to our previous procedures [10,12,23]. In brief, seed shells of the Japanese horse chestnut (100 g fresh weight) were ground well with a mill into powder and immersed in 300 ml of 70% acetone at room temperature for 3 days. The resulting extracts were evaporated to dryness by a rotary evaporator. After the reconstitution of the mixtures in distilled water, the debris was removed by the fil-