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Interactions of starch with a cyanidin–catechin pigment (vignacyanidin) isolated from *Vigna angularis* bean

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ABSTRACT

A cyanidin–catechin pigment isolated from adzuki bean (vignacyanidin) interacted with starch. The pigment had absorption maxima at 530 and 540 nm at pH 2.0 and 6.8, respectively, and starch (10 and 100 mg ml^{-1}) increased the absorbance, shifting the absorption maxima to longer wavelengths. Nitrite oxidised vignacyanidin at pH 2.0, and the oxidation resulted in the production of nitric oxide (NO). Rates of the oxidation and the NO production were enhanced by starch. Vignacyanidin inhibited α-amylase-catalysed digestion of starch at pH 6.8, and amylose digestion was more effectively inhibited than amylopectin digestion. The above results suggest (i) that binding of the pigment to starch increased the accessibility of nitrous acid to the pigment, and (ii) that the binding reduced the digestibility of starch by α-amylase. Possible functions of the pigment in the stomach and the intestine are postulated, taking the above results into account.

1. Introduction

The seed coat of adzuki bean, *Vigna angularis* (Willd.) Ohwi et Ohashi, contains purplish-red vignacyanidin (Fig. 1) and its isomer that have hydrophobic properties (Takahama, Yamauchi, & Hirota, 2013). The pigments may contribute to the purplish-red or dark-purple colour of Japanese and Chinese sweets prepared using the paste of adzuki bean. When adzuki bean is cooked together with glutinous rice, the rice is made purplish red. The colour of these foods is supposed to due to the binding of the above pigments to starch. Hydrophobic properties of vignacyanidin and its isomer may allow these pigments to bind to starch. This idea is deduced from the reports that components with hydrophobic properties, such as lipids, bile salts, and flavonoids, can bind to starch (Crowe, Seligman, & Copeland, 2000; Holm et al., 2006; Kwasniewska-Karolak, Nebesny, & Rosicka-Kaczmarek, 2008; Takahama & Hirota, 2010, 2011, 2013). Adzuki bean contains both amylose and amylopectin (Durov, 2003; Tang, Watanabe, & Mitsunaga, 2002), and approximately 99% of the starch of glutinous rice is amylopectin (Osawa & Inoue, 2007). These data suggest that vignacyanidin and its isomer can bind to amylopectin, as well as amylose.

When foods prepared using adzuki bean are ingested, free forms of adzuki pigments and adzuki pigments bound to starch will be mixed with saliva in the oral cavity, and then swallowed into the stomach. Because saliva contains nitrite (Doel, Benjamin, Hector, Rogers, & Allaer, 2005) and salivary nitrite is transformed to nitrous acid (pK_a = 3.3) in the stomach, reduction of nitrous acid by adzuki pigments, to produce nitric oxide (NO), is possible in the stomach. It has been reported that nitrous acid can oxidise ascorbic acid (Iijima, Fyfe, & McColl, 2003; Licht, Tannenbaum, & Deen, 1988) and some phenolic compounds (Peri et al., 2005; Pietraforte et al., 2006; Takahama, Oniki, & Hirota, 2002; Takahama, Yamamoto, Hirota, & Oniki, 2003) in the stomach to produce NO.

This paper deals with the interactions of vignacyanidin with starch. In addition, this paper deals with the starch-dependent enhancement of oxidation of vignacyanidin by nitrous acid and the inhibition of α-amylase-catalysed digestion of starch by vignacyanidin. Taking the results of the above studies into account, (i) manner of interaction between starch and vignacyanidin, (ii) ability of vignacyanidin to produce NO in the stomach, and (iii) usefulness of vignacyanidin on the prevention of starch digestion in the intestine are now considered.

2. Materials and methods

2.1. Reagents and plant materials

Vignacyanidin was isolated from adzuki bean by acidic methanol extraction, ethyl acetate extraction, and preparative reversed phase HPLC, as reported (Takahama et al., 2013). Soluble starch and pancreatin, from hog pancreas, were obtained from Wako Pure

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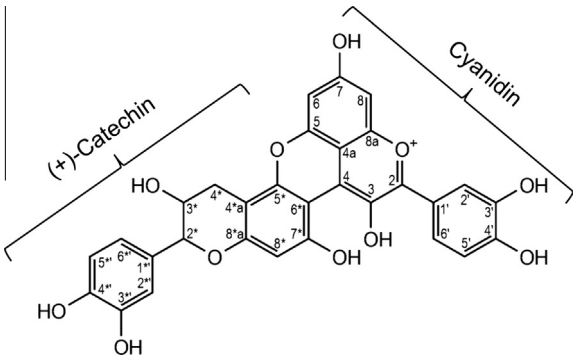

Fig. 1. Structure of vignacyanidin.

Chemical Industries (Osaka, Japan). Amylose (molecular weight, about 2800) and amylopectin were from Tokyo Kasei Kogyo (Tokyo, Japan), and a NO-generating reagent, 1-hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl-1-triazene (NOC 7) (purity >90%), was from Dojindo (Kumamoto, Japan).

2.2. Preparation of solutions

Isolated vignacyanidin was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM. Potassium iodide (1.5 g) was dissolved in 12.5 ml of water. Iodine (0.635 g) was added to the solution, and the volume of the mixture of potassium iodide and iodine was brought to 50 ml by adding water. The solution (equivalent to 100 mM iodine) was kept in the dark, to use as iodine solution. Pancreatin (3.3 mg ml^{-1}) was dissolved in water. Soluble starch (10 and 100 mg ml^{-1}), amylose (10 mg ml^{-1}), and amylopectin (10 mg ml^{-1}) were prepared in 50 mM KCl–HCl (pH 2.0) and 20 mM sodium phosphate (pH 6.8). The suspensions were gelatinised in gently boiling water. After cooling, starch suspensions were used for experiments.

2.3. Nitrite-induced oxidation of vignacyanidin

Nitrite-induced oxidation of vignacyanidin was studied using a spectrophotometer (UV-2450, Shimadzu) equipped with an integrating sphere (ISR-240A, Shimadzu; Kyoto, Japan). The light path of the measuring beam was 4 mm. The reaction mixture (1 ml) contained 20 μM vignacyanidin in 50 mM KCl–HCl (pH 2.0), with and without soluble starch, amylose, or amylopectin. Because the mixtures that contained starch were turbid, the base line was compensated for each measurement. Changes in the absorption spectra, after the addition of 1 mM sodium nitrite, were recorded by repeat scanning.

2.4. Measurement of NO production

Nitrite-induced NO production was studied at 30 °C, using a Clark-type electrode (Rank Brothers, Cambridge, UK) (Takahama & Hirota, 2012). The polarising voltage was −0.7 V. All reactions were run in 2 ml of 50 mM KCl–HCl (pH 2.0), with and without starch. After excluding air from the above reaction mixtures by bubbling argon gas, NO production was recorded by successive addition of 1 mM nitrite and 10, 20, and 40 μM vignacyanidin. NO is produced stoichiometrically in nitrite/ascorbic acid systems as follows (Iijima et al., 2003; Licht et al., 1988):

$$2HNO_2 + \text{ascorbic acid} \rightarrow 2NO + \text{dehydroascorbic acid} + 2H_2O \quad (1)$$

Therefore, the production of NO in nitrite/vignacyanidin systems could be calibrated using a nitrite/ascorbic acid system at pH 2.0. In addition, NOC 7 (0.1 mM) was used to calibrate NO production (Takahama & Hirota, 2012).

2.5. Formation of starch/iodine complexes and starch digestion

Formation of starch/iodine complexes and starch digestion were studied using a 557 spectrophotometer equipped with an end-on type photomultiplier (Hitachi, Tokyo, Japan). The light path of the measuring beam was 4 mm. The mixture (1 ml), to study effects of vignacyanidin (10 μM) on the formation of starch/iodine complexes, contained 0.1 mg of soluble starch in 20 mM sodium phosphate (pH 6.8). An aliquot (0.9 ml) of the mixture was mixed with 0.1 ml of iodine solution, and absorption spectra of starch/iodine complexes were recorded. In addition, effects of 10 μM vignacyanidin on pancreatin-induced digestion of soluble starch were also studied, using the above reaction mixture. The concentration of pancreatin added was 3.3 μg ml^{-1}.

Effects of vignacyanidin concentration on pancreatin-induced digestion of starch were studied as follows: a starch suspension (5 ml), which contained 0.5 mg of soluble starch, amylose, or amylopectin, was prepared using 20 mM sodium phosphate (pH 6.8) that contained 60 mM NaCl. An aliquot (0.9 ml) of the suspension was withdrawn to mix with 0.1 ml of iodine solution, and the absorption spectrum of the mixture was memorised. Four microlitre of pancreatin (3.3 mg ml^{-1}) were added to the remaining starch suspension (4.1 ml), and an aliquot (0.9 ml) of the suspension was withdrawn after incubation for defined periods at room temperature (about 25 °C) to mix with 0.1 ml of iodine solution. Degree of starch digestion was estimated from the difference spectra, after and before incubation with pancreatin. To study the effects of vignacyanidin on starch digestion, the pigment was added to the above starch suspension that contained 0.1 mg ml^{-1} of soluble starch, amylose, or amylopectin suspensions. Pancreatin-induced starch digestion was terminated by iodine solution (Takahama & Hirota, 2010, 2011).

2.6. Statistics

When required, data were shown as means ± SDs and were evaluated statistically using the Student's *t*-test. A *P*-value < 0.05 was regarded as statistically significant. When statistical analysis was not required, typical data or averages of two experiments were shown.

3. Results and discussion

3.1. Effects of starch on absorption spectra of vignacyanidin

Fig. 2 shows effects of soluble starch on ultraviolet–visible (UV–vis) absorption spectra of vignacyanidin. Starch increased absorbance of vignacyanidin around 415 and 530 nm and the absorption maximum at 530 nm shifted to longer wavelength with the increase in starch concentration at pH 2.0. At pH 6.8, starch increased the absorbance of vignacyanidin, shifting the absorption peaks at 419 and 540 nm to longer wavelengths, although the degree of the increase at 540 nm was smaller at pH 6.8 than at pH 2.0. If vignacyanidin molecules associated with each other in aqueous solutions because of its hydrophobic property, the absorbance increase by starch could be explained by its binding to starch to decrease the concentration of associated viganacyanidin. Starch has hydrophobic properties (Crowe et al., 2000; Holm et al., 2006; Kwasniewska-Karolak et al., 2008), therefore, vignacyanidin may be able to interact with starch hydrophobically. Starch-induced