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Stabilization of natural anthocyanin by intercalation into montmorillonite

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ABSTRACT

Because of its non-toxicity, naturally occurring anthocyanin has a potential for the utilization as a harmless coloring material. Its color instability under alkaline condition or light irradiation limits its practical use. Anthocyanin can be stabilized by the intercalation into montmorillonite. The anthocyanin–montmorillonite maintains the original color at basic condition of even pH 11. The intercalated anthocyanin also exhibits enhanced stability against visible light irradiation compared with the anthocyanin simply adsorbed on the external surface of the mineral particles. The stability enhancement is attributed to the electrostatic host–guest interaction and to steric protection from the attack of atmospheric oxygen. The intercalated complex can be a candidate of an environmentally friendly coloring material.

1. Introduction

Anthocyanins are found in many flower petals and fruits (Goto and Kondo, 1991). Their non-toxicity is suitable for the utilization of food dyeing. However, their color durability is not satisfactory for practical use. They are easily discolored on exposure to light (Bakowska et al., 2003; Torskangerpoll and Andersen, 2004) or slightly basic conditions (Brouillard and Delaporte, 1977; Brouillard and Dubois, 1977). As shown in Scheme 1, flavylium form **1** of the anthocyanin is converted to quinonoidal base **2** in mild alkaline conditions, and finally to non-colored chalcone **4** via carbinol pseudobase form **3**. All these conversions are accompanied by a marked color change. The problem about the stability is very serious and prevents the wide use of the anthocyanin as a colorant other than for food dyeing.

On the other hand, incorporation of organic dyes into inorganic host materials is widely investigated in recent days (Gomez-Romero and Sanchez, 2005; Takagi et al., 2006a,b), and there have been many reports concerning the enhancement of the stability of organic dyes by complexation with inorganic host materials such as clays (Bujdák et al., 2002; Endo et al., 1989; Saito et al., 2005; Shichi and Takagi, 2000) or zeolites (Calzaferri, 1998; Hölderich et al., 2000; Hoppe et al., 1994; Ramamurthy, 2000; Telbiz et al., 2001). We have reported that a synthetic cationic flavylium dye can be stabilized by intercalation into montmorillonite K10 (Kohno et al., 2007) or incorporation into the pores of zeolites (Kohno et al., 2008). The flavylium dye has the same core structure as anthocyanins (2-phenylbenzopyrylium), and is considered as a model compound of anthocyanins (Iacobucci and Sweeny, 1983; Ito et al., 2002; Jurd, 1969; Mazza and Brouillard, 1987).

In this study, we report the stabilization of a naturally occurring anthocyanin by intercalation into montmorillonite. The anthocyanin assumes the cationic flavylium form under an acidic condition, and is expected to be intercalated by the ion exchange reaction. Therefore, the complexation of the anthocyanin with clay minerals may be a key technology for the development of an environmentally friendly coloring material.

Lima et al. (2007) have reported the adsorption of anthocyanin on several inorganic hosts including clay minerals. In their study, montmorillonite has been confirmed to adsorb anthocyanin, but the effect of the stabilization by adsorption has not been investigated in detail, and the adsorption condition has not been fully optimized for intercalation. In the present study, we try to confirm the enhancement of the stability of the anthocyanin by intercalation of the anthocyanin. As described above, we have already found that the model compound, flavylium dye, is stabilized by the adsorption between the layers of montmorillonite (Kohno et al., 2007). We show in this study that intercalated anthocyanin was sufficiently stabilized against an alkaline environment or visible light irradiation.

2. Experimental

2.1. Materials

We used montmorillonite, a synthetic non-swelling mica and silica. Kunipia F montmorillonite (designated as KF) was supplied from

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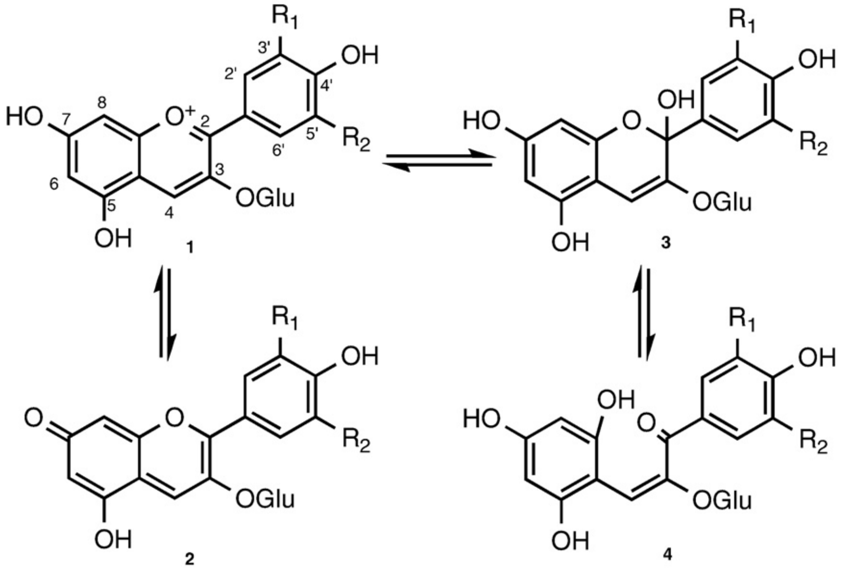

Scheme 1. Several forms of anthocyanin. **1**: flavylium, **2**: quinonoidal base, **3**: pseudobase, **4**: chalcone.

the Clay Science Society of Japan (JCSS-3101) and was used as received. The synthetic non-swelling mica (designated as NSM) was supplied from Wako Chemical Co. and used as received. Silica (designated as SIO) was supplied from Catalysis Society of Japan (JRC-SIO-9A).

KF is a purified natural montmorillonite. The cation exchange capacity of KF was 1.15 meq/g. NSM does not show cation exchange because the K^+ ions are strongly bound in the interlayer space.

As an anthocyanin dye, we used commercially available powdered Grape Color BC-120 supplied from Kiriya Chemical Co., extracted from grape skins. The anthocyanin BC-120 was purified as described in the literature (Baublis et al., 1994) using an adsorption column XAD-7. The dye was dissolved in a 5% aqueous solution of formic acid, and passed through the XAD-7 column for three times. The eluate was concentrated, and mixed with an excess amount of diethyl ether to precipitate the purified dye. The precipitate was air-dried at room temperature. Thus purified anthocyanin was denoted as AN. AN was analyzed by HPLC using Mightysil as packing material, with an aqueous solution of acetic acid and acetonitrile as eluent. The purity and the composition of AN was determined by UV and ESI-MS (Table 1). AN was found to be a mixture of five anthocyanins, and the main component was malvidin-3-glucoside. The composition was in agreement with other studies concerning the analysis of the anthocyanins extracted from grape skins (Baublis et al., 1994; Kammerer et al., 2005; Vivar-Quintana et al., 2002).

2.2. Adsorption of AN

A given amount of AN was dissolved in 2 ml of methanol, and 100 ml of the 5% aqueous formic acid solution was added. The pH of the solution was 2.5. Anthocyanins are reported to be stable for about 15 days at pH 1 to 3 (Cabrita et al., 2000). The solution was mixed with 1 g of KF and kept in the dark. After 24 h, the resulting sediment was separated from the solution by filtration, dried at 353 K, and powdered (sample AN/KF). The same procedure was used for AN and NSM (AN/NSM). Unless otherwise noted, the amount of AN in AN/KF and AN/NSM was 10 mg per 1 g of the host materials.

AN was not adsorbed on SIO by simply mixing the AN solution with SIO. Therefore, AN was loaded on the surface of SIO by evaporating the solvent of the mixture of the AN solution and SIO. AN powder of 30 mg was mixed with 1 g of SIO in a mortar, and then 1 ml of the 5% aqueous formic acid solution was dropped to the mixture. To evaporate the solvent, the paste was kept in the dark at room temperature in the air for 24 h. After drying, the solid was powdered (AN/SIO).

2.3. Measurements

XRD patterns were measured with the Rigaku MiniFlex X-ray diffractometer (CuKα, step angle and scan speed 0.05° and 2°/min, 15 mA, 30 kV).

UV–Vis spectra were collected with the JASCO V-550 spectrophotometer. To measure diffuse reflectance spectra, the ISV-469 integrating sphere was equipped to the spectrometer.

To investigate the durability of the sample against alkaline environment, the pH of the AN solution and the AN/KF dispersion was adjusted to 11 by adding a small amount of 1 M aqueous NaOH. Immediately after the pH was raised, the spectral changes of both samples were recorded.

To investigate the light endurance of AN/KF, AN/NSM and AN/SIO, visible light was irradiated to the samples under ambient temperature and pressure. The color fading was measured by the decrease of the absorption in the diffuse reflectance spectra. A 100 W halogen lamp (Schott Megalight 100) equipped with a 30 cm length flexible light guide was used as a visible light source. The powder samples were loaded in a plastic cell covered with a thin glass, and placed at the position of 2 cm from the aperture of the light guide. In this condition, the increase in the temperature of the sample under irradiation was only within 7 K, so that the influence of the temperature on the stability could be neglected. When the sample was irradiated under N_2 atmosphere, the sample cell was packed in a gas barrier film filled with nitrogen.

Table 1
Components of AN and their ingredients

Compound name	Substituents[a]		Ingredient (%)[b]
	R_1	R_2	
Malvidin-3-glucoside	OMe	OMe	49
Peonidin-3-glucoside	OMe	H	20
Petunidin-3-glucoside	OMe	OH	15
Delphinidin-3-glucoside	OH	OH	13
Cyanidin-3-glucoside	OH	H	3

[a] For positions of R_1 and R_2, see Scheme 1.
[b] Total amount of anthocyanin in AN was estimated to be 14%.