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Enhanced stability of natural anthocyanin incorporated in Fe-containing mesoporous silica

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ABSTRACT

Anthocyanin is known as a safe coloring material although its poor stability often limits more extended use. In this study, we tried the enhancement of the photostability of the anthocyanin by the incorporation into the pore of HMS type mesoporous silica containing various metal ions. The anthocyanin was adsorbed on the HMS by pouring a highly concentrated anthocyanin solution to the dried HMS, urging the incorporation of the dye into the mesopore by a capillary condensation. Improved stability of the anthocyanin against visible irradiation was achieved by the adsorption onto the HMS containing Fe^{3+}, although other metal ions in the HMS showed less effect on the stabilization. From the UV–vis spectroscopy, the Fe^{3+} species effective for the stabilization of the anthocyanin was found to be the tetrahedral one highly dispersed in the silica framework. The decrease in the absorptivity of the anthocyanin on the Fe-HMS compared to other metal-containing HMS suggested that the Fe-HMS promoted the aggregate formation of the anthocyanin molecules. The highly dispersed Fe^{3+} species had an ability to gather the anthocyanin molecule close to itself, resulting in tight incorporation of the dye inside the mesopore. The incorporation and aggregate formation of the anthocyanin was expected to contribute to the improvement of the stability against irradiation.

1. Introduction

From the viewpoint of safety, natural dyes are generally superior to the synthetic ones. Therefore, they are suitable for the use as a colorant of foods and cosmetics, where direct contact with human body cannot be avoided [1,2]. In addition, the naturally occurring dyes are better than the synthetics in regard to the consumer perception because they are not artificial materials. Among the natural dyes, anthocyanin is one of the most common dyes and generally found in the body of many plants [3]. The anthocyanin, having the structure with the flavylium ring [4], is thought to be definitely nontoxic compound since it has been used as a colorant of foods for a long time [5]. In fact, the anthocyanin is one of the most used natural food additives in the present days, and the importance is growing further. In addition, anthocyanin is known to show bioactivities such as anticancer and antioxidant properties, mainly due to its quenching effect of reactive oxygen species [6].

However, the natural dyes are commonly poor in the stability [7]. Especially, the anthocyanin easily loses its color under neutral to slightly basic conditions or under irradiation [8]. The instability hinders more extensive use of the natural dyes [9].

Incorporation of organic molecules into inorganic host materials is known as a promising method to improve the stability of the organic molecules [10]. For instance, inclusion of dyes into the clay interlayers [11–13], zeolites [14,15], and mesoporous silicates [16–18] have been reported to enhance the stability of the guest materials. The ancient Maya Blue is also a good example of the composite materials, where the guest indigo dye is greatly stabilized by the incorporation into the channel of the palygorskite clay [19–23]. In addition, the inorganic host has also been used as a vessel for the photochemical conversion system between the chalcone and the flavylium dye [24,25], which is a model compound of the natural anthocyanin with the same chromophore.

We have investigated the stability enhancement of various natural dyes by making composites with nontoxic inorganic host materials. In a previous report, we showed that the anthocyanin was successfully intercalated and stabilized in the interlayer space of the cation-exchangeable clay (montmorillonite) since the

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anthocyanin was a cationic dye [26]. The synthetic flavylium dye was also stabilized by the incorporation into the pore of the mesoporous silicates containing metal ions such as Al^{3+} [27,28]. However, the natural anthocyanin itself was not significantly adsorbed onto the HMS type of the mesoporous silica without metal addition, and only a limited enhancement of the stability was observed by the incorporation of the natural anthocyanin into the Al-containing HMS [4].

In this study, we report the methods and conditions to enhance the stability of the natural anthocyanin by the incorporation into the pore of the mesoporous silica. The HMS type of the mesoporous silica is used as a host material, because the preparation is relatively easy under mild conditions [29]. The effect of the incorporation on the stability of the anthocyanin is investigated in detail using the HMS containing several metal ions. The stabilization effect is evaluated from the light fastness of the dye under visible light irradiation.

2. Experimental

2.1. Materials

Anthocyanin was purchased from Kanto Chemical as "Grape Skin Color". The as-received anthocyanin dye was purified by a similar method described earlier [26,30] using an adsorption column XAD-7. Briefly, the dye was dissolved in a 5% aqueous solution of formic acid and passed through the column. The eluate was concentrated and mixed with an excess amount of diisopropyl ether to precipitate the purified dye. The precipitate was air-dried at room temperature. Thus purified anthocyanin was denoted as AN.

HMS was prepared based on the literature [31]. Dodecylamine weighed 0.91 g (Wako Chem.) was dissolved in 5 cm^3 of ethanol, mixed with 45 cm^3 of water, and heated up to 333 K. Tetraethoxysilane (Wako Chem., denoted as TEOS) weighed 4.6 g was dropped to the solution, and the mixture was kept at 333 K for 22 h. The sample was filtered, washed with water, dried in an oven and calcined at 903 K for 6 h under dry air stream. The molar composition of the source materials was 1.0 dodecylamine/4.5 TEOS/511 water/17 ethanol.

To prepare the Al- and B-containing HMS, aluminum isopropoxide and boric acid, (Wako Chem.) were used as the source of Al^{3+} and B^{3+}, respectively. The nitrate salts of Ni^{2+} ($Ni(NO_3)_2{\cdot}6H_2O$, Kishida Chem.), Mg^{2+} ($Mg(NO_3)_2{\cdot}6H_2O$, Wako Chem.), Ga^{3+} ($Ga(NO_3)_3{\cdot}nH_2O$, Wako Chem.) and Fe^{3+} ($Fe(NO_3)_3{\cdot}9H_2O$, Wako Chem.) were used as the sources of each metal-containing HMS. As for the Fe-containing HMS, tris(2,4-pentanedionato)iron(III) purchased from Dojindo Chem. (denoted as $Fe(acac)_3$) was used as well as the above-mentioned iron(III) nitrate.

The preparation method of the HMS containing various metal ions is as follows. Unless otherwise noted, the amount of the metal ions was adjusted to 1.0 mol% toward Si in the crude mixture. On using the boric acid or nitrate salts as a metal ion source, given amount of the source compound was dissolved in 5 cm^3 of water, and the solution was added dropwise to the dodecylamine solution with TEOS by turns. On the other hand, when the aluminum isopropoxide or $Fe(acac)_3$ was used as a metal source, it was dissolved in 3 cm^3 of ethanol, mixed with TEOS, and then poured onto the dodecylamine solution. After mixing the source compounds, the preparation procedure was the same as that of pure HMS described above. In the following, the metal-containing HMS is denoted as M-HMS (M = Al, B, Ni, Mg, Ga, Fe). The FeHMS is referred to as Fe(N*x*)HMS or Fe(A*x*)HMS, where N and A denotes the Fe sources (N: $Fe(NO_3)_3$, A: $Fe(acac)_3$), and *x* represents the Fe/Si molar ratio of the starting mixture expressed in percentage.

The HMS and M-HMS powder samples were characterized by XRD and nitrogen adsorption isotherm (see supplementary data). In the XRD pattern, all the samples showed a single reflection peak at around $2\theta = 2°$. The BET specific surface area of the samples was fairly large (681–1075 m^2/g). In addition, the samples exhibited a narrow pore size distribution with the mean pore diameter between 2.8 and 3.2 nm. These results showed that the samples had a mesoporous structure.

The composite materials of each M-HMS and AN were prepared by mixing the AN solution and the M-HMS. The AN weighed 0.6 mg was added to 0.075 cm^3 of methanol, mixed with 0.075 cm^3 of 5% formic acid aqueous solution, and dissolved by ultra-sonication for 20 min. The AN solution looked strongly dark-red because of the high concentration. Meanwhile, the M-HMS powder was dried at 393 K for 24 h in an oven before mixing with the AN. Then, all of the prepared AN solution (0.15 cm^3) was added dropwise to the previously dried M-HMS powder (100 mg) using a micropipette. This preparation method was expected to promote the incorporation of AN into the mesopore by the capillary condensation. The mixture was dried for 2 h at 313 K under reduced pressure. Thus-obtained composite samples were denoted as AN/M-HMS.

2.2. Measurements

Diffuse reflectance UV–vis spectra were collected with JASCO V-550 spectrophotometer with ISV-469 integrating sphere equipped to the spectrometer. Sodium sulfate was used as a reference material. On measuring spectra, the powder samples were loaded in a plastic cell covered with a thin glass.

To investigate the light endurance of each sample, visible light was irradiated to the samples under ambient temperature and pressure. 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 held in the same cell as that used for the UV–vis measurement, and placed at the position of 1.0 cm from the aperture of the light guide. The light intensity at the sample position was adjusted to 210 klux. The degree of the color fading was determined by the decrease in the main absorption peak of the AN on the diffuse reflectance spectra of each composite material.

To investigate the effect of various metal ions on the AN molecule in the solution, an aqueous ethanolic solution (1:1 v/v, 10 cm^3) containing 5.2 mg of AN was mixed with 20 μmol of the nitrate salt of each metal ion. The pH of the solution was adjusted to 1.4 by HCl. The influence of the added metal ions on the AN was evaluated by the change in the transmittance UV–vis spectra collected with Shimadzu UV-1700 spectrophotometer. To determine whether the Fe^{2+} ion was produced in the mixture or not, 1,10-phenanthroline was used as an indicator.

3. Results and discussion

3.1. UV–vis spectra of the composite materials

Fig. 1 illustrates the diffuse reflectance UV–vis spectra of various AN/M-HMS samples. A single peak at around 540 nm was assigned to the AN in the composite sample. The peak was observed at 538 nm in the AN/HMS sample without metal ions. Since the AN solution gave the absorption peak at almost the same wavelength of 536 nm (see first row of Table 1), it can be said that the interaction between the AN and the HMS was weak. Meanwhile, the AN/M-HMS samples showed an absorption peak between 536 and 546 nm. Therefore, the absorption peak did not show any significant shift by the existence as well as the kind of the metal ions included in the HMS. This result does not imply the formation of any complexes between the AN and the metal ions