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Complexation of Some Aromatic Compounds with Cyclic Nigerosyl–(1→6)–Nigerose

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The fluorescence of hydrophylic and hydrophobic aromatic compounds in aqueous solution was investigated in the presence of cyclic nigerosyl–(1→6)–nigerose (CNN). The fluorescence intensity of vanillin was enhanced by the addition of CNN and the intensity increased with increasing the CNN concentration. Benesi–Hildebrand (BH) plot based on the fluorescence data showed the linear relationship between $1/(I–I_0)$ and $1/[CNN]_0$, where I_0 and I are the fluorescence intensities in the absence and presence of CNN, respectively, and $[CNN]_0$ is initial concentration of CNN. The plot suggested that 1:1 vanillin–CNN complex would be formed under the experimental condition. The binding constant of vanillin to CNN was estimated as 7.1×10^1 M^{-1}. Similar results were obtained on the complexations of cinnamaldehyde and eugenol. Both BH plots for these compounds showed the linear relationship, while the plot for raspberry ketone gave two defferent lines. It suggested that 1:1 and 1:2 guest–CNN complexs would be formed by depending on the CNN concentration. For these complexs, the binding constants were estimated 3.8×10^2 and 3.7×10^1 M^{-1}, respectively. In addition, the linear relationship between the binding constant of the hydrophobic compounds and their log P values was observed, and thus, indicated that the hydrophobic interaction would be the predominant factor on the formation of guest–CNN complex.

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides, which composed of six, seven or eight D–glucose units linked by 1, 4–α–glucosidic bond and are referred to respectively as α–, β–, and γ–CD. CDs are chemically and physically stable molecules formed by the enzymatic modification of starch, and the cavity of CD is relatively hydrophobic while the external faces are hydrophilic. Hence, hydrophobic molecules have a high affinity with the CD's cavity in aqueous solution. In food industry, microencapsulation with CDs is widely applied to control flavor release, mask odor and tastes, stabilize color and protect ingredients from oxidation (Singh *et al*., 2002). Recently, the production of a novel cyclic tetrasaccharide, cyclic nigerosyl–(1→6)–nigerose (CNN), by 1, 6–glucosyltransferase and 1,3–α–isomaltosyltransferase from *Bacillus globisporus* was reported by Aga *et al*. (2002 and 2003). CNN has a unique structure consisting of four glucose residues connected by alternate α–(1→3)– and α–(1→6)– linkages. The crystal properties of CNN and the stability of powdered functional oils using CNN were investigated by Oku *et al*. (2007). They reported that ethanol and propanol were complexd with CNN, and vitamin D3, vitamin E, and eicosapentanoic acid were stabilized by powdering with CNN. It was suggested that CNN is a useful material for the microencapsulation (powdering) of food ingredients.

In the present study, the complexation of some aromatic compounds, vanillin, cinnamaldehyde, eugenol, and raspberry ketone, with CNN were investigated to maintain the aroma and/or their functional property during the storage of food products. Vanillin (4–hydroxy–3–methoxybenzaldehyde) is the major constituent of natural vanilla and widely used as a flavoring agent in food industry (Walton *et al*., 2003). Fitzgerald *et al*. (2003) reported the antimicrobial potential of vanillin against the growth of the yeasts associated with food spoilage. Raspberry ketone (4–(4–hydroxyphenyl) butan–2–one), which is a major aromatic compound of red raspberry (*Rubus idaeus*), exerts anti–obese actions and alter the lipid metabolism (Morimoto *et al*., 2005). The complexation profile of the aromatic compounds with CNN was investigated by fluorescent analysis because it is one of the effective methods for the complexation behavior with CD in an aqueous system. Then, the binding constants of the compounds against CNN were estimated from each of the Benesi–Hildebrand (BH) plot based on the fluorescence data. In addtion, we examined the effect of hydrophobic property of the compounds on the complexation with CNN.

MATERIALS AND METHODS

Materials

CNN was supplied by Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). Vanillin, cinnamaldehyde, eugenol were all purchased from Nacalai Tesque Inc. (Kyoto, Japan). Raspberry ketone was purchased from Tokyo Chemical Industry Co., LTD. (Tokyo, Japan). These compounds were used without further purification. All other chemicals used in this study were of analytical reagent grade.

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Complexation method of the aromatic compounds with CNN

Vanillin was dissolved in deionized water at the concentration of 0.4 mM and other aromatic compounds were dissolved in 20% ethanol solution at the same concentration. Each of the solution (2 mL) was added to 2 mL of the CNN solution and then vortex vigorously for 1 min. The resulting solution was subjected to the fluorescent spectral analysis.

Fluorescent spectral analysis

All steady–state fluorescence measurements were carried out with a fluorescence spectrophotometer (Model 5300, Shimadzu Co., Ltd., Kyoto, Japan). Fluorescence spectra of the aromatic compound and/or their CNN complex were measured after the pre–incubation of sample solution at 25 ˚C for 10 min. For all measurements, excitation wavelength was set at 360 nm and fluorescence emission spectra were obtained at 400–450 nm.

RESULTS AND DISCUSSION

Complexation of vanillin

To determine the interaction of CNN to the hydrophilic aromatic compound, the complexation of vanillin was investigated investigated by the fluorescent spectral analysis. Vanillin itself exhibited weak fluorescence emission with maximum wavelength of 410 nm at 360 nm for the excitation. The addition of CNN caused a remarkable increase of vanillin fluorescence. Fig. 1 shows the effect of CNN concentration on the fluorescence intensity of vanillin at 410 nm at 25 ˚C. The CNN concentration was set at 5–80 mM. The intensity was gradually enhanced with increasing CNN concentration. The changes were due to the interaction between CNN and vanillin, implying the formation of CNN–vanillin complexes. As suggested on the complexation with cyclodextrin (Ishiwata and Kamiya, 1997), the increase in intensity is attributed to a decrease in rotational freedom in the medium of the hydrophobic CNN cavity in which the aromatic ring of the compound is adsorbed and shielded from quenching processes of its excited state.

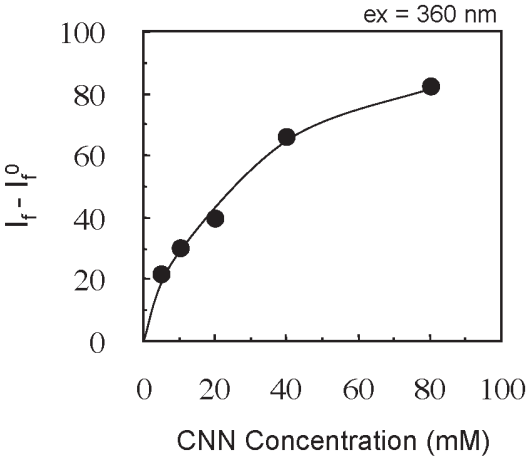

Fig. 1. Effect of CNN concentration on the fluorescence emission intensity of vanillin at 410 nm. Excitation wavelength was set at 360 nm. The final concentration of vanillin was set at 0.2 mM.

The binding constant of vanillin against CNN can be obtained from BH plot, which is double reciprocal type plot, of $1/(I–I_0)$ vs $1/[CNN]_0$, where I_0 and I are the fluorescence intensities in the absence (only vanillin) and presence of CNN (CNN–vanillin complex), respectively, and $[CNN]_0$ is initial concentration of CNN. The binding constant (K) was calculated based on the following BH equation.

$$1/(I–I_0) = 1/(I'–I_0) + 1/K(I'–I_0)[CNN]_0$$

where I' is the limiting intensity of fluorescence. Thus, the K value was obtained from the slope and the intercept of the plot. Fig. 2 shows the BH plots of $1/(I–I_0)$ as a function of $1/[CNN]_0$ based on the fluorescence data of vanillin in the presence of CNN as shown in Fig. 1. The BH plot of vanillin showed the linear relationship between $1/(I–I_0)$ and $1/[CD]_0$, and the relation coefficient was 0.997. It indicated that vanillin and CNN was formed to 1:1 complex under the experimental conditions. Then, the biding constant (K) of vanillin to CNN was determined from the BH plot, that is, it estimated as 7.1×10^{1} (M^{-1}) by dividing the intercept value (1.3×10^{-2}) by the slope value (1.8×10^{-1}). The binding constant of CNN is 4 times smaller than that of β–CD reported in our previous study (Ishikawa *et al.*, 2007), indicating that the cavity of CNN is too small for the tight binding to vanillin. The X–ray structure of CNN was reported by Bradbrook *et al.* (2000). They suggested that CNN adopts a plate–like overall shape with a very shallow depression on one side. Thus, the shallow depression of CNN would be weakly interacted with the aromatic ring of vanillin.

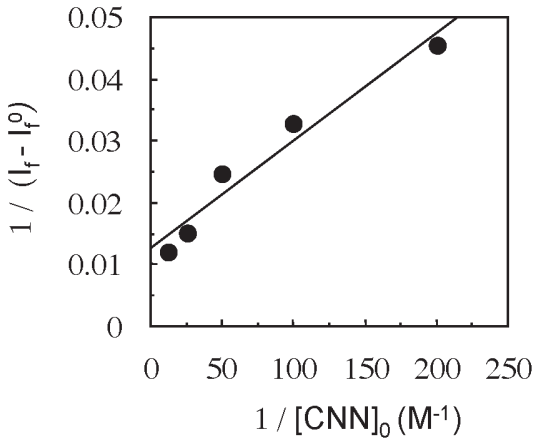

Fig. 2. The BH plot for the complexation of vanillin with CNN. I_0 and I are the fluorescence intensities in the absence (only vanillin) and presence of CNN (CNN–vanillin complex), respectively, and $[CNN]_0$ is initial concentration of CNN.

Complexations of cinnamaldehyde, eugenol, and raspberry ketone

The complexation behaviors of hydrophobic compounds with CNN were investigated in the similar manner for vanillin. Fig. 3 shows the effect of CNN concentra-

tion on the fluorescence intensity of cinnamaldehyde, eugenol, and raspberry ketone at 25 ˚C. The emission wavelength was set at 410 nm for cinnamaldehyde and eugenol, and at 425 nm for raspberry ketone. The complexation process was carried out at 3–100 mM CNN in 10% ethanol solution. As a result, the enhancement of fluorescence intensity was observed for all compounds. The intensity of cinnamaldehyde and raspberry ketone significantly increased over the range of 3–100 mM CNN concentration, while the intensity of eugenol reached to plateau at 12.5 mM CNN concentration. Then, the binding constants and stoichiometric ratios of the guest–CNN complex determined from the BH plots. Fig. 4 shows the BH plots of $1/(I–I_0)$ as a function of $1/[CNN]_0$ based on the fluorescence data of cinnamaldehyde and eugenol in the presence of CNN. Both plots for cinnamaldehyde and eugenol exhibited good linearity. This implies the formation of inclusion complexes with a stoichiometry of 1:1 (guest : CNN). The slope and the intercept of the BH plot were obtained from the linear graph, and biding constants for cinnamaldehyde and eugenol were estimated as 4.1×10^1 and 6.3×10^2 (M^{-1}), respectively (table 1). On the other hand, the BH plot for raspberry ketone suggested the formation of complexes of different guest–CNN stoichiometry. Fig. 5 shows the BH plot based on the fluorescence data of raspberry ketone in the presence of CNN. The plot is described by two linear segments. The initial linear portion represents the formation for the 2:1 raspberry ketone–CNN complex, while the final linear portion represents that for the 1:1 complex. The binding constants for the 1:2 and 1:1 complex were calculated as described above, and estimated as 3.7×10^1 and 3.8×10^2 (M^{-1}), respectively (table 1).

The effect of ethanol on the binding constant of cinnamaldehyde to CNN was determined (Fig. 6). The complexation was performed in 10–40% ethanol solution. The slope and the intercept of the BH plot were obtained in the similar manner, and the binding constant for CNN were estimated. As a result, a marked decrease in the binding constant was observed with increasing methanol concentration and the value in 30% ethanol was about 4 times smaller than in 10% one. This may result from the

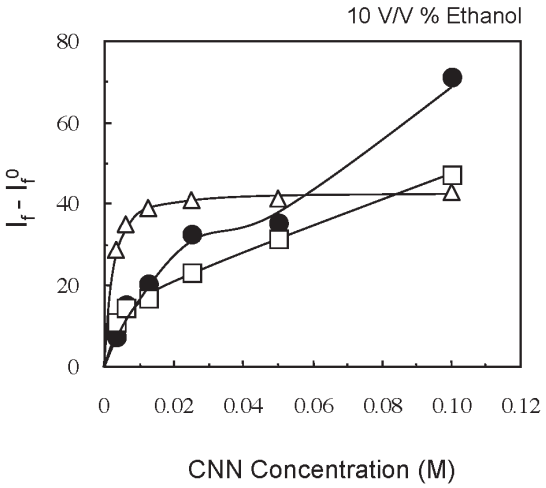

Fig. 3. Effect of CNN concentration on the fluorescence emission intensity of cinnamaldehyde, eugenol, and raspberry ketone. Excitation wavelength was set at 360 nm. The final concentration of the compounds was set at 0.2 mM.
Cinnamaldehyde (●); Eugenol (△); Raspberry ketone (□).

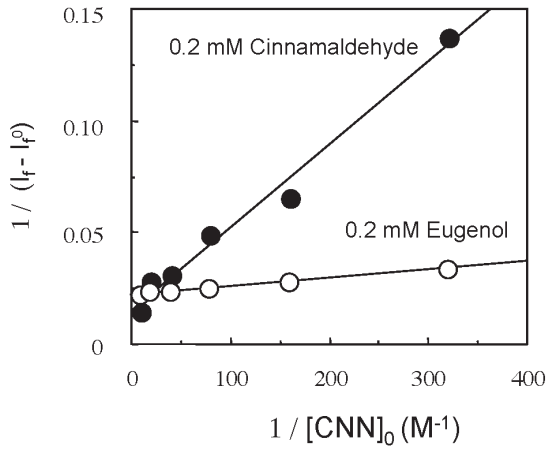

Fig. 4. The BH plot for the complexation of cinnamaldehyde and eugenol with CNN.
Cinnamaldehyde (●); Eugenol (○).

Table 1. Binding constants (K) of cinnamaldehyde, eugenol, and raspberry ketone to CNN

Cinnamaldehyde

Guest : CNN	y–intercept	slope	r	K (M^{-1})
1 : 1	1.5×10^{-2}	3.7×10^{-4}	0.991	4.1×10^1

Eugenol

Guest : CNN	y–intercept	slope	r	K (M^{-1})
1 : 1	2.3×10^{-2}	3.6×10^{-5}	0.997	6.3×10^2

Raspberry ketone

Guest : CNN	y–intercept	slope	r	K (M^{-1})
1 : 1	4.9×10^{-2}	1.3×10^{-4}	0.996	3.8×10^2
1 : 2	1.9×10^{-2}	5.2×10^{-4}	0.999	3.7×10^1

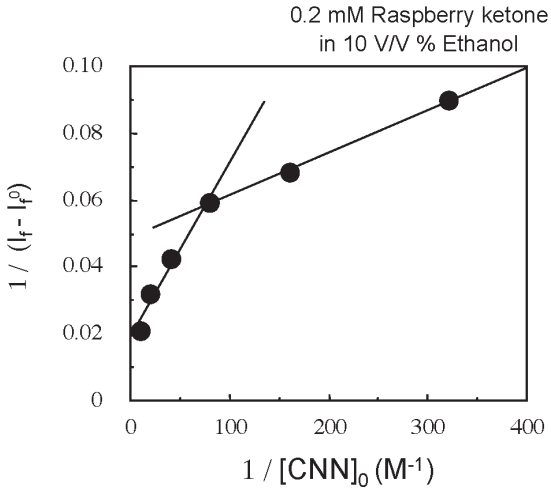

Fig. 5. The BH plot for the complexation of raspberry ketone with CNN.

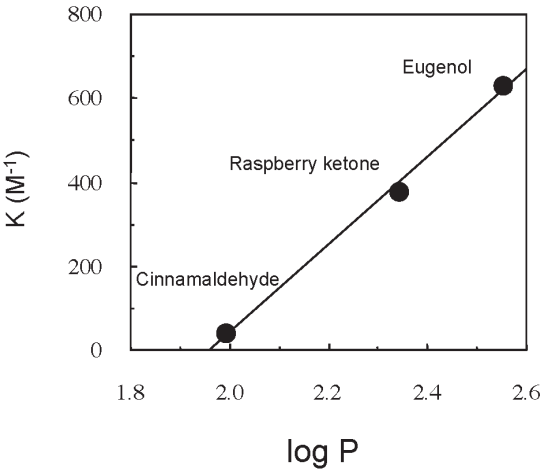

Fig. 7. Effect of hydrophobic property of cinnamaldehyde, eugenol, and raspberry ketone on the CNN complexation.

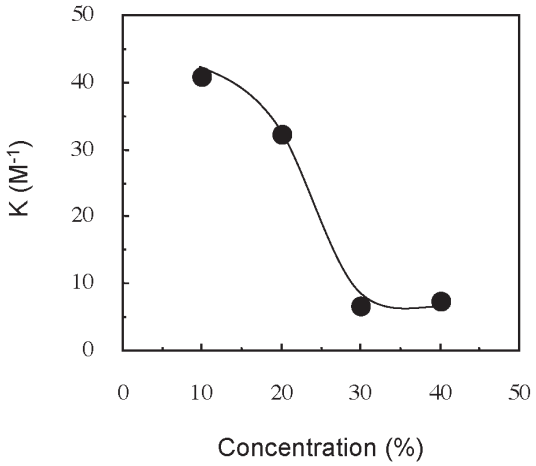

Fig. 6. Effect of ethanol concentration on the complexation of cinnamaldehyde with CNN.

increase in the affinity of cinnamaldehyde to the surrounding solvent due to an increase in the solvent hydrophobicity. In addition, it was suggested that ethanol molecules interact with CNN (Oku *et al.*, 2007), and thus, the hindering of the CNN complexation with ethanol molecules would also result in lowering the binding constant of cinnamaldehyde with CNN.

Effect of hydrophobic property of guest molecule on the complexation with CNN

To clarify the effect of hydrophobic property of cinnamaldehyde, eugenol, and raspberry ketone on the CNN complexation, their binding constants to CNN were ploted against the logarithm of the octanol–water partition coefficient, log P, of these compounds (Fig. 7). Log P value is the most frequently used hydrophobicity parameter, and it was estimated by using online Marvin software (version 3.5.1, Chem Axon Ltd., Hungary) in the present study. As a result, the binding constant was greatly influenced by log P of the compound, and thus, its hydrophobic property. A marked increase in the binding constant was observed with increasing the log P value, and the linear relationship between the binding constants and the log P values was obtained. It was suggested that the hydrophobic interaction would be the predominant factor on the formation of the guest–CNN complex.

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