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https://doi.org/10.5109/9287

出版情報：九州大学大学院農学研究院紀要. 52 (1), pp.87-90, 2007-02-28. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **52** (1), 87–90 (2007)

Complexation of Vanillin and Ethylvanillin with α–, β–, and γ–Cyclodextrin

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(*Received November 8, 2006 and accepted December 1, 2006*)

The fluorescence of vanillin and ethylvanillin in aqueous solution was investigated in the presence of α–, β–, and γ–cyclodextrin (CD). The fluorescence intensity of vanillin was enhanced with increasing CD concentration in the order of γ < β < α–CD. Benesi–Hildebrand (BH) plot based on these fluorescence data showed the linear relationship between $1/(I–I_0)$ and $1/[CD]_0$, where I_0 and I are the fluorescence intensities in the absence and presence of CD, respectively, and $[CD]_0$ is initial concentration of CD. The results indicated that 1:1 vanillin–CD inclusion complex was formed under the experimental condition. The binding constants of vanillin against CDs from the BH plot were in the order of α < γ < β–CD. The binding constant of vanillin to β–CD was estimated as 2.7×10^2 M^{-1}. The binding constants of ethylvanillin were 4–5 times smaller than those of vanillin. The binding ability of vanillin to β–CD became reduced with increasing the concentration of methanol.

INTRODUCTION

Microencapsulation technique has been applied to stabilize the core material, to control the release of the core material and to separate reactive or incompatible components of formulation (Balassa and Fanger, 1971; Lasic, 1998). Liquid droplets, solid particles, or gaseous materials with physiological activity are packaged into the shells that protect and control their release at controlled rates under desired conditions (Dziezak, 1988). In food industry, microencapsulation with cyclodextrins (CDs), which are chemically and physically stable molecules formed by the enzymatic modification of starch, are widely applied to control flavor release, mask odor and tastes, stabilize color and protect ingredients from oxidation (Singh *et al.*, 2002). 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. The cavity of CDs is relatively hydrophobic while the external faces are hydrophilic. Hence, hydrophobic molecules have a high affinity with the CD's cavity in aqueous solution.

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). Both synthetic vanillin and vanillin from natural vanilla have been used in food products. Synthetic vanillin is prepared from lignin, eugenol, or guaiacol. In addition, vanillin has been reported to act as an antioxidant to maintain the quality of food products (Burri *et al.*, 1989). Recently, its antimicrobial activity has been reported. Fitzgerald *et al.* (2003) reported the antimicrobial potential of vanillin against the growth of three yeasts associated with food spoilage, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii*. Moreover, they investigated the mode of antimicrobial action of vanillin against *Escherichia coli*, *Lactobacillus plantarum* and *Listeria innocua*, and showed that vanillin is primarily a membrane–active compound, resulting in the dissipation of ion gradients and the inhibition of respiration (Fitzgerald *et al.* (2004). In the series of their studies, the authors suggested that the aldehyde moiety in the vanillin structure is essential for its antimicrobial activity.

To maintain the aroma and the antimicrobial activity of vanillin during the storage of food products, it is necessary to prevent its oxidation by protecting its aldehyde group. Hence, we tried to encapsulate vanillin and ethylvanillin with α–, β–, and γ–CD to protect the aldehyde group from oxidation. Fluorescent analysis was used in the present study because it is one of the effective methods for the investigation of the CD complexation behavior of aromatic compound in an aqueous system. Then, the biding constants of vanillin and ethylvanillin against CDs were estimated from each of the Benesi–Hildebrand (BH) plot based on the fluorescence data. In addtion, the effect of methanol on the binding constant of vanillin against β–CD was also investigated.

MATERIALS AND METHODS

Materials

Vanillin, ethylvanillin and CDs (α–, β–, and γ–CD) were all purchased from Nacalai Tesque Inc. (Kyoto, Japan), and used without further purification. All other chemicals used in this study were of analytical reagent grade.

Complexation method of vanillin and ethylvanillin with cyclodextrin

Each of vanillin and ethylvanillin was dissolved in an aqueous solution at the concentration of 0.4 mM. The solution (2 mL) was added to 2 mL of the CD solution

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(1.2–40 mM) 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 vanillin and CD–vanillin (ethyl vanillin) in aqueous solution 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

Fluorescence spectra of vanillin

Fig. 1 shows the fluorescence spectra of vanillin in the absence (only vanillin) or the presence of α–CD at 25°C. The concentration of vanillin was set at 0.2 mM (final conc.) and α–CD was added to the solution over the range from 0.6 to 20 mM (final conc.). Vanillin itself exhibited weak fluorescence emission with maximum wavelength of 410 nm at 360 nm for the excitation. Addition of CD caused a remarkable increase of vanillin fluorescence. The changes were due to the interaction between CD and vanillin, implying the formation of CD–vanillin inclusion complexes. As suggested by Ishiwata and Kamiya (1997), the increase in intensity is attributed to a decrease in rotational freedom in the medium of the hydrophobic CD cavity in which the aromatic ring of the compound is included and shielded from quenching processes of its excited state. The fluorescence intensity was gradually enhanced with increasing CD concentration. The addition of 20 mM CD exhibited ~twice stronger intensity than that of only vanillin. Similar behaviors were observed in other CD, and the enhancement of fluorescence intensity of vanillin was compared in the presence of α–, β–, and γ–CD. Fig. 2 shows the increase in fluorescence emission intensity of vanillin at 410 nm in the presence of α–, β–, and γ–CD. The CDs concentration was set at over the range from 0.6 to 20 mM (final conc.), as a result of which the extent of the fluorescence enhancement was in the order of γ < β < α–CD.

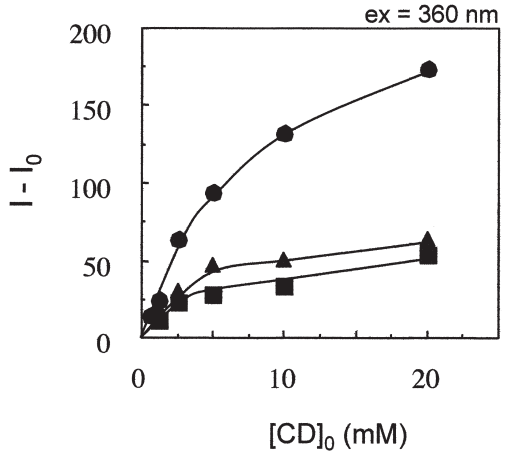

Fig. 2. Fluorescence emission intensity of vanillin at 410 nm in the presence of α–, β–, and γ–CD at 25°C. Excitation wavelength was set at 360 nm. I_0 and I are the fluorescence intensities in the absence (only vanillin) and presence of CD (CD–vanillin complex), respectively, and $[CD]_0$ is initial concentration of CD. The final concentration of vanillin was set at 0.2 mM.
α–CD (●); β–CD (▲); γ–CD(■).

Estimation of binding constants of vanillin and ethylvanillin to α–, β–, and γ–CD

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

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

where I' is the limiting intensity of fluorescence. Thus, the K value was obtained from the slope and the intercept of the plot.

Fig. 3 shows the BH plots of $1/(I–I_0)$ as a function of

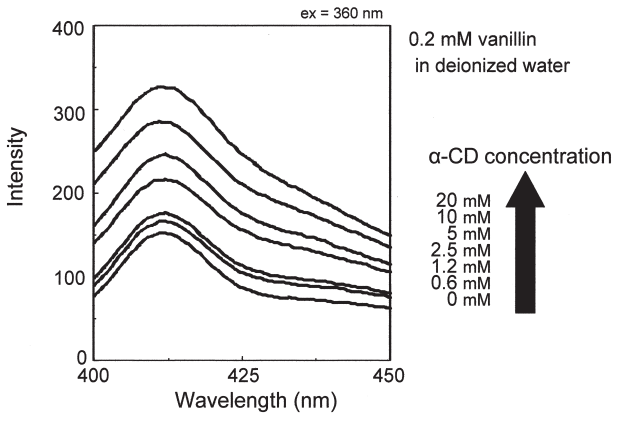

Fig. 1. Fluorescence spectra of vanillin in the absence (only vanillin) and the presence of α–CD at 25°C.

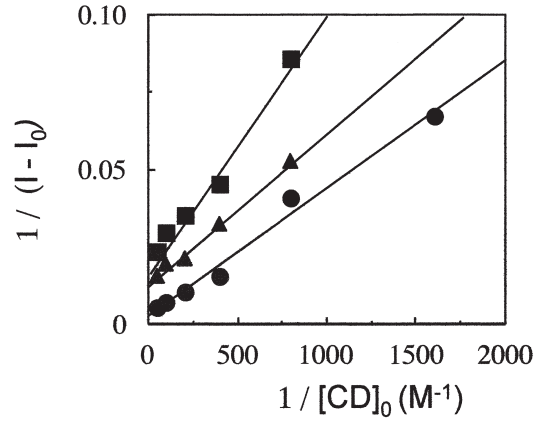

Fig. 3. The BH plots of $1/(I–I_0)$ as a function of $1/[CD]_0$.
α–CD (●); β–CD (▲); γ–CD(■).

$1/[CD]_0$ based on the fluorescence data of vanillin in the presence of α–, β–, and γ–CD as shown in Fig. 2. In the case of α–, β–, and γ–CD, BH plot showed the linear relationship between $1/(I–I_0)$ and $1/[CD]_0$, and their relation coefficients were 0.993, 0.996, and 0.989, respectively (Table 1). The results indicated that vanillin and CD were formed to 1:1 inclusion complex. Next, we estimated the binding constants of vanillin to α–, β–, and γ–CD and compared their values. The slope and the intercept of the BH plot were obtained from the linear graph of $1/(I–I_0)$ vs. $1/[CD]_0$, and biding constants (K) were estimated from the ratio of intercept /slope (Table 2). Hence, the binding constants to α–, β–, and γ–CD were 0.73×10^2, 2.7×10^2, and 2.0×10^2 M^{-1}, respectively. The binding constant to α–CD is smaller than that of β–CD, indicating that the cavity of α–CD (internal diameter of 5.7 Å) is too small for vanillin to be inserted. The binding constant to β–CD is larger than that of γ–CD, while the cavity of β–CD (i. d.=7.8 Å) is smaller than that of γ–CD (i. d.=9.5 Å). The result suggests that the cavity of β–CD would be a tight fit to aromatic ring of vanillin. The binding constant of vanillin to β–CD has been studied by Divakar (1990). The value was determined by ^{1}H–NMR measurements of vanillin H–5' (ortho to phenolic OH) signal, in which, the shifts of H–5' were monitored when increasing amounts of β–CD were added to vanillin. The binding constant was estimated as 1.11×10^4 M^{-1} by a Scatchard plot of NMR data. This value was higher than that of the fluorescent method in the present study, indicating that the binding constant is dependent on the method of determination. In addition, the binding constants of benzene derivatives to β–CD were determined by fluorescence method (Hoshino *et al.*, 1981). In this study, the values of benzene and phenol were reported as 196 and 40 M^{-1}. Thus, the binding ability of vanillin to β–CD would be higher than those of benzene and phenol.

The binding constants of ethylvanillin to α–, β–, and γ–CD were also determined in the similar manner for vanillin. The enhancement of fluorescence of ethylvanillin was also observed by the addition of CDs and the BH plots for α–, β–, and γ–CD exhibited good linearity. This implies the formation of inclusion complexes with a stoichiometry of 1:1 (ethylvanillin : CD). The slope and the intercept of the BH plot were obtained from the linear graph, and biding constants for CDs were estimated as the order of α < γ < β–CD (Table 3). This result suggests that the binding ability ethylvanillin to β–CD would be higher than those of α–, and γ–CD for vanillin, while the binding constants for ethylvanillin were 4–5 times smaller than those of vanillin. Divakar (1990) reported the structure of vanillin–β–CD inclusion complex by using NMR. It was revealed by 1D difference NOE experiments that vanillin exists with the phenolic end nearer to the narrower end the aldehyde end to the wider end of the torus–shaped β–CD molecule. In the case of ethylvanillin, therefore, its ethoxyl group at the narrower end of CD molecule would hinder its ability to form the inclusion complex.

Table 1. Binding constants (K) of vanillin to α–, β–, and γ–CD in aqueous solution

	y–intercept	slope	r	K_1 (M^{-1})
α–CD	3.0×10^{-3}	4.1×10^{-5}	0.993	0.73×10^2
β–CD	1.3×10^{-2}	4.9×10^{-5}	0.996	2.7×10^2
γ–CD	1.7×10^{-2}	8.4×10^{-5}	0.989	2.0×10^2

Table 2. Binding constants (K) of ethylvanillin to α–, β–, and γ–CD in aqueous solution

	y–intercept	slope	r	K_1 (M^{-1})
α–CD	9.8×10^{-4}	8.5×10^{-5}	0.990	0.16×10^2
β–CD	3.1×10^{-3}	5.3×10^{-5}	0.956	0.59×10^2
γ–CD	5.4×10^{-3}	1.2×10^{-4}	0.977	0.44×10^2

Table 3. Effect of methanol on the binding constant (K) of vanillin to β–CD

MeOH (%)	y–intercept	slope	r	K (M^{-1})
0	1.7×10^{-3}	5.0×10^{-5}	0.981	2.7×10^2
10	1.2×10^{-3}	6.0×10^{-5}	0.992	2.0×10^1
20	1.2×10^{-3}	8.0×10^{-5}	0.996	1.5×10^1
30	0.5×10^{-3}	6.0×10^{-5}	0.999	0.8×10^1

Effect of methanol on the formation of the complex of vanillin–CD

The effect of methanol on the binding constant of vanillin to β–CD was determined. The slope and the intercept of the BH plot were obtained in the similar manner, and the binding constant for β–CD in methanol solution (10, 20 and 30%) was estimated as listed in Table 3. As a result, a marked decrease in the binding constant was observed with increasing methanol concentration and the value in 30% methanol was about 30 times smaller than in 0% one. This may result from the increase in the affinity of vanillin to the surrounding solvent due to an increase in the solvent hydrophobicity.

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