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PROPYL GALLATE-β-CYCLODEXTRIN COMPLEXES. SPECTROSCOPIC AND THERMODYNAMIC STUDIES*

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Abstract. The spectral and temperature dependence of enhanced fluorescence of propyl gallate complexes with β-cyclodextrin in aqueous solutions was investigated. From the temperature dependence data in the range of 278-333 K, equilibrium constant and thermodynamic parameters like free enthalpy, enthalpy and entropy for the complexation of PG with CD were calculated. At 293 K those values are K_{eq} = 452 M^{-1} at 25 C, ΔG = –62.4 kJ M^{-1}, ΔH = –113.1 kJ M^{-1} and ΔS = –173 J M^{-1} K^{-1}, respectively. Characterization of the fluorescence enhancing effect and structure of the forming complex were discussed on the basis of molecular interaction mechanisms.

Keywords: cyclodextrins, propyl gallate, fluorescence, inclusion complex

INTRODUCTION

Many plants possess excellent antioxidant properties and such effects are connected with phenolic and flavonoid compounds. Gallic acid is one of the most abundant phenols having strong antioxidant properties. Also its derivatives, including propyl gallate, are widely used in food as antioxidants [6,7,10,11,16]. However, solubility of these compounds in water is low, which causes that their antioxidant action is limited. At higher concentrations the formation of associates is observed [2]. In order to extend the applications of the antioxidant properties of propyl gallate (PG) in aqueous solutions we tested the use of cyclodextrins.

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Enzyme-modified starch derivatives composed of six, seven or eight glucopyranose units are called α-, β- or γ-cyclodextrins (CD), respectively. The main interest in CD lies in their ability to form inclusion complexes with different types of compounds. Its molecular structure creates a molecule with a hydrophilic exterior, soluble in water, and hydrophobic interior where appropriate compounds may be accommodated. This leads to the formation of so called host-guest or inclusion complexes. An entrapped molecule mostly exhibits different physico-chemical properties compared to its free state. All toxicity studies have shown that orally administered CD is practically non-toxic [5]. CDs are widely used in foods, mainly as carriers of flavour or colorants [13,14], as additives reducing cholesterol in low cholesterol butter, or as emulsifiers for chocolate [1,3,4,8,9,12,15].

In this work we investigate the spectral and temperature dependence of the enhanced fluorescence of propyl gallate complexes with β-cyclodextrin in aqueous solutions. From the temperature dependence data in the range of 278-333 K, equilibrium constant and thermodynamic parameters for the complexation of PG with CD were calculated. The obtained results were used to characterize the fluorescence enhancing effect of CD in terms of the molecular mechanism of complex formation with PG.

MATERIALS AND METHODS

Methyl, ethyl and propyl gallate, purum >98% (HPLC) were obtained from Fluka, α,β,γ-cyclodextrins were obtained from Aldrich. Doubly distilled water was used as solvent, Stock solutions of gallate derivatives in double distilled water at 1 mM concentration were stored at 25°C. Stock solutions of cyclodextrins were prepared at concentration of 10 mM. Before measurements the solution was stirred and equilibrated at 298 K for 60 min.

Absorption spectra were taken in 1 cm quartz cuvette, fluorescence data were obtained using Shimadzu RF 5000 PC spectrofluorimeter with thermostatic-controlled cell compartment.

RESULTS AND DISCUSSION

The absorption spectra of PG in β-CD show some minor changes compared to those in water. A slight blue shift by 2-3 nm and a small decrease (10%) in the molar absorption coefficient is observed upon the addition of CD to the solution. The existence of isosbestic point at 292 nm indicates the equilibrium between free, monomeric and complexed PG what suggests 1:1 stoichiometry for the formed complex. In order to confirm the formation of complexes and to distinguish between spectral modification produced by interactions between PG

and glycozydic units or changes in solvent properties arising from the added CD the same set of experiments were carried out in the presence of D –(+)-glucose. Comparable amount of D–(+)-glucose caused only a barely observable shift in the absorption maximum, by about 1 nm, whereas the fluorescence was not influenced at all. Those results are good indications that the spectral changes observed in the presence of PG and CD arise from the complex formation between those two components.

Due to the fact that gallic acid and its derivatives exhibit fluorescence, we applied this property to estimate the interactions between α-, β- and γ-cyclodextrins and gallate derivatives. In water the fluorescence of gallate derivatives is low, whereas in organic solvents with lower dielectric constant the fluorescence intensity increases significantly (2-3 times) compared to water [2]. One may expect that when gallate forms an inclusion complex with hydrophobic interior of cyclodextrin, the fluorescence intensity should increase. The results presented in Figure 1 indicate that the most significant increase was observed for methyl gallate in α-cyclodextrin and for propyl gallate in β-cyclodextrin. For the other combinations of gallates with α-, β- and γ-cyclodextrins the observed fluorescence was 4-5 times lower. These data indicate that complex formation is observed in two cases. First, when methyl gallate fits the α-cyclodextrin cavity with estimated diameter of about 5 A. Second, for propyl gallate with β-cyclodextrin with diameter of 6.5 A. The fact that ethyl gallate does not fit into those three cyclodextrins shows the importance of the dimensional relations during inclusion complex formation. The above experiments showed that PG in the presence of β-cyclodextrin forms inclusion complexes and, from the fact that propyl gallate is widely used in food as an antioxidant, our further investigations regarding the thermodynamic properties of such complexes were performed on this system.

Figure 2 presents the fluorescence changes of 100 μM PG at different concentrations of CD measured at different temperatures. One of the important physicochemical parameters which describe the inclusion complex is equilibrium constant K_{eq} or dissociation constant K_d. In order to calculate K_{eq}, given by formula 1, we applied the fluorescence changes observed during titration with β-cyclodextrin. From the obtained graph the equilibrium constant K_{eq} was calculated.

$$K_{eq} = [\text{PG-CD}] / \{[\text{PG}]\,[\text{CD}]\} \quad (1)$$

To retrieve information regarding stoichiometry and binding strength the data has been transferred to Benesi-Hildebrandt plots. The obtained linear plot confirms 1:1 association complex formation between PG and CD. The equilibrium constants at different temperatures, estimated using the linear regression analysis method, are listed in Table 1.

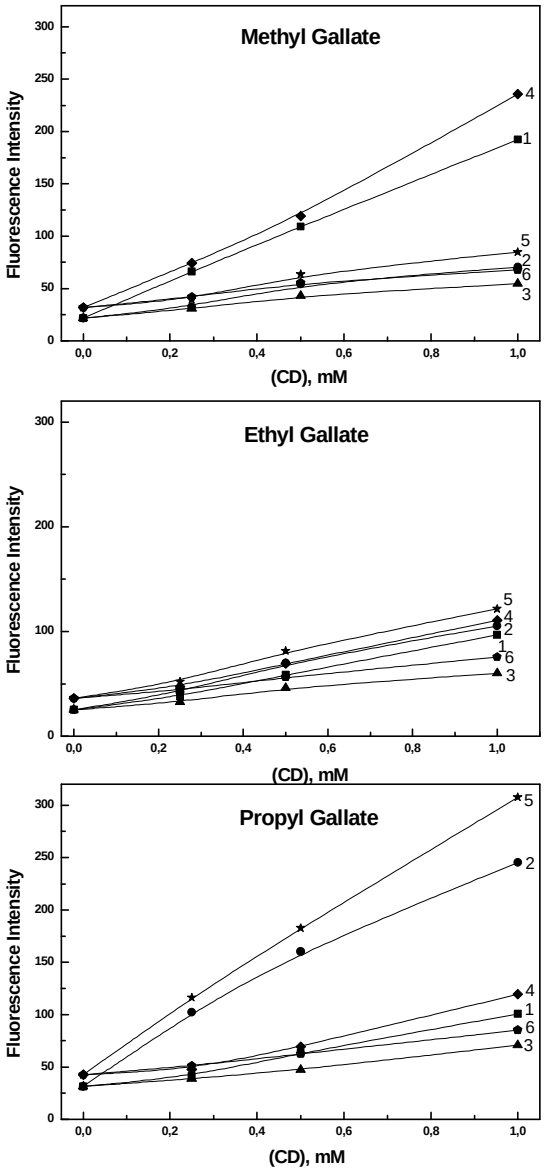

Fig. 1. Fluorescence maxima of gallates in the presence of α-, β- and γ-cyclodextrins. Curves assignment: **1** 50 μM PG + α–CD; **2** 50 μM PG + β–CD; **3** 50 μM + γ–CD; **4** 100 μM PG + α–CD; **5** 100 μM PG + β–CD; **6** 100 μM PG + γ–CD

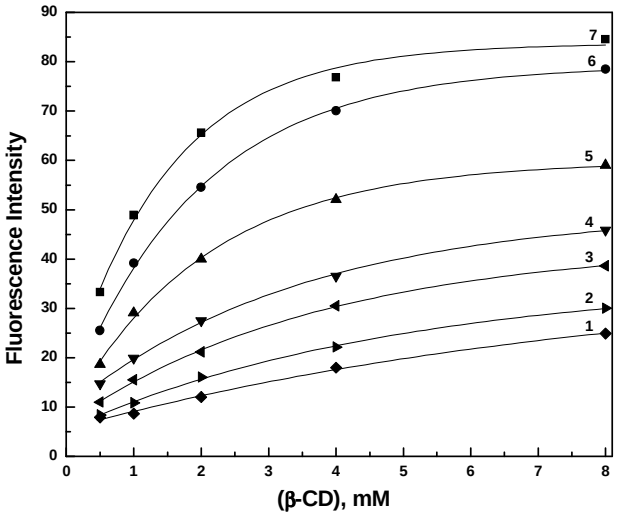

Fig. 2. Fluorescence maximum intensity changes of 100 μM PG measured at different concentrations of α-CD at different temperatures. Curves assignment: **1** – 333 K, **2** – 323 K, **3** – 313 K, **4 –**303 K, **5** – 293 K, **6** – 283 K, **7 –** 278 K

Table 1. Thermodynamic parameters of PG-CD complex, equilibrium constant K_{eq} (M^{-1}), enthalpy ΔH (kJ M^{-1}), free enthalpy ΔG (kJ M^{-1}) and entropy ΔS (J K^{-1})

Temp. (K)	K (M^{-1})	ΔH (kJ M^{-1})	ΔG (kJ M^{-1})	ΔS (J M^{-1} K^{-1})
278	832,9	–121.1	–64.9	–202.8
283	669,6	–119.4	–64.1	–196.0
293	452,6	–113.1	–62.4	–173.0
303	336,4	–103.9	–61.2	–139.5
313	249,3	–98.9	–59.9	–124.4
323	185,6	–91.8	–58.7	–100.1
333	152,1	–74.6	–58.2	–46.9

The thermodynamic parameters like standard free energy change ΔG, eq. 2, the enthalpy change ΔH, eq. 4, and standard entropy change ΔS, eq. 3, can be obtained from the temperature dependency of the equilibrium constant K_{eq}. The free energy change is calculated from the equilibrium constant K_{eq} according to the formula

$$\Delta G = -RT \ln K_{eq} \quad (2)$$

The enthalpies were calculated from the plot of ln K_{eq} versus inverse temperature where the slope provides the enthalpy data. The entropy of complexation can be calculated from the equation

$$\Delta G = \Delta H - T\Delta S \tag{3}$$

Figure 3 presents the plot of ln K_{eq} versus inverse of absolute temperature that gives a straight line with the slope given by the formula (4)

$$-\Delta H/ RT \tag{4}$$

which allows the calculation of the enthalpy change ΔH. The calculated thermodynamic parameters are collected in Table 1. All calculated thermodynamic parameters exhibit negative values. Usually, complex formation is associated with large negative values of thermodynamic parameters. Hydrophobic interactions are usually associated with small positive ΔH and large ΔS because those are entropy- driven interactions. The data given in Table 1 suggest that the complex formation between PG and CD is an enthalpy driven process. However, the observed fluorescence enhancement indicates that the fitted PG molecule interacts with the hydrophobic interior of cyclodextrin cavity. To explain this phenomenon, which has also been observed in other cyclodextrin-ligand systems, it is considered that during complex formation water bound inside the cavity releases enthalpy, which lowers the energy of the system when the guest molecule replaces the water molecule [1].

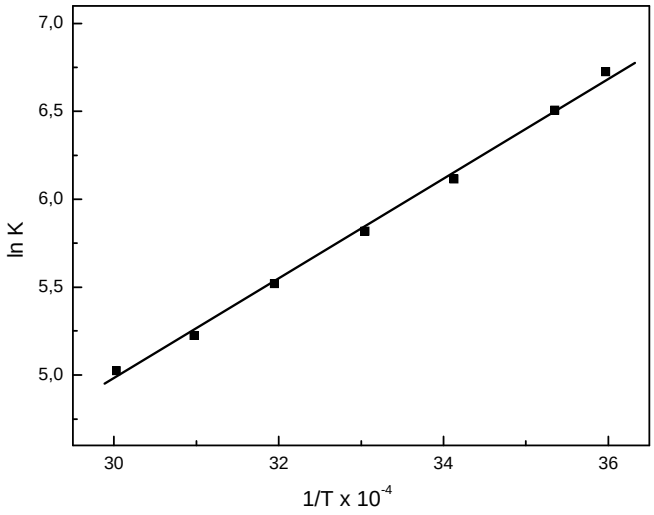

Fig. 3. Plot of ln K_{eq} versus inverse of absolute temperature

Calculated enthalpy ΔH values – Table 1 – are similar to those reported for the other inclusion complexes with CD. This indicates the compensation process where the guest molecule that may form electrostatic or hydrogen bonding interactions with atoms inside the cavity replaces the water molecules from the cavity. The negative entropy increase obtained during the complex formation indicates that this process significantly reduces the configurational freedom of the PG. This suggests that the PG molecule is anchored in the CD cavity by its nonpolar propyl chain. From our unpublished semiempirical quantum mechanical calculations it is known that transitions responsible for the fluorescence are located on the aromatic part of the PG molecule. The fluorescence maximum of PG in the complex is only a few nm shifted compared to the maximum observed in water. It indicates that the aromatic part still remains in the aqueous phase while the propyl chain is included into the CD cavity. Such a picture is also supported by an additional experiment where increasing temperature increased the fluorescence intensity of PG in glycerin, data not shown, which reflects the change in viscosity of the environment. The increased viscosity decreases the rotational motion of the propyl chain, which significantly increases fluorescence. However, taking into account the dimensional relations between the diameter of the cavity and the size of the aromatic part of the PG molecule and the observed a few nm shift of emission maximum of the complex, we cannot exclude that the trihydroxy benzoic part of the PG molecules may enter the CD cavity.

CONCLUSION

In aqueous solutions, in the presence of CD, the PG molecule forms a complex with a stoichiometry of 1:1. This inclusion produces modifications in the absorption and fluorescence spectra of the PG that were used to evaluate the equilibrium constant of the formed PG-CD complex. The obtained thermodynamic parameters were used to calculate the equilibrium constant K_{eq} and also showed that temperature is an important factor that determines the stability of complex formation.

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KOMPLEKS GALUSANU PROPYLU Z β-CYKLODEKSTRYNĄ. BADANIA SPEKTROSKOPOWE I TERMODYNAMICZNE

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S t r e s z c z e n i e. Celem badań był określenie zależności temperaturowych oraz własności spektroskopowych fluorescencji kompleksu galusanu propylu z β-cyklodekstryną w roztworach wodnych. Z otrzymanych zależności temperaturowych w zakresie od 278 K do 333 K obliczono stałą równowagi kompleksu K_{eq} oraz parametry termodynamiczne kompleksu GP z β-CD takie jak entalpia swobodna ΔG, entalpia ΔH i entropia S. W temperaturze 293 K wartości te wynoszą: K_{eq} = 452 M^{-1}, ΔG = –62,4 kJ·M^{-1}, ΔH = –113,1 kJ· M^{-1} i ΔS = –173 J·M^{-1}·K^{-1}. Zjawisko wzrostu natężenia fluorescencji PG w utworzonym kompleksie oraz strukturę tego kompleksu przedyskutowano na podstawie molekularnego mechanizmu oddziaływań pomiędzy PG a hydrofobowym wnętrzem cząsteczki β-cyklodekstryny.

S ł o w a k l u c z o w e: cyklodekstryny, galusan propylu, fluorescencja, kompleks inkluzyjny