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ORIGINAL ARTICLE

Thermodynamic, IR spectral and X-ray diffraction studies of the "*β*-cyclodextrin-*para*-aminobenzoic acid" inclusion complex

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Abstract The inclusion complex with stoichiometric composition 1:1 was formed as a result of intermolecular interactions between *para*-aminobenzoic acid and *β*-cyclodextrin. The stability constant of "*β*-cyclodextrin-*para*-aminobenzoic acid" inclusion complex at 289, 292 and 313 K was calculated by the Ketelar equation. The influence of temperature on the stability of "*β*-cyclodextrin-*para*-aminobenzoic acid" inclusion complex was also examined, and thermodynamic parameters involved in the complex formation (ΔG, ΔH, ΔS) were calculated. Supramolecular complex formation between *para*-aminobenzoic acid and *β*-cyclodextrin was confirmed by X-ray diffraction and IR spectroscopy studies.

Keywords *β*-Cyclodextrin · *para*-Aminobenzoic acid · Inclusion complex · Thermodynamics · IR spectroscopy · X-ray diffraction

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides converted from starch [1]. *β*-CD is the most widely used cyclodextrin. It forms inclusion compounds with organic molecules which have hydrophobic moieties and suitable geometry due to hydrophilic exterior and hydrophobic interior of toroidal *β*-CD molecule [2, 3]. Organic compounds may enter partly or entirely into the hydrophobic cavity of *β*-CD simultaneously expelling the few "high-enthalpy water" molecules from the inside [4–8]. Solubility, bioavailability, and stability of various drug molecules can increase due to its incorporation into the *β*-CD cavity [4–8].

para-Aminobenzoic acid (*p*-ABA) is used as an intermediate in bacterial synthesis of folate [9]. *p*-ABA, as an antioxidant, provides protection against ozone, smoking, and other air pollutants which damage cell structures and membranes through oxidative stress [10, 11]. It has some therapeutic effect against vitiligo, scleroderma, herpes and arthritis [9, 12–14]. It was possible to expect that the therapeutic effect from use of *p*-ABA as complexes with cyclodextrins will be enhanced. However the literature data about conditions of complex formation and its stability are significantly distinguished [15, 16]. Therefore in this work interaction between *β*-CD and *p*-ABA has been investigated in details by use of UV- and IR-spectroscopy, and also X-ray diffraction analysis.

Experimental

Materials and chemicals

β-CD (from Fluka, purity ≥ 99%), *p*-ABA (from Merk, purity ≥ 99%), citric acid, sulfuric acid, sodium hydroxide, disubstituted sodium phosphate (all from Reakhim, pure analytical) were used without additional purification.

Methods and instruments

UV spectra of *p*-ABA solutions were recorded in the 220–350 nm spectral range with a Specord M-40. Quarts cells with 10 mm pathlength were used. All spectroscopic measurements were made with temperature-controlled cell holder and water bath.

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pH of *p*-ABA buffer solutions were measured by an Ionometer I-120.1.

IR spectra of *β*-CD, *p*-ABA and inclusion complex "*β*-CD-*p*-ABA" were recorded in the frequency range from 4000 to 1200 cm^{-1} with a Thermo Nicollet NEXUS FT-IR spectrophotometer using KBr pelleting.

X-ray diffraction spectra were registered by use of a diffractometer DRON-4-02 (emission of Cu K_α, $\lambda = 1.54178$ Å) with nickel filter.

Procedure of study of interaction between *β*-CD and *p*-ABA

Aliquots (on 1 mL) of *p*-ABA (1.0×10^{-4} mol L^{-1}) solution were placed into a 10 mL volumetric flasks and an appropriate amounts of *β*-CD solution (1.0×10^{-2} mol L^{-1}) prepared in pH = 3.60 buffer solution were added. The obtained solutions were diluted up to constant volume (10 mL) with buffer solution (mixture of 0.2 M Na_2HPO_4 and 0.1 M citric acid solutions with pH = 3.60). The starting concentration of *β*-CD was varied from 0.0012 to 0.0072 mol L^{-1}. Then the prepared solutions were shaken at 289, 292 and 313 K for 24 h up to attainment of equilibrium. The solutions were prepared just before taking measurements. Twice-distilled water was used for preparation of aqueous solutions. The reference solutions contained the same concentration of reagents, but no *p*-ABA.

Procedure of synthesis of inclusion complex of *p*-ABA with *β*-CD

Batches of *β*-CD and *p*-ABA were weighed in a 1:1 M ratio and dissolved singly in twice-distilled water. Then the *β*-CD solution was gradually dropped to the *p*-ABA solution and continuously stirred with an electromagnetic stirrer at 293 K for 24 h. The binary solution was stored at 277 K for 48 h. The white precipitate was filtered, washed with twice-distilled water and dried in an oven at 333 K for 24 h.

Results and discussion

Stability constant of "*β*-CD-*p*-ABA" complex

It has been known [15, 16] that *β*-CD produces complexes with molecular, anionic and cationic forms of *p*-ABA. However complex of *β*-cyclodextrin with uncharged form of *para*-aminobenzoic acid is firmer [16]. Therefore complex formation between *p*-ABA and *β*-CD was studied in buffer solutions at pH = 3.60, where *p*-ABA exists preferably in molecular form. As *p*-ABA absorbs in the same interval of waves lengths as complex formed in the solution, the values of stability constant were calculated by the Ketelar relation [17]:

$$\frac{1}{A - A_o} = \frac{1}{a} + \frac{1}{aK_s[\beta - CD]_o}, \quad (1)$$

where A and A_o—*p*-ABA absorbance with and without *β*-CD, respectively; a—the constant which is the difference between extinction coefficients of "*β*-CD-*p*-ABA" complex and *p*-ABA at the same wave length; K_s—stability constant (binding constant) of "*β*-CD-*p*-ABA" complex; $[\beta\text{-CD}]_o$—the starting concentration of *β*-CD in a solution.

Good linear relationship ($R = 0.997$–0.999) between spectral characteristics of *p*-ABA and *β*-CD content in the coordinates $1/(A - A_o) = f\ (1/[\beta\text{-CD}]_o)$ was obtained. It confirms the formation of a 1:1 complex between *p*-ABA and *β*-CD over studied temperature interval. The stability constants of "*β*-CD-*p*-ABA" complex formation at variable temperatures were calculated from the tangent of the slope of the straight lines (Table 1).

Complex thermodynamics

The temperature dependence of binding constants was used for calculation of thermodynamic parameters involved in the complex formation: changes of the Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS) [18]. The values of the Gibbs free energy change at various temperatures were found by the van't Hoff equation [18]:

$$\Delta G = -RT \ln K_s. \quad (2)$$

The values of entropy and enthalpy changes depending on the variation of the stability constant with the temperature were calculated graphically from the intercept and tangent of the slope, respectively, by use of the equation [18]:

$$\ln K_s = \frac{\Delta S}{R} - \frac{\Delta H}{RT}. \quad (3)$$

The obtained thermodynamic parameters are given in Table 1.

The negative values of ΔG testify that the complex formation is a spontaneous and thermodynamically favored process. Four driving forces for the inclusion of the "guest" molecules into cyclodextrins were proposed [19] including the hydrogen binding between the hydroxyl groups of cyclodextrins and the "guest", van der Waals forces between "host" and "guest" molecules, hydrophobic interactions, and the release of "high-enthalpy water" molecules from the inner CD-cavities to bulk water. The value of negative enthalpy change (Table 1) is typical for low-energy interactions [19]. As the change of entropy is also negative, the complex formation is entropically unfavourable [18]. The negative entropy changes are attributable to decrease in translational and rotational degrees of

Table 1 Stability constant and thermodynamic parameters of "β-CD-*p*-ABA" complex

Temperature (K)	K_s (L mol^{-1})	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
289	176 ± 28	−12.4	−19.3	−24
292	158 ± 25	−12.3		
313	97 ± 15	−11.8		

freedom of complex formation molecules as compared with the free ones on giving more ordered system [19]. Thus, negative values of thermodynamic parameters indicate that complex is formed as a result of *p*-ABA incorporation into the cavity of β-CD.

IR spectral study

Inclusion complex formation may be confirmed by IR spectroscopy because bands resulting from the incorporated "guest" molecule are generally shifted or their intensities are altered.

In the IR spectrum of *p*-ABA (Fig. 1, curve 1) the valence vibrations of the N–H bonds in the primary amino group and the C–H bonds in the aromatic ring with maxima at 3460, 3362, 3231 and 3051 cm^{-1}, respectively, are registered. The band of valence vibrations of the C=O bond in the carboxyl group is observed at 1661 cm^{-1}. The absorption bands with maxima at 1600, 1573 and 1524 cm^{-1} belong to the valence vibrations of the C=C bonds in the benzene ring. The absorption band of the valence vibrations of the C–N bond in the amino group connected with the benzene ring is observed at 1310 cm^{-1}. The bands of the deformation vibrations of the N–H bonds in the amino group and the C–H bonds in the benzene ring are registered at 1625, 890 cm^{-1} and 1173, 1131, 844 cm^{-1}, respectively [20].

In the IR spectrum of β-CD (Fig. 1, curve 2) the wide band is registered with the absorption maximum at 3320 cm^{-1}, which is caused by the valence vibrations of the O–H bonds in the primary hydroxyl groups (C-6–OH group) connected by the intermolecular hydrogen bonds or in the secondary hydroxyl groups connected by the intramolecular hydrogen bonds (the C-2–OH group of one glucopyranose unit and C-3–OH group of adjacent glucopyranose unit) [2]. Also, in the IR spectrum of β-CD the absorption band with maximum at 2926 cm^{-1} is observed. It belongs to the valence vibrations of the C–H bonds in the CH and CH_2 groups. In the region 1400–1200 cm^{-1} the absorption bands of the deformation vibrations of the C–H bonds in the primary and secondary hydroxyl groups of β-CD (1411, 1368, 1335, 1301, 1246 cm^{-1}), and in the region 1200–1030 cm^{-1} the absorption bands of the valence vibrations of the C–O bonds in the ether and hydroxyl groups of β-CD (1080 and 1027 cm^{-1}) are registered. The absorption bands in the region 950–700 cm^{-1} belong to the deformation vibrations of the C–H bonds and the pulsation vibrations in glucopyranose cycle.

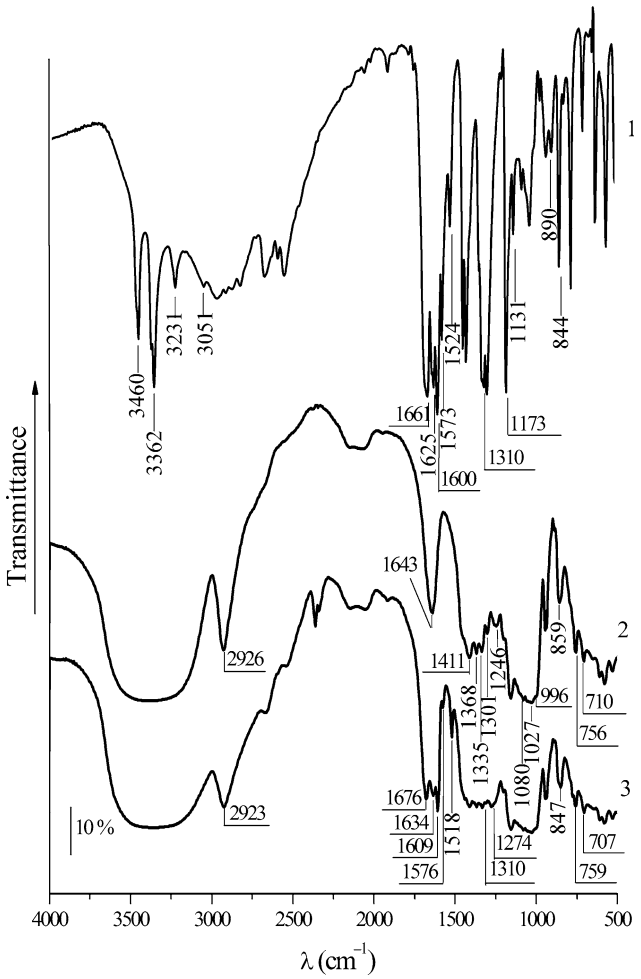

Fig. 1 IR spectra of *p*-ABA (1), β-CD (2) and "β-CD-*p*-ABA" inclusion complex (3)

The IR spectrum of the inclusion complex of β-CD with *p*-ABA differs from the IR spectra of *p*-ABA and β-CD (Fig. 1, curve 3). The band of the valence vibrations of the C=O bond in the carboxyl group of *p*-ABA is shifted to higher frequency in spectral pattern of the inclusion complex (1676 cm^{-1}). At the same time, the band of the deformation vibrations of the N–H bonds in the amino group is shifted to lower frequency—1634 cm^{-1}. The absorption bands of the valence vibrations of the C–O bonds in the ether and hydroxyl groups of β-CD are slightly broadened. Moreover, the absorption bands of the valence vibrations of the C=C bonds in the benzene ring are shifted to 1609, 1576 and 1518 cm^{-1}; the peak at 1246 cm^{-1} in the spectrum of β-CD which belongs to the

deformation vibrations of the C–H bonds in the hydroxyl groups is shifted to the 1274 cm^{-1} and greatly widened. In the IR spectrum of the inclusion complex (Fig. 1, curve 3), characteristic absorption bands maxima of glucopyranose cycle are assigned to 847, 759 and 707 cm^{-1}. These results indicate that the vibrations and bends of the "guest" molecule are restricted due to the encapsulation of *p*-ABA into the *β*-CD cavity.

X-ray diffraction study

Diffraction patterns of the "*β*-CD-*p*-ABA" inclusion complex and individual compounds are different. Encapsulation of "guest" molecule into the cavity of *β*-CD results in the appearance of new intensive diffraction peaks at 12.39, 19.28 and 20.54 2Θ in comparison with precursors (*β*-CD: 12.62, 22.54, 34.70 2Θ; *p*-ABA: 13.73, 15.19, 21.74 2Θ). Essential decrease of the inclusion complex crystallinity is due to the formation of supramolecular compound [21–23]. In accordance with the data obtained by the single-crystal X-ray diffraction [24] location of amino and carboxyl groups of *p*-ABA on the wide and narrow edge of the *β*-CD molecule, respectively, is the most likely.

Conclusions

In this work spectrophotometric study of intermolecular interactions between *para*-aminobenzoic acid and *β*-cyclodextrin was carried out. The complexation behavior of *p*-ABA in citric buffer solution has been investigated with and without *β*-CD. The Ketelar equation was used to calculate the stability constant of "*β*-CD-*p*-ABA" complex at 289, 292 and 313 K. The stability constant decreases from 176 to 97 L mol^{-1} with increase in temperature. Calculation of the thermodynamic parameters involved in the complex formation was made by the van't Hoff equation. Negative values of thermodynamic parameters (ΔG, ΔH, and ΔS) indicate that the complex is formed as the result of *p*-ABA incorporation into the cavity of *β*-CD. From the results obtained by IR spectroscopy it may be concluded that vibrations and bends of the included "guest" molecule are restricted due to encapsulation of *p*-ABA into the *β*-CD cavity. Interaction between *p*-ABA and *β*-CD gave rise supramolecular complex with new crystalline structure.

References

1. Schradinger, F.: Uber thermophile Bakterien aus verschiedenen Speisen und Milch sowie uber einige Umsetzungsprodukte derselben in kohlenhydrathaltigen Nahrlosungen, darunter kristallisierte Polysaccharide Dextrin aus Starke. Z. Unters. Nahr. u. Genussm. **6**, 865–880 (1903)
2. Szejtli, J.: Introduction and general overview of cyclodextrin chemistry. Chem. Rev. **98**, 1743–1754 (1998)
3. Liu, L., Guo, Q.-X.: The driving forces in the inclusion complexation of cyclodextrins. J. Incl. Phenom. **42**, 1–14 (2002)
4. Zouvelekis, D., Yannakopoulou, K., Mavridis, I.M., Antoniadou-Vyza, E.: The self-association of the drug acemetacin and its interactions and stabilization with *β*-cyclodextrin in aqueous solution as inferred from NMR spectroscopy and HPLC studies. Carbohydr. Res. **337**, 1387–1395 (2002)
5. Li, N.B., Luo, H.Q., Liu, S.P.: Resonance Rayleigh scattering study of the inclusion complexation of chloramphenicol with *β*-cyclodextrin. Talanta **66**, 495–500 (2005)
6. Zhang, Q.-F., Jiang, Z.-T., Guo, Y.-X., Li, R.: Complexation study of brilliant cresyl blue with *β*-cyclodextrin and its derivatives by UV–vis and fluorospectrometry. Spectrochim. Acta. **69**, 65–70 (2008)
7. Hergert, L.A., Escandar, G.M.: Spectrofluorimetric study of the *β*-cyclodextrin–ibuprofen complex and determination of ibuprofen in pharmaceutical preparations and serum. Talanta **60**, 235–246 (2003)
8. Redenti, E., Szente, L., Szejtli, J.: Cyclodextrin complexes of salts of acidic drugs. Thermodynamic properties, structural features, and pharmaceutical applications. J. Pharm. Sci. **90**, 979–986 (2001)
9. Scholar, E.M., Pratt, W.B.: The Antimicrobial Drugs. University Press, Oxford (2000)
10. Xavier, S., MacDonald, S., Roth, J., Caunt, M., Akalu, A., Morais, D., Buckley, M.T., Liebes, L., Formenti, S.C., Brooks, P.C.: The vitamin-like dietary supplement *para*-aminobenzoic acid enhances the antitumor activity of ionizing radiation. Int. J. Radiat. Oncol. Biol. Phys. **65**, 517–527 (2006)
11. Pearson, D., Shaw, S.: Life extension: a practical scientific approach. Warner Books, New York (1982)
12. Zarafonetis, C.J., Dabich, L., Skovronski, J.J., DeVol, E.B., Negri, D., Yuan, W., Wolfe, R.: Retrospective studies in scleroderma: skin response to potassium *para*-aminobenzoate therapy. Clin. Exp. Rheumatol. **6**, 261–268 (1988)
13. Sieve, B.F.: Clinical achromotrichia. Science **94**, 257–258 (1941)
14. Serpone, N., Salinaro, A., Emeline, A.V., Horikoshi, S., Hidaka, H.: An in vitro systematic spectroscopic examination of the photostabilities of a random set of commercial sunscreen lotions and their chemical UVB/UVA active agents. Photochem. Photobiol. Sci. **1**, 970–981 (2002)
15. Stalin, T., Shanthi, B., Rani, P.V., Rajendiran, N.: Solvatochromism, prototropism and complexation of *para*-aminobenzoic acid. J. Incl. Phenom. Macrocycl. Chem. **55**, 21–29 (2006)
16. Shuang, S.M., Yang, Y., Pan, J.H.: Study on molecular recognition of *para*-aminobenzoic acid species by α-, *β*- and hydroxypropyl-*β*-cyclodextrin. Anal. Chim. Acta **458**, 305–310 (2002)
17. Sverdlova, O.V.: Electronic Spectra in Organic Chemistry. Khimiya, Leningrad (1973). (in Russian)
18. Atkins, P.W.: Physical Chemistry. Oxford University Press, Oxford (1978)
19. Rekharsky, M.V., Inoue, Y.: Complexation thermodynamics of cyclodextrins. Chem. Rev. **98**, 1875–1918 (1998)
20. Nakanishi, K.: Infrared absorption spectroscopy-practical. Nankodo Company Ltd, Tokyo (1962)
21. Von Plessing Rossel, C., Carreno, J.S., Rodriguez-Baeza, M., Alderete, J.B.: Inclusion complex of the antiviral drug acyclovir with cyclodextrin in aqueous solution and in solid phase. Quim. Nova. **23**, 749–752 (2000)

22. Patil, A., Pore, Y., Kuchekar, B.: Effect of L-arginine on bicalutamide complexation with hydroxypropyl-β-cyclodextrin. Dig. J. Nanomater. Biostress **3**, 89–98 (2008)
23. Ammar, H.O., Salama, H.A., Ghorab, M., Mahmoud, A.A.: Inclusion complexation of glimepiride in dimethyl-β-cyclodextrin. Asian J. Pharm. Sci. **2**, 44–55 (2007)
24. Zhang, Y., Yu, S., Bao, F.: Crystal structure of cyclomaltoheptaose (β-cyclodextrin) complexes with *p*-aminobenzoic acid and *o*-aminobenzoic acid. Carbohydr. Res. **343**, 2504–2508 (2008)