Thermodynamics of the effects of substituent, degree of substitution, and pH on complex formation of hydroxypropyl-α- and hydroxypropyl-β-cyclodextrins with ascorbic acid

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The interaction of ascorbic acid with hydroxypropyl-α- and hydroxypropyl-β-cyclodextrins of different degree of substitution was studied at 298.15 K and different pH using solution calorimetry. In an aqueous solution, only hydroxypropyl-β-cyclodextrins form weak molecular complexes with the nonionized form of ascorbic acid. The thermodynamic functions of complex formation and stability constants of the complexes were calculated. The systems with weak intermolecular interaction without complex formation were characterized by enthalpic virial coefficients. On the basis of the obtained thermodynamic characteristics it was shown that the selectivity of complex formation of hydroxypropyl-α- and hydroxypropyl-β-cyclodextrins with ascorbic acid is determined by the size of the macrocyclic cavity, the presence of the hydroxypropyl substituent, and the medium acidity. The degree of substitution of hydroxypropyl-β-cyclodextrins exerts no substantial effect on the thermodynamic parameters of interaction with ascorbic acid.

Key words: hydroxypropyl-α-cyclodextrin, hydroxypropyl-β-cyclodextrin, ascorbic acid, host—guest complexes, thermodynamics of complex formation, intermolecular interactions, enthalpic virial coefficients.

Ascorbic acid (AA) is classified as a water-soluble vitamin and plays an important role in the living activity of an organism through participation in the synthesis of collagen, normalization of capillary permeability, and control of redox processes of carbohydrate metabolism and blood coagulability.[1,2]

Ascorbic acid is readily oxidized and transformed into dehydroascorbic acid, which is less stable and transformed into diketogulonic acid having no biological activity.[3] Ascorbic acid encapsulation by cyclodextrins can be used to prevent its oxidation. Cyclodextrins (CDs) are natural cyclic oligosaccharides containing from six to twelve D-glucopyranose residues linked "head-to-tail" by the α(1→4)-glycoside bond. Cyclodextrins can act as host molecules toward different guest molecules due to their large hydrophobic cavity, which can accommodate organic molecules according to their size and shape.[4,5] The property of CDs to form host—guest complexes (or inclusion complexes) allows the practical use of CDs as encapsulating materials preserving a "guest" from external factors (air oxygen, moisture, light, *etc.*) and the preparation of more stable medicines and drugs with prolonged effect.[6]

We have previously[7,8] studied the interaction of AA with α- and β-CDs and their fragments, *viz.*, glucose and maltose. In this work, we continued to study the interaction of AA with modified CDs, namely, hydroxypropyl-α- and hydroxypropyl-β-cyclodextrins, in aqueous solutions at different pH of the medium. Hydroxypropyl-α- and hydroxypropyl-β-cyclodextrins (HP-CDs) were chosen due to their higher solubility in water, low toxicity, and high selectivity in complex formation with "guest" molecules.[9,10] The effect of a substituent, degree of substitution, and pH of the medium on the thermodynamic parameters of interaction of different CDs with AA in aqueous solutions can be examined on the basis of new experimental data and earlier obtained results.[8]

Experimental

Commercial samples of HP-α-CD and HP-β-$CD_{(4.2)}$ (Aldrich) with an average degree of substitution of 4.2 were used. The water content in HP-α-CD and HP-β-$CD_{(4.2)}$, being 6.4 and 27.6%, respectively, was taken into account in calculation of the concentration.

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A general procedure[11] of preparation of hydroxypropylated derivatives of β-CD was somewhat modified and used for the synthesis of HP-β-$CD_{(2.1)}$ with the degree of substitution 2.1. The synthesis was carried out in a strongly alkaline medium (30% aqueous solution of NaOH), which favored regioselective hydroxypropylation only at the primary hydroxy groups of CDs.[12] β-Cyclodextrin (17.03 g, 15.0 mmol) was dissolved with stirring in a warm (50 °C) 30% solution of NaOH (55 mL). After homogenization, the solution was cooled to 0 °C. Propylene 1,2-oxide (2.91 g, 50.1 mmol) was added to the solution with stirring for 30 min at the temperature from 0 to −3 °C, and then the reaction mixture was stored for 19 h at 20 °C. The alkaline solution was neutralized with concentrated hydrochloric acid to pH 7—7.5 and then concentrated *in vacuo* to 1/2 of the initial volume. A residue was dissolved in 95% EtOH (300 mL), and the solution was stirred for 1.5 h. A precipitate of NaCl that formed was filtered off and thoroughly washed with 95% EtOH. Combined solutions were concentrated *in vacuo* to dryness, and DMF (100 mL) was added. The mixture was stirred, and a precipitate of NaCl was filtered off and washed with DMF. Combined extracts were concentrated *in vacuo* to form a viscous syrup. The latter was repeatedly triturated with acetone in a porcelain mortar. A solid powder that formed was filtered off, washed with acetone, and dried *in vacuo*. A white powder of HP-β-$CD_{(2.1)}$ was obtained in 60% yield (11.30 g), m.p. 228—231 °C (decomp.), R_f 0.90 (95% EtOH—H_2O, 1 : 3), 0.70 (95% EtOH—$CHCl_3$, 9 : 1). ^{1}H NMR (D_2O) of HP-β-$CD_{(2.1)}$ (Bruker WM-250 spectrometer, working frequency 250 MHz, δ): 1.19 (d, 6.3 H, $C\underline{H}_3CH$, $^3J_{HCCH}$ = 6.4 Hz); 3.48—4.15 (m, 48.3 H, $CH_3(OH)C\underline{H}CH_2$, $CH_3CHC\underline{H}_2$, C(2)H—C(5)H, $C(6)H_2$); 5.13 (d, 7 H, C(1)H, $^3J_{HCCH}$ = 10.0 Hz).

Ascorbic acid (reagent grade) was recrystallized from an aqueous-ethanol solution and dried before use at 363 K for several days to constant weight.

All solutions were prepared by gravimetry using freshly bidistilled water.

Thermal effects of dissolution of crystalline CD samples in water and aqueous solutions of AA were measured at 298.15 K using a solution calorimeter with the isothermal shell. The design of the calorimeter was described in more detail elsewhere.[13] The CD concentration remained unchanged: $(1.00\pm0.05)\cdot10^{-3}$ mol kg^{-1}. The concentration of AA solutions was varied within 0—0.65 mol kg^{-1}. The equilibrium composition of an H_2O—AA system was calculated using the RRSU computer program based on the Brinkley method.[14,15] The computation showed that the nonionized form of AA (H_2A) was predominant in the concentration range under study. This is also indicated by the pH values of the solutions (pH 2.2—2.5) determined on an ionometer with an accuracy of ±0.01. To create pH 7.5 at which the ionized AA form (HA^-) predominates, 1 *M* KOH was used.

The enthalpy of transfer (ΔH_{tr}(w→w+y)) of CD from water (w) to an aqueous solution of AA (w+y) was calculated from the experimentally determined solution enthalpies of CDs in water (ΔH_s(w)) and in an AA solution (ΔH_s(w+y))

$$\Delta H_{tr}(w\rightarrow w+y) = \Delta H_s(w+y) - \Delta H_s(w). \qquad (1)$$

The shape of the concentration plot $\Delta H_{tr}(w\rightarrow w+y) = f(m_{AA})$ is a criterion for the occurrence of complex formation. The linear plots $\Delta H_{tr}(w\rightarrow w+y) = f(m_{AA})$ with a negative or positive slope (Fig. 1, *a*) are characteristic of systems with weak intermolecular interactions.[16] The weak interactions, which are not accompanied by complex formation, were described on the basis of virial coefficients calculated from the expression of the McMillan—Mayer theory[17,18]

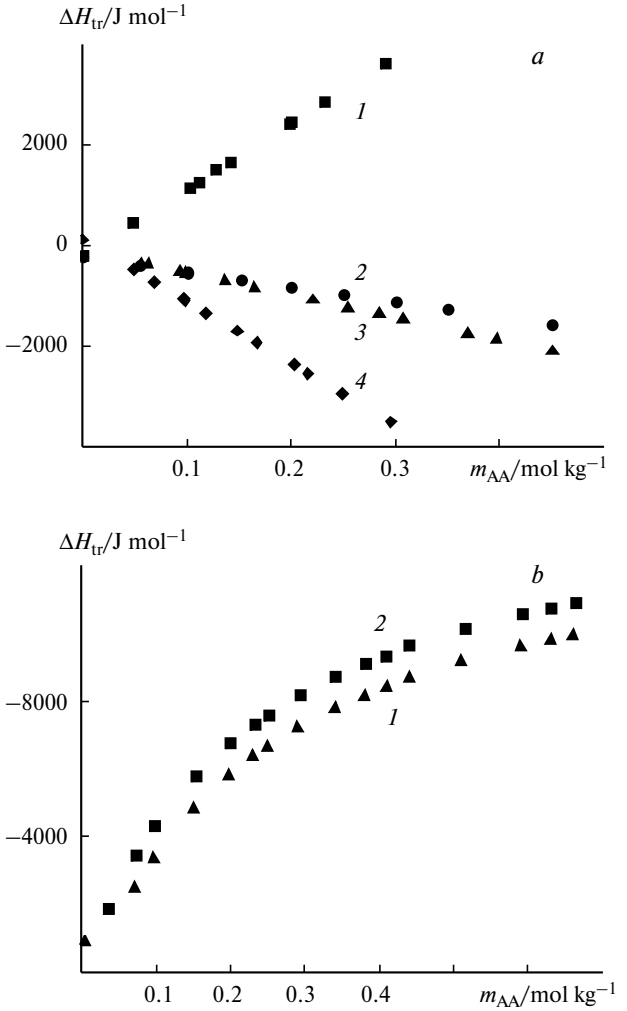

Fig. 1. Enthalpies of transfer of hydroxypropyl-α- and hydroxypropyl-β-cyclodextrins from water to aqueous solutions of ascorbic acid *vs.* ascorbic acid concentration (T = 298.15 K) for the systems with weak intermolecular interactions (*a*) and systems with complex formation (*b*); *a*: *1*, HP-α-CD—HA^-; *2*, HP-β-$CD_{(2.1)}$—HA^-; *3*, HP-β-$CD_{(4.2)}$—HA^-; *4*, HP-α-CD—H_2A; *b*: *1*, HP-β-$CD_{(2.1)}$—H_2A; *2*, HP-β-$CD_{(4.2)}$—H_2A.

$$\Delta H_{tr}(w\rightarrow w+y)/m_y = 2h_{xy} + 3h_{xyy}m_y + 3h_{xxy}m_x + ..., \qquad (2)$$

where m_y and m_x are the concentrations of AA and CD, respectively; h_{xy}, h_{xyy}, and h_{xxy} are the enthalpy coefficients of pair and triple interactions, which are the result of all energy changes in the system caused by two main types of interactions: solute—solute and solute—solvent.[16] Since the CD concentration is very low ($m_x \rightarrow 0$), the last term in Eq. (2) can be neglected. The h_{xy} values calculated by the linear least-squares method are given in Table 1.

Table 1. Enthalpy coefficients of pair interactions for cyclodextrins with ascorbic acid in water at 298.15 K

System (x—y)	h_{xy}* /J kg mol^{-2}
HP-α-CD—H_2A	−5204 (102)
HP-α-CD—HA^-	5651 (74)
β-CD—H_2A**	−1800 (900)
HP-β-$CD_{(2.1)}$—HA^-	−2248 (146)
HP-β-$CD_{(4.2)}$—HA^-	−2649 (39)

* The errors calculated from Student´s criterion with allowance for the 95% confidence interval are given in parentheses.
** Data of the previous study.[8]

Table 2. Thermodynamic parameters for the complex formation* of cyclodextrins with ascorbic acid in water at 298.15 K

Complex	K /kg mol^{-1}	$-\Delta G_c^0$	$-\Delta H_c^0$	$-T\Delta S_c^0$
		kJ mol^{-1}		
α-CD—H_2A**	1.9 (0.2)	1.6 (0.3)	6.0 (0.3)	4.4 (0.7)
HP-β-$CD_{(2.1)}$—H_2A	4.1 (0.3)	3.5 (0.3)	12.9 (0.6)	9.4 (1.2)
HP-β-$CD_{(4.2)}$—H_2A	4.3 (0.4)	3.6 (0.3)	16.2 (0.6)	12.6 (1.7)

* The errors calculated from Student´s criterion with allowance for the 95% confidence interval are given in parentheses.
** Data of the previous study.[8]

The plots presented in Fig. 1, *b* characterize the systems with complex formation. In this case, the thermodynamic characteristics of complex formation log$K$, ΔG_c, ΔH_c, and ΔS_c were calculated using the HEAT computer program (Table 2). The stoichiometry of the 1 : 1 complex, thermal transfer effects, and total concentrations of CD and AA in single entries were introduced into the HEAT program. Based on these data, the search for unknown parameters log$K$ and ΔH_c was reduced to the numerical minimization of the F functional by the desired parameters[19]

$$F = \sum_1^N w_i(\Delta H_i^{exp} - \Delta H_i^{calc})^2, \qquad (3)$$

where ΔH_i is the thermal effect of the ith reaction, N is the number of entries, and $w_i = A/(\delta\Delta H_i)^2$ are the weight factors (A is the coefficient, which is chosen from the condition $\Sigma w_i = N$, *i.e.*, the sum of weights is equal to the number of entries; $\delta\Delta H_i$ is the absolute error of ΔH_i measurement).

Results and Discussion

As known,[4,5] CD complexes with guest molecules are formed due to noncovalent interactions (*viz.*, hydrophobic and van der Waals interactions, hydrogen bonding), and the complementarity principle is substantial for the complex formation. We have earlier found[8] that only α-CD with a smaller macrocyclic cavity forms a molecular complex with AA, although the interactions of α- and β-CDs with AA are accompanied by an exothermic effect, *i.e.*, they are favorable by enthalpy. However, the observed selectivity in interactions cannot be extended over the systems with hydroxypropyl-substituted CDs.

As can be seen from the data in Fig. 1, *a*, HP-α-CD forms no complexes with the nonionized form of AA. The enthalpy coefficient of pair interaction h_{xy} for this system is given in Table 1. The h_{xy} value is high and negative, which is related to the predomination of the exothermic contribution from intermolecular interactions between AA and HP-α-CD. These can be van der Waals interactions and weak hydrogen bonds between the polar groups of AA and the hydroxypropyl substituents in α-CD. Although the interaction is energetically favorable, HP-α-CD forms no complex with AA. Probably, the bulky substituents of HP-α-CD localized on the external surface of its molecule create steric hindrance for the penetration of a "guest" into the "host" cavity.

An opposite effect of the hydroxypropyl substituents was found for HP-β-CD. The plots shown in Fig. 1, *b* indicate that only HP-β-$CD_{(2.1)}$ and HP-β-$CD_{(4.2)}$ form complexes with a molecule of ascorbic acid (H_2A). When treating the experimental data for these two systems, the calculation program was based on several most probable (according to published data[4,5]) models of interaction (1 : 1, 1 : 2, and 2 : 1). The best agreement between the calculation results and experimental data was achieved for the 1 : 1 model. Therefore, the thermodynamic parameters of complex formation are attributed to the reaction

$$H_2A + \text{HP-β-CD} \rightleftharpoons H_2A/\text{HP-β-CD}.$$

The thermodynamic parameters of complex formation are given in Table 2. They show that the complexes are weak and the complex formation is accompanied by negative changes in the enthalpy and entropy. The enthalpy term of the Gibbs energy makes the main contribution to the stability constant of the complexes.

Higher negative values of the change in the enthalpy and entropy of the complex formation were obtained for the HP-β-$CD_{(4.2)}$—H_2A complex, *i.e.*, the interaction of HP-β-$CD_{(4.2)}$ with AA is more favorable from the viewpoint of enthalpy and is accompanied by the formation of more structured complexes. A comparison of the complexation abilities of HP-β-CD and unsubstituted β-CD, which forms no complex with AA,[8] also indicates a substantial role of the hydroxypropyl substituents in complex formation. Bulky substituents of HP-β-CD surrounding the cavity can form hydrogen bonds with the polar groups of AA thus retaining the AA in the complex. According to published data,[20—25] the role of the hydroxypropyl substituents can be (1) additional protection of a "guest" included into the "host" cavity, (2) formation of hydrogen bonds with polar groups of a "guest"; (3) creation of steric hindrance for the penetration of a "guest" into the macro-

cyclic cavity. The predominant influence of each of the three factors is determined, in turn, by the structure of a guest molecule. For instance, in the case of large and immobile guest molecules, hydroxypropyl substituents exert most often steric hindrance for insertion into the CD cavity. The stability constant of the phenolphthalein complex with HP-β-CD decreases with an increase in the degree of substitution of the macrocycle, whereas small and conformationally mobile "guests" (for example, *p*-nitrophenol) form more stable inclusion complexes with HP-β-CD with a higher degree of substitution.[23] The complexes of 10-methylbenzophenothiazine, harman, and harmine with HP-β-CD are characterized by higher stability constants than those for β-CD.[24,25]

The obtained thermodynamic characteristics enable us to compose the following series of increasing the complexing ability of natural and modified CDs toward the nondissociated form of AA:

$$\text{HP-β-CD}_{(4.2)} > \text{HP-β-CD}_{(2.1)} > \text{α-CD} > \text{HP-α-CD} > \text{β-CD}.$$

As follows from the data in Fig. 1, *a*, the AA (HA^-) ionized by the first step forms no complexes with HP-CDs. Analysis of the h_{xy} values (see Table 1) shows that the interaction of HA^- with the HP-CDs under study is characterized by the same regularities as those revealed for their complex formation with H_2A: $\text{HP-β-CD}_{(4.2)}$ and $\text{HP-β-CD}_{(2.1)}$ form complexes with H_2A, and the complex formation with $\text{HP-β-CD}_{(4.2)}$ is energetically more favorable. Similarly, the interaction of $\text{HP-β-CD}_{(4.2)}$ with HA^- is more favorable from the viewpoint of enthalpy, and this system has the highest and most negative enthalpic virial coefficient (see Table 1). The interaction between HP-α-CD and HA^- has a positive h_{xy} value, indicating that the endothermic effects related to dehydration processes prevail.

The solute—solute and solute—solvent interactions are two competitive processes. The anionic form of AA forms no complexes with HP-CDs because of a stronger interaction between HA^- and water molecules.

It should be mentioned in conclusion that the selectivity of complex formation of HP-α- and HP-β-CDs with AA is determined by the size of the macrocyclic cavity, the presence of a hydroxypropyl substituent, and acidity of the medium, which determines the predomination of the molecular or ionized form of the acid in an aqueous solution. The degree of substitution of HP-β-CDs exerts no substantial effect on their interactions with ascorbic acid.

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