Thermodynamic parameters of the interaction of cryptand[222] with amino acids in water at 298.15 K

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The interaction of cryptand[222] with amino acids in water, which is weak for most amino acids and controlled by the solvent effect in the case of non-polar amino acids, was studied at 298.15 K by the calorimetric method. Cryptand[222] undergoes selective complex formation with some polar and aromatic amino acids. The thermodynamic functions and equilibrium constants of complex formation of the macrocyclic ligand with L-histidine, L-threonine, and L-glutamine were calculated.

Key words: amino acids, cryptand[222], thermodynamics of complex formation.

It is known that cryptands increase the solubility of amino acids in organic solvents.[1] Therefore, they are used in membrane transport,[2,3] which includes processes of "molecular recognition." The concept of the development of novel high-efficiency medicines is based on the ability of macrocycles to readily penetrate through biological membranes. Since amino acids are the components of antibiotics and play an important role in living organisms, the study of specific features of their interaction with such a macrocyclic carrier as cryptand[222] (CR) is of special interest for investigations of complex processes of membrane transport.

In this work, which is a continuation of our previous studies,[4] we considered the specific features of the interaction of CR with amino acids in water at 298.15 K on the basis of calorimetric data.

Experimental

Amino acids (Reanal, Hungary) were additionally purified by recrystallization from water—alcohol solutions and dried in a vacuum desiccator at 350 K for 72 h to a constant weight.

Cryptand[222] was synthesized in the IREA (Institute of Reactants, Moscow, Russia). The purity of this compound was determined on a DSK calorimeter by the method of sample melting and amounted to ≥99.1 mol.%. Before calorimetric experiments, the CR was dried *in vacuo* at 350 K for 72 h. Solutions were prepared with bidistilled water. The pH of solutions before and after calorimetric experiments was determined on an I-130 ionometer.

Calorimetry. A weighed sample of crystalline CR ($(1.10\pm0.01)\cdot10^{-2}$ g) was dissolved in water and in an aqueous solution of an amino acid whose concentration was varied from 0.05 to 0.25 mol kg^{-1}. Thermal effects of dissolution were measured on an isothermic calorimeter with a cell volume of 17 mL at 298.150±0.005 K. The calorimetric installation consists of a calorimetric cell containing a temperature gage, a heater, an ampule holder, a mercury seal to prevent heat losses, systems of air and liquid thermostatting, and a system for measuring the temperature in the cell and thermostat and maintaining the isothermic regime of the work.

To estimate the accuracy and reliability of the operation of the calorimetric installation, we carried out a series of measurements of the enthalpy of dissolution of KCl in water at 298.15 K. The values obtained agree well with the reference data. For example, the thermal effect of dissolution of KCl in water was 17265±96 J mol^{-1} at a molality of KCl of $4.368\cdot10^{-3}$ mol kg^{-1}, which agrees with the published data[5] (17339 J mol^{-1}); therefore, the measurements can be considered as reliable.

Processing of experimental data

Information about the interactions of molecules of the substances in a solution can be obtained by processing of the experimental data by the McMillan—Mayer theory[6] using polynomials with respect to powers of the concentration, whose coefficients reflect the contributions from the interaction of molecules of the components of the mixture between each other[7]:

$$\Delta H_{tr} = 2m_y h_{xy} + 3h_{xyy}m_y^2 + 3h_{xxy}m_x m_y + \ldots \quad (1)$$

or

$$\Delta H_{tr}/m_y = 2h_{xy} + 3h_{xyy}m_y + 3h_{xxy}m_x + \ldots \quad (2)$$

Here m_y and m_x are the molalities of the amino acid and CR, respectively; h_{xy}, h_{xyy}, and h_{xxy} are the enthalpy coefficients of pair and triple interactions; ΔH_{tr} is the enthalpy of transfer of CR from water to aqueous solutions of amino acids calculated from the equation

$$\Delta H_{tr} = \Delta H_s(AA) - \Delta H_s(H_2O),$$

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based on the enthalpy of dissolution of CR in water ($\Delta H_s(H_2O)$) and that in a solution of amino acid ($\Delta H_s(AA)$) obtained directly from the calorimetric experiment. Since we used dilute solutions and the concentration of CR was constant and very low ($m_x \to 0$), the last term in Eqs. (1) and (2) can be neglected. The h_{xy} coefficients calculated by the linear least-squares method on the basis of Eq. (2) are presented in Table 1.

In the study of the interactions of CR with amino acids in aqueous solutions, we assumed that the interactions between the molecules of these compounds can be either weak or strong. To find the strong interactions resulting in the complex formation, the dependence $\Delta H_s(AA) = f(m_y/m_x)$ was plotted for each system (Fig. 1). In the case of formation of the CR—amino acid complex, after a strong increase the $-\Delta H_s(AA)$ values should become unchanged with further increase in the m_y/m_x ratio (see Fig. 1, curve *2*). Thus, we revealed the formation of the complexes in the CR—L-histidine, CR—L-glutamine, and CR—L-threonine systems, for which the standard thermodynamic parameters ($\log K$, $\Delta G_c°$, $\Delta H_c°$, and $\Delta S_c°$) of the process were calculated and presented in Table 2. A hypothetical 1 *M* ideal solution of the ligand was accepted as the standard state. For calculation of the equilibrium constants ($\log K$) and changes in the standard enthalpies ($\Delta H_c°$) of complex formation from the obtained ΔH_{tr} values, we used the HEAT computer program,[8] in which the search for unknown parameters ($\log K$ and $\Delta H_c°$) is reduced to the numerical minimization of the F functional by the desired parameters:

$$F = \sum_{i=1}^{N} w_i (\Delta H_{i,exp} - \Delta H_{i,calc})^2,$$

where ΔH_i is the thermal effect of the i-th reaction, N is the number of experiments, w_i are the weighted factors calculated as $w_i = A/(\delta\Delta H_i)^2$ (where A is the coefficient chosen from the condition $\sum w_i = N$, *i.e.*, the sum of the weights is equal to the number of experiments; and $\delta\Delta H_i$ is the absolute error of the measurement of ΔH_i).

Amino acids and CR can participate in the acid-base equilibria in aqueous solutions:

$$NH_3^+CH(R)COOH \rightleftharpoons NH_3^+CH(R)COO^- + H^+, \quad (3)$$

$$NH_3^+CH(R)COO^- \rightleftharpoons NH_2CH(R)COO^- + H^+, \quad (4)$$

$$CR + H^+ \rightleftharpoons CRH^+, \quad (5)$$

$$CRH^+ + H^+ \rightleftharpoons CRH_2^{2+}, \quad (6)$$

$$H_2O \rightleftharpoons H^+ + OH^-. \quad (7)$$

Based on the equilibrium constants of reactions (3)—(7) and the pH of the solutions after the calorimetric experiment (see Table 1), we can assume the occurrence of one or another process of (3)—(7) and its influence on the

Table 1. Enthalpy coefficients of interactions of CR with amino acids in water (h_{xy}) at 298.15 K

Amino acid	h_{xy}* /kJ kg mol^{-2}	pH
Glycine	2.07 (0.19)	7.97
L-α-Alanine	9.52 (0.15)	7.92
L-Valine	13.08 (0.18)	7.00
L-Leucine	2.17 (0.34)	8.06
L-Isoleucine	11.57 (0.22)	8.03
DL-Methionine	16.57 (0.13)	7.88
L-Phenylalanine	39.22 (0.78)	7.82
L-Proline	4.10 (0.09)	8.41
L-Histidine	−26.29 (0.80)	7.90
L-Threonine	24.03 (0.30)	7.35
L-Serine	25.76 (0.12)	7.79
L-Asparagine	−46.98 (0.32)	6.88
L-Glutamine	26.98 (0.28)	7.82

* The errors calculated from the Student criterion taking into account the 95% confidence interval are presented in parentheses.

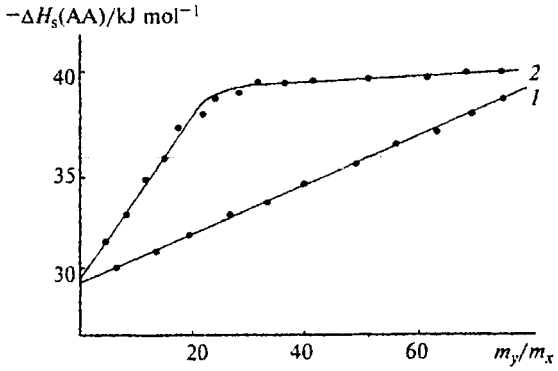

Fig. 1. Enthalpy of dissolution of CR in an aqueous solution of amino acid ($\Delta H_s(AA)$) as a function of the ratio of molalities of the amino acid (m_y) and cryptand (m_x): *1*, complex formation does not occur; *2*, a complex is formed.

Table 2. Thermodynamic functions of complex formation of CR with amino acids in water at 298.15 K

Amino acid	$\log K$	$\Delta G_c°$	$\Delta H_c°$	$\Delta S_c°$
		kJ mol^{-1}		/J mol^{-1} K^{-1}
L-Histidine	1.41	−8.0	−37.9	−100.0
	(0.33)	(1.6)	(7.5)	(20.0)
L-Threonine	1.94	−11.1	−2.6	28.2
	(0.35)	(2.1)	(0.5)	(5.4)
L-Glutamine	1.88	−10.7	−2.4	27.9
	(0.30)	(1.7)	(0.4)	(4.4)

Note. The values of the 95% confidence interval are given in parentheses.

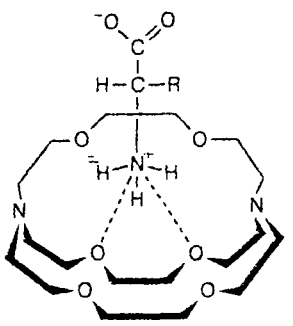

Fig. 2. Scheme of formation of the CR—amino acid complex in alcohol solutions (according to Ref. 12).

experimental values of the thermal effects. To eliminate the contribution of processes (3)—(7) to the desired $\log K$ and ΔH_c° values, the equilibrium constants and thermal effects of "side" reactions (3)—(7) were additionally introduced into the calculation program.[9,10]

Results and Discussion

According to the published data,[11,12] the interaction of CR with amino acids in methanol and ethanol is accompanied by the formation of 1 : 1 complexes due to the penetration of the terminal NH_3^+ group of the amino acid into the cryptand cavity and the formation of one hydrogen and two electrostatic bonds[11] (Fig. 2).

In our case, the reaction medium is water, which possesses stronger solvating properties than alcohols. This favors the situation in which the interactions of cryptand with amino acids are weak and not always accompanied by complex formation. In addition, the interaction of amino acids with CR in an aqueous medium is accompanied by the protonation of one or two N atoms of the macrocyclic ligand ($\log K_1$ = 9.71 and $\log K_2$ = 7.31).[10] It has been established[13] by the study of the structure of protonated CR molecules in aqueous and methanol solutions that in water the H^+ ions are located inside the macrocyclic cavity and in methanol they are outside it. It can be assumed that in the case of a protonated macrocycle, H^+ ions create steric hindrances for the efficient penetration of amino acid into the macrocyclic cavity, thus preventing complex formation. Therefore, the interaction of cryptand with the amino acids under study does not result, in most cases, in the formation of complexes, except for the CR—L-histidine, CR—L-threonine, and CR—L-glutamine systems.

The complex formation of CR with L-histidine (L-His) is due, in our opinion, to the specific features of the structure of the lateral chain of histidine. Its main lateral group serves as a proton acceptor; therefore, a bond can be formed between the N atom in the lateral chain of L-His and the H atom of the monoprotonated form of cryptand. Calculation of the equilibrium composition for this system, using the computer program based on the modified Brinckley method,[14] showed that the monoprotonated form of the macrocyclic ligand ($\alpha(CRH^+)$ = 37% and $\alpha(CRH_2^{2+})$ = 18%) predominates in the solution. The pH measured after the calorimetric experiment is ~8.0 (see Table 1), which also confirms the assumption that the equilibrium of the protonation of cryptand is shifted toward the formation of CRH^+, and histidine interacts with the monoprotonated ligand. Therefore, we attribute the thermodynamic functions of complex formation (see Table 2) to a process which is described by the following equation:

$$NH_3^+CH(R)COO^- + CRH^+ \rightleftharpoons$$

$$\rightleftharpoons NH_3^+CH(R)COO^- \cdot CRH^+ + \Delta H_c^\circ. \qquad (8)$$

It was similarly confirmed that Eq. (8) is valid for the complex formation of CR with polar amino acids, L-threonine and L-glutamine. Since L-threonine and L-glutamine molecules contain the polar OH and NH_2 groups capable of forming H bonds with molecules of the ligand and solvent, it is possible that the interaction is accompanied, in these cases, by the reorganization of the solvent (the cleavage of old and formation of new H bonds).

Weak interaction of CR with amino acids, which is not accompanied by complex formation, can be described using the enthalpy coefficients of pair interactions h_{xy} (see Table 1), which provide information on a relationship between the solvation of molecules of the reactants and their ability to interact with each other.[15]

Nonpolar amino acids (glycine, L-alanine, L-valine, and DL-methionine) are characterized by low positive

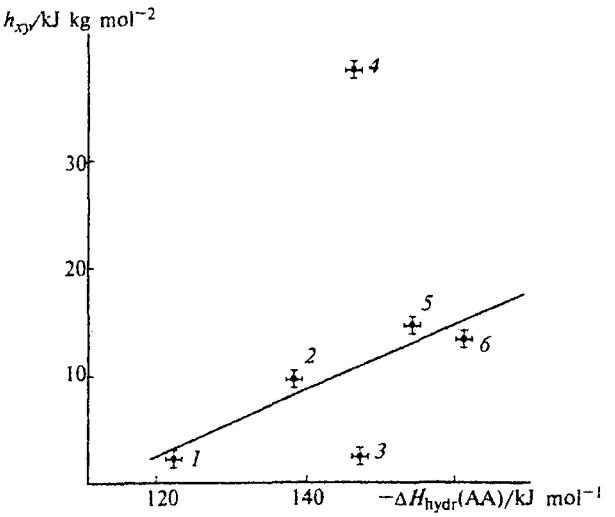

Fig. 3. Dependences of the coefficients of pair interactions of CR with amino acids in water (h_{xy}) on the enthalpy of hydration of amino acids[17] (ΔH_{hydr}(AA)): *1*, glycine; *2*, L-α-alanine; *3*, L-leucine; *4*, L-phenylalanine; *5*, DL-methionine; and *6*, L-valine.

h_{xy}. This indicates that a decrease in the hydration of molecules of dissolved substances (the endothermic effect) makes the main contribution to the interaction of these amino acids with CR. The predominant solvent effect in the processes is confirmed by a linear correlation between the tendency of amino acids (glycine, L-α-alanine, L-valine, and DL-methionine) to interact with cryptand and their solvate (hydrate) parameters (Fig. 3). L-Phenylalanine and L-leucine do not obey the presented linear dependence $h_{xy} = f(\Delta H_{hydr}(AA))$. Therefore, it can be said that the solvent does not play a considerable role in the interaction of these amino acids with CR. It is seen in Fig. 3 that higher h_{xy} coefficients correspond to more negative enthalpies of hydration of amino acids ($\Delta H_{hydr}(AA)$). Thus, it is confirmed that the more strongly solvated the molecules of substance, the lower its ability to interact with other particles in the solution.[15]

L-Leucine and L-isoleucine are structural isomers, and their h_{xy} coefficients noticeably differ. The number of water molecules that form a clathrate-like network around the alkyl group of L-leucine (L-Leu) is lower[16] than that for L-isoleucine. In this connection, the contribution from the dehydration of leucine to the enthalpy coefficient of its interaction with CR is lower than that for isoleucine, which results in a lower h_{xy} for the CR—L-Leu system.

The interaction of CR with such a polar amino acid as L-asparagine is characterized by a high negative h_{xy} coefficient (see Table 1). Calculation of the equilibrium composition of this system showed that the formation of biprotonated cryptand (the exothermic effect) plays an important role in this case, which explains the abnormally negative value of h_{xy}. The pH of 6.88 for this system also indicates shift of the equilibrium toward the formation of LH_2^{2+}.

Thus, CR selectively interacts with amino acids, which can be an example of the ability of a macrocyclic ligand for molecular recognition of various "guest"-molecules in aqueous solutions.

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