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INCLUSION COMPOUNDS IN WATER: THERMODYNAMICS OF THE INTERACTION OF CYCLOMALTOHEXAOSE WITH AMINO ACIDS AT 25°*

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ABSTRACT

The interaction in water of some α-amino acids (alanine, 2-aminobutyric acid, valine, norvaline, leucine, isoleucine, norleucine, methionine), *N*-acetyl-L-valinamide, and *N*-acetyl-L-leucinamide with cyclomaltohexaose (α-cyclodextrin) has been studied by calorimetry at 25°. The results indicate that inclusion occurs only with those amino acids bearing longer alkyl chains, the complexes formed are weak, and α-cyclodextrin does not discriminate between the D and L forms of the amino acids. The association constants are smaller than those for alcohols having comparable side-chains; hence, the presence of the zwitterion inhibits the inclusion process.

INTRODUCTION

Complexes formed, in solution[1–8] or in the solid state[9,10], by cyclomalto-oligosaccharides (cyclodextrins, CDs) with a variety of organic substances have technological, pharmaceutical, and biological applications[10]. However, despite the extensive literature on these complexes[11–19], much remains to be learned of the forces involved in these interaction processes, the changes in hydration and conformation, and the kinetics of the inclusion processes.

In the solid state, the smallest of the cyclodextrins (cyclomaltohexaose, αCD) has two molecules of water in the cavity, hydrogen-bonded to each other and to two glucopyranose rings[12]. When the guest molecule penetrates into the cavity, these water molecules are displaced and the hydration cosphere is modified. In turn, the conformation of the αCD relaxes[20].

We have studied binary, aqueous solutions of αCD[21] and its interaction with alcohols[21], alkylureas[22], and other organic molecules[23,24]. The behaviour of the macrocyclic αCD is different from that of other saccharides, and resembles that of

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the higher polyhydric alcohols[25,26]. We now report on the interaction of αCD with L-alanine, DL-2-aminobutyric acid, D-valine, D- and L-norvaline, L-leucine, L-isoleucine, D- and L-norleucine, L-methionine, *N*-acetyl-L-valinamide (NAVA), and *N*-acetyl-L-leucinamide (NALA), using calorimetry at 25°.

EXPERIMENTAL

Materials. — αCD and the amino acids employed were crystalline commercial products and were used without further purification. *N*-Acetyl-L-valinamide (NAVA) and *N*-acetyl-L-leucinamide (NALA) were prepared as reported[27]. Solutions were prepared freshly by weight before each run, using doubly distilled water. The concentration of αCD was determined from the $[\alpha]_D$ value.

Calorimetry. — An LKB 10700-1 flow microcalorimeter was used at 25°. The values of the experimental heats were obtained from

$$\Delta H = -(dQ/dt)/P_w \qquad (1)$$

where dQ/dt is the heat flux and P_w is the total mass-flow rate of water through the calorimeter. Details of the calorimetric experiments have been reported extensively[21–24,28–33].

Treatment of the data. — Where inclusion does not occur, the calorimetric data were treated according to the McMillan–Mayer theory for real solutions[34–36]. Thus, each of the thermodynamic excess properties, J^E, can be defined, independently of the process occurring (self association of a solute, association between different species, weak non-bonding interactions, *etc.*), by equation 2,

$$J^E = J - J_w^\circ - \Sigma m_x \overline{J_x^\circ} - J^{ID} \qquad (2)$$

where J and J^E are the absolute and the excess values, respectively, of each property of a solution containing 1 kg of water and m_x moles of each solute species, J_w° is the standard property of 1 kg of water, $\overline{J_x^\circ}$ is the limiting partial molal quantity of each solute, and J^{ID} its ideal part. J^E (excess enthalpy, free energy, entropy, volume, *etc.*) can be represented as a power expansion of the molality. For instance, for enthalpy (the ideal value of which is zero), it results in

$$H^E = \Sigma\Sigma h_{xy} m_x m_y + \Sigma\Sigma\Sigma h_{xyz} m_x m_y m_z + \cdots \qquad (3)$$

The free energy and entropy coefficients are related to the h_{xy} coefficients by the classical relationships

$$h_{xy} = \left[\frac{\partial(g_{xy}/T)}{\partial(1/T)} \right]_p \qquad (4)$$

and

$$h_{xy} = g_{xy} + Ts_{xy} \tag{5}$$

The values of the experimental heats of dilution of binary aqueous solutions can be used to fit the expansion

$$(1/m)\Delta H^{dil}(m_i \rightarrow m) = h_{xx}(m - m_i) + h_{xxx}(m^2 - m_i^2) + \cdots \tag{6}$$

where ΔH^{dil} (J per kg of solvent in the final solution) is the heat of dilution from the initial (m_i) to the final molality (m). Knowledge of the coefficients of equation 6 is necessary in order to determine the contributions to the total enthalpy changes from the dilution of each of the solutes during the mixing of two binary solutions or during the dilution of a ternary solution.

The enthalpy of formation of a 1:1 complex (αCD–L) between αCD and a guest molecule (L) or, in general, the enthalpy of interaction between solutes[35], ΔH^*, is related to the heat of mixing of two binary solutions, ΔH^{mix}, and to the heats of dilution experienced by the two solutes x and y, ΔH^{dil}, as follows[28]

$$\Delta H^* = \Delta H^{mix}[(m_{ix})(m_{iy}) \rightarrow (m_x, m_y)] - \Delta H^{dil}(m_{ix} \rightarrow m_x) - \Delta H^{dil}(m_{iy} \rightarrow m_y) \tag{7}$$

The standard molar enthalpy of inclusion, ΔH_B°, of the guest molecule is obtained simply from the relation

$$\Delta H_B^\circ = \Delta H^*/m_{\alpha CD \cdot L} \tag{8}$$

where $m_{\alpha CD \cdot L}$ is the aquomolality of the adduct formed. In the presence of a large excess of the guest molecule, $m_{\alpha CD \cdot L} \rightarrow m_{\alpha CD}$, and at saturation

$$\Delta H_B^\circ = (\Delta H^*/m_{\alpha CD})_{sat}. \tag{9}$$

The enthalpy of inclusion of one guest molecule by αCD has the same significance as the standard molar enthalpy of binding per independent site for the binding of small molecules to proteins or biopolymers, and the same symbolism[37] is used here. ΔH^*, normalized for the total molality, $m_{\alpha CD}$, can be related to the actual molality of the guest molecule, m_L^f, to the saturation value of ΔH^*, and to the apparent association constant, K_B', through the following relationship[37]

$$\frac{\Delta H^*}{m_{\alpha CD}} = \frac{K_B' m_L^f \Delta H_B^\circ}{1 + K_B' m_L^f}. \tag{10}$$

Equation *10* can be rewritten in a linear form that is useful for fitting the data, as follows

$$\frac{m_{\alpha CD}}{\Delta H^*} = \frac{1}{\Delta H_B^\circ} + \frac{1}{\Delta H_B^\circ K_B' m_L^f}. \qquad (11)$$

For each value of ΔH^*, the actual concentration of the guest molecule is given by

$$m_L^f = m_L - \Delta H^*/\Delta H^*(\text{sat}) m_{\alpha CD} \qquad (12)$$

where m_L is the total stoichiometric molality of the guest. ΔH_B° and K_B' are obtained from equations *11* and *12* by an iterative least-squares method. The condition of best fit was assumed to be achieved when the difference between two successive values of ΔH_B° was <2%. The values of ΔG_B° and $T\Delta S_B^\circ$ were then obtained from

$$\Delta G_B^{\circ\prime} = -RT \ln K_B'; \qquad T\Delta S_B^{\circ\prime} = \Delta H_B^\circ - \Delta G_B^{\circ\prime} \qquad (13)$$

The absence of any information about the activity coefficients leads to association parameters that are not defined exactly. Only an apparent constant can be determined and, consequently, the standard free energy and entropy suffer from the same limitation.

When the formation of a complex does not occur, the above association model is inadequate to describe the system studied: the iterative least-squares do not converge and saturation is not detected easily. Information about the weak interactions of hydrated solutes can be obtained then from ΔH^{**} (*J* per kg of solvent)[28], the difference between the heats of dilution of ternary and binary solutions given by

$$\Delta H^{**} = \Delta H^{dil}[(m_{ix},m_{iy}) \rightarrow (m_x,m_y)] - \Delta H^{dil}(m_{ix} \rightarrow m_x) - \Delta H^{dil}(m_{iy} \rightarrow m_y). \qquad (14)$$

Expressing the dilution enthalpies as a function of the self- and cross-interaction coefficients, the following relation is obtained

$$\frac{\Delta H^{**}}{m_y(m_x - m_{ix})} = 2\,h_{xy} + 3\,h_{xxy}(m_{ix} + m_x) + 3\,h_{xyy}(m_{iy} + m_y) + \cdots. \qquad (15)$$

The values of the coefficients appearing in equation *15* were evaluated by a least-squares method: the polynomial of highest degree was chosen, the coefficients of which still exceeded their own 95% confidence limits.

RESULTS

In Table I, *h* coefficients are shown for binary aqueous solutions of αCD, and compared with those of some selected saccharides and polyhydric alcohols[25,26,29,38,39]. According to the above-mentioned criterion, only the second coefficient is significant, due to the small range of concentrations because of the low solubility of αCD.

TABLE I

SELECTED VALUES OF THE COEFFICIENTS[a] OF THE EXCESS ENTHALPIES OF BINARY AQUEOUS SOLUTIONS OF SOME SACCHARIDES AND POLYOLS AT 25°

Solute	h^b_{xx}	h^c_{xxx}
αCD[d]	−3920 (65)	—
D-Galactose[e]	133 (8)	—
D-Mannose[e]	207 (14)	−14 (5)
D-Glucose[f]	343 (10)	−13
D-Fructose[e]	264 (18)	−7 (4)
Lactose[e]	506 (32)	—
Sucrose[f]	577 (6)	−33 (8)
Maltose[g]	483 (7)	—
Cellobiose[g]	756 (14)	—
Raffinose[e]	811 (50)	—
Melezitose[g]	607 (25)	—
Glycerol[h]	251	—
Erythritol[h]	358 (22)	−11 (10)
Ribitol[h]	295 (5)	—
D-Arabinitol[h]	187 (3)	—
Xylitol[h]	80 (11)	5 (4)
D-Mannitol[h]	66 (12)	20 (12)
D-Glucitol[h]	−11 (5)	24 (3)
Galactitol[h]	−132 (50)	222 (138)
Perseitol[h]	−299 (20)	122 (58)
myo-Inositol[i]	−800 (29)	188 (28)

[a]Figures in parentheses are the 95% confidence limits. [b](J/mol)/(mol/kg). [c](J/mol)/(mol/kg)^2. [d]Ref. 21. [e]Ref. 29. [f]Ref. 38. [g]Ref. 39. [h]Ref. 25. [i]Ref. 26.

As can be seen, the h_{xx} values for the saccharides are positive and increase at increasing molecular weight. The negative h_{xx} value for αCD can be compared with those of the higher polyhydric alcohols which become negative with increasing molecular weight and depend on their stereochemistry.

The pair-wise interaction coefficients for NALA[40] and for the amino acids under examination[41] are reported in Table II. These data are from the literature with the exception of those for methionine, the second enthalpic coefficient of which was determined from the experimental heats of dilution through equation *6*. The h coefficients of the substances were used to calculate the ΔH^* and ΔH^{**} values.

In Table III, the thermodynamic parameters are reported for the interactions of αCD with norvaline, norleucine, methionine, and *N*-acetyl-L-leucinamide. The ΔH°_B and K'_B values were evaluated through the iterative least-squares method. The enthalpies are negative and increase with increasing length of the alkyl chain. The substitution of a methylene group by sulphur, as in methionine, lowers the association constant and the enthalpy becomes more negative with respect to that of norleucine. Also reported in Table III, for purposes of comparison, are the values of the thermodynamic parameters relative to some alcohols[21] and alkylureas[22].

TABLE II

VALUES OF THE ENTHALPIC INTERACTION COEFFICIENTS[a] FOR THE AMINO ACIDS EMPLOYED AND FOR *N*-ACETYL-L-LEUCINAMIDE AT 25°

Solute[b]	h^c_{xx}
L-Alanine	215 (4)
DL-2-Aminobutyric acid	528 (33)
D-Valine	846 (34)
D-Norvaline	927 (25)
L-Norvaline	927 (25)
L-Leucine	1269 (18)
L-Isoleucine	1307 (27)
D-Norleucine	1424 (24)
L-Norleucine	1424 (24)
L-Methionine	576 (22)
N-Acetyl-L-leucinamide	1969 (24)

[a]Figures in parentheses are the 95% confidence limits. [b]Except for methionine, the data for NALA are from ref. 40, and those for the amino acids are from ref. 41. [c](J/mol)/(mol/kg).

TABLE III

THERMODYNAMIC PARAMETERS FOR THE ASSOCIATION BETWEEN αCD AND VARIOUS GUEST MOLECULES AT 25°

Guest	K'_B[a,b]	$-\Delta H^\circ_B$[b,c]	$-\Delta G^\circ_B$[c,d]	$T\Delta S^\circ_B$[c,e]
D-Norvaline	12(5)	3(1)	6.2(1.1)	3.2(2.2)
L-Norvaline	12(7)	2(1)	6.2(1.6)	4.2(2.6)
D-Norleucine	46(7)	9.3(0.8)	9.5(0.3)	0.2(1.1)
L-Norleucine	46(2)	8.9(0.3)	9.5(0.1)	0.6(0.4)
L-Methionine	9(2)	14(3)	5.4(0.6)	−8.6(3.6)
L-NALA	20(4)	6.1(0.8)	7.4(0.5)	1.3(1.3)
Ethanol[f]	6.7	2.5	4.7	2.2
1-Propanol[f]	27	6.1	8.2	2.1
1-Butanol[f]	99.9	9.9	11.4	1.5
sec-Butyl alcohol[f]	28.4	9.0	8.3	−0.7
Isobutyl alcohol[f]	21.9	9.4	7.7	−1.7
Monoethylurea[g]	2.8	7.4	2.6	−4.8
Monopropylurea[g]	12.8	11.2	6.3	−4.9
Monobutylurea[g]	125.6	13.0	12.0	−1.0

[a]kg/mol. [b]Figures in parentheses are the standard deviations as obtained by fitting the data to equation *11*. [c]kJ/mol. [d]Errors are half the range of $\Delta G^\circ_B{}'$ calculated from the upper and lower error in K'_B. [e]Errors are the sum of the errors on free energy and enthalpy. [f]Ref. 21. [g]Ref. 22.

NALA could be considered as a tridentate ligand and, in the αCD/NALA system, there is the possibility of binding through the leucyl, acetyl, or amide moieties. However, when calorimetry was carried out at constant [αCD], then, at constant [NALA], the equality of the saturation enthalpies indicated a 1:1 complex.

TABLE IV

ENTHALPIC INTERACTION COEFFICIENTS[a] FOR AQUEOUS SOLUTIONS OF αCD AND VARIOUS AMINO ACIDS AT 25°

Substance	h_{xy}^b
L-Alanine	−548 (272)[c]
DL-2-Aminobutyric acid	476 (41)
D-Valine	1432 (92)
L-Leucine	−9540 (494)
L-Isoleucine	−20626 (1800)[c]

[a]Figures in parentheses are the 95% confidence limits. [b](J/mol)/(mol/kg). [c]The fitting of the data required the triplet coefficients (6.5 ±2.2) × 10^4 and (1.4 ±0.7) × 10^4 (J/mol)/(mol/kg)^2 for isoleucine and (2.6 ±0.9) × 10^3 and −(1.5 ±0.8) × 10^3 for alanine.

In Table IV, the cross-enthalpic interaction coefficients are reported for the system involving αCD and alanine, 2-aminobutyric acid, valine, leucine, and isoleucine. On mixing binary solutions at constant [αCD], the formation of inclusion complexes did not occur. Hence, the calorimetric data were treated according to equations *14* and *15*. The enthalpic cross-interaction coefficients were negative and high for isoleucine and leucine, positive and low for valine and aminobutyric acid, and negative and low for alanine.

DISCUSSION

Aqueous solutions of αCD. — Calorimetric data, treated in terms of excess enthalpies and then in terms of enthalpic interaction coefficients, gave[21] the negative h_{xx} coefficient for αCD reported in Table I and opposite in sign of those of mono- and oligo-saccharides. Studies of polyhydric alcohols[25,26] showed a trend of the excess enthalpy coefficients from positive to negative, depending on the molecular weight and on the stereochemistry, reaching the highest negative value with *myo*-inositol[33]. There is a less clear, but similar trend for the virial coefficients of the excess free energies[42]. For αCD and *myo*-inositol, negative values can be calculated (−1430 and −260 (J.mol^{-1})(mol.kg^{-1}), respectively[43,26]) for g_{xx} from the experimental osmotic coefficients. From these data and from the values of h_{xx} reported in Table I, the following values were obtained for the entropic contributions Ts_{xx} at 25°: for αCD −2490 and for *myo*-inositol −540 (J.mol^{-1})(mol.kg^{-1}). According to a classification proposed for non-electrolytes on the basis of the signs of these coefficients, αCD and *myo*-inositol should be considered, at least at 25°, as "structure breakers" of the water organization. In fact, this kind of solute shows negative coefficients for the properties considered.

The coefficients become less positive (or more negative) in a series of isomeric polyhydric alcohols if the hydrophilic and hydrophobic domains of the surfaces of the solutes are relatively separated[25]. The co-existence of many polar

groups, each capable of binding strongly to water, on a given domain of the solute molecule can disrupt the ice-like structure of water, and the properties of the corresponding aqueous solutions will resemble those of such chaotropic solutes as urea.

Miyajima *et al.*[43] proposed a dimerization model in order to rationalize the osmotic coefficients of aqueous solutions of αCD and γCD, and calculated for the former a self-association constant of 0.66 kg/mol. We have shown that this kind of model, which can be used for solutions having negative deviations from Henry's law, may give results devoid of physical meaning, as with aqueous solutions of urea and thiourea[28,44]. Significant aggregation of solute molecules in solution requires the existence of positive free energy and enthalpy third coefficients[45]. For αCD, these coefficients cannot be detected up to the solubility limits[21,43].

A model based on the coalescence of the hydration cospheres of structure-breaking solutes can explain better the existence of negative and relatively small values of the three h_{xx}, g_{xx}, and Ts_{xx} coefficients[32,40]. According to this model, the solute–solute interactions, at least in dilute aqueous solutions of simple organic molecules, are overwhelmed by the variation of solute–solvent and solvent–solvent interactions with respect to the standard state (infinitely dilute solution). Then, for αCD, the negative values of the coefficients account for the perturbations of the external hydration cosphere.

Contrasting suggestions were made by Miyajima *et al.*[43] for other properties of CD solutions, such as the apparent limiting molar volume, the viscosity B-coefficient (which can be considered as a measure of the hydrodynamic volume), and the dependences of these properties on the temperature. In particular, from the dB/dT values, these authors hypothesized that CDs are structure makers. However, these parameters express the interactions of the solute with the solvent at infinite dilution. Since the hydration of αCD is complex because of the prevailingly hydrophobic cavity, it is possible that these parameters depend essentially on this kind of structured water.

Studies of the structure of the αCD-hexahydrate[12] have shown that, in the crystal form, two water molecules are located inside the cavity, hydrogen-bonded to each other and to two oxygen atoms. The macrocycle is in a "tense" conformation and is less symmetrical than that observed when a molecule other than water fits into the cavity. However, the internal hydration cosphere should remain almost unchanged on interaction with other hydrated molecules of αCD, a process involving only the external, mainly destructured shell of water. Hence, it is possible that limiting and excess properties give only apparently contrasting results.

The formation of inclusion compounds. — The complexes formed from αCD and norleucine, norvaline, methionine, and NALA are weak, as for most of the adducts involving small non-electrolytes. They are even weaker than those formed with alcohols and alkylureas. For alcohols, inclusion is possible even for isobutyl and *sec*-butyl alcohol, but the apparent association constants are about four times lower than that of 1-butanol, probably because of steric hindrance. For the amino acids examined, the presence of the zwitterion lowers the value of the association

by ~50%, *e.g.*, ~100 kg/mol for 1-butanol and ~50 kg/mol for norleucine, which has a similar alkyl chain. The effect of the zwitterion is particularly evident on comparing isoleucine and leucine with *sec*-butyl and isobutyl alcohol, respectively. No significant inclusion could be detected for the amino acids. For norvaline, the side chain of which is similar to that of 1-propanol, association is possible even though characterized by a low constant. Hence, it can be inferred that, for amino acids, inclusion is possible only for those that have normal and longer alkyl chains. Another destabilizing effect on the formation of the complex is the substitution of a methylene group by sulphur, as in methionine. Sulphur disturbs the hydrophobic hydration of the alkyl chain and reduces the value of the constant.

The ΔH_B° characterizing the formation of these inclusion complexes is always negative, as for all the complexes reported in the literature. In general, the value of the enthalpy is small and it is the sum of several and contrasting effects. The hydration cospheres of both solutes are modified greatly during the association. For αCD, water molecules in the cavity are displaced to the bulk, whereas there are rearrangements in the external hydration cosphere. At the same time, the hydration shell of the hydrophobic part of the guest molecule loses some water molecules on entering the cavity. These two effects are endothermic. However, there are other effects which make the value of the enthalpy negative, namely, the reconstitution of the hydration shell of the complex, dipole-induced dipole and "host–guest" interactions, and the decrease in energy when a hydrophobic residue fills the cavity. The small value obtained for the substances bearing the shortest alkyl chains is due to the poor fitting of the cavity to the hydrophobic residue: interactions cannot be optimized if the alkyl chain is affected too much by the vicinity of the zwitterion, as in norvaline (ΔH_B° ~2 kJ/mol). The same occurs for ethanol, monoethylurea, and other small molecules. Of the substances examined, the highest value for the enthalpy was obtained for methionine. Sulphur has a structure-breaking action, and the hydration cosphere of the alkyl chain then has structured and destructured regions. When inclusion occurs, water relaxed from the destructured domain gives a further negative contribution to the value of the enthalpy.

Hydrophobic interactions do not always play the major role in the formation of inclusion complexes. The values of $\Delta S_B^\circ{}'$ are positive or negative and a plot of ΔH_B° against $\Delta S_B^\circ{}'$ for many complexes is almost linear[46]. Hence, there is an enthalpy–entropy compensation, a phenomenon frequently observed in water, and ascribed to the modifications experienced by the solvent in the hydration cospheres of the interacting substances. The cratic contribution is about 10 kJ/mol at 25°, and this quantity must be added to the values reported for evaluating the changes in entropy involved primarily in the expulsion of water from the hydration cospheres of both solutes, which, in turn, overwhelm the losses of degree of freedom experienced by the guest molecule.

One of the most important applications of CDs relies on their ability to discriminate chiral forms of optically active substances[10]. Unfortunately, αCD

cannot distinguish between D and L forms of norleucine and norvaline, probably because of the low values of the binding constants.

In considering the structure of the αCD–*N*-acetyl-L-leucinamide inclusion complex, the amide group can be neglected since it is similar to a urea residue, which is not included[22]. In its crystal[47], NALA assumes a conformation that leaves the leucyl residue unhindered by the remainder of the molecule. Hence, in the absence of the zwitterion, this leucyl residue should behave as the alkyl residue of isobutyl alcohol and this is indicated by the values of the constants (22 for isoBuOH and 20 kg/mol for NALA). The acetyl residue probably does not participate in the inclusion, otherwise the inclusion would be similar for *N*-acetyl-L-valinamide: for this substance, instead, no thermal effects were observed. It is then concluded that inclusion occurs mainly through the alkyl side-chain.

The data for the αCD/non-including substance systems can be discussed in terms of reciprocal perturbations of the hydration cospheres of both solutes. Only the enthalpic interaction coefficients can be considered, since it is not possible to obtain the free energy and entropic contributions. Table IV shows that the coefficients characterizing the interaction of αCD with isoleucine and leucine are negative and high, which could indicate that a somewhat specific interaction is occurring, resembling the inclusion process. Probably, for steric reasons, the interactions of these amino acids with the cavity cannot be optimized and a stable complex cannot be formed. This situation is more evident for isoleucine, which is characterized by a negative and larger cross-coefficient, that reflects the presence of the longer alkyl chain. For other amino acids, only the interaction with the external cosphere of αCD seems to be possible. For valine and 2-aminobutyric acid, the cross-coefficients are positive, thus indicating that the release of water molecules from the hydrophobic hydration domain of the cospheres of the amino acids is the prevailing process. For alanine, the hydrophilic–hydrophobic interaction is not enough to overcome the hydrophilic–hydrophilic interaction, and the cross-coefficient is negative, reflecting release of destructured water to the bulk.

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