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# Water-mediated interactions between benzene rings Calorimetric studies of aromatic model compounds in aqueous solutions at 298 K

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**Abstract**

Pairwise enthalpic interaction coefficients of the virial expansion of the excess enthalpies were determined at 298 K by measuring the enthalpies of dilution in aqueous solution of binary aqueous solutions containing 4-hydroxyphenylacetic acid, 2-phenylethanol, 3-phenylpropanol, 3-phenylpropionic acid, L-tyrosine and L-phenylalanine. Coefficients obtained are compared with those already reported in the literature for other aromatic substances in aqueous solutions. Not withstanding the similarity of the substances employed, the values of the enthalpic coefficients range from highly negative to highly positive, an indication that the interactions between the benzene rings are largely dependent on the nature of the functional groups. For hydroxylated substances, enhanced hydrophobic interactions are operating, probably for the simultaneous interaction between the benzene rings and the alkyl chains, forced by the hydroxyl group. On the contrary, the strength of hydrophobic interactions in the solutions of the amino acids depend on the pH of the medium and on the presence of hydroxyl group on the aromatic ring. The data are discussed according to an interaction model which assumes the presence of a preferential configuration between two hydrated molecules.


*Keywords:* Aromatic substances; Microcalorimetry; Excess enthalpy; Hydrophobic interactions; Hydrophilic interactions; Preferential configuration

## 1. Introduction

It is generally accepted that the amino acid sequence of a protein uniquely determines its native conformation that, for small globular proteins, is expected to represent the thermodynamic minimum free energy conformation accessible during the time scale of the folding process [1]. Hence, the interactions of the side chains of constituent amino acids with the solvent and with each other largely determine the folding process. Hydrophobic interactions are known to play a major role in these processes [2–4]; however, other studies put in evidence that hydrophilic interactions are strong, highly dependent on orientation and on the properties of the solvent, and probably they are as important as the hydrophobic ones in many different biological processes [5–8].

It is difficult to make prediction of protein structure since suitable potentials for calculating the free energies of different conformations are missing. Some studies report conformational free energies as given by the sum of pairwise interactions between amino acid residues evaluated from the statistical analyses of available structures [9–11]. Another approach assumes that the free energy of a given conformation can be evaluated from the solvent-accessible surface areas of aliphatic, aromatic and polar residues [12–15]. However, no potential proposed to date has proven to be sufficiently accurate for the realistic folding simulations.

Experimental studies with the model compounds help in treating this problem. A method is reported in the literature for quantitating the energetics of pairwise interactions between

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amino acid side chains using high-pressure liquid chromatography [16,17]. In the same way, pairwise interaction coefficients of the virial expansion of the excess thermodynamic properties are very useful parameters to get information about the mechanism through which two hydrated molecules interact in solution. The physical meaning of the pair interaction coefficients of an excess property is linked to the variation of a thermodynamic property when two hydrated molecules are brought from an infinite distance, where solute–solvent interactions prevail, to a finite distance where solute–solute, water-mediated interactions are operating.

Preceding studies from this laboratory on the aqueous solutions of model molecules of biological interest allowed to establish that the cooperativity of hydrophobic interactions depend on the presence of interactions between hydrophilic functional groups on the same molecule [18]. In particular, the analysis of the signs and values of the pairwise enthalpic interaction coefficients have allowed us to explain the behaviour in solution of a variety of hydroxylated substances [18,19], α-aminoacids [20], carboxylic acids [21], amines [21] and the mixture of these solutes [19,20]. An interaction model has been proposed postulating the presence of preferential configurations in aqueous solution determined by the interactions between hydrophilic groups. This interaction allows the juxtaposition of hydrophobic groups, making their interaction more effective and different from a simple statistical one.

The present investigation deals with the pairwise interaction coefficients of the excess enthalpies evaluated from the enthalpies of dilution in water of binary aqueous solutions of substances bearing benzene rings: 4-hydroxyphenylacetic acid, 2-phenylethanol, 3-phenylpropanol, 3-phenylpropionic acid, L-tyrosine and L-phenylalanine. We aim to ascertain whether the preferential configurations can be hypothesised for these systems, to gain more information about the various contributions acting in the interaction between hydrated molecules, and to study the influence of the different functional groups on the hydrophobic interactions between the benzene rings. The comparison with the data already reported in the literature about other model molecules allows to ascertain whether the aromatic–aromatic, water-mediated interaction is different from the other interactions acting in these solutions. It must be emphasized that our analysis will be based on enthalpic interaction coefficients. In fact, obtaining Gibbs free energy data presents many experimental difficulties because of enthalpy–entropy compensation effects which would lead only to small Gibbs energy interaction coefficients.

## 2. Experimental

The solutes employed, Sigma or Aldrich products, were of the highest commercially available purity (98–99.5% minimum). They were dried on phosphorus pentoxide under reduced pressure. Solutions were prepared by weight: water was twice distilled and filtered on a Millipore membrane. Measurements of the heats of dilution were carried out using a Thermal Activity Monitor (TAM) from Thermometric, equipped with a GP 10 gradient programmer, a 500 μL mixing chamber, a PSV 50 electrovalve and a P3 peristaltic pump (all from Pharmacia) for the authomatic preparation and for the pumping of solutions into the cells of the calorimeter. The method has been tested through known systems. Enthalpies of dilution in water of urea and hexane-1,2-diol have been determined, and the evaluated pairwise enthalpic interaction coefficients ($h_{xx} = -331 \pm 3\ \mathrm{J\,kg\,mol^{-2}}$ for urea and $h_{xx} = 2999 \pm 46\ \mathrm{J\,kg\,mol^{-2}}$ for hexane-1,2-diol) were in agreement with the literature values ($h_{xx} = -350 \pm 13\ \mathrm{J\,kg\,mol^{-2}}$ for urea [22] and $h_{xx} = 2955 \pm 46\ \mathrm{J\,kg\,mol^{-2}}$ for hexane-1,2-diol [23]. The values of the dilution enthalpies, $\Delta H_{dil}$, were obtained from:

$$\Delta H_{dil}(m_x^i \rightarrow m_x^f) = \frac{(dQ/dt)}{P_w}$$

where $(dQ/dt)$ is the heat evolved or absorbed per unit time, $P_w$ the total mass flow rate of water per unit time, and $m_x^i$ and $m_x^f$ are the initial and final molalities, respectively. $\Delta H_{dil}$ is given in J $kg^{-1}$ of solvent in the final solution.

## 3. Results

According to the treatment of solution properties originally proposed by McMillan-Mayer [24] and specifically applied to those of aqueous solutions of nonelectrolytes by Kauzmann [25] and other authors [26,27], an excess thermodynamic property can be expressed as a function of molalities through a virial expansion of pair and higher order interaction coefficients, $j$, as follows:

$$J^E = \sum_{i=1}\sum_{k=1} j_{ik}\, m_i m_k + \text{higher terms} \quad (1)$$

For two-component solutions containing a solute x and water, virial coefficients of the power series of the excess enthalpies, $h$, as a function of molalities can be easily derived from the enthalpies of dilution, $\Delta H_{dil}$, as follows:

$$\Delta H_{dil}(m^i \rightarrow m^f) = h_{xx} m^f (m^f - m^i) + h_{xxx}\, m^f (m^{f2} - m^{i2}) + \text{higher terms} \quad (2)$$

where $m_x^i$ and $m_x^f$ are the molalities of the x solute before and after the dilution process, respectively. The $h$ coefficients appearing in Eq. (2) represent the enthalpic contributions to the Gibbs free energy coefficients characterising the interaction between pairs, triplets, or higher order interactions. They implicitly account also for all the variations of solvent–solvent and solute–solvent interactions. The values of the self coefficients for each solute are obtained by dilution of binary solutions. To determine the coefficients, a least square procedure was used. Owing to the limited range of concentrations explored, only pairwise coefficients were

found to be necessary for the best fitting of experimental data.

For all of the investigated systems, dilution is an exothermic process and, consequently, the derived enthalpic interaction coefficients are positive. The only exception is 4-hydroxyphenylacetic acid whose dilution is endothermic; then, the corresponding enthalpic coefficient is negative. Measurements have been carried out using aqueous solutions of hydrochloric acid as solvent; this is necessary in order to avoid the dissociation of some of the solutes employed.

In Table 1, the coefficients are reported for 4-hydroxyphenylacetic acid, 2-phenylethanol, 3-phenylpropanol, 3-phenylpropionic acid, L-tyrosine and L-phenylalanine, together with those characterizing the aqueous solutions of other aromatic compounds, different in the functional group and in the length of the alkyl chain protruding from the benzene ring. In the same table, the structures are reported for the substances employed in the present study. Concentration range was 0.1–0.015 mol kg$^{-1}$ for 4-hydroxyphenylacetic acid, 0.03–0.015 mol kg$^{-1}$ for 2-phenylethanol and 3-phenylpropionic acid, 0.02–0.01 mol kg$^{-1}$ for 3-phenylpropanol, 0.065–0.0098 mol kg$^{-1}$ for phenylalanine, and 0.029–0.0086 mol kg$^{-1}$ for tyrosine. Coefficients are positive; they increase at increasing length of the alkyl chain. There is a 1000 units increase passing from 2-phenylethanol to 3-phenylpropanol, while 3-phenylpropionic acid has almost the same coefficient as 2-phenylethanol. Only the coefficient for 4-hydroxyphenylacetic acid is large and negative. The coefficient for phenylalanine in acid solution is much higher than the one in water, while the coefficient for tyrosine in HCl is much smaller than the one for phenylalanine in the same experimental conditions.

## 4. Discussion

It is reported in the literature that the group contribution to the overall pairwise coefficient of the virial expansion of the Gibbs free energy, obtained through a group additivity approach, is negative for the interactions between groups having the same effect on the structure of water and positive for mixed interactions ($G_{\text{hydrophilic–hydrophilic}} < 0$, $G_{\text{hydrophobic–hydrophobic}} < 0$, $G_{\text{hydrophilic–hydrophobic}} > 0$) [33,34]. Thus, two interacting molecules prefer to be oriented in a configuration where favourable interactions between like groups are maximized. Mixed interactions are less probable because of the positive contribution to the Gibbs free energy. On this ground and on the basis of thermodynamic and spectroscopic studies on polyhydroxylated substances, we proposed the "preferential configuration" model [18,35]. Namely, the presence of a "side-on", preferential configuration was hypothesised, which allows the simultaneous juxtaposition of the maximum number of hydrophilic and hydrophobic domains. Other configurations, in which hydrophilic–hydrophobic interactions could occur, cannot be excluded but are less probable.

The aromatic substances presently examined are built up by several groups: the aromatic ring, the alkyl chain and the hydrophilic functional groups. According to the above cited model, the interaction occurs through the juxtaposition of the aromatic rings and the alkyl chains, forced by the interaction between the hydrophilic functional groups. The pairwise enthalpic interaction coefficient increases by 1000 units on going from 2-phenylethanol to 3-phenylpropanol. However, the difference in the length of the alkyl chain, due to an added methylene group, does not account for that increase since the pairwise enthalpic interaction coefficient is 243 J kg mol$^{-2}$ for ethanol [27] and 559 J kg mol$^{-2}$ for propanol [27]. The

Table 1
Enthalpic self-interaction coefficients, $h_{xx}$, for aromatic compounds in HCl 0.1 mol L$^{-1}$ aqueous solutions at 298 K

| Substance | $h_{xx}$[a] |
|---|---|
| 4-Hydroxyphenylacetic acid, (OH)$C_6H_4CH_2COOH$ | −2032 (134) |
| Phenol, $C_6H_5OH$ | 816 (8)[b] |
| 2-Phenylethanol, $C_6H_5CH_2CH_2OH$ | 2622 (46) |
| 3-Phenylpropanol, $C_6H_5CH_2CH_2$ $CH_2OH$ | 3628 (142) |
| 3-Phenylpropionic acid, $C_6H_5CH_2CH_2COOH$ | 2316 (48) |
| L-Phenylalanine, ($C_6H_5CH_2CH(COOH,NH_3^+)$) | 2750 (238) |
| L-Phenylalanine, ($C_6H_5CH_2CH(COO^-,NH_3^+)$) | 1140 (30)[c] |
| *N*-Acetylphenylalanine, $C_6H_5CH_2CH(COOH)NHCOCH_3$ | 2267 (86)[d] |
| *N*-Acetylphenylalanineamide, $C_6H_5CH_2CH(CONH)NHCOCH_3$ | 1049 (53)[e] |
| L-Tyrosine, (OH)$C_6H_4CH_2CH(COOH,NH_3^+)$ | 563 (75) |
| L-Proline, $C_4H_8NH^+COO^-$ | 409 (8)[f] |
| *cis*-L-Hydroxyproline, (OH)$C_4H_7$ $NH^+COO^-$ | −528 (14)[f] |
| *trans*-L-Hydroxyproline, (OH)$C_4H_7$ $NH^+COO^-$ | −156 (7)[f] |

[a] Units: J kg mol$^{-2}$. Number in parentheses represent the 95% confidence limits.
[b] Determined in water, Ref. [28].
[c] Determined in water, Ref. [30].
[d] Determined in HCl 0.01 mol L$^{-1}$, Ref. [29].
[e] Determined in water, Ref. [31].
[f] Ref. [32].

same thing is observed when passing from phenol to 2-phenylethanol: a jump of about 1800 J kg mol$^{-2}$ in the values of coefficients is observed (see Table 1), assigning in the average 900 J kg mol$^{-2}$ to the successive addition of a methylene group. This enhanced effectiveness of hydrophobic interactions between the alkyl chains probably occurs for the simultaneous interaction between the aromatic rings and the alkyl chains, forced by the hydroxyl groups. The coefficient for 3-phenylpropionic acid is, instead, 1000 units less than 3-phenylpropanol and similar to the coefficient for 2-phenylethanol. This is not unexpected since the number of aliphatic carbon atoms is the same for 3-phenylpropionic acid and 2-phenylethanol.

The coefficient for phenylalanine in water is much smaller (1140 J kg mol$^{-2}$) than those for other similar substances: in acidic solution, it jumps to 2750 J kg mol$^{-2}$, thus indicating an enhanced cooperativity of hydrophobic interactions. The model of preferential configuration well rationalizes these data. Among the various favourable configurations of two interacting molecules having the same chirality, the interaction between the opposite charges of zwitter ions is supposed to prevail in water, lowering the cooperativity of hydrophobic interaction. Preceding works, indeed, have shown that zwitter ions are the worst promoters of hydrophobic interactions, α,ω-aminoacids showing the lowest cooperativity [21]. In contrast, in HCl, the electrostatic contributions are repulsive because of the protonation of the carboxyl group. A new, preferential configuration prevails, the side-on, stabilized by the simultaneous juxtaposition of the aromatic rings, the aliphatic parts and the carboxyl groups. α-Aminoacids bearing unsubstituted residues behave similarly. In water, their enthalpic homochiral coefficients depend linearly on the first power of the number of aliphatic carbon atoms in the alkyl chain, $n_c$, thus indicating that the $CH_2$–$CH_2$ interaction is not cooperative. In acidic solution, the coefficients depend linearly on the second power of $n_c$: that characterizes a behaviour typical of prevailingly hydrophobic solutes. The results for the two derivatives of phenylalanine, namely *N*-acetylphenylalanine in HCl and *N*-acetylphenylalanineamide in water, show that their coefficients are very similar to those for phenylalanine in acidic solution and in water, respectively. Hence, the two molecules behave as the *N*-acetyl group were not present. Probably, the simultaneous hydrophobic interactions between the aromatic rings and the alkyl chains prevail on the possible interactions between the *N*-acetyl group. It is worth to remember that interactions between benzene rings, stronger than those between zwitter ions, are hypothesized to be the cause of the lack of chiral recognition in the aqueous solutions of free phenylalanine [30].

The presence of a hydroxyl group on the benzene ring causes the coefficient to decrease or even to become negative. The pairwise coefficient for 4-hydroxyphenylacetic acid is large and negative; from the enthalpic point of view, this substance behaves as a prevailingly destructuring hydrophilic solute [18]. Preceding studies on ternary aqueous systems allowed to determine that the COOH carboxyl group is the most effective promoter of hydrophobic interactions [21]. However, the values of the enthalpic coefficients for the lower terms of the carboxylic acid series, namely succinic, glutaric and adipic acids, are negative and almost invariant while those for longer terms unravel that COOH is more effective than the OH group in promoting hydrophobic interactions. Probably, the steric hindrance of the carboxyl group prevents the effective juxtaposition of a short alkyl chain, causing the interaction between hydrophilic domain to dominate. Then, the negative sign of the pairwise coefficient for 4-hydroxyphenylacetic acid could originate from the length of the hydrophobic part between the two functional groups, not enough to overwhelm the effect of the steric hindrance of the carboxyl group. The forced interaction between the hydrophilic domains leads to the large and negative value of the coefficient.

The coefficient for L-tyrosine is much smaller than that for phenylalanine in the same experimental conditions. A similar behaviour is shown by 4-hydroxyprolines whose enthalpic interaction coefficients are negative, against a positive value for proline [32]. The trend of the enthalpic coefficients for these systems, built up by the contributions originating from interactions between zwitterionic, hydrophilic and hydrophobic domains, is well rationalized through the model of preferential configuration. Accordingly, a configuration prevails sustained by the concurrent hydrophilic interactions between zwitter ions and OH groups, and by the juxtaposition of hydrophobic domains. The positive value of the coefficient indicates that the interaction between the hydrophobic parts is strong enough to prevail on the hydrophilic ones. For both hydroxyphenylacetic acid and tyrosine, there is a further, common contribution that makes the coefficients to decrease, namely the rigidity of the aromatic ring. The forced interaction between the hydrophilic domains reduces the number of juxtaposing hydrophobic hydrogen atoms, leading to a decreased value of the coefficient.

As a conclusion, the preferential configuration model allows to rationalise the values of the enthalpic interaction coefficients, assigning to the interaction between hydrophilic groups the role of promoter of hydrophobic interactions. The knowledge of these interaction mechanisms is especially important when studying the main driving forces stabilizing the conformations of biological macromolecules in aqueous solution, or the possible mode of action of other molecules, such as chemical denaturants, on the globular proteins [36–40].

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