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Enthalpic interactions of amino acids with imidazole in aqueous solutions at 298.15 K

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Abstract

The enthalpies of mixing of aqueous imidazole solution and six kinds of amino acid solution (glycine, L-alanine, L-serine, L-valine, L-proline, L-threonine) have been determined at 298.15 K using a 2277 flow microcalorimetry. The results have been analyzed in terms of the McMillan–Mayer model to obtain the heterotactic enthalpic interaction coefficients. The pairwise interactions between amino acids and imidazole have been studied by a group additivity approach of savage and wood (abbreviated as SWAG). The results show that the zwitteriomic groups and different side-groups of the amino acids make their different contribution to enthalpic pair interactions.

Keywords: Amino acids; Imidazole; Enthalpy of mixing; Heterotactic enthalpic interaction; Solute–solute interaction

1. Introduction

The native structure of proteins is ruled by weak, non-bonding interactions between the amino acid residues and between these residues and the aqueous environment. Among these weak interactions, the hydrophobic interactions have been long invoked to explain many biochemical processes [1–3], while a minor role is assigned to hydrophilic interactions. However, in more recent studies, hydrophilic interactions have been demonstrated to be as important as the hydrophobic ones in processes such as protein folding and molecular recognition [4–10].

In our previous studies, the enthalpies of dilution of amino acids in aqueous solution of DMF, ethanol, glucose, sucrose, urea and sodium halide and the enthalpies of mixing of amino acids and urea and monomethylurea in aqueous solutions were determined by mixing flow microcalorimetry [11–18]. As a continuation of this work, the present study is aimed at examining the heterotactic enthalpic interactions between glycine, L-alanine, L-serine, L-valnine, L-proline and L-threonine and imidazole in aqueous solutions.

Heterocyclic compounds are those that have a cyclic structure with two, or more, different kinds of atom in the ring. Heterocyclic compounds are very distributed in Nature and are essential to life; they play a vital role in the metabolism of all living cells [19].

2. Experimental

Biochemical reagent grade glycine, L-alanine, L-serine, L-valine, L-proline and L-threonine were used after recrystallization from methanol–water mixture and drying in vacuum over P_2O_5 at room temperature for at least 72 h. Analytical reagent grade imidazole was used without further purification. Water was deionized and distilled using a quartz sub-boiling purifier. Both the amino acid solutions and imidazole solution were prepared by mass using a Mettler AE 200 balance with a precision of ±0.0001 g. All the solutions were degassed and used within 12 h after preparation to avoid possible bacterial contamination.

The heat of mixing was measured by a mixing-flow microcalorimeter (2277 Thermal Activity Monitor made in Sweden). All the measurements were carried out at 298.15 K. The solutions were pumped through the mixing cell at constant rates by a microperpex peristaltic pump with a pair of wheel (VS2-10R MIDI 2,5-50PRM). The

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flow rates were determined by weighing samples delivered in 5 min. The variation in flow rates was less than 0.1% both before and after a complete experiment. The process of experiment is in the following sequence:

(a) A (water) + B (water)—baseline determined.
(b) A (aqueous amino acid solution) + B (aqueous imidazole solution)—mixing thermal power determined.
(c) A (aqueous amino acid solution) + B (water)—dilution thermal power determined.
(d) A (aqueous imidazole solution) + B (water)—dilution thermal power determined.
(e) A (water) + B (water)—baseline re-established.

The dilution enthalpies ΔH_{dil} were calculated from the equation

$$\Delta H_{\mathrm{dil}} = \frac{P}{f_{\mathrm{A}} + f_{\mathrm{B}} - m_{x,i} M_x f_{\mathrm{A}}} \tag{1}$$

where P is the dilution thermal power of solute, $m_{x,i}$ the molality of solution before dilution, f_{A} the flow rate of solution, f_{B} the flow rate of solvent water, and M_x is the molar mass of solute.

The final molality m_{f} was calculated from the equation

$$m_{\mathrm{f}} = \frac{m_{x,i} f_{\mathrm{A}}}{f_{\mathrm{B}}(m_{x,i} M_x + 1) + f_{\mathrm{A}}}$$

The mixing enthalpies ΔH_{mix} of aqueous amino acid solutions and aqueous imidazole solution were calculated from the equation

$$\Delta H_{\mathrm{mix}} = \frac{P^*}{f_{\mathrm{A}} + f_{\mathrm{B}} - m_{x,i} M_x f_{\mathrm{A}} - m_{y,i} M_y f_{\mathrm{B}}} \tag{3}$$

where P^* is the mixing thermal power (μW), f_{A}, f_{B} are the flow rate of solution x and y, respectively, $m_{x,i}$, $m_{y,i}$ are the morality of solution x and y before mixing.

3. Results and discussion

The results are analyzed in terms of the McMillan–Mayer theory [20]. The excess enthalpy $H^{\mathrm{E}}(m_x, m_y)$ of a solution containing two solute species x and y, can be obtained by

$$\frac{H^{\mathrm{E}}(m_x, m_y)}{wl} = H(m_x, m_y) - h^*_{\mathrm{w}} - m_x H^{\infty}_{x,\mathrm{m}} - m_y H^{\infty}_{y,\mathrm{m}} \tag{4}$$

where $H^{\mathrm{E}}(m_x, m_y)$ is the excess enthalpy of a solution containing m_x mol of x and m_y mol of y species and wl kg of water, $H(m_x, m_y)$ is the total enthalpy of a solution containing wl kg of water and m_x mol of x and m_y mol of y. h^* is the standard enthalpy of 1 kg of water, and $H^{\infty}_{x,\mathrm{m}}$ and $H^{\infty}_{y,\mathrm{m}}$ are the limiting partial molar enthalpies of species x and y.

The excess enthalpy can be expressed in terms of a virial expansion:

$$\frac{H^{\mathrm{E}}(m_x, m_y)}{wl} = h_{xx} m_x^2 + 2h_{xy} m_x m_y + h_{yy} m_y^2 + h_{xxx} m_x^3 + 3h_{xxy} m_3^2 m_y + 3h_{xyy} m_x m_y^2 + h_{yyy} m_y^3 + \cdots \tag{5}$$

In which h_{xx}, h_{yy}, h_{xy} and h_{xxx}, h_{xyy}, h_{xxy} and h_{yyy} are the second and third virial coefficients characterizing like and unlike pair and triplet interactions.

For a binary solution containing one solute species, we can show:

$$\frac{H^{\mathrm{E}}(m_x)}{wl} = h_{xx} m_x^2 + h_{xxx} m_x^3 + \cdots \tag{6}$$

by conducting mixing and dilution calorimetric determinations, ΔH_{mix}. $\Delta H_{\mathrm{dil}}(x)$ and $\Delta H_{\mathrm{dil}}(y)$ have been determined. To make easier the calculation, an auxiliary function ΔH^* was introduced:

$$\Delta H^* = \Delta H_{\mathrm{mix}} - \Delta H_{\mathrm{dil}} - \Delta H_{\mathrm{dil}} \tag{7}$$

from Eqs. (4) and (5), it follow that:

$$\frac{\Delta H^*}{wl} = 2h_{xy} m_x m_y + 3h_{xxy} m_x^2 m_y + 3h_{xyy} m_x m_y^2 \tag{8}$$

or

$$\frac{\Delta H^*}{wl m_x m_y} = 2h_{xy} + 3h_{xxy} m_x + 3h_{xyy} m_y + \cdots \tag{9}$$

The experimental values ΔH_{mix} of amino acid solutions with imidazole solution together with ΔH^* are given in Table 1.

The data were fitted to Eq. (8) using a least-squares procedure to obtain the heterotactic enthalpic interaction coefficients (Table 2).

As there are some difficulties in the interpretation of the higher h coefficient, only the pairwise interactions are considered.

The enthalpic pair interaction coefficients are regarded as a measure of the heat effects (i.e. the enthalpy of interaction) when two solute particles approach each other [21]. This process is companied by overlapping of solvation co-sphere of the solute molecules, resulting in a partial reorganization of the solvation co-spheres and a change of the solute-solvent interaction. Therefore, the enthalpic pair interaction coefficients h_{xy} are the results of solvation effects and direct solute–solute interaction effects.

For the amino acid–imidazole–water system discussed here, there are three types of interaction:

1. Partial dehydration of the amino acid molecules (endothermic effect). The dehydration is caused by mutual penetration of the hydration shells of interacting molecules in the aqueous medium.
2. Partial dehydration of the imidazole molecule (endothermic effect).

Table 1
Enthalpies of dilution and mixing of aqueous amino acids solution and aqueous imidazole solution at 298.15 K

$m_{x,i}$ (mol kg^{-1})	$m_{y,i}$ (mol kg^{-1})	$m_{x,f}$ (mol kg^{-1})	$M_{y,f}$ (mol kg^{-1})	$\Delta H_{dil(x)}/wl$ (J kg^{-1})	$\Delta H_{dil(y)}/wl$ (J kg^{-1})	$\Delta H_{mix}/w1$ (J kg^{-1})	$\Delta H^*/w1$ (J kg^{-1})
Gly + imidazole							
0.1000	0.1000	0.0510	0.0487	1.00	−0.77	13.19	12.97
0.1500	0.1500	0.0636	0.0856	2.34	−0.95	21.95	20.56
0.1800	0.1800	0.0763	0.1026	3.30	−1.23	27.55	25.48
0.2000	0.2000	0.0847	0.1139	4.11	−1.69	31.26	28.83
0.2200	0.2200	0.0931	0.1253	4.90	−1.72	34.12	30.95
0.2500	0.2500	0.1056	0.1422	6.38	−1.99	39.46	35.06
0.2800	0.2800	0.1181	0.1592	7.83	−2.64	44.93	39.74
0.3000	0.3000	0.1265	0.1704	9.11	−3.16	49.07	43.11
0.3200	0.3200	0.1348	0.1817	10.41	−3.81	52.70	46.10
0.3500	0.3500	0.1472	0.1985	11.67	−4.39	59.35	52.06
0.3800	0.3800	0.1596	0.2154	14.16	−4.67	63.47	53.98
0.4000	0.4000	0.2211	0.1734	15.87	−5.90	70.12	60.14
0.4200	0.4200	0.2320	0.1819	17.06	−6.44	74.91	64.29
0.4500	0.4500	0.2483	0.1947	20.14	−7.22	83.04	70.12
0.5000	0.5000	0.2090	0.2824	23.47	−8.89	89.66	75.08
L-Alanine + imidazole							
0.1000	0.1000	0.0510	0.0487	−0.53	−0.77	13.79	15.09
0.1500	0.1500	0.0636	0.0856	−1.24	−0.95	19.84	22.03
0.1800	0.1800	0.0762	0.1026	−1.70	−1.23	23.52	26.44
0.2000	0.2000	0.0845	0.1139	−2.00	−1.69	28.27	31.96
0.2200	0.2200	0.0929	0.1253	−2.48	−1.72	30.36	34.56
0.2500	0.2500	0.1054	0.1422	−3.17	−1.99	35.59	40.75
0.2800	0.2800	0.1179	0.1592	−3.96	−2.64	40.39	46.99
0.3000	0.3000	0.1262	0.1704	−4.75	−3.16	42.78	50.68
0.3200	0.3200	0.1344	0.1817	−5.42	−3.81	44.73	53.96
0.3500	0.3500	0.1468	0.1985	−6.14	−4.39	50.22	60.75
0.3800	0.3800	0.1592	0.2154	−7.34	−4.67	55.73	67.73
0.4000	0.4000	0.2201	0.1738	−8.54	−5.90	60.95	75.38
0.4200	0.4200	0.2310	0.1823	−9.56	−6.44	64.88	80.88
0.4500	0.4500	0.2472	0.1951	−10.81	−7.22	69.77	87.80
0.5000	0.5000	0.2082	0.2824	−12.40	−8.89	78.28	99.57
L-Serine + imidazole							
0.1000	0.1000	0.0511	0.0485	1.61	−0.77	23.31	22.48
0.1500	0.1500	0.0635	0.0856	3.74	−0.95	34.73	31.94
0.1800	0.1800	0.0761	0.1026	5.50	−1.23	43.37	39.10
0.2000	0.2000	0.0844	0.1139	6.57	−1.69	48.96	44.08
0.2200	0.2200	0.0927	0.1252	8.51	−1.72	53.36	46.58
0.2500	0.2500	0.1052	0.1422	10.16	−1.98	63.42	55.25
0.2800	0.2800	0.1176	0.1591	12.69	−2.64	72.34	62.29
0.3000	0.3000	0.1259	0.1704	14.93	−3.16	79.12	67.35
0.3200	0.3200	0.1341	0.1816	16.77	−3.81	85.81	72.85
0.3500	0.3500	0.1414	0.2035	19.75	−4.39	92.48	77.11
0.3800	0.3800	0.1533	0.2208	23.35	−4.67	106.37	87.69
0.4000	0.4000	0.2203	0.1730	25.77	−5.90	113.23	93.36
0.4200	0.4200	0.2311	0.1815	28.82	−6.44	121.23	98.86
0.4500	0.4500	0.2473	0.1942	31.36	−7.22	131.07	106.93
0.5000	0.5000	0.2073	0.2823	36.59	−8.89	147.60	119.90
L-Valine + imidazole							
0.1000	0.1000	0.0511	0.0484	−2.15	−0.77	16.35	19.27
0.1500	0.1500	0.0625	0.0865	−4.56	−0.94	25.30	30.81
0.1800	0.1800	0.0748	0.1037	−6.71	−1.23	30.93	38.87
0.2000	0.2000	0.0830	0.1151	−8.03	−1.69	34.14	43.86
0.2200	0.2200	0.0912	0.1266	−10.23	−1.72	37.80	49.76
0.2500	0.2500	0.1035	0.1438	−12.61	−1.99	44.47	59.07
0.2800	0.2800	0.1156	0.1609	−15.75	−2.64	50.41	68.81
0.3000	0.3000	0.1237	0.1723	−19.06	−3.16	54.78	77.00
0.3200	0.3200	0.1318	0.1836	−21.05	−3.81	59.08	83.94
0.3500	0.3500	0.1439	0.2007	−25.06	−4.39	62.36	91.81
0.3800	0.3800	0.1559	0.2177	−30.41	−4.67	70.75	105.83

Table 1 (*Continued*)

$m_{x,i}$ (mol kg^{-1})	$m_{y,i}$ (mol kg^{-1})	$m_{x,f}$ (mol kg^{-1})	$M_{y,f}$ (mol kg^{-1})	$\Delta H_{dil(x)}/wl$ (J kg^{-1})	$\Delta H_{dil(y)}/wl$ (J kg^{-1})	$\Delta H_{mix}/w1$ (J kg^{-1})	$\Delta H^*/w1$ (J kg^{-1})
0.4000	0.4000	0.2197	0.1732	−34.25	−5.90	76.65	116.79
0.4200	0.4200	0.2304	0.1817	−38.37	−6.44	81.66	126.47
0.4500	0.4500	0.2465	0.1945	−45.59	−7.22	88.89	141.71
0.5000	0.5000	0.2035	0.2855	−50.98	−8.89	99.46	159.32
L-Proline + imidazole							
0.1000	0.1000	0.0417	0.0578	−0.84	−0.70	3.11	4.66
0.1500	0.1500	0.0624	0.0866	−1.56	−0.95	3.42	5.93
0.1800	0.1800	0.0747	0.1039	−2.60	−1.23	4.37	8.20
0.2000	0.2000	0.0829	0.1154	−3.20	−1.68	4.50	9.39
0.2200	0.2200	0.0910	0.1268	−3.99	−1.72	4.98	10.69
0.2500	0.2500	0.1032	0.1440	−5.02	−1.99	5.22	12.23
0.2800	0.2800	0.1154	0.1611	−6.43	−2.64	5.57	14.65
0.3000	0.3000	0.1235	0.1726	−7.64	−3.16	5.88	16.67
0.3200	0.3200	0.1316	0.1840	−8.05	−3.81	6.19	18.05
0.3500	0.3500	0.1436	0.2010	−9.24	−4.39	6.47	20.10
0.3800	0.3800	0.1556	0.2181	−10.69	−4.67	6.85	22.21
0.4000	0.4000	0.2200	0.1729	−13.36	−5.90	7.03	26.29
0.4200	0.4200	0.2308	0.1814	−15.81	−6.44	7.29	29.54
0.4500	0.4500	0.2469	0.1941	−17.34	−7.22	7.76	32.33
0.5000	0.5000	0.2032	0.2860	−17.69	−8.89	8.37	34.95
L-Threonine + imidazole							
0.1000	0.1000	0.0418	0.0578	−0.21	−0.70	20.42	20.92
0.1500	0.1500	0.0625	0.0865	−0.50	−0.95	31.46	31.91
0.1800	0.1800	0.0748	0.1037	−0.80	−1.23	38.59	39.02
0.2000	0.2000	0.0830	0.1152	−0.93	−1.69	43.57	44.32
0.2200	0.2200	0.0912	0.1266	−1.23	−1.72	49.62	50.12
0.2500	0.2500	0.1034	0.1438	−1.42	−1.99	54.52	55.08
0.2800	0.2800	0.1156	0.1609	−1.71	−2.64	64.20	65.13
0.3000	0.3000	0.1237	0.1723	−2.00	−3.16	69.99	71.14
0.3200	0.3200	0.1318	0.1836	−2.10	−3.81	75.05	76.75
0.3500	0.3500	0.1438	0.2007	−2.51	−4.39	82.58	84.46
0.3800	0.3800	0.1558	0.2177	−2.66	−4.67	90.69	92.70
0.4000	0.4000	0.2196	0.1732	−3.12	−5.90	97.86	100.63
0.4200	0.4200	0.2303	0.1817	−3.22	−6.44	102.19	105.42
0.4500	0.4500	0.2464	0.1945	−3.60	−7.22	112.60	116.22
0.5000	0.5000	0.2034	0.2855	−4.77	−8.89	133.72	137.83

3. Direct interactions of amino acid molecule and imidazole molecule.

Among these interactions, the effect (2) can be assumed as being constant, the observed differences in h_{xy} values depend on the competitive equilibrium between (1) effect and (3) effect.

The interactions of different amino acids and imidazole molecule can be explained using a group additivity approach of savage and wood (SWAG) [22]. The principle is assumed that each functional group on one molecule interacts with every functional group on the other molecule and that each of these interactions has a characteristic effect on the enthalpy that is independent of the position of the functional group in the two molecules. The enthalpic interaction coefficient is the sum of all the various interactions between functional groups on the two solutes:

$$h_{xy} = \sum_{i,j} n_i^x n_j^y H_{ij} \tag{10}$$

Table 2
Heterotactic enthalpic interaction coefficients between amino acids and imidazole in aqueous solutions at 298.15 K

Solutes ($x + y$)	h_{xy} (J kg mol^{-2})	h_{xxy} (J kg^2 mol^{-3})	h_{xyy} (J kg^2 mol^{-3})	S.D.	R^2
Glycine + imidazole	1066	−620	−781	1.32	0.9960
L-Alanine + imidazole	1165	−494	−624	1.06	0.9986
L-Serine + imidazole	1599	−1071	−932	1.29	0.9984
L-Valine + imidazole	1784	−584	−824	1.45	0.9991
L-Proline + imidazole	362	32	−228	0.45	0.9983
L-Threonine + imidazole	1595	−1098	−528	1.75	0.9978

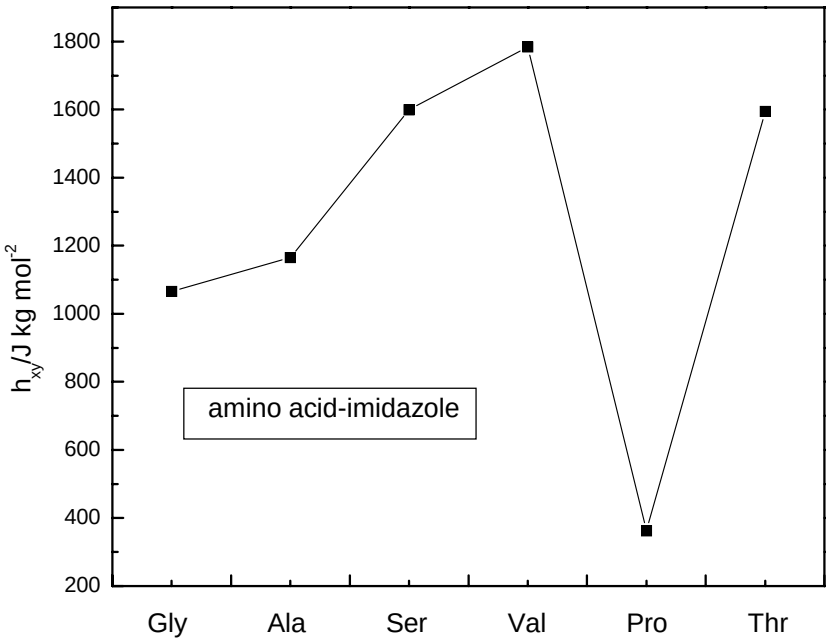

Fig. 1. Comparison of heterotactic enthalpic pairwise interaction coefficients for amino acids and imidazole in the aqueous solutions at 298.15 K.

where n_i^x is the number of groups i on molecule x, n_j^y is the number of groups j on molecule y, and H_{ij} is the characteristic contribution to the enthalpy of i group interacting with j group. The sum is over all group i on molecule x and all group j on molecule y.

For amino acids and imidazole, each molecule in the set was divided into a number of functional groups. We have elected to treat the NH_3^+ and COO^- functional groups as one unit, which was symbolized E. The hydrocarbon functional group is based on the methylene unit. We have assumed that a CH_3 group is equivalent to $1.5CH_2$ group, a CH group is equivalent to $0.5CH_2$ groups, and H is also counted as $0.5CH_2$ groups. OH group is assumed as one unit. The molecule of imidazole is planar and aromatic, features which favor the possible intermolecular interaction by vertical stacking of imidazole rings. Besides, intermolecular hydrogen bonding is feasible by the existence of both donor and acceptor sites. The imidazole molecule can self-associate extensively in both non-polar solvents and aqueous solution [23–25]. So, the molecule of imidazole can be assumed as a hydrophobic group, symbolized I. Using this scheme and the H_{ij} values in Table 2, the resulting relationships are:

$$H_{\mathrm{E-I}} = 926(\pm 43), \qquad H_{\mathrm{CH_2-I}} = 225(\pm 31),$$
$$H_{\mathrm{OH-I}} = 331(\pm 33)$$

Although the principle has limitations [26–28], it has been applied with reasonable success to a wide range of solutes including amides [29], alcohols [30,31], amines [32], *N*-acetyl amino acid amide [33,34], amino acids [35], and even some electrolytes [36,37].

The enthalpy of the E–I interaction is large and positive, this indicate that the interaction between zwitterionic head group on amino acid molecule and imidazole molecule make a positive contribution to the enthalpy pair interaction coefficients. The interactions of both the hydrophobic group (CH_2) and hydrophilic group (OH) and the imidazole molecule make positive contribution to h_{xy}. So, all h_{xy} values are positive and this show the hydrophobic interaction dominant in the mixing processes of amino acids and imidazole.

Glycine is the simplest amino acid in nature. The replacement of a hydrogen atom in the molecule of glycine by a methyl group causes an increase in the enthalpy pair interaction coefficient of L-alanine with imidazole. The extension of amino acid side chain with alkyl groups of L-valine causes the enthalpy pair interaction coefficient values of the interaction between L-valine and imidazole molecule more positive.

The side chains of L-serine and L-threonine have a hydroxyl group respectively. The h_{xy} value of L-serine and imidazole molecule has no much difference with that of L-threonine and imidaole molecule. This can been seen from Fig. 1.

L-Proline is the only native amino acid with a pyrrole ring. Its special structural characteristics make it important in determining the properties of polypeptides. The analogy of cyclic structure of L-proline with the imidazole molecule diminishes the interactions between them. So the h_{xy} value of L-proline and imidazole is less positive than that of other amino acids and imidazole molecule.

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