Studies on the Interactions of L-Valine and L-Threonine with Saccharides in Aqueous Solutions at 298.15 K

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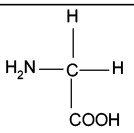
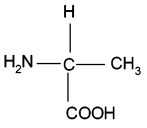
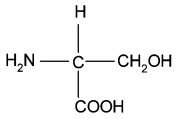
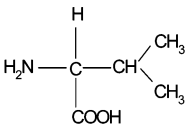
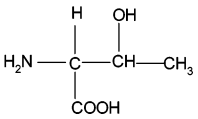
Mixing enthalpies of aqueous L-valine and L-threonine solutions with aqueous sorbose solution and aqueous fructose solution and their dilution enthalpies have been determined with a Thermometric-2277 thermal activity monitor at 298.15 K. The experimental data have been analyzed in terms of McMillan−Mayer formalism to obtain the enthalpic virial coefficients for heterotactic interaction. The enthalpic pairwise interaction coefficients h_{xy} of L-valine with saccharides are positive and those of Lthreonine are negative. The variations of the enthalpic pairwise interaction coefficients are interpreted in terms of solute−solute interactions.

Introduction

Saccharides are very important in physiological processes. They not only are the basic material for energy metabolism in organisms but also play a significant role in the configuration of biological molecules.[1,2] In particular, interactions between model molecules of protein and saccharide can help in elucidating either the intramolecular interactions between the amino acid residues of a glycoprotein or the intermolecular interactions between the saccharide part of a glycoprotein and those proteolytic enzymes against which a protective action is explained. Moreover, interactions between such model systems are very important factors that determine the folded conformation of a globular protein since saccharide and polyhydric alcohols can increase the thermal stability of proteins or reduce the extent of their denaturation by other reagents.[3−7]

As an extension to our previous study,[8−10] we report the calorimetric measurement results of the non-covalent bonding interactions between the amino acids L-valine and L-threonine and the saccharides sorbose and fructose. The enthalpic coefficients of interaction of the systems have been calculated according to the McMillan−Mayer model.[11] Table 1 depicts the chemical structures of the five amino acids studied in this paper and our previous work.[8]

Table 1. Molecular Formulas of α-Amino Acids Studied in This Work and Our Previous Work

Amino Acids	molecular formula
Glycine	
L-Alanine	
L-Serine	
L-Valine	
L-Threonine	

Materials and Methods

L-Valine and L-threonine (biochemical reagent, > 99.0 %, from Shanghai Chemical Co.) were used after recrystallization from methanol−water mixtures and drying in vacuum over P_2O_5 at room temperature for at least 72 h. Sorbose and fructose (biochemical reagent, from Shanghai Chemical Co.) were used without further purification. The water used for the preparation of solution was deionized and distilled using a quartz subboilingpurifier. Both the aqueous amino acid solutions and aqueous saccharide solutions were prepared by mass using a Mettler AE 200 balance with a precision ± 0.0001 g. All the solutions were degassed and used within 12 h after preparation to minimize possible bacterial contamination.

The enthalpies of dilution and mixing were carried out at (298.15 ± 0.01) K by an LKB flow microcalorimeter. Details of the apparatus and experimental procedures used have been given elsewhere.[8] Experimental errors in the determination of the molar enthalpies of dilution and mixing were estimated to be < 1 %. Every dilution and mixing experiment was repeated three times, and the average of the three measured values was calculated.

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Table 2. Enthalpies of Dilution and Mixing for Amino Acid (*x*) + Fructose (*y*) and Amino Acids (*x*) + Sorbose (*y*) in Aqueous Solutions 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}	$\Delta H_{dil(x)}/w1$ / J·kg^{-1}	$\Delta H_{dil(y)}/w1$ / J·kg^{-1}	$\Delta H_{mix}/w1$ / J·kg^{-1}	$\Delta H^*/w1$ / J·kg^{-1}
			L-Valine + Fructose				
0.1000	0.1000	0.0509	0.0484	−2.15	0.13	−0.51	1.51
0.1500	0.1500	0.0622	0.0862	−4.42	−0.57	−2.00	2.99
0.1800	0.1800	0.0745	0.1032	−6.90	−0.93	−3.49	4.33
0.2000	0.2000	0.0826	0.1145	−7.98	−1.59	−4.01	5.56
0.2200	0.2200	0.0908	0.1258	−9.95	−2.00	−4.35	7.59
0.2500	0.2500	0.1030	0.1426	−12.53	−2.36	−5.99	8.90
0.2800	0.2800	0.1151	0.1594	−15.66	−2.74	−7.43	10.96
0.3000	0.3000	0.1231	0.1705	−18.95	−3.58	−8.27	14.26
0.3200	0.3200	0.1312	0.1816	−20.93	−4.23	−9.44	15.72
0.3500	0.3500	0.1432	0.1982	−24.91	−5.61	−12.48	18.04
0.3800	0.3800	0.2104	0.1593	−30.85	−7.33	−15.12	23.05
0.4000	0.4000	0.1631	0.2257	−32.04	−8.32	−16.20	24.17
0.4200	0.4200	0.2321	0.1754	−38.47	−9.92	−20.13	28.26
0.4500	0.4500	0.2467	0.1891	−45.72	−10.03	−24.64	31.11
0.5000	0.5000	0.2025	0.2800	−50.67	−10.06	−27.28	33.46
			L-Threonine + Fructose				
0.1000	0.1000	0.0509	0.0496	0.43	0.13	−1.13	−1.69
0.1500	0.1500	0.0617	0.0740	0.50	−0.57	−2.20	−2.13
0.1800	0.1800	0.0738	0.0886	0.80	−0.93	−3.42	−3.29
0.2000	0.2000	0.0819	0.0982	0.93	−1.59	−4.56	−3.90
0.2200	0.2200	0.0900	0.1079	1.23	−2.00	−5.21	−4.44
0.2500	0.2500	0.1021	0.1222	1.42	−2.36	−7.11	−6.18
0.2800	0.2800	0.1141	0.1366	1.71	−2.74	−8.83	−7.80
0.3000	0.3000	0.1221	0.1461	2.00	−3.58	−9.92	−8.34
0.3200	0.3200	0.1300	0.1555	2.10	−4.23	−11.24	−9.11
0.3500	0.3500	0.1419	0.1697	2.51	−5.61	−13.93	−10.83
0.3800	0.3800	0.2108	0.1837	3.04	−7.33	−16.63	−12.35
0.4000	0.4000	0.1616	0.1930	3.06	−8.32	−18.97	−13.71
0.4200	0.4200	0.2325	0.2023	3.22	−9.92	−21.47	−14.764
0.4500	0.4500	0.2471	0.2162	3.60	−10.03	−24.98	−18.56
0.5000	0.5000	0.2007	0.2392	4.77	−10.06	−27.49	−22.19
			L-Valine + Sorbose				
0.1000	0.1000	0.0512	0.0481	−2.15	−1.41	−1.37	2.19
0.1500	0.1500	0.0834	0.0650	−4.65	−1.94	−1.655	4.94
0.1800	0.1800	0.0999	0.0777	−6.84	−3.03	−2.41	7.47
0.2000	0.2000	0.0828	0.1144	−7.98	−2.35	−1.52	8.81
0.2200	0.2200	0.1220	0.0945	−10.44	−4.34	−3.72	11.05
0.2500	0.2500	0.1031	0.1424	−12.53	−3.64	−1.82	14.35
0.2800	0.2800	0.1153	0.1592	−15.66	−3.89	−2.09	17.46
0.3000	0.3000	0.1233	0.1703	−18.95	−7.03	−4.60	21.38
0.3200	0.3200	0.1314	0.1814	−20.93	−7.92	−5.61	23.23
0.3500	0.3500	0.1434	0.1979	−24.91	−10.00	−6.80	28.11
0.3800	0.3800	0.2104	0.1593	−30.84	−13.63	−10.46	34.01
0.4000	0.4000	0.1634	0.2254	−32.04	−13.27	−9.12	36.20
0.4200	0.4200	0.2321	0.1754	−38.46	−15.68	−13.80	40.34
0.4500	0.4500	0.2466	0.1891	−45.71	−17.10	−16.54	46.27
0.5000	0.5000	0.2029	0.2797	−50.67	−19.13	−11.85	57.95
			L-Threonine + Sorbose				
0.1000	0.1000	0.0413	0.0580	0.21	−0.76	−0.65	−0.10
0.1500	0.1500	0.0618	0.0866	0.50	−0.92	−0.71	−0.29
0.1800	0.1800	0.0740	0.1037	0.80	−1.55	−1.22	−0.47
0.2000	0.2000	0.0821	0.1151	0.93	−2.35	−2.11	−0.70
0.2200	0.2200	0.0901	0.1264	1.23	−2.69	−2.41	−0.95
0.2500	0.2500	0.1022	0.1433	1.42	−3.64	−3.40	−1.18
0.2800	0.2800	0.1143	0.1602	1.71	−3.89	−3.47	−1.29
0.3000	0.3000	0.1223	0.1713	2.00	−7.02	−6.42	−1.40
0.3200	0.3200	0.1302	0.1825	2.10	−7.92	−7.33	−1.51
0.3500	0.3500	0.1422	0.1992	2.51	−10.00	−9.16	−1.66
0.3800	0.3800	0.2108	0.1589	3.04	−13.63	−12.37	−1.78
0.4000	0.4000	0.1619	0.2268	3.06	−13.27	−12.41	−2.20
0.4200	0.4200	0.2326	0.1749	3.22	−15.68	−14.78	−2.32
0.4500	0.4500	0.2471	0.1885	3.60	−17.10	−15.93	−2.44
0.5000	0.5000	0.2010	0.2814	4.77	−19.13	−16.92	−2.56

An excess thermodynamic property can be expressed as a virial expansion of pair and triplet interaction coefficients, which account for all the variations of the solute−solute and solute−solvent interactions according to the McMillan−Mayer theory.[11−13] The thermodynamic procedures used have been described previously,[8−10] and only a summary will be given here.

The excess enthalpy of a solution is defined as

$$H^{E}(m_x, m_y)/w1 = H(m_x, m_y) - h_w^* - m_x H_{x,m}^{\infty} - m_y H_{y,m}^{\infty} = h_{xx} m_x^2 + 2h_{xy} m_x m_y + h_{yy} m_y^2 + h_{xxx} m_x^3 + 3h_{xxy} m_x^2 m_y + 3h_{xyy} m_x m_y^2 = + h_{yyy} m_y^3 + \ldots \quad (1)$$

Table 3. Heterotactic Enthalpic Interaction Coefficients h_{xy} between Amino Acids (x) + Fructose (y) and Amino Acids (x) + Sorbose (y) in Aqueous Solutions at 298.15 K

solutes $x + y$	h_{xy} / J·kg²·mol⁻³	h_{xxy} / J·kg²·mol⁻³	h_{xyy} / J·kg²·mol⁻³	SD	R^2
L-Val + fructose	432.15 ± 34.35	15.59 ± 60.30	−308.62 ± 51.03	0.61	0.9974
L-Thr + fructose	−269.67 ± 38.77	649.02 ± 68.38	−612.79 ± 101.01	0.60	0.9960
L-Val + sorbose	471.86 ± 25.69	28.39 ± 45.24	69.75 ± 38.21	0.46	0.9994
L-Thr + sorbose	−57.06 ± 6.02	46.54 ± 10.60	44.79 ± 8.79	0.11	0.9855

where $H^E(m_x, m_y)/w1$ and $H(m_x, m_y)/w1$ represent the excess and the absolute enthalpy, respectively, of a solution containing 1 kg of water; m_x is mole of x; m_y is mole of y; h^*_w is the standard enthalpy of 1 kg of pure water; and $H^\infty_{x,m}$ and $H^\infty_{y,m}$ are the limiting partial molar enthalpies of species x and y, respectively. h_{ij} and h_{iij} are the enthalpic virial coefficients characterizing the pairwise and triplet interactions of the solvated species, and m_x and m_y are the molalities of the solutes x and y, respectively. To make the calculation easier, an auxiliary function ΔH^* was introduced:

$$\Delta H^* = \Delta H_{mix} - \Delta H_{dil}(x) - \Delta H_{dil}(y) = H^E(m_x, m_y) - H^E(m_x) - H^E(m_y) \quad (2)$$

Thus, eq 1 can be rewritten as

$$\Delta H^*/w1 = 2h_{xy}m_xm_y + 3h_{xxy}m_x^2m_y + 3h_{xyy}m_xm_y^2 + ... \quad (3)$$

Results and Discussion

The enthalpies of dilution and mixing for the solutions studied in this work are listed in Table 2. The enthalpic interaction coefficients for the solutions, which were obtained by least-squares analysis with eq 3, are reported in Table 3.

As there are some difficulties in the interpretation of higher h coefficients, only the pairwise coefficients h_{xy} are considered here. The h_{xy} coefficients are considered as enthalpic contributions to the coefficients of the excess Gibbs free energy and a measure of the global effect constituting a sum of the following processes: the partial dehydration of the solutes and the further direct interaction caused by the short-range molecular forces.[14] The partial dehydrations of the hydration shell of the amino acid zwitterions, fructose and sorbose, are all endothermic processes. The direct solute−solute interaction between amino acids and fructose or sorbose plays the dominant role in the process of interaction. As mentioned in the literature,[8] the direct interaction of α-amino acid with fructose and sorbose comprises three kinds of interactions: the hydrophilic−hydrophilic interaction, the hydrophobic−hydrophobic interaction, and the hydrophobic−hydrophilic interaction.

The enthalpic interaction coefficients, h_{xy}, represent the result of the competitive equilibrium between the above effects. Among these effects, the direct interactions between amino acid and saccharide molecules play the dominant role during the overall interaction processes. The values of h_{xy} of L-valine with saccharides are positive and those of L-threonine with saccharides are negative. The discrepancies of h_{xy} are mainly dependent on the differences in the structure of the two interacting solutes.

The magnitude tendency of the h_{xy} coefficients of amino acids with saccharides has been analyzed concretely as follows:

(i) Heterotactic enthalpic pairwise interactions of L-valine and L-threonine with same saccharide in aqueous solution. The differences of h_{xy} coefficients between amino acids and fructose and sorbose are dramatically contingent on the discrepancies of the structure of amino acids. The enthalpic pairwise interaction coefficients h_{xy} of L-valine with saccharides are positive and those of L-threonine are negative. The experimentally observed values of h_{xy} testify to the predominance of endothermic processes over exothermic processes for L-valine with saccharides, while for L-threonine with saccharides the exothermic processes play the dominant role. As can be seen from Table 1, the side chain of L-threonine can be considered as a substitute for one hydroxyl group of one of the methyl groups of L-valine. The main differences of the interactions of L-valine and L-threonine with fructose and sorbose lie in the following: there exist hydrophobic−hydrophobic and hydrophobic−hydrophilic interactions (both making positive contributions to h_{xy}) of the methyl group of L-valine with the apolar group and the OH groups of fructose and sorbose. There also exist hydrophilic−hydrophobic (making positive contributions to h_{xy}) and hydrophilic−hydrophilic interactions (making negative contributions to h_{xy}) of the hydroxyl group on the side chain of L-threonine with the apolar groups and the OH groups of fructose and sorbose molecule. So the comparative magnitude of h_{xy} (L-valine) and h_{xy} (L-threonine) depends on the competitive balance of the above varied interactions.

(ii) Heterotactic enthalpic pairwise interactions of same amino acids with sorbose and fructose in aqueous solution. From Table 3, it can be clearly seen that the rule of the h_{xy} coefficients between amino acids and fructose is similar to that of the h_{xy} coefficients between amino acids and sorbose. In the meantime, there exist some distinctions in their interaction behaviors. These can be attributed primarily to the similarities and discrepancies in the structures of fructose and sorbose. Sorbose and fructose are ketohexoses, and they are prevalent in a six-membered ring conformation, called pyranose. The sequence of the h_{xy} values for them seems to depend on the number and position of equatorial (e) and axial (a) -OH groups in the anomeric form, which is predominant in solution. The extent of hydration of saccharide molecules depends on their conformations and configurations of their hydroxyl groups, and e-OH groups are more readily hydrated than a-OH groups.[15] The saccharide molecule, which has a larger number of e-OH groups, has a stronger stabilizing effect on water structure. Any other property that depends on the saccharide hydration are thought to correlate well with the mean number of e-OH group in saccharide molecules.[16] The less negative values of h_{xy} are shown by L-threonine with sorbose, which bears only an a-OH group. The more negative values of h_{xy} are shown by L-threonine with fructose, which bear two a-OH groups. The extension of nonpolar side chain increases to a great extent the hydrophobic quality of L-valine, and the values of h_{xy} are positive. Fructose bears more a-OH groups, which result in the hydrophilic effect larger than sorbose in the process of interaction with L-valine. For the L-valine molecule, the experimental results is h_{xy} (sorbose) > h_{xy} (fructose).

(iii) Comparison between the interactions of L-valine and L-threonine with saccharides and those of other amino acids (glycine, L-alanine, and L-serine) with saccharides. In this continuing series of investigation,[8] the rules of the interactions of L-valine and L-threonine with saccharides have been found to be more complicated than those of glycine, L-alanine, and

L-serine with saccharides owing to discrepancies of the structure of amino acids. Since there is one methyl on the side chain of L-threonine as compared to L-serine, eqs 3 and 4 (both making positive contributions to h_{xy}) are reinforced. In consequence, we can see that h_{xy} values of L-threonine with saccharides are less negative than those of L-serine with saccharides. The extension of nonpolar side chain increases to a great extent the hydrophobic quality of L-valine, and the values of h_{xy} are more positive as compared to those of L-alanine.

Conclusion

The heterotactic pair enthalpic pairwise interaction coefficients h_{xy} are determined by measuring the mixing enthalpies of ternary system α-amino acids (L-valine and L-threonine) + monosaccharide (fructose and sorbose) + H_2O and their dilution enthalpies, respectively, with a Thermometric-2277 thermal activity monitor at 298.15 K. Mixing enthalpy is recognized as a basic thermodynamic parameter to study the interactions between two solute molecules occurring in solutions. But little data concerning mixing enthalpies of amino acids with other model components are available.

Enthalpic interaction coefficients have been proven to be very useful to explain the behavior of hydroxylated substances,[17,18] α-amino acids,[19,20] carboxydric acids,[21] amine,[21] and a mixture of these solutes. In the present work, the experimental values of h_{xy} of L-valine with saccharides are positive, and those of L-threonine with saccharides are negative. The variations of the enthalpic pairwise interaction coefficients are interpreted in terms of solute−solute interactions. In the ternary solutions under investigation, there are mainly three kinds of interactions that are expected to contribute to h_{xy} coefficient, where the direct solute−solute interaction between amino acids and fructose or sorbose plays the dominant role. The experimentally observed values of h_{xy} testify to the predominance of endothermic processes over exothermic processes for L-valine with saccharides, while for L-threonine with saccharides, the exothermic processes play the dominant role. At present, many studies focus on the interaction mechanism of amino acids with nonelectrolytes occurring in solutions. Amino acids with polar or apolar side chains and some special amino acids such as L-proline need to draw more attention in order to deepen the understanding of solute−solute interactions.

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