Enthalpic Interactions of Amino Acids with Saccharides in Aqueous Solutions at 298.15 K

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The enthalpies of mixing of three kinds of aqueous amino acid solutions (glycine, L-alanine, and L-serine) with aqueous sorbose solution and aqueous fructose solution and their respective enthalpies of dilution have been determined at 298.15 K using a 2277 flow microcalorimetry. The experiment data have been analyzed in terms of McMillan−Mayer formalism to obtain the enthalpic virial coefficients for heterotactic interaction. The results have been interpreted from the point of view of solute−solute interactions.

Introduction

Many investigations have shown that sugars and polyhydric alcohols increase the thermal stability of proteins or reduce the extent of their denaturation by other reagents.[1−5] A successful explanation about the mechanism of the stabilization seems to be that the stabilization ability of these additives may be dominantly mediated through the changes in solvent properties or alteration of water structure.[1,2,6−9] Although some trends correlating the stabilizing potency of sugars and polyols with the number or configuration of the hydroxyl groups have been noted,[2] there are numerous exceptions,[10,11] and all proteins and enzymes do not respond equally to a given compound. Thus our understanding of the mechanism of stabilization of proteins by these additives is still incomplete.

To understand the nature of interactions of sugars and polyols with proteins in aqueous solutions, we have studied the thermodynamics of mixing varieties of amino acids solutions with aqueous saccharide solutions where the mixing process is accompanied by a change in solute−solute interaction and solute−solvent interaction.

Virial coefficients of the power series of the excess enthalpies as a function of the molalities permit access to information about the interactions between hydrated molecules.[12] They can be easily derived from enthalpies of dilution and mixing of solutions containing *n* solutes.

As an extension to our previous studies,[13−18] we report here the enthalpies of mixing aqueous solutions of glycine, L-alanine, and L-serine with aqueous solutions of the sorbose and fructose and their respective enthalpies of dilution. These results serve as a basis for calculation of the heterotactic enthalpic coefficients of interactions between the amino acids and saccharides in aqueous solutions according to the McMillan−Mayer theory.[19] These coefficients reflect the sum of the enthalpic effects of interactions between the components in aqueous solutions.

Material and Methods

Glycine, L-alanine, and L-serine (biochemical reagent, >99.0%, from Shanghai Chem. Co.) were used after recrystallization from methanol−water mixtures and drying in a vaccum over P_2O_5 at room temperature for at least 72 h. Sorbose and fructose (biochemical reagent, from Shanghai Chem. Co.) were used without further purification. The water used for the preparation of solution was deionized and distilled using a quartz sub-boiling purifier. Both the aqueous amino acid solutions and aqueous saccharide solutions 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 enthalpy of mixing was measured by a mixing-flow microcalorimeter (2277 ThermalActivity Monitor, made in Sweden). All the measurements were carried out at 298.15 K. The solutions were pumped through the mixing vessel of the calorimeter at constant rates using a microperpex peristaltic pump with a pair of wheels (VS2-10R MIDI 2,5-50PRM). The 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:

1. A(water) + B(water)—baseline determined.
2. A(aqueous amino acid solution) + B(aqueous saccharide solution)—mixing thermal power determined.
3. A(aqueous amino acid solution) + B(water)—dilution thermal power determined.
4. A(water) + B(aqueous saccharide solution)—dilution thermal power determined.
5. A(water) + B(water)—baseline re-established.

Each dilution and mixing experiment was repeated three times, and the average of three measured values was given.

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

$$\Delta H_{dil} = P/(f_A + f_B - m_{x,i}M_x f_A) \quad (1)$$

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

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

$$m_{x,f} = \frac{m_{x,i}f_A}{f_B(m_{x,i}M_x + 1) + f_A} \quad (2)$$

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

$$\Delta H_{mix} = P^*/(f_A + f_B - m_{x,i}M_x f_A - m_{y,i}M_y f_B) \quad (3)$$

where P^* is the mixing thermal power (μw), f_A and f_B are the flow rates of solutions x and y, respectively, $m_{x,i}$ and $m_{y,i}$ are the initial molarilities of solutions x and y before mixing.

The excess enthalpy $H^E(m_x,m_y)$ of a solution containing two solute species x and y can be analyzed using the McMillan−Mayer theory,[19−21] which allows the separation of effects arising from molecular pairwise, triplet, and higher interactions between the two solutes

$$H^E(m_x,m_y)/wl = H(m_x,m_y) - h_w^* - m_x H_{x,m}^\infty - m_y H_{y,m}^\infty \quad (4)$$

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

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

$$H^E(m_x,m_y)/wl = h_{xx}m_x^2 + 2h_{xy}m_x m_y + h_{yy}m_y^2 + h_{xxx}m_x^3 + 3h_{xxy}m_x^2 m_y + 3h_{xyy}m_x m_y^2 + h_{yyy}m_y^3 + \ldots \quad (5)$$

In which h_{xx}, h_{yy}, and 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

$$H^E(m_x)/wl = h_{xx}m_x^2 + h_{xxx}m_x^3 + \ldots \quad (6)$$

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

$$\Delta H^* = \Delta H_{mix} - \Delta H_{dil}(x) - \Delta H_{dil}(y) \quad (7)$$

from eqs 4 and 5; it follows that

$$\Delta H^*/wl = 2h_{xy}m_x m_y + 3h_{xxy}m_x^2 m_y + 3h_{xyy}m_x m_y^2 \quad (8)$$

or

$$\Delta H^*/(wl m_x m_y) = 2h_{xy} + 3h_{xxy}m_x + 3h_{xyy}m_y + \ldots \quad (9)$$

Experimental Results and Discussion

The experimental values ΔH_{mix} and ΔH_{dil} of aqueous amino acid, sorbose, and fructose solutions are given in Table 1 together with ΔH^*.

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

The enthalpic pairwise-interaction coefficients are regarded as a measure of the heat effect (i.e., the enthalpy of interaction) when two solute particles approach each other. The physical meaning of the pair interaction coefficients of an excess property is linked to the variation of the 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.[22] The process is supposed to occur by the overlap of hydration cospheres with the consequent release of water molecules from these cospheres to the bulk, the sign of the thermodynamic properties of the water molecules in the cospheres and in the bulk.[23]

Since there are some difficulties in the interpretation of the higher h coefficients, only the pairwise coefficient h_{xy} is considered here. The enthalpic interaction coefficients, h_{xy}, as representatives of the enthalpic properties of the solution and a measure of interactions between two hydrated solutes, depend on the interactions between solute molecules under investigation and solvent water. Thus the overall effect reflects the following three superimposed processes:

The first is the partial dehydration of the hydration shell of the amino acid zwitterions (an endothermic process and lead to a positive contribution to h_{xy}). The dehydration is caused by mutual penetration of the hydration shells interaction molecules in the aqueous medium.

The second is the partial dehydration of the hydration shell of sorbose or fructose molecules (an endothermic process that leads to a positive contribution to h_{xy}).

The third is the direct interaction between the molecules of the amino acids and sorbose and fructose. Generally speaking, the types of interactions occurring between the amino acid molecules and saccharide molecules can be classified as follows:

(1) Hydrophilic−ionic interactions between the OH group of the saccharides and the zwitterionic centers of the amino acids.

(2) Hydrophobic−hydrophobic interactions between the nonpolar side groups of the saccharide molecules and the amino acids.

(3) Hydrophilic−hydrophilic interactions between the OH group of the saccharide molecules and the OH group of the amino acids.

(4) Hydrophilic−hydrophobic interactions between the OH group of the saccharide molecules and the nonpolar side group of the amino acids.

The resulting sign of h_{xy} characterizing excess enthalpic properties of aqueous solution containing two different solute series would be a consequence of the competitive equilibrium between the above effects. Among these effects, the direct interactions between amino acid and saccharide molecules are considered as the most important interaction and play the dominant role during the interaction processes.

The discrepancies of h_{xy} are mainly dependent on the differences in the structure of the two interacting solutes.

1. Heterotactic Enthalpic Interactions of the Same Kind of α-Amino Acid with Sorbose and Fructose in the Aqueous Solutions. For the same kind of amino acid series, the discrepancies of h_{xy} are mainly ascribed to the difference in the configuration of sorbose and fructose.

A way to analyze the pairwise coefficients is the Savage and Wood group contribution approach (SWAG).[24] This approach, however, cannot distinguish isomers. The occurrence of nonbonding interactions in aqueous solutions was largely emphasized, but the influence of the OH group in different sugar molecules was not completely elucidated since sugars present in the solutions as a mixture of interconverting isomers and anomers.

Saccharide in aqueous solution exhibits a quite complex behavior. Each monosacchride exists in aqueous solution

Table 1. Enthalpies of Dilution and Enthalpies of Mixing for α-Amino Acids(x) + Sorbose(y) and α-Amino Acids(x) + Fructose(y) in Aqueous 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}	ΔH_{dil}(x)/J·kg^{-1}	ΔH_{dil}(y)/J·kg^{-1}	ΔH_{mix}/J·kg^{-1}	ΔH/J·kg^{-1}
			Glycine + Sorbose				
0.1000	0.1000	0.05105	0.0483	1.00	−1.41	−1.88	−1.46
0.1500	0.1500	0.062912	0.0857	2.34	−0.92	−1.64	−3.06
0.1800	0.1800	0.075397	0.1026	3.30	−1.55	−2.62	−4.37
0.2000	0.2000	0.083702	0.1138	4.11	−2.35	−4.61	−6.38
0.2200	0.2200	0.091994	0.1250	4.90	−2.69	−5.12	−7.33
0.2500	0.2500	0.104404	0.1418	6.38	−3.64	−6.70	−9.44
0.2800	0.2800	0.116782	0.1584	7.83	−3.90	−7.16	−11.10
0.3000	0.3000	0.125016	0.1695	9.11	−7.03	−11.04	−13.12
0.3200	0.3200	0.133236	0.1805	10.41	−7.92	−13.24	−15.74
0.3500	0.3500	0.14554	0.1970	11.67	−10.00	−15.59	−17.26
0.3800	0.3800	0.212161	0.1591	14.58	−13.63	−21.41	−22.36
0.4000	0.4000	0.165977	0.2243	15.18	−13.27	−22.05	−23.95
0.4200	0.4200	0.234191	0.1751	17.06	−15.68	−27.51	−28.89
0.4500	0.4500	0.248987	0.1887	20.14	−17.10	−32.45	−35.49
0.5000	0.5000	0.206591	0.2783	23.47	−19.13	−32.56	−36.90
			L-Alanine + Sorborse				
0.1000	0.1000	0.0511	0.0483	−0.53	−1.41	−1.74	0.20
0.1500	0.1500	0.0631	0.0854	−1.24	−0.92	−1.69	0.47
0.1800	0.1800	0.0756	0.1023	−1.69	−1.55	−2.57	0.67
0.2000	0.2000	0.0839	0.1135	−2.00	−2.35	−3.61	0.74
0.2200	0.2200	0.0922	0.1247	−2.47	−2.69	−4.32	0.84
0.2500	0.2500	0.1046	0.1413	−3.16	−3.64	−5.87	0.94
0.2800	0.2800	0.1170	0.1579	−3.95	−3.89	−6.80	1.04
0.3000	0.3000	0.1252	0.1690	−4.73	−7.03	−10.43	1.33
0.3200	0.3200	0.1335	0.1800	−5.41	−7.92	−11.75	1.57
0.3500	0.3500	0.1457	0.1964	−6.12	−10.00	−14.23	1.90
0.3800	0.3800	0.2113	0.1594	−7.77	−13.63	−19.35	2.05
0.4000	0.4000	0.1660	0.2237	−8.21	−13.27	−19.33	2.15
0.4200	0.4200	0.2332	0.1755	−9.56	−15.68	−22.95	2.29
0.4500	0.4500	0.2479	0.1892	−10.81	−17.10	−25.45	2.46
0.5000	0.5000	0.2066	0.2775	−12.36	−19.13	−28.86	2.63
			L-Serine + Sorbose				
0.1000	0.1000	0.0512	0.0481	1.67	−1.41	−1.22	−1.48
0.1500	0.1500	0.0838	0.0646	4.04	−1.94	−1.11	−3.20
0.1800	0.1800	0.1005	0.0773	5.69	−3.03	−1.66	−4.32
0.2000	0.2000	0.0835	0.1138	6.57	−2.35	−1.32	−5.54
0.2200	0.2200	0.1226	0.0941	8.50	−4.34	−2.88	−7.05
0.2500	0.2500	0.1040	0.1417	10.16	−3.64	−1.50	−8.02
0.2800	0.2800	0.1163	0.1584	12.69	−3.89	−1.65	−10.45
0.3000	0.3000	0.1244	0.1694	14.93	−7.03	−3.30	−11.20
0.3200	0.3200	0.1326	0.1805	16.77	−7.92	−4.71	−13.56
0.3500	0.3500	0.1447	0.1969	19.27	−10.00	−5.72	−14.98
0.3800	0.3800	0.2115	0.1587	23.50	−13.63	−8.90	−18.77
0.4000	0.4000	0.1649	0.2242	24.78	−13.27	−7.52	−19.02
0.4200	0.4200	0.2333	0.1747	28.81	−15.68	−11.46	−24.59
0.4500	0.4500	0.2480	0.1883	31.35	−17.10	−13.92	−28.17
0.5000	0.5000	0.2049	0.2782	36.59	−19.13	−11.99	−29.45
			Glycine + Fructose				
0.1000	0.1000	0.0508	0.0486	1.00	0.13	−0.82	−1.94
0.1500	0.1500	0.0628	0.0858	2.34	−0.57	−3.46	−5.23
0.1800	0.1800	0.0753	0.1027	3.30	−0.93	−5.85	−8.21
0.2000	0.2000	0.0836	0.1140	4.11	−1.59	−7.03	−9.55
0.2200	0.2200	0.0918	0.1252	4.90	−2.00	−7.91	−10.81
0.2500	0.2500	0.1042	0.1419	6.38	−2.36	−11.14	−15.16
0.2800	0.2800	0.1166	0.1586	7.83	−2.74	−14.02	−19.11
0.3000	0.3000	0.1248	0.1697	9.11	−3.58	−15.66	−21.19
0.3200	0.3200	0.1330	0.1807	10.41	−4.23	−18.34	−24.52
0.3500	0.3500	0.1453	0.1972	11.67	−5.61	−21.36	−27.43
0.3800	0.3800	0.2122	0.1591	14.58	−7.33	−26.50	−33.76
0.4000	0.4000	0.1657	0.2246	15.18	−8.32	−28.43	−35.29
0.4200	0.4200	0.2342	0.1751	17.06	−9.92	−34.32	−41.46
0.4500	0.4500	0.2490	0.1887	20.14	−10.03	−36.72	−46.82
0.5000	0.5000	0.2063	0.2787	23.47	−10.06	−40.76	−54.16
			L-Alanine + Frucose				
0.1000	0.1000	0.0507	0.0486	−0.53	0.13	−0.73	−0.33
0.1500	0.1500	0.0627	0.0858	−1.24	−0.57	−2.57	−0.77
0.1800	0.1800	0.0752	0.1027	−1.70	−0.93	−4.07	−1.44
0.2000	0.2000	0.0834	0.1140	−2.00	−1.59	−5.46	−1.87
0.2200	0.2200	0.0917	0.1252	−2.48	−2.00	−6.77	−2.30
0.2500	0.2500	0.1040	0.1419	−3.17	−2.36	−8.64	−3.11
0.2800	0.2800	0.1163	0.1586	−3.96	−2.74	−10.60	−3.91
0.3000	0.3000	0.1245	0.1697	−4.75	−3.58	−12.71	−4.38
0.3200	0.3200	0.1327	0.1807	−5.42	−4.23	−14.72	−5.07

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}	ΔH_{dil}(x)/J·kg^{-1}	ΔH_{dil}(y)/J·kg^{-1}	ΔH_{mix}/J·kg^{-1}	ΔH/J·kg^{-1}
			L-Alanine + Frucose (Continued)				
0.3500	0.3500	0.1449	0.1972	−6.14	−5.61	−17.34	−5.58
0.3800	0.3800	0.2113	0.1594	−7.77	−7.33	−20.91	−5.82
0.4000	0.4000	0.1652	0.2246	−8.23	−8.32	−22.73	−6.18
0.4200	0.4200	0.2332	0.1755	−9.56	−9.92	−26.35	−6.87
0.4500	0.4500	0.2479	0.1892	−10.81	−10.03	−29.11	−8.26
0.5000	0.5000	0.2054	0.2787	−12.40	−10.06	−33.50	−11.04
			L-Serine + Fructose				
0.1000	0.1000	0.0508	0.0484	1.61	0.13	−0.38	−2.12
0.1500	0.1500	0.0627	0.0858	3.74	−0.57	−1.72	−4.89
0.1800	0.1800	0.0751	0.1027	5.50	−0.93	−3.01	−7.58
0.2000	0.2000	0.0833	0.1139	6.57	−1.59	−3.50	−8.48
0.2200	0.2200	0.0915	0.1251	8.51	−2.00	−4.18	−10.69
0.2500	0.2500	0.1038	0.1419	10.16	−2.36	−5.77	−13.58
0.2800	0.2800	0.1161	0.1586	12.69	−2.74	−7.33	−17.28
0.3000	0.3000	0.1242	0.1696	14.93	−3.58	−8.10	−19.45
0.3200	0.3200	0.1324	0.1807	16.77	−4.23	−9.79	−22.33
0.3500	0.3500	0.1395	0.2023	19.75	−5.61	−11.39	−25.53
0.3800	0.3800	0.2115	0.1587	23.50	−7.33	−13.90	−30.07
0.4000	0.4000	0.1647	0.2245	24.78	−8.32	−14.67	−31.13
0.4200	0.4200	0.2334	0.1747	28.82	−9.92	−18.34	−37.24
0.4500	0.4500	0.2480	0.1883	31.36	−10.03	−19.69	−41.01
0.5000	0.5000	0.2046	0.2786	36.59	−10.06	−21.82	−48.35

Table 2. Heterotactic Enthalpic Interaction Coefficients for α-Amino Acids with Sorbose and Fructose in Aqueous Solutions at 298.15 K

solute x + y	h_{xy}/J·kg·mol^{-2}	h_{xxy}/J·kg^2·mol^{-3}	h_{xyy}/J·kg^2·mol^{-3}	std dev	R^2
glycine + sorbose	−276	−330	136	0.76	0.9966
L-alanine + sorbose	48	−29	−38	0.10	0.9878
L-serine + sorbose	−275	−199	192	0.50	0.9976
glycine + frucose	−560	−14	218	0.55	0.9991
L-alanine + fructose	−108	78	−26	0.34	0.9875
L-serine + fructose	−525	64	196	0.59	0.9986

either as a six-membered ring, called pyranose, or as a five-membered ring, called furanose. The noncyclic forms are very low. Each ring contains one chiral carbon atom, which allows the existence of two (α and β) anomers. In fact, the aqueous solutions of monosaccharide contain mixtures of anomers and isomers in chemical equilibrium.

Sorbose and fructose are ketohexoses, and they are prevailingly in the pyranose chair conformation. For both of these substances, the −OH (2) is maintained in the axial position by the presence of the more hindering $-CH_2OH$, linked to the same carbon atom on the ring.[24] First, the sequence of the h_{xy} values for them seems to depend on the number and position of equatorial and axial −OH groups in the anomeric form that is predominant in solution. Pairs of equatorial −OH groups can promote the formation of labile cages of solvent molecules, but the presence of an axial −OH group can reduce this capability. The less negative values of h_{ij} are shown by sorbose, which bears only an axial −OH group; the more negative values of h_{ij} are shown by fructose, which bear two axial −OH groups. The difference in the values of h_{xy} of the two sets obtained seems to be significant, beyond the fitting uncertainty. This fact differentiates further the behavior of the OH group in the two classes of saccharide. Second, it can also be attributed to intramolecular contributions, mainly the inductive effect due to the hemiacetal oxygen. Ring closure is also important. Finally a role must be played by the specific stability of the hydration shell of each solute molecule.

2. Heterotactic Enthalpic Interactions of Different Kinds of α-Amino Acids with Sorbose and Fructose in the Aqueous Solutions. Here we consider the amino acid series; the discrepancies of heterotactic enthalpic interactions coefficients mainly depend on the differences in the structures of the amino acids studied.

Glycine is the simplest amino acid in nature. In aqueous solutions, the interactions between glycine and saccharides include types 1 and 4, of which the former is dominant. This results in negative values of $h_{ij.}$ L-Alanine has one hydrogen atom of the α-carbon replaced by a methyl compared to glycine, which increases the hydrophobic properties of L-alanine. So the interactions between L-alanine and saccharides include types 1, 2, and 4. Thus, the enthalpic interaction coefficients between L-alanine and saccharides are larger than those of glycine. But for two kinds of saccharides considered, the sign of values h_{ij} is converse. This fact showed that the behavior of the −OH group in these two classes of monosaccharides solutes is different.

The side chain of L-serine has polar group $-CH_2OH$, which makes the enthalpies of mixing more complex. Interactions between L-serine and saccharides include all four types, and the effect of 1 and 3 screen over the effect of 2 and 4. In addition, it is possible for the L-serine molecule to interact with saccharide molecules side by side. This means the zwitterionic groups and −OH group of L-serine molecule can interact with different OH group of the same saccharide molecule. In this situation, the nonpolar groups of L-serine and saccharide become closer and the hydrophobic interactions are enhanced. So the h_{xy} of L-serine interacting with saccharide is less negative than that of glycine interacting with saccharide, but the introduction of −OH leads to the more negative values than those of L-alanine.

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