Interaction Between Some Monosaccharides and Aspartic Acid in Dilute Aqueous Solutions

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Received: 16 November 2007 / Accepted: 21 January 2008 /

Published online: 8 March 2008

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Abstract Interaction between aspartic acid and D-glucose, D-galactose, and D-fructose has been studied by isothermal titration calorimetry, calorimetry of dissolution, and densimetry. It has been found that D-glucose and D-fructose form thermodynamically stable associates with aspartic acid, in contrast to D-galactose. The selectivity in the interaction of aspartic acid with monosaccharides is affected by their stereochemical structures.

Keywords Monosaccharide · Aspartic acid · Associate formation · Calorimetry · Densimetry

1 Introduction

Protein–carbohydrate interactions are the basis of very important biochemical processes occurring in living organisms, such as viral and bacterial infections [1, 2], immunological response [3], metastatic spread and growth [4], some enzymatic reactions [5], etc. Therefore, these interactions have been the subject of intense investigations over recent years. But in spite of this fact, the mechanism and nature of the interactions responsible for delicate molecular recognition of carbohydrates by proteins remains a much-debated area of inquiry. Studies of the protein–carbohydrate interactions are not an easy task due to the particular complexity of these large biomolecules. Therefore, one useful approach that can be used to understand the nature and mechanism of protein–carbohydrate interactions is to study model compounds which are low-molecular-weight solutes that incorporate some of structural features of such complex molecules. The aim of this work was to study the interactions between some isomeric monosaccharides (D-glucose, D-galactose, and D-fructose) and L-aspartic acid in aqueous solution. Aspartic acid is a functional group of reaction centers of some enzymes and lectins. Monosaccharides are monomer units of the oligosaccharide chains of glycoproteins and glycolipids.

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Analysis of theoretical and experimental data [3, 5–9] shows that, in general, intermolecular interactions between large organic particles in aqueous medium are non-covalent and accompanied by small energy changes. This is because they are characterized by the confluence of contributions of many weak polar and non-polar intermolecular forces which often compensate each other. In addition, monosaccharides and amino acids are highly hydrated, and their ability to interact intermolecularly has to compete with water. That is why to obtain reliable conclusions about such interactions, a combination of three experimental methods has been applied: isothermal titration calorimetry, calorimetry of dissolution, and densimetry.

2 Experimental

2.1 Materials

L-Aspartic acid, β -D-fructose (Fru; ICN Biomedicals, >99% purity), D-glucose, D-galactose (Fluka) were used without further purification. The samples of the reagents were dried in a vacuum at 353 K for several days before use. Solutions were prepared by weight using doubly distilled degassed water. pH measurements were performed with digital pH meter OP 211/1 (Hungary).

2.2 Methods

2.2.1 Isothermal Titration Calorimetry

The heats of interactions were measured with an automatic titration differential calorimeter [10] at 298.15 K. The measurement technique and procedure of treatment of the experimental data have been reported earlier [11]. The initial concentration of an aqueous solution of amino acid placed into the calorimetric cell was 2.51 mM. The initial concentration of an aqueous solution of monosaccharide placed into the syringe was 0.20–0.23 M. The volume of an injection was $7,644 \times 10^{-5} \text{ dm}^3$. Control experiments were performed by identical injections of the monosaccharide solution into the cell containing water. Appropriate corrections of the heats of interactions relative to the heats of dilution were made. During the analysis of the calorimetric results, 1:1, 2:1, and 1:2 models of interaction between aspartic acid and monosaccharide were considered. However, the best fitting of the experimental data was observed using the 1:1 model. The calorimetric titration curves for the aspartic acid/D-glucose/water and aspartic acid/D-fructose/water systems are presented in Fig. 1. For the aspartic acid/D-galactose system, no difference between the titration heat effects and the heat effects of dilution was detected.

At first approach, the determined thermodynamic characteristics can be considered as standard because all the data are obtained in dilute solutions at the temperature 298.15 K, and no concentration dependences of these characteristics for the monosaccharide and amino acids in the studied range were observed. The stability constant values and the thermodynamic characteristics of binding of the studied monosaccharides with aspartic acid are presented in Table 1.

2.3 Calorimetry of Dissolution

The enthalpies of solution of aspartic acid ($\Delta_{\text{sol}}H_{\text{aa}}$) in water–monosaccharide mixtures were measured with an airtight isoperibol calorimeter. The titan calorimetric cell, of

Fig. 1 Integral titration curves of aqueous solution of aspartic acid (2.51 mM) by aqueous solutions of monosaccharides (0.22–0.23 M) at 298.15 K: 1, D-glucose; 2, D-fructose

capacity approximately 70 cm^3 , was equipped with a calibration heater ($R=11.56 \text{ } \Omega$), a termistor ($R=8.65 \text{ } \Omega$), an ampoule holder, and stirrer. A temperature change of approximately $3 \times 10^{-5} \text{ K}$ could be detected. The calorimeter was placed in a hermetically closed jacket and immersed in a water thermostat. A double thermostating system allows maintaining the temperature stability better than $5 \times 10^{-3} \text{ K}$. Samples of aspartic acid were placed into a thin-walled glass ampoule. The error in the calorimetric measurements was estimated to be $\pm 0.5\%$. The solute molality in all cases was estimated to be $2 \times 10^{-3} \text{ mol kg}^{-1}$, which can be considered as infinitely dilute, and the measured enthalpies of solution were regarded as standard enthalpies of solution ($\Delta_{\text{sol}} H_{\text{aa}}^0$). The monosaccharide concentration was varied from 0 to 0.15 mol per kilogram of solvent.

2.4 Densimetry

Density measurements were performed with a magnetic float densimeter. The description of the densimeter, the measurement technique, and the procedure of treatment of the experimental data have been reported earlier [12]. The uncertainty in ρ was estimated to be $\pm 2 \times 10^{-2} \text{ kg m}^{-3}$. The densities of the ternary solutions of aspartic acid–monosaccharide–water were measured at fixed concentration of the amino acid ($m_{\text{aa}}=0.04 \text{ mol kg}^{-1}$). They are presented in Table 2.

Apparent molar volumes of the studied monosaccharides were calculated with the equation

$$\Phi_{\text{vs}} = \frac{M}{\rho} - \frac{1000(\rho - \rho_0)}{m_s \rho \rho_0} \quad (1)$$

Table 1 Stability constants and thermodynamic parameters of 1:1 associate formation (kJ mol^{-1}) of some monosaccharides with aspartic acid in aqueous solutions at 298.15 K

	D-glucose (pH=2.99)	D-fructose (pH=3.00)	D-galactose (pH=2.96)
K	1566 ± 374	513 ± 42	No associate formation
ΔH	-1.15 ± 0.27	-2.29 ± 0.21	
$T\Delta S$	17.1	13.0	

Table 2 Experimental density data in aspartic acid–monosaccharide–water ternary systems at 298.15 K

Aspartic acid/D-glucose		Aspartic acid/D-galactose		Aspartic acid/D-fructose	
$m_{aa}=0.04038$		$m_{aa}=0.04008$		$m_{aa}=0.03997$	
m_s	ρ (kg m ⁻³)	m_s	ρ (kg m ⁻³)	m_s	ρ (kg m ⁻³)
0.00000	999.61	0.00000	999.61	0.00000	999.62
0.02033	1000.98	0.01651	1000.76	0.02031	1001.05
0.20810	1001.55	0.01998	1001.00	0.03009	1001.17
0.03996	1002.39	0.02507	1001.36	0.03704	1002.23
0.04719	1002.84	0.02996	1001.69	0.03899	1002.47
0.05509	1003.28	0.03510	1002.05	0.04007	1002.61
0.06084	1003.66	0.04029	1002.40	0.04155	1002.71
0.07116	1004.33	0.04995	1003.09	0.04499	1002.90
0.08180	1005.03	0.05484	1003.42	0.05093	1003.21
		0.06114	1003.87	0.06055	1003.82
		0.07023	1004.48	0.07974	1005.14
		0.08018	1005.16		

where M is the molar mass of the monosaccharide; m_s is its molality; ρ is the density of the three component solutions; ρ_0 is the density of the aqueous solution of the amino acid ($m_{aa}=0.04$ mol kg⁻¹). For a binary solution, ρ and ρ_0 are the densities of aqueous monosaccharide solution and water, respectively.

The uncertainty in apparent molar volume was calculated as:

$$d\Phi_{vs} = \left| -\left(M + \frac{1000}{m_s} \right) \frac{\delta\rho}{\rho^2} \right| + \left| \frac{1000\delta m_s}{m_s^2} \left(\frac{\rho - \rho_0}{\rho\rho_0} \right) \right| + \left| \frac{1000\delta\rho_0}{m_s\rho_0} \right|. \quad (2)$$

However, for the given system, ρ_0 is constant. Moreover, the error in solution molality is low (typically not greater than 0.03%) and its contribution to uncertainty in $d\Phi_{vs}$ much smaller than that arising from the error in density. Thus, it has been assumed that the second and the third terms in (2) are negligible. Therefore, if the uncertainty in the solution density is 2×10^{-2} kg m⁻³, typical values of $d\Phi_{vs}$ at $m_s=0.02$, 0.04, and 0.08 mol kg⁻¹ were 1×10^{-6} , 0.5×10^{-6} , and 0.25×10^{-6} m³mol⁻¹, respectively.

3 Discussion

The data presented in Table 1 show that, in contrast to D-galactose, D-glucose and D-fructose form thermodynamically stable associates with aspartic acid. Moreover, the stability constant of the associate formation of the amino acid with D-glucose is about three times greater than that with D-fructose. These results indicate selective binding of the monosaccharides under study with aspartic acid. There are at least two reasons for such selectivity in binding. Firstly, it may be hydration of the monosaccharide molecules. It is well known that interaction between solutes in aqueous dilute solution is realized through overlap of their hydration shells and partial dehydration. Strong hydration can weaken or even prevent associate formation. However, according to literature data on volumetric properties [13], g_{xx} [14], and the viscosity B -coefficient [15], the extent of hydration decreases in the following order: glucose > fructose > galactose.

The second reason may be an effect of the stereochemical structure of the monosaccharide molecules. The mentioned monosaccharides are stereoisomers. It should

be noted that, in aqueous solution, they exist in equilibrium of a few tautomeric forms [14, 16–18]. Literature data indicate that carbohydrates can exhibit α/β anomer selectivity with respect to some molecular ligands [19, 20]. However, as is well known, the preponderant tautomer in the equilibrium mixture is the β -pyranose because this tautomer is the most thermodynamically favorable in water [16, 18]. According to the literature data, the percentage ratio of α/β anomers for D-glucose and D-galactose is approximately equal. Thus, the aldohexoses D-glucose and D-galactose differ from each other only by spatial orientation of the OH group at the fourth carbon atom (C4; Fig. 2). It is likely that an axial orientation of the OH group at C4 in the D-galactose molecule prevents formation of the associate between this monosaccharide and aspartic acid. The crucial role of spatial orientation of the OH group at C4 for the interactions between carbohydrates and molecular ligands as well as proteins has been stressed in a series of studies [9, 12, 21–23].

D-Fructose is ketohexose (Fig. 2). This monosaccharide has a quite different stereochemical configuration in comparison with D-glucose and D-galactose, besides the equilibrium mixture of D-fructose in water at room temperature which contains a significant amount of furanose forms [18]. Probably, the selectivity in the binding of aspartic acid is connected with these factors.

As can be seen from Table 1, the associate formation process is accompanied by small exothermic effects and large positive entropy changes. It is well known that monosaccharides and amino acids are extensively hydrated in aqueous solutions. Partial dehydration of the interacting particles upon the binding and solvent reorganization contributes significantly to the stability of the monosaccharide–aspartic acid associates due to the favorable entropic nature of the process.

Aspartic acid is a negatively charged (or acid) amino acid. The aspartic acid molecule contains two carboxylic groups. Its isoelectric point is $pI=2.97$. According to the measured pH (see Table 1) in the studied solutions, it exists as zwitterions. In our previous works [11, 23–24], we have studied interactions between zwitterionic forms of aliphatic, aromatic, polar amino acids, and some mono- and oligosaccharides. It is interesting to study the behavior of zwitterions of negatively charged aspartic acid with respect to monosaccharides. Major sources of negative enthalpy changes are specific as well as van der Waals interactions. Hydrogen bonding and ion–dipole interactions are possible types of specific interactions between the studied monosaccharides and amino acid. Small negative ΔH values result from competition of all the processes mentioned above.

The interactions between the monosaccharides and aspartic acid become apparent from the dependences of the enthalpies of solution of aspartic acid in water–monosaccharide

Fig. 2 Schematic representation of stereochemical structures of the studied monosaccharides (Dominant in aqueous solution form; *a* axial, *e* equatorial hydroxyl group)

Fig. 3 Standard enthalpies of solution of aspartic acid in mixtures of water with monosaccharides as function of the solvent composition at 298.15 K: 1, D-glucose; 2, D-fructose; 3, D-galactose

mixtures on their composition (Fig. 3). Because we discuss the dissolution process of aspartic acid in different solvents, the contribution of the crystal lattice energy of the amino acid to the $\Delta_{\text{sol}}H_{\text{aa}}^0$ is constant. As can be seen from Fig. 3, the $\Delta_{\text{sol}}H_{\text{aa}}^0$ values become more negative upon addition of small amounts of D-glucose and D-fructose, up to definite concentrations of the monosaccharides. It means that dissolution of aspartic acid in these mixtures becomes enthalpically more favorable than that in pure water. This effect may be explained by dominant specific interactions of aspartic acid molecules with their environment and associate formation of the amino acid with D-glucose and D-fructose. No remarkable effect on $\Delta_{\text{sol}}H_{\text{aa}}^0$ is observed at additions of D-galactose. The shape of the $\Delta_{\text{sol}}H_{\text{aa}}^0 = f(m_s)$ curves presented in Fig. 3 may be associated with a competition of the specific interactions and probably changes in the monosaccharide hydration due to reducing the amount of water.

Volumetric properties of solutions are directly connected with interactions between their components and structure changes accompanying these interactions. Thus, the associate formation should affect the volume changes in the systems under study. Experimental

Fig. 4 Dependences of apparent molar volumes of monosaccharides on their concentration in aqueous solution of aspartic acid fixed concentration ($m_{\text{aa}} = 0.04 \text{ mol kg}^{-1}$) at 298.15 K: D-glucose; 2-D-fructose; 3-D-galactose

density data of the studied systems are listed in Table 2. Dependences of apparent molar volumes of the studied monosaccharides (Φ_{vs}) on the m_s/m_{aa} ratio in aqueous solution of aspartic acid of fixed concentration are presented in Fig. 4. As can be seen from this figure, the dependences exhibit minima for the systems where the associate formation occurs. The position of the minima corresponds the 1:1 stoichiometry of the formed associates. D-Galactose exhibits no such minimum. The concentration dependences of Φ_{vs} usually are interpreted in terms of a destructive overlap of hydration shells of interacting solutes [25, 26]. According to this model of solute–solute interactions, a negative volume change results from the overlap of the shells of a hydrophilic group and a hydrophobic group or two hydrophobic groups. The associate formation of aspartic acid with D-glucose and D-fructose is accompanied by a decrease of the apparent molar volume of the monosaccharides. This means that the interaction involving a hydrophobic group is dominant in this process. However, the authors of works [27, 28] believe that an additional negative contribution to the volume change may arise if one of the solutes has strong structure-breaking influence on the water structure. Aspartic acid is a strong hydrophilic structure-breaker as are glycine, urea, and thiourea. The stability of the hydration shell of the monosaccharides toward a more powerful water-destroying agent plays a key role. Due to the stronger breaking effect of the charged centers of aspartic acid on the hydration shells of hydrophilic groups of the monosaccharide, the extent of hydration of the monosaccharides decreases, resulting in the negative contribution to the volume change.

4 Concluding Remarks

In this work, we have reported the results of an experimental study of the interactions between aspartic acid and some monosaccharides in dilute aqueous solutions. The data obtained by titration calorimetry, calorimetry of solution, and densimetry are in good agreement. The calorimetric and densimetric studies show that, in contrast to D-galactose, D-glucose and D-fructose form thermodynamically stable associates with aspartic acid. The associate formation is mostly entropy-driven. From a biological point of view, this study may elucidate some aspects of molecular recognition carbohydrates by proteins. Aspartic residues of proteins play an important role in binding of carbohydrates by lectins and enzymes. Our results show binding specificity of the aspartic residues with respect to D-glucose and D-fructose in dilute aqueous solutions, but not to D-galactose. Thus, the interaction between some proteins and carbohydrates with a D-galactose as terminal residue may be weakened. These interactions are affected by stereochemical structure of the monosaccharides.

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