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DISCOVERY OF BOUND AND UNBOUND WATERS IN CRYSTALLINE AMINO ACIDS REVEALED BY THERMAL ANALYSIS

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The thermal analytical study of most hydrophobic and hydrophilic *D*/*L* amino acids reveals significant hydropathy index correlation between the presence of water and crystalline amino acids. The TG derivative mass profiles for arginine and lysine (hydrophilic acids) at various time intervals of atmospheric exposure, show two distinct peaks, one between 50 and 60°C (unbound water), and one close to 100°C (bound-like water). The DSC heat-cool profiles for lysine and arginine confirmed the presence of these multiple waters with two heats of vaporization. The absence of these patterns from the TG and DSC for cysteine and phenylalanine (hydrophobic acids) further supports the conclusions.

Keywords: *dielectric analysis (DEA), DSC, DTG, heat of crystallization, heat of fusion, heat of vaporization, hydropathy index, TG*

Introduction

We performed thermal analytical study of 20 amino acids. We focused on four of the *L* and *D* amino acids of arginine, lysine, cysteine and phenylalanine. These crystalline amino acids were obtained from Sigma-Aldrich (St. Louis, Mo.). Experimental conditions and all procedures were standardized to eliminate environmental factors from influencing the analyses. There is a detailed study done on water and its properties under various instruments such as the DEA, DSC and the TG. When the amino acids were observed under the same instruments, a unique pattern similar to that of water was observed among the crystalline hydrophilic amino acids but not among the hydrophobic acids.

A recent article in the Science Times on water was very illuminating. It points out that 'water behaves very differently from other small molecules' [1]. It also stated that 'if you want something else with similar properties, you would end up with something much bigger and more complex, and then you would loose the advantages that water has in being smaller'. Water is drawn together by the hydrogen bonds, water molecules become stable, able to absorb huge amounts of energy before initiating a major phase change from solid ice to liquid water or liquid to gas. As a result, water has surprisingly a high boiling and melting temperature. Water is also an exceptional solvent with the ability to dissolve more chemicals than any other liquid. It can behave as an acid, a base and is the focus of this study on amino acids hydration.

Amino acids are composed of a central carbon atom that is bound to an amino group, a carboxylic acid group, a hydrogen atom and a side chain of varied structure. The side chains are commonly classified as non-polar, polar non-charged and polar charged, with each residue displaying varied chemical and physical characteristics that influence their aqueous interactions. These amino acid–water interactions and their subsequent water–protein interface are critical in the behavior of biological systems [2]. Further defining these aqueous interactions will help elucidate protein properties, including their structural folding patterns [3].

The non-random distribution of bound water molecules surrounding amino acids residues in the hydration patterns of proteins has been identified as a key to the modeling of protein structures [4]. Almost all amino acids exist in mirror-image forms, called the *L* isomer and *D* isomer, only one of which (typically the L form) is biologically active. TG and DTG have proven to be effective methods of identifying and quantifying the bulk and bound water in a sample [5]. The thermal analytical study of hydrophobic and hydrophilic *D* and *L* amino acids reveals the presence of multiple kinds of waters, bound and unbound, in the crystalline structure of hydrophilic amino acids. A number representing the hydrophilic and hydrophobic properties of these acids is the hydropathy index. The larger the number is the more hydrophobic is the sample. Negative indices indicate hydrophilic characteristics.

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Experimental

Material and methods

An Olympus BX60 microscope and camera were used to capture black and white photographic images of four pairs of *D* and *L* amino acids at 4 and 40× magnification.

A Thermal Analytical Instrument (TAI) 2970 DEA was used to determine the electrical conductivity profile of the amino acids. For each amino acid, a sample of approximately 10 mg was placed on a single surface gold ceramic interdigitated electrode in an isolated nitrogen rich dry atmosphere. The samples were ramped at a rate of 10°C min^{-1} from room temperature (24°C) to just above melting. Conductivity measurements were recorded at controlled interval frequencies ranging from 0.10 to 10000 Hz for all temperatures.

A TAI 2920 DSC was used to characterize melting and crystallization properties of the samples. Aluminum pans and lids were prepared with samples weighing between 7 and 14 mg and subjected to a cool and heat series cycling between –50 and 150°C at a rate of 10°C min^{-1} in an isolated nitrogen atmosphere. Heat flow (W g^{-1}) values *vs.* time and temperature were generated.

A TAI 2950 TGA was used to measure the percent (%) mass loss of the amino acids when heated to temperatures below the melting point, but above the boiling point of water. Samples were loaded into platinum pans and heated in an isolated nitrogen environment to 150°C. Isothermal conditions were then maintained to ensure reaction completion.

DSC heat and cool profiles of water reveal three distinct peaks. The first peak is the heat of crystallization at about 0°C, followed by the heat of fusion at about 10°C, and finally the heat of vaporization of 2200 J g^{-1} at about 112°C, as shown in Fig. 1.

The TG and derivative TG (DTG, % $°C^{-1}$) profiles for arginine (–4.5 hydropathy index) [5] and lysine (–3.9) show two distinct peaks, one between 50 and 60°C, a lower evaporation temperatures characteristic of free water, and one around 100°C, characteristic of bound water. A sequence of TG profiles obtained after varying intervals (5 min to 24 h) of atmospheric exposure (relative humidity of 60%) illustrates the uptake patterns of these water complexes. Figures 2 and 3 below display the peaks observed for D and L arginine by the DTG.

DSC heat and cool profiles (–50 to 150 to –50°C) for lysine and arginine confirmed the presence of bound and unbound waters with two heats of vaporization. The first peak in Figs 4 and 5 is observed around the 50–60°C range and the second peak is observed within the 100–110°C range,

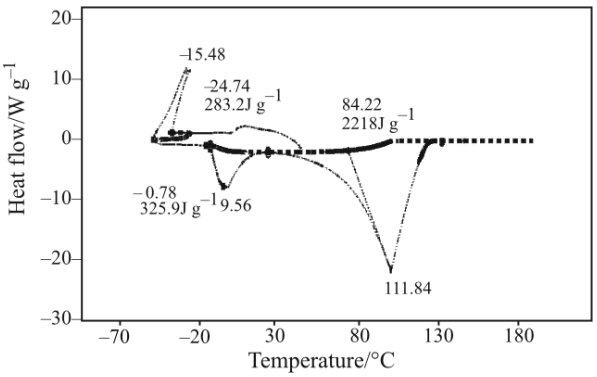

Fig. 1 Study of water by the DSC

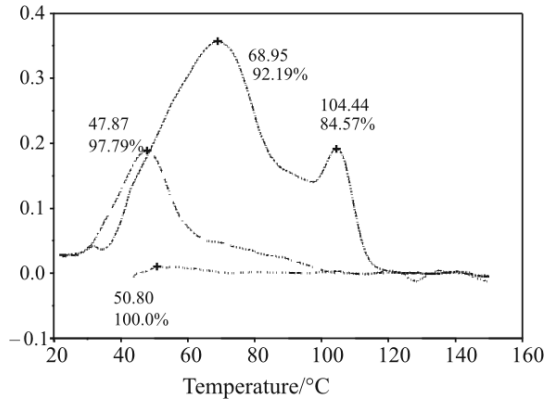

Fig. 2 DTG curve of *D* arginine. From the — – bottle, --- – 5 min atm. exposure and –·–· – 30 min atm. exposure

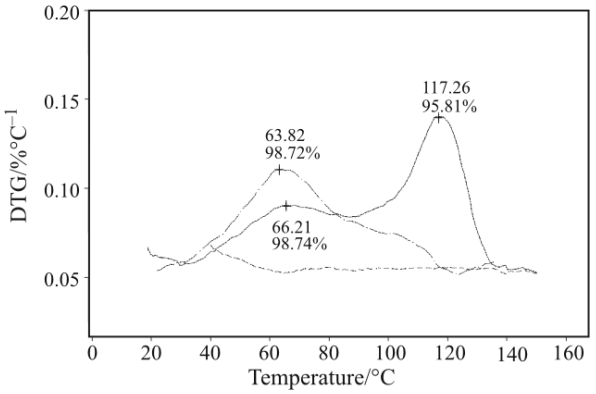

Fig. 3 DTG curve of *L* arginine. From the — – bottle, --- – 5 min atm. exposure and –·–· – 30 min atm. exposure

indicating the presence of the heat of fusion and the heat of vaporization.

The moisture's impact can also be seen in dielectric analysis (DEA), Fig. 6, where conductivity is correlated with mass percent moisture; the movement of absorbed water to the amino acid surface can also be observed in comparisons of the DEA profiles

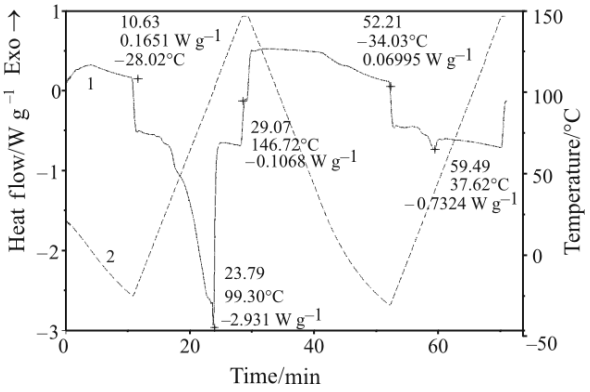

Fig. 4 1 – DSC curve of *D* arginine, 2 – temperature

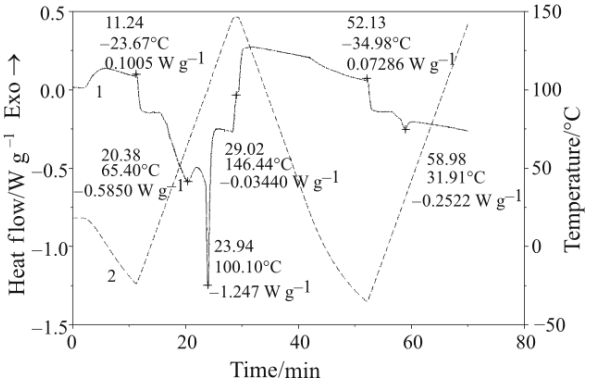

Fig. 5 1 – DSC curve of *L* arginine, 2 – temperature

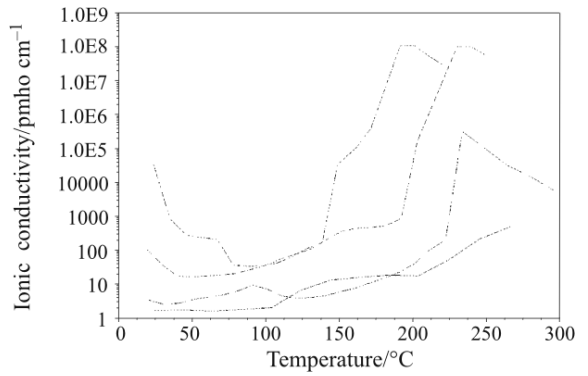

Fig. 6 DEA overlay of 4 amino acids. –·–· – *L* cysteine, --- – *L* lysine, –·–· – *L* arginine and — – *L* phenylalanine

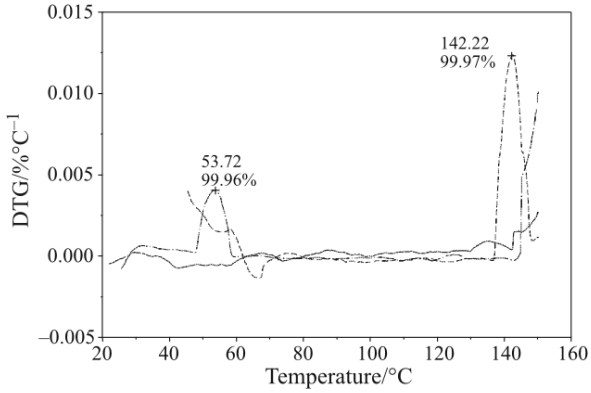

Fig. 7 DTG curve of *D* phenylalanine. From the — – bottle, --- – 5 min atm. exposure and –·–· – 30 min atm. exposure

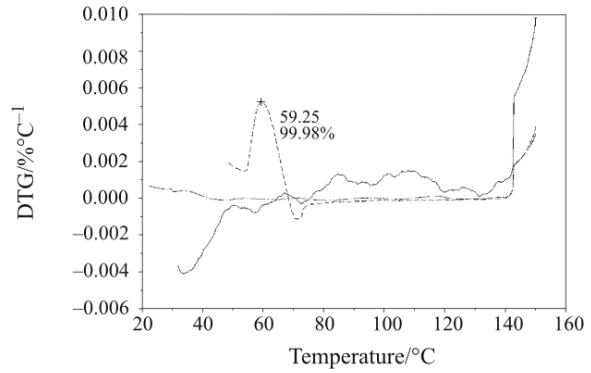

Fig. 8 DTG curve of *L* phenylalanine. From the — – bottle, --- – 5 min atm. exposure and –·–· – 30 min atm.

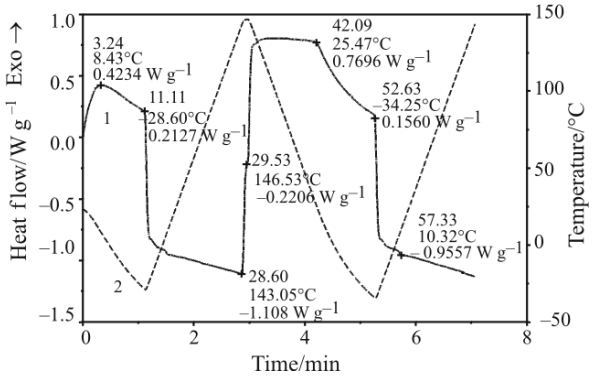

Fig. 9 1 – DSC curve of *D* phenylalanine, 2 – temperature

at an applied frequency of 1.00 Hz (surface analysis) and 1000 Hz (bulk analysis).

These patterns are absent amongst the hydrophobic amino acids, including cysteine (hydropathy index +2.5) and phenylalanine (+2.8) from the TG, DSC and the DEA. *D* and *L* isomers of phenylalanine in Figs 7 and 8, displayed only minor peaks with a DTG rate of less than 10% indicating minimal to almost no water. There is a major peak observed at around 142°C for the D isomer but the rate calibration at that point lead to a DTG rate of only 0.3% water loss. This calibration, therefore, leads to the understanding that the relative DTG rate for hydrophobic acids is less than 10% whereas for hydrophilic acids is more than 10%.

Similar results were obtained when the phenylalanine samples were studied under the DSC, Figs 9 and 10. No peaks were observed indicating the absence of water. These results correlate with the positive hydropathy index of phenylalanine and its hydrophobic characteristics.

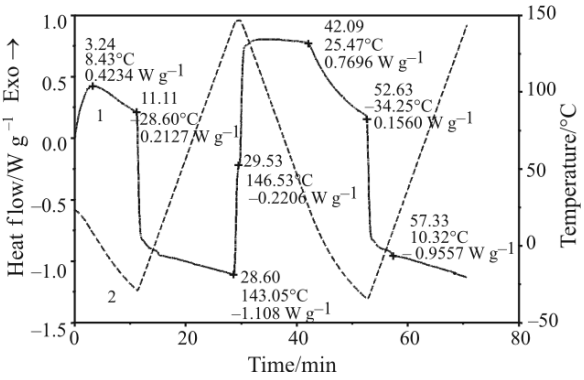

Fig. 10 1 – DSC curve of *L* phenylalanine, 2 – temperature

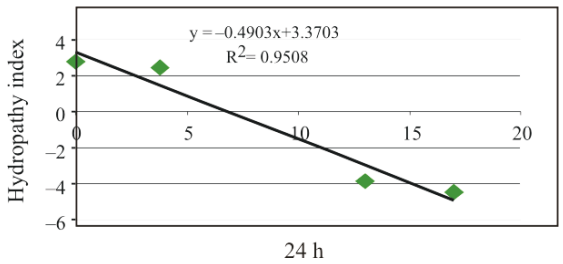

Fig. 11 Average mass loss of water (24 h) on amino acids *vs.* hydropathy index. R2=0.96 for controlled moisture exposure. RH 60%

The three peaks discussed for the DTG samples of arginine and phenylalanine are summarized in Table 1 according to the time of atmospheric exposure and display the rate in terms of % °C^{-1} for the individual samples. Table 1 also focuses on the comparison of the average of the samples exposed for a period of 24 h to the hydropathy index. This comparison is presented in the form of a graph with the average of 24 h on the *y*-axis and the hydropathy index on the *x*-axis in Fig. 11, displaying a coefficient of 0.98. Therefore this information sheds light on the data and proves that amino acids with a low hydropathy index (hydrophilic acids) contain more water whereas amino acids with a high hydropathy index (hydrophobic acids) contain less amount of water comparatively.

The peaks in Tables 2 and 3 are arbitrarily assigned variables such as A, a, B, b, C and c. The physical forms of these peaks are unknown. These tables provide supporting evidence to the DTG curves for the two amino acids mentioned above.

When the phenylalanine rates are compared to the arginine rates, the % water loss is calculated by assuming 0.36% °C^{-1} as 100% water loss since this is the highest rate observed and then calculating the % water loss of the different peaks relative to this value. The results of these calculations are revealed in Table 4. The table also analyzes the types of water found based on the peak's temperature range.

Table 1 Water content in *D* and *L* amino acids plus hydropathy index

Sample	Hydropathy index	Rates/% °C^{-1}						Average *D*–*L* rates/ % °C^{-1}	
		5 min	10 min	30 min	60 min	1440 min	Neat	1440 min	Neat
D arginine	–4.5	0.2	0.9	5.1	14	17	17	17	11
L arginine		0.3	1.1	3.5	7.8	17	5.1		
D lysine	–3.9	1.1	2.3	6.1	4.4	14	9.1	13	10
L lysine		2.1	2.7	3.9	4.6	11	11		
D cysteine	2.5	0.7	1.5	3.0	1.3	6.2	5.9	3.8	6.1
L cysteine		0.2	0.4	0.6	1.0	1.5	0.4		
D phenylalanine	2.8	0.093	0.060	0.018	0.011	0.014	0.026	0.02	0.03
L phenylalanine		0.050	0.012	0.010	0.010	0.031	0.030		

Table 2 Arginine water type as peak values observed in DTG

Sample	Exposure	Water type					
		A unbound		B unbound		C bound	
		T/°C	% °C^{-1}	*T*/°C	% °C^{-1}	*T*/°C	% °C^{-1}
D arginine	neat	48	0.019	70	0.36	104	0.19
	30 min						
L arginine	neat			63	0.05	119	0.14
	30 min			67	0.09	107	0.03

Table 3 Phenylalanine water type as peak values observed in DTG

Sample	Exposure	Water type					
		A unbound		B unbound		C bound	
		T/°C	% °C^{-1}	*T*/°C	% °C^{-1}	*T*/°C	% °C^{-1}
D phenylalanine	neat	35	0.01	66	0.01	137	0.01
	30 min			54	0.01		
L phenylalanine	neat			68	0.01	102/141	0.1/0.01
	30 min					94	0.01

Table 4 Relative rates of % water loss based on peaks from the DTG curves of neat arginine and phenylalanine

Sample	Water type			
	Unbound		Bound	
	T/°C	Rel. rate	*T*/°C	Rel. rate
D arginine	70	0.36=100%	104	0.19=53%
L arginine	63	0.05=14%	119	0.14=39%
D phenylalanine	66	0.01=3%	137	0.01=3%
L phenylalanine	68	0.01=3%	102	0.01=3%

Table 5 Water type as peak value observed in DSC curve (neat samples)

Sample	Hydropathy index	Water type				DSC	TG
		A unbound		B bound			
		T/°C	W g^{-1}	*T*/°C	W g^{-1}	ΔH_{vap}	water/%
D arginine	–4.5	66	0.59	99	2.9	953	47
L arginine				100	1.2	553	26
D lysine	–3.9	63	2.2	104	1.5	332	17
L lysine		68	2.5	105	1.4	324	16
D cysteine	2.5	69	2.5	65	0.25	133	6.7
L cysteine		56	0.56			38	2.8
D phenylalanine	2.8	n	n	n	n	n	n
L phenylalanine		n	n	n	n	n	n

n=not observed

There is also a unique relation observed among the neat samples of amino acids between their molar heat of vaporization observed from the DSC curves and the % water loss observed from the TG curves. There is a direct proportionality observed between the molar heat of vaporization and the % water loss to the hydrophilic or hydrophobic characteristics of the amino acids. Hydrophilic amino acids are observed to have a high molar heat of vaporization and a high % water loss. This trend decreases from hydrophilic amino acids towards the hydrophobic amino acids as seen in Table 5. This comparison clearly focuses on the trend between the samples' hydrophilic or hydrophobic characteristics with the % water loss observed within the sample. There is also a clear correlation of the % water loss with the stated hydropathy index of the sample.

Results and discussion

Two interesting analytical results have been discovered with this study. First, there is an excellent correlation between the hydropathy index [6] and the TG as well as the DSC analysis of % water absorbed and bound to 4 sets of *D* and *L* amino acids (Tables 1–5 and Figs 1–11). The increase in water content ranged from a hydrophobic amino acid like phenylalanine with no absorbed water to a hydrophilic amino acid like arginine (26% by DSC and 17% by TG) which

readily absorbs water from the atmosphere. Lysine's water by DSC was 16% and TG 11%.

The second observation is that there were different forms of water fixed to these 4 sets of amino acids. There was clearly unbound water peaking in the TG and DSC at 60–65°C. However, the DSC revealed an interesting anomaly: unbound water typically crystallizes from –10 to –20°C and melts at about 0°C on cooling; in these amino acids only the heat of fusion was observed with no observed crystallization or melting. The DSC heat mode revealed multiple waters peaks absent on reheating. DTG exposed at least three kinds of water peaks at 30–60, 60–70 and 90–105°C. The former two peaks are probably due to desorption of water with varying binding strengths. With more energy needed to remove the higher temperature water peaks at about 100°C probably related to bound water or even more interaction with the amino acid than the waters desorbing at 30–70°C. The DTG curve overlays for the various *D* and *L* amino acids clearly demonstrate the varying water types and relative amounts as measured by the DTG peak value in % $°C^{-1}$ a rate value. Desorption and vaporization of water is typically viewed in the DSC as a broad diffuse peak with a peak temperature of about 100°C. The DSC peaks of water loss from the amino acids have sharp narrow peaks that are usually associated with the loss of bound water. Therefore, these two complimentary methods have demonstrated that these amino acids have unusual binding properties to water.

Conclusions

A major step in understanding the kinds and binding of water that are associated with D and L amino acids has been accomplished in this study. The hydropathy index can be determined by TG and supported by DSC analysis. There are number of kinds of water associated with these amino acids. The hydrophilic and hydrophobic sites on these acids and on proteins will be explored in future research.

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