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BOMB CALORIMETRIC AND NMR STUDIES ON CRYSTALLINE HEXAGLYCINE *

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ABSTRACT

The enthalpy of combustion of crystalline hexaglycine ($C_{12}H_{20}N_6O_7$,cr) has been determined by combustion bomb calorimetry. The molar enthalpy of combustion at 298.15 K for the following reaction: $C_{12}H_{20}N_6O_7(cr) + 13.5O_2(g) = 12CO_2(g) + 10H_2O(l) + 3N_2(g)$ is $\Delta_c H_m^\circ = -(5930.3 \pm 11.3)$ kJ mol^{-1}; the corresponding enthalpy of formation at 298.15 K is $\Delta_f H_m^\circ = -(1650.1 \pm 11.7)$ kJ mol^{-1}. Because of the difficulty in establishing the moisture content of the sample, the enthalpy of combustion is based upon the mass of carbon dioxide formed in each calorimetric experiment rather than upon the mass of sample prior to combustion. A variety of NMR measurements have been made, both ^{1}H NMR and CP–MAS ^{13}C, to characterize hexaglycine and several other oligoglycines, and to determine the water content of the hexaglycine. The energy contribution to the enthalpy of formation of crystalline hexaglycine for the repeating unit, $-(CH_2-CO-NH)-$, is compared to similar values derived from related peptides and amino acids in the solid phase.

INTRODUCTION

A comprehensive picture of the energetics and stabilities of proteins can be derived from the thermodynamic properties of amino acids and peptides.

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Information of this kind is needed for a variety of applications involving proteins in biotechnology. Testing elementary hypotheses regarding the enthalpy and stability of the peptide bond or molecular groupings adjacent to the peptide bond is essential in order to establish the basic features of this comprehensive picture for proteins. The determination of the enthalpy of combustion and formation is a property of prime interest and is necessary to understand the energetics and stability of proteins.

This paper describes the determination of the enthalpy of combustion of crystalline hexaglycine using bomb calorimetry. The results of NMR measurements are described and provide valuable information regarding the structure and the presence of water in hexaglycine. In conjunction with this work, an evaluation was made of the existing data in the literature on the enthalpies of combustion and formation of crystalline glycine, diglycine, triglycine, tetraglycine, and pentaglycine. An examination of the hydration properties of these substances was also made.

Since 1884, there have been ten reported determinations [1–10] for the enthalpy of combustion and formation of crystalline glycine. The value for $\Delta_f H_m^\circ$ of glycine, $-(528.5 \pm 0.5)$ kJ mol^{-1}, has been selected by Pedley et al. [11], and is based on the calorimetric results of Huffman et al. [8] and Ngauv et al. [10]. Three determinations of the enthalpy of combustion of diglycine have been made; two were reported in 1904 by Fischer and Wrede [5] and by Landrieu [12], and a careful study was described by Huffman [13] in 1942. When Huffman's diglycine value, $-(744.9 \pm 0.6)$ kJ mol^{-1}, is corrected for atomic weight changes and a more current value for the energy of combustion of benzoic acid, one obtains $-(746.9 \pm 0.6)$ kJ mol^{-1}; Pedley et al. [11] select the value $-(747.7 \pm 0.6)$ kJ mol^{-1}. In the studies by Huffman et al. [8] and Ngauv et al. [10] on glycine, considerable care was taken to insure that the sample burned in the calorimetric experiments was dry. Similar care was followed by Huffman [13] to insure dryness without thermal transition in the diglycine samples used in his calorimetric studies. All of his samples were heated prior to combustion to at least 85 °C, since Hughes in an unpublished investigation found that when any of the three crystal forms of diglycine, as found by Bernal [14], is heated to 100 °C, it is converted to the alpha form. Combustion results on samples which had been dried at temperatures less than 85 °C were low. In 1910, Wrede [6] reported enthalpy of combustion determinations for triglycine and tetraglycine, but no mention was made of the water content or drying procedures in preparing samples for combustion calorimetry. Correction of the combustion data to present-day energy units gave $\Delta_f H_m^\circ$ values of $-(966.0)$ and $-(1191.8)$ kJ mol^{-1} for triglycine and tetraglycine, respectively. Since 1910, no subsequent combustion calorimetry has been performed on triglycine or tetraglycine. There are no data in the literature for $\Delta_c H_m^\circ$ or $\Delta_f H_m^\circ$ for pentaglycine.

Examination of the literature shows that the ability of proteins to absorb

water has been a topic of considerable study. The work of Mellon et al. [15] indicated that glycine, diglycine, and triglycine show a complete lack of absorption of water vapor at all humidities up to 93% relative humidity (R.H.). However, they found that tetraglycine, pentaglycine, and hexaglycine absorb significant amounts of water vapor as the number of peptide residues increases. These authors concluded that the peptide chain of proteins is responsible for a large portion of the water vapor absorption of proteins, but were not able to distinguish between absorptions of the imino and carbonyl groups. The properties of polyglycine esters show that the peptide linkage must be responsible for most of the water absorption by these materials. At 93.3% relative humidity and 303.15 K, polyglycine II (molar mass 3400 g mol^{-1}) holds about 35.0 moles of water per mole of peptide compared to 1.04 moles of water per mole of peptide for hexaglycine. More information on the hydration of proteins and polypeptides can be found in a review by Kuntz and Kauzmann [16]. Studies on semicrystalline polybutadiene [17] and poly(oxymethylene) [18], show the heat capacity dependence with crystallinity. Also, studies on the thermal properties of polypropylene [19] show the dependence of different crystallinities on the enthalpy of fusion data, enthalpy, entropy and Gibbs energies. Our NMR studies on hexaglycine point to the unresolved structure and non-crystalline regions of this peptide, which suggest that variations in the enthalpies of combustion and formation can be expected from such features.

EXPERIMENTAL

Materials

The hexaglycine and all of the other oligoglycines in this study were purchased from Sigma Chemical Co. * and were used without further purification. The polyethylene tubing used for encapsulation of the sample was manufactured by the USI Chemical Corporation. The hexaglycine used directly from the bottle was very difficult to pellet. (The sample geometry required in combustion bomb calorimetric measurements is to press the crystalline solid into pellet form). It was found that materials which have a higher water content pelleted more easily. The sample was therefore stored

* Certain commercial equipment, instruments, or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

in a 53% relative humidity chamber (saturated KSCN solution at ambient room temperature, 295.75 K) [20] and only removed just prior to pelleting.

Sample characterization

Elemental analyses were necessarily performed on two samples of hexaglycine, the sample stored in the relative humidity chamber at 53% relative humidity and the other vacuum dried for 24 h at 323 K. These data were required in order to determine whether there had been a change in the crystalline material that was analyzed, as compared with the sample that was actually burned in the calorimeter. Storing the crystalline material in the moist environment did not alter the structure of the peptide, with the results of the elemental analyses showing satisfactory agreement with theoretical values for carbon, hydrogen, nitrogen, and direct oxygen. Results for the two samples are reported on a dry basis in Table 1.

A hexaglycine sample was also submitted for powder X-ray diffraction. The resulting pattern was diffuse with no sharp peaks, indicative of a material that is poorly crystalline and probably contains a significant quantity of water.

NMR analyses

Proton solid state ^{1}H NMR spectra were obtained in order to gain insight into the amount and mobility of the water present in the hexaglycine. Solid state ^{13}C spectra were also taken in order to seek independent evidence as to whether or not water was incorporated into the crystal lattice of hexaglycine. The spectrometer employed was a Bruker CXP-200; r.f. field strengths for both protons and ^{13}C nuclei corresponded to precession frequencies of about 70 kHz. Samples were contained in ceramic, 7 mm O.D. spinners and spinning frequencies were about 2.5 kHz.

Three samples were analyzed by solid state NMR: one was from the original bottle as received from the vendor, the second was a sample dried at 323 K for 24 h under vacuum, and the third was stored in a 53% relative humidity chamber.

TABLE 1

Results of elemental analyses (mass%)

	Theoretical	24 h vac. 323 K	53% R.H. chamber
C	40.00	40.00	40.00
H	5.59	5.59	5.66
N	23.32	23.15	23.33
O	31.08	30.83	31.13

TABLE 2

Results of moisture analysis by ^1H NMR spectroscopy

Sample [a]	f_N [b]	Narrow linewidth (kHz)	% H_2O [c]
1	0.11 ±0.01	1.6	5.8±0.6
2	0.034±0.008	3.8	1.7±0.4
3	0.11 ±0.01	1.5	5.8±0.6

[a] 1 Sample as-received from vendor. 2 Dried 24 h under vacuum at 323 K. 3 Stored in 53% R.H. chamber.

[b] Fraction of total ^1H intensity in narrow line.

[c] If all narrow line is due to water protons.

The data summarized in Table 2 were obtained from the ^1H NMR lineshapes, two of which are displayed in Fig. 1. It was assumed that the narrow portions of the lines corresponded entirely to water protons. The sample stored in the 53% relative humidity chamber and the as-received

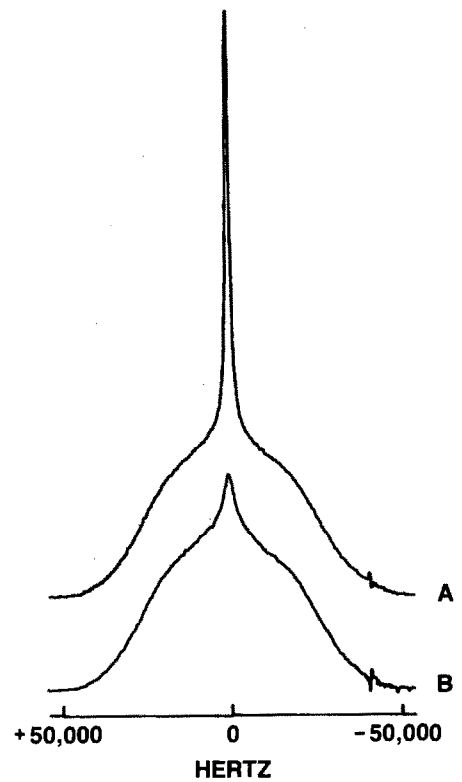

Fig. 1. 200 MHz proton spectra of two of the hexaglycine samples: (A) equilibrated at 53% relative humidity and (B) equilibrated in vacuum at 323 K for 24 h (lower). The original hexaglycine sample possessed a spectrum nearly identical to (A).

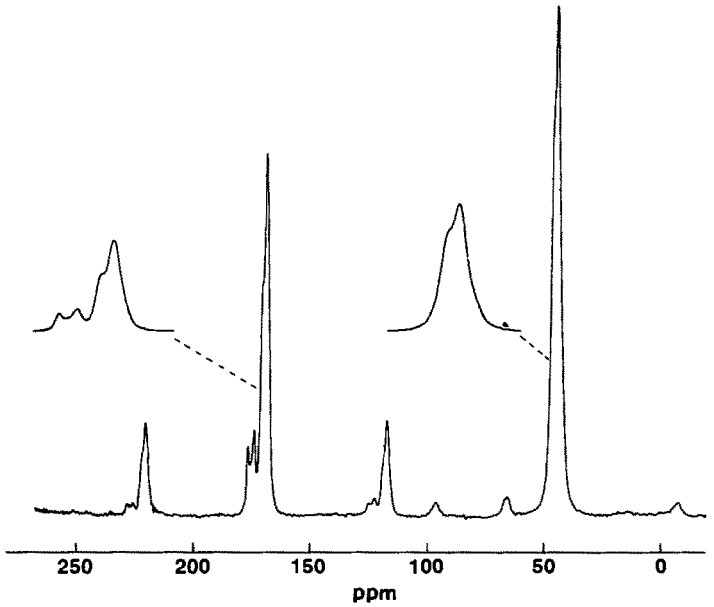

Fig. 2. 50.3 MHz ^{13}C CP–MAS spectrum of the sample equilibrated at 53% relative humidity. MAS frequency is about 2500 Hz, so spinning sidebands appear in integral multiples of 50 ppm on either side of the central resonances. Insets for the two center bands are expanded three times along the frequency axis and attenuated vertically by a factor of 4. All hexaglycine samples showed identical spectra.

sample had water contents of $5.8 \pm 0.6\%$. The 50 °C vacuum dried sample still retained $1.7 \pm 0.4\%$ water.

A representative ^{13}C NMR spectrum is shown in Fig. 2. In spite of the fact that the samples differed in water content, there was no change in the ^{13}C NMR spectra of the three samples.

Solution NMR spectra were acquired by use of the Bruker DISR86 program (versions 860101.0 and 860101.1) with a Bruker Instruments Model WM-400 spectrometer that was equipped with an Aspect 3000 data system and process controller. The samples of di-, tri-, tetra-, penta-, and hexaglycine (13–27 mg each) were examined as their solutions in trifluoroacetic acid-d_1 (CF_3CO_2D, 0.5 cm^3), except for hexaglycine (27 mg) which was also studied as its solution in trifluoroacetic acid: acetic acid-d_3 (CF_3CO_2H: CD_3CO_2H, 9:1 v/v, 0.5 cm^3). Tetramethylsilane was used as an internal reference.

Karl Fischer and vacuum oven determinations

Karl Fischer (K.F.) determinations were first made on samples placed directly into the methanol-based K.F. reagent. Even though hexaglycine is not completely soluble in methanol, a water determination was still feasible.

TABLE 3

Water analyses on oligoglycines

Oligoglycine	Water content (mass%)	Method
Hexaglycine	3.25	K.F. (cr, methanol)
	5.80	K.F. (cr/TFA/methanol)
	3.86 (pellet)	Vac. oven (24 h 323 K)
	3.38 (crystal)	Vac. oven (72 h 323 K)
	3.40 (crystal)	Vac. oven (68 h 296 K)
	5.80	^{1}H NMR
Diglycine	0.023	K.F. (cr, methanol)
Triglycine	0.11	K.F. (cr, methanol)
Tetraglycine	1.40	K.F. (cr, methanol)
Pentaglycine	0.36	K.F. (cr, methanol)

An average value of 3.25 mass% was obtained. K.F. measurements were also made on the humidified crystalline material, by first pre-solubilizing the hexaglycine in trifluoroacetic acid. An aliquot of this solution was weighed into the K.F. titrator with the resulting average water content being 5.80 ± 0.08 mass%. A blank correction had to be made for a possible secondary reaction, esterification of the methanol by trifluoroacetic acid, that was taking place and producing additional water. This was indicated by the continual high sloping baseline on the output of the strip chart. K.F. analyses for the other oligoglycines were also performed. The water contents (solid material directly into the K.F. titrator) for the di-, tri-, tetra-, and pentaglycines were 0.23, 0.11, 1.40 and 0.36 mass%, respectively.

Vacuum oven drying techniques were also employed to determine the water content. Three hexaglycine samples were dried at temperatures from 296.15 to 373.15 K. Plotting the data showed that at 323.15 K the maximum water loss was 3.4 mass%, with sample degradation occurring at higher temperatures. Obviously, in vacuum drying, all of the water, though not tightly bound, cannot be removed as is indicated by the lower water contents found. The results of the water analyses for all of the oligoglycines are presented in Table 3. Close agreement was obtained between the water content of 5.8 ± 0.6 mass% determined by solid state ^{1}H NMR and the 5.8 ± 0.1 mass% found by K.F. analysis in trifluoroacetic acid. Because of the difficulties that would be encountered in trying to remove this amount of water from the peptide molecule without deterioration and thus begin combustions with a perfectly dry sample, the determination of the enthalpy of combustion had to be based upon the mass of carbon dioxide evolved during the calorimetric experiment rather than upon the mass of sample introduced into the bomb before a measurement.

Combustion calorimetry

The combustion measurements were made in an isoperibol (constant temperature jacket) oxygen bomb calorimeter. The reaction vessel is submerged in a thermostatted water bath at 301 K controlled to ± 0.003 K. The temperature rise caused when a measured amount of sample is burned is compared with that caused when a measured amount of Standard Reference Material (NBS SRM 39i) benzoic acid is burned in the same calorimetric system. Samples were removed from the 53 per cent relative humidity chamber when needed, pressed into approximate 1 g pellets, and returned to the chamber for storage. The pellets were very fragile and some loss occurred upon removing them from the pellet die. A benzoic acid pellet, in the order of 0.77 g, was used to aid in the initiation of combustion of the sample. Each pellet was weighed individually, quickly placed into a preweighed polyethylene bag of approximately 0.16 g and lightly heat sealed. The bag was used in order to protect the hexaglycine from any moisture change while in the bomb prior to ignition. Both samples and bag were handled with polyethylene gloves. The polyethylene bag was made from lay-flat tubing, 2.54 cm wide and 0.0635 mm thick. The supplier reported that it contained about 2.4 weight per cent EVA (a copolymer of ethylene and vinyl acetate), added to enhance its physical characteristics, but that would reduce its crystallinity, and therefore lower its density to approximately 0.91 g cm^{-3}. The internal energy of combustion of the polyethylene was determined in this lab to be $-(45691.4 \pm 4.0)$ J g^{-1}, (one SDM). The combustion of the auxiliary substances, benzoic acid and polyethylene, accounted for 46 and 17 per cent, respectively, of the measured energy change in each experiment. The encapsulated sample was placed into a preweighed platinum crucible, placed on the crucible support of the bomb head, and placed in contact with a 10 cm length of 0.075 mm platinum fuse wire. The bomb has an internal volume of 0.342 dm^3. Distilled water (2 cm^3) was added to the bomb to provide an atmosphere saturated with water and to ensure that water formed as a combustion product would be in a liquid state. The sealed bomb was charged with high purity oxygen to a pressure of 3.10 MPa at $T = 295$ K (ambient room temperature), placed in the calorimeter, and the energy of combustion of hexaglycine was measured. Analysis for CO_2 was carried out on the gaseous products of combustion for hexaglycine with its auxiliary combustion substances. CO_2 recoveries were also carried out individually on the polyethylene material and benzoic acid used as auxiliary materials. The average CO_2 recovery for the polyethylene is equal to 99.76 ± 0.13 per cent of the theoretical. The benzoic acid gave CO_2 recoveries equal to 100.02 ± 0.01 per cent of the theoretical. The uncertainties reported are the standard deviations.

CALORIMETRIC RESULTS

NBS Standard Reference Material benzoic acid (SRM 39i) has a certified specific energy of combustion of $-(26434 \pm 3)$ J g^{-1} [21] at 298.15 K under standard bomb conditions. The mean effective energy equivalent of the calorimeter E(EEE-std) and its standard deviation of the mean were determined from six benzoic acid combustions and found to be $-(14746.7 \pm 1.4)$ J K^{-1}. The thermochemical values and physical constants used in the calculations appear in Table 4. Table 5 contains the details of seven combustion experiments for hexaglycine. The headings listed in the table were explained in a previous publication from this laboratory [22]. The results of the seven energy of combustion measurements for the reaction

$$C_{12}H_{20}N_6O_7(cr) + 13.5O_2(g) = 12CO_2(g) + 10H_2O(l) + 3N_2(g) \quad (1)$$

and the values derived from these results are summarized in Table 6. The quantity $\Delta C_{p,m}\Delta T$ is a heat capacity correction which includes the calculated change of heat capacity of the calorimetric system from 298.15 to 301.15 K. Combining the molar internal energy of combustion, $\Delta_c U_m^\circ(301.15\ K) = -(5931.71 \pm 10.66)$ kJ mol^{-1}, $\Delta nRT = +3.76$ and $\Delta C_{p,m}\Delta T = -2.39$ kJ mol^{-1}, the molar enthalpy of combustion $\Delta_c H_m^\circ(298.15\ K)$ is calculated to be $-(5930.34 \pm 11.26)$ kJ mol^{-1}. The molar enthalpy of formation $\Delta_f H_m^\circ(C_{12}H_{20}N_6O_7, cr, 298.15\ K) = -(1650.07 \pm 11.71)$ kJ mol^{-1}.

The molar mass of hexaglycine, $C_{12}H_{20}N_6O_7$, and CO_2 used in this study was 360.3268 g mol^{-1} [28] and 44.0098 g mol^{-1} [28], respectively. The $\Delta_f H_m^\circ$ values for $CO_2(g)$ and $H_2O(l)$ were taken to be $-(393.509 \pm 0.130)$ kJ mol^{-1} [29] and $-(285.83 \pm 0.042)$ kJ mol^{-1} [29], respectively.

The total uncertainty assigned to $\Delta_c H_m^\circ$ and $\Delta_f H_m^\circ$ was obtained by the method of Olofsson [30]. The final result was obtained by combining (square root of the sum of the squares) random errors associated with the combustion measurements, auxiliary materials, benzoic acid calibrations, errors in the certified value of benzoic acid, errors in weighings, errors due to the uncertainty in the water determination, and reasonable estimates of all other

TABLE 4

Physical constants used in calculations

	$C_{p,m}$ (J K^{-1} mol^{-1})	ρ (g cm^{-3})
Hexaglycine	94.0 [23]	1.2 [27]
Polyethylene	32.22 [24]	0.91 [24]
Benzoic acid	147.65 [25]	1.32 [34]
O_2 (g)	29.355 [26]	
CO_2(g)	37.112[26]	
H_2O(l)	75.291 [26]	
N_2(g)	29.125 [26]	

TABLE 5

Experimental results for the internal energy of combustion of hexaglycine

	Experiment number						
	1	2	3	4	5	6	7
E(EEE-std, 301.15 K) ($J\ K^{-1}$)	−14746.70	−14746.70	−14746.70	−14746.70	−14746.70	−14746.70	−14746.70
C(contents) ($J\ K^{-1}$)	21.72	21.60	21.61	16.54	21.69	21.46	21.59
E(EEE-act) ($J\ K^{-1}$)	−14768.42	−14768.30	−14768.31	−14763.24	−14768.40	−14768.16	−14768.30
ΔT (K)	2.959675	2.849541	2.981485	2.988705	2.939280	2.819612	2.955551
Q(tot) (J)	−43709.71	−42082.88	−44031.51	−44122.97	−43408.45	−41640.50	−43648.46
Q(ign) (J)	1.62	1.69	1.49	1.39	1.28	1.69	1.16
Q(HNO_3) (J)	127.49	127.16	112.32	117.93	126.79	107.67	112.08
Q(WC) (J)	37.39	35.37	36.71	34.23	37.34	33.99	36.32
Q(corr. to 301.15 K) (J)	−0.02	0.21	−0.10	−0.25	0.05	0.33	−0.07
Q(aux, $C_6H_5CO_2H$) (J)	15064.55	16098.99	20444.37	20530.77	14692.37	20477.51	20462.87
Q(aux, C_2H_4) (J)	7440.97	6892.83	7750.66	8006.65	7536.40	7519.33	7684.33
Q(react) (J)	−21037.71	−18926.63	−15686.06	−15432.25	−21014.22	−13499.98	−15351.77
Mass CO_2 (total) (g)	3.81786	3.69540	3.88226	3.88047	3.78692	3.66530	3.84219
Mass CO_2 (benzoic acid) (g [a])	1.43888	1.53769	1.95274	1.96099	1.40334	1.95590	1.95450
Mass CO_2 (polyethylene) (g [a])	0.49869	0.46196	0.51945	0.53660	0.50509	0.50395	0.51443
Mass CO_2 (EVA) (g)	0.00799	0.00740	0.00833	0.00860	0.00810	0.00808	0.00824
Mass CO_2 (hexaglycine only) (g)	1.87230	1.68835	1.40174	1.37428	1.87040	1.19737	1.36502
$\Delta_c U^\circ$ (301.15 K) ($J\ g^{-1}$)	−11236.29	−11210.13	−11190.42	−11229.34	−11235.15	−11274.69	−11246.55

[a] We have assumed that all of the benzoic acid and polyethylene have burned completely.

TABLE 6

Summary of results for hexaglycine

$\Delta_c U^\circ$ ($C_{12}H_{20}N_6O_7$, 301.15 K)	$-(11231.80 \pm 10.09)$ [a]	J g^{-1}
$\Delta_c U_m^\circ$ ($C_{12}H_{20}N_6O_7$, 301.15 K)	$-(5931.71 \pm 10.66)$ [a,b]	kJ mol^{-1}
ΔnRT	$+3.76$	kJ mol^{-1}
$\Delta_c H_m^\circ$ ($C_{12}H_{20}N_6O_7$, 301.15 K)	$-(5927.95 \pm 10.66)$ [c]	kJ mol^{-1}
$\Delta C_{p,m} \Delta T$	-2.39	kJ mol^{-1}
$\Delta_c H_m^\circ$ ($C_{12}H_{20}N_6O_7$, 298.15 K)	$-(5930.34 \pm 11.26)$ [c]	kJ mol^{-1}
$\Delta_f H_m^\circ$ ($C_{12}H_{20}N_6O_7$, 298.15 K)	$-(1650.07 \pm 11.71)$	kJ mol^{-1}

[a] Uncertainty is the standard deviation of the mean.

[b] The molecular weight for $12(CO_2) = 528.1176$ g mol^{-1} was used.

[c] Uncertainty is twice the assigned uncertainty.

sources of error. The total uncertainty is equal to twice the overall standard deviation unless otherwise stated.

It is possible to derive a value for $\Delta_f H_m^\circ$ for the repeating unit ($-CH_2-CO-NH-$) in hexaglycine from the value for $\Delta_f H_m^\circ$ for glycine itself and the following equation

$$\Delta_f H_m^\circ(\text{c, oligoglycine}) = \Delta_f H_m^\circ(\text{c, glycine}) + (n) \times [\Delta_f H_m^\circ(\text{c, } -CH_2-CO-NH-)] \quad (2)$$

where n is the number of repeating units present in the oligoglycine. For hexaglycine, n is five, and the value of the repeating unit is -224.3 kJ mol^{-1}. Using the value for $\Delta_f H_m^\circ$ (c, glycine) $= -(528.5 \pm 0.5)$ kJ mol^{-1} [11], one can derive values for the repeating unit ($-CH_2-CO-NH-$) from other oligoglycines whose enthalpies of combustion and formation have been determined using combustion bomb calorimetry. These values are listed in Table 7.

Other approaches can be tried which lead to a value for the $\Delta_f H_m^\circ$ glycine repeating unit. For example, the value for the $\Delta_f H_m^\circ$ (c, glycine anhydride) divided by two yields a value for the repeating unit: [(glycine anhydride/2) $= -(446.5 \pm 1.3/2) = -(223.3)$] [11]. The difference between a dipeptide containing a glycine residue and the amino acid which corresponds to the other component of the dipeptide also allows one to calculate the value of $\Delta_f H_m^\circ$ for the glycine repeating unit. The average value for the $\Delta_f H_m^\circ$ for the glycine repeating unit obtained from the values listed in Table 7 is $-(220.0 \pm 2.0)$ kJ mol^{-1}. (The error quoted is twice the SDM). The agreement between the latter value for the glycine repeating unit and that derived from our calorimetric measurements $-(224.3 \pm 5.2)$ kJ mol^{-1} shows overlap and is reasonable.

The uncertainty between the experimentally derived values for the $\Delta_f H_m^\circ$ glycine repeating unit is larger than that obtained from an analysis of previously determined calorimetric data and is related to a variety of factors

TABLE 7

Values of $\Delta_f H_m^\circ$ for glycine repeating unit

	$\Delta_f H_m^\circ$(c, 298.15 K) (kJ mol^{-1})	n	$\Delta_f H_m^\circ$(c, $-CH_2-CO-NH-$) (kJ mol^{-1})	Ref.
Values from oligoglycines				
gly–gly	−747.7 ± 0.7	1	−219.2	11
gly–gly	−746.9 ± 0.6	1	−218.4	13
gly–gly	−753.1 ± 6.0	1	−224.6	5
gly–gly–gly	−966.0 ± 12.0	2	−218.8	6
gly–gly–gly–gly	−1191.8 ± 18.0	3	−221.1	6
Values from different dipeptides				
glycylvaline − (DL-valine)				
−835.0 ± 0.5 − (−617.9 ± 1.6)		1×3	−217.1	11
glycylphenylalanine − (DL-phenylalanine)				
−684.4 ± 1.8 − (−460.6 ± 2.6)		1×3	−223.8	11
DL-alanylglycine − (DL-alanine)				
−777.8 ± 0.9 − (−563.6 ± 0.6)		1×3	−214.2	11
DL-leucylglycine − (DL-leucine)				
−859.8 ± 1.3 − (−640.6 ± 1.0)		2	−219.2	11
Value from glycine anhydride	−446.5 ± 1.3		−223.3	
Average value			−220.0 ± 2.0	

which include: the partial crystallinity of the hexaglycine sample and its influence on the enthalpy of combustion, uncertainties in the determinations for carbon dioxide, and the requirement for auxiliary combustion substances to assist in attaining as complete a combustion as possible.

NMR RESULTS

Important characterizational information on the oligoglycines can be obtained from an NMR investigation. These studies can provide insight regarding the location of the water within the hexaglycine crystal lattice and can identify the presence of other structural forms of hexaglycine, such as the cyclic form. The following NMR results detail these findings.

The fact that the water resonances in the ^1H NMR spectra in Fig. 1 are narrow means that the water molecules are rotationally and translationally mobile on a timescale of a few microseconds. Thus, water is not tightly bound in a crystal lattice. The reduction of the water content in vacuum also implies that the water is not tightly bound. The linewidths observed for

water protons in hexaglycine and the concentration dependence of these linewidths is parallel to what we have observed (unpublished results) in cellulosic materials, e.g. cotton. In cotton, the water molecules have many opportunities to interact via hydrogen bonding with the cellulose chains. At the same time, deuterium exchange studies [31] indicate that the water molecules do not penetrate the interior of the crystalline regions. Presumably, the water has access to the non-crystalline regions and the surfaces of the crystallites whose lateral dimensions are typically in the 3.5 nm range [32]. From the mobility of the water molecules and from the non-stoichiometric amounts of water present in these three hexaglycine samples, we conclude that water is not likely to be in the hexaglycine crystal lattice. However, the question remains, particularly in view of the fact that wide angle X-ray powder scattering profiles give no distinct peaks, as to whether or not the hexaglycine is in the crystalline state. This latter observation suggests either that the hexaglycine is non-crystalline or that the crystallites are very small, i.e. a few nm in minimum dimension. To address this question, we turn to the ^{13}C NMR spectra.

As mentioned, the ^{13}C CP–MAS spectra of the three hexaglycine samples are identical. We focus on the centerband, near 170 ppm in Fig. 2, associated with the carbonyl carbons, in order to comment on the condensed state of the hexaglycine. This resonance consists of a rather intense upfield region and a poorly resolved doublet of weaker intensity about 6 ppm downfield. The intensity ratio of the upfield line to the weaker downfield doublet is close to 5 : 1 so that it seems reasonable that the latter doublet should be assigned to one of the six carbonyl carbons of hexaglycine. Its unique chemical shift relative to the other five carbonyl carbons strongly suggests its assignment to the carbonyl of the carboxy terminus of the hexaglycine molecule. Furthermore, the fact that this resonance is a doublet suggests one of the following: (a) the crystal structure of hexaglycine contains two magnetically inequivalent sites, (b) the hexaglycine regions may be both ordered and disordered, i.e. some regions could be crystalline and other regions non-crystalline, or (c) this carbon could have a strong dipolar interaction with a ^{14}N nucleus whose quadrupolar interaction could split that carbon resonance line [33]. If the assignment is correct, option (c) is unlikely because the carboxy terminus has no bonded nitrogen. (This is, however, the most likely explanation for the unresolved structure apparent in the more intense upfield portion of the carbonyl line as all of the other carbonyl carbons are bonded to nitrogens.) At this point we cannot intelligently choose between options (a) and (b). Therefore, there may be regions of disorder. There are two observations which favor explanation (a) over (b) although the resulting arguments are not strong. First, if (a) is the correct explanation, the intensity ratio of the doublet should be a ratio of small whole numbers since the stoichiometry of the unit cell dictates this. The doublet is approximately in a 1 : 1 ratio (see inset in Fig. 2). Second, if there

Fig. 3. Proton lineshapes of the original hexaglycine sample following variable spin-lock times of (A) 0.05 ms, (B) 5 ms and (C) 15 ms. Spectra have been normalized to the same intensity for the broad hexaglycine resonance to emphasize that the narrow water signal is decaying faster than the hexaglycine signal. The relative rates of decay, however, are not the same during the first five as opposed to the last ten ms indicating that the two kinds of protons are very likely coupled by spin diffusion.

are disordered regions, it is likely that water molecules could penetrate these regions. Under these circumstances, it is reasonable to expect that the oxygens on the carboxy terminus would be sites of interaction via hydrogen bonding and this, in turn, might influence the chemical shift of these carbons. Such a shift was not observed; however, this may not be an entirely fair test as some water was present in all of the samples.

In a final attempt to comment on the morphology of the hexaglycine as it relates to the distribution of water molecules, we ran two proton experiments on the original hexaglycine. First, we looked at proton lineshapes as a function of the proton spin locking time in a $T_{1\rho}$ experiment. This is shown in Fig. 3 where lineshapes, renormalized to the same intensity for the broad portion, are given for spin lock times of 0.05, 5, and 15 ms. Relative to the broad signal, the decay rate for the water protons is much greater between 0.05 and 5 ms than between 5 and 15 ms. This is a strong indication that the

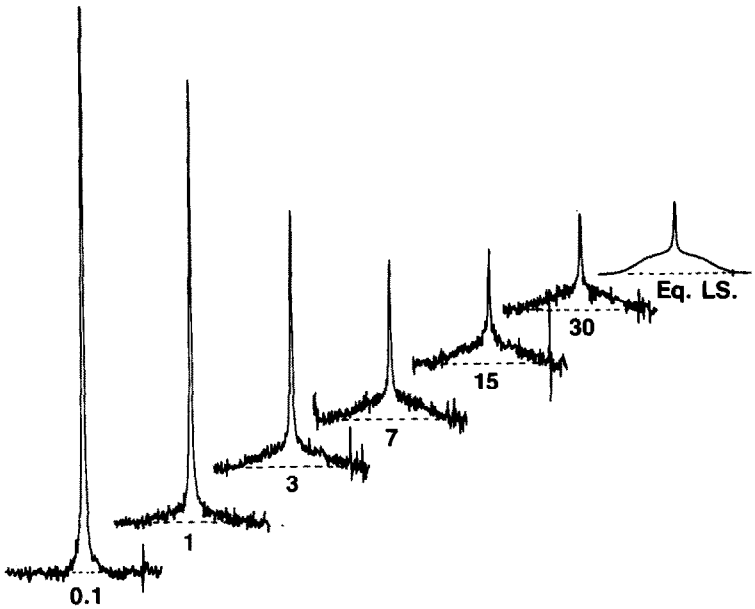

Fig. 4. Demonstration of polarization transfer between the water and the hexaglycine protons in the original hexaglycine sample after the indicated spin diffusion times. The initial polarization condition, given by the left-hand spectrum, shows that all of the polarization is found among the water protons. Most of the transfer has occurred after 7 ms of spin diffusion; however, after 30 ms, the equilibrium lineshape (right-hand spectrum) is not yet obtained. The rate of this transfer implies that the minimum dimension describing the majority of hexaglycine domains has an upper limit of about 6 nm.

polarization of the water protons is coupled by spin diffusion to the polarization of the hexaglycine protons. In order to clarify this coupling to a greater extent, a spin diffusion experiment, shown in Fig. 4 was undertaken. Starting at time zero, following a modified Carr–Purcell train of five 180° pulses having a spacing of 50 μs, the polarization resides exclusively amongst the water protons. Then, following variable periods of spin diffusion, polarization flows from the water protons to the hexaglycine protons. The rate at which this happens can give an indication of the domain size of the hexaglycine domains, provided that the flow of polarization across the water/hexaglycine interface is not rate-limiting (a tenuous assumption in view of the narrow water proton resonance). With this assumption, the data leads to minimum domain dimensions of about 6 nm, although the fact that an equilibrium lineshape has not yet been achieved after 30 ms of spin diffusion could also imply that there are some larger regions present. However, if the flow of polarization across the interface is very rate-limiting, then a whole range of possibilities exists from a partially crystalline morphology with water interpenetrating the non-crystalline hexaglycine molecules, to fully crystalline morphologies with water having access only to the

TABLE 8

^{1}H NMR chemical shifts (δ) and coupling constants J (Hz) of oligoglycines [a]

Compound	Parameter	CH_2ND	CH_2ND_3	NH [b]	NH_3 [c]
Diglycine	δ	4.35	4.28	7.89	7.51
	J			5.7	
Triglycine	δ	4.32 4.38	4.28	7.71 8.09	7.49
	J			5.6 5.5	
Tetraglycine	δ	4.33 4.35 4.40	4.32	7.74 8.00 8.09	7.49
	J			5.5 5.3 5.6	
Pentaglycine	δ	4.33 4.34 4.34 4.38	4.28	7.67 7.91 7.96 8.04	7.48
	J			5.7 5.8 5.8 5.7	
Hexaglycine	δ	4.32 4.34 4.34 4.34 4.38	4.28	7.68 7.89 7.91 7.95 8.04	7.48
	J			5.6 5.7 6.2 6.1 5.5	
		CH_2NH	CH_2NH_3	NH	NH_3
Hexaglycine [d]	δ	4.32 4.34 4.34 4.34 4.38	4.28	7.71 7.93 7.94 7.98 8.06	7.44
	J			5.7 5.3 6.0 5.9 5.7	5.7

[a] Measured at 400 MHz for solutions in CF_3CO_2D, unless stated otherwise; $J = {}^3J_{HCNH}$, i.e. the spin coupling constant between the CH_2 protons and their vicinal NH proton(s).

[b] Residual triplets for undeuterated NH groups.

[c] Residual broad singlets for undeuterated NH_3 groups.

[d] Solution in $CF_3CO_2H:CD_3CO_2H$ (9:1 v/v).

surfaces of crystallites. In the latter case, minimum domain dimensions for the majority of crystallites would be less than 6 nm.

Thus, there remains an ambiguity as to the condensed state of the hexaglycine in these samples, and also, to what extent the hexaglycine is crystalline. Therefore, the enthalpies of formation and combustion for hexaglycine reported herein may have a small uncertainty linked to the reference state for the solid.

Finally, we turn to the question of whether there is any cyclic hexaglycine, existing as an impurity, in these samples. All of the oligoglycines (degree of polymerization, $n = 2$–6) were tested for correct structure and suitable purity by high resolution ^1H NMR of CF_3CO_2D solutions. The possible existence of the hexaglycine material in a cyclic structure was of particular concern, and so this material was studied in greater detail than the lower homologs; specifically by ^1H NMR of its solution in 9:1 CF_3CO_2H:CD_3CO_2H, a solvent system that yields a greater wealth of spin coupling information than does CF_3CO_2D. In these acidic solvent systems, protonation (or deuteronation) of the terminal amino group of each oligoglycine was expected to occur, but protonation of amide nitrogen atoms was not anticipated. However, in CF_3CO_2D, both types of nitrogen groups were expected to undergo deuterium exchange of the nitrogen-bound protons, thus leading to simplification of the spectra.

The ^1H NMR spectra of the oligoglycine solutions in each case showed only one major component, and no impurity peaks were detected, even by long term signal averaging of up to 5000 scans. The spectra of the solutions in CF_3CO_2D showed n CH_2 singlets, one of which was always broad and at highest field (δ 4.28 in most cases, see Table 8). This broad CH_2 singlet was assigned to the N-terminal CH_2 group, the signal of which was expected to be split into a complex, but unresolved septet by coupling of the CH_2 protons with the deuterons of the adjacent ND_3 group. Confirmation of this assignment was obtained from the spectrum of hexaglycine in CF_3CO_2H:CD_3CO_2H which displayed the signal at highest field as a quartet ($^3J_{HCNH}$ 5.7 Hz), and the remaining CH_2 signals as doublets ($^3J_{HCNH}$ 5.5–5.7 Hz).

The spectra of the solutions of the oligoglycines in CF_3CO_2D also showed $n - 1$ triplets that represent the amide NH protons and their spin coupling in each case to two *vicinal* CH_2 protons, and a broad singlet at δ 7.5 (see Table 8) that was assigned to the NH_3 protons on the basis that its

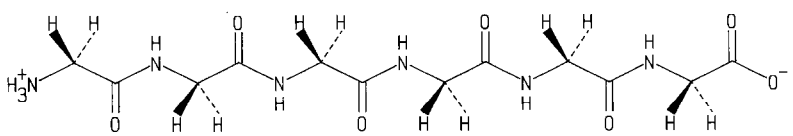

Fig. 5. Structure of the linear hexaglycine molecule.

integrated intensity was invariably three times that of any one of the NH triplets.

These results (summarized in Table 8) indicate that all of the oligoglycines studied have acyclic structures. Figure 5 presents the acyclic structure of the hexaglycine molecule. In particular, the detection of five NH protons and three protons from NH_3 for hexaglycine means that this sample cannot have a cyclic structure, for which a total of only six NH protons would be expected.

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