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Partial molar heat capacities of the side chains of some amino acid residues in aqueous solution

The influence of the neighboring charges

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Partial molar heat capacities of the side chains of some amino acid residues (Ala, Val, Leu, Ile, Ser) have been determined over a broad temperature range from calorimetric heat capacity measurements of the corresponding tripeptides and cyclodipeptides. The data obtained are compared with those determined earlier from the heat capacities of analog compounds. It is shown that in amino acids and even tripeptides of the Gly-X-Gly type, the influence of the end charges on the heat capacity of the side chain is rather significant even in buffered solutions of high ionic strength.

1. Introduction

The importance of the thermodynamic parameters of a polypeptide chain with non-interacting amino acid residues follows from the fact that the corresponding random coiled state is commonly used as a reference state in a theoretical consideration of the formation of the native protein structure. The heat capacity of the unfolded random coiled polypeptide chain can be estimated by a simple summation of the partial heat capacities of the constituent components [1]. This in itself requires a knowledge of the partial heat capacities of all amino acid residues constituting the polypeptide chain.

There have been several attempts to evaluate the partial molar heat capacity of amino acid residues in aqueous solution [2,3]. However, all of those studies were carried out only at 25 °C without checking whether the model compounds used were indeed proper models for studying amino acid residues. In addition, these critical confirmatory tests were not performed in our previous paper devoted to the determination of the partial molar heat capacity of amino acid residues in a broad temperature range [4]. To clarify this point, it was necessary to undertake a detailed comparative study of the suitability of various model compounds for the determination of heat capacities of given amino acid residues over a wide temperature range.

In this paper, we analyze the values of the partial molar heat capacities of some amino acids derived from a calorimetric study of various model compounds, namely, tripeptides (Gly-X-Gly, where X = Gly, Ala, Val, Leu, Ile, Ser) and cyclodipeptides (c(Gly-Gly), c(Ala-Gly), c(Ala-Ala), and compare them with the data which we

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obtained earlier from the partial heat capacities of simple organic analogs of amino acid side chains [4].

2. Materials and methods

Cyclodipeptides c(Gly-Gly), c(Ala-Gly) and c(Ala-Ala) were obtained from Biochem Biosciences and used without further purification.

The procedures for synthesis, purification, analysis, solution preparation and concentration determination of the peptides Gly-Ala-Gly, Gly-Val-Gly, Gly-Ile-Gly, Gly-Leu-Gly, Gly-Ser-Gly, Gly-Pro-Gly, Gly-Met-Gly, Gly-His-Gly were those described in our previous papers [4,5]. Cyclic dipeptides were studied in two different solvents: pure water and 0.5 M sodium acetate, pH 4.0. The tripeptides were studied only in the sodium acetate buffer.

The apparent heat capacity difference, $\Delta C_{p,\phi}$, between the solvent and the solution was evaluated using a DASM-4 scanning microcalorimeter (Bureau of Biological Instrumentation, Academy of Sciences of the U.S.S.R.). The apparent molar heat capacity, $C^{o}_{P,\phi}$, was calculated as described in ref. 6, using the formula:

$$C_{p,\phi} = C_{p,1} \cdot \frac{V^{o}_{\phi}}{V_1} + \frac{\Delta C_{p,\phi}}{m} \qquad (1)$$

where $C_{p,1}$ and V_1 represent the partial heat capacity and partial volume of the solvent, respectively, m is the mass of the solute in a calorimetric cell, and V^{o}_{ϕ} denotes the partial volume of the solvent. The data on the partial molar volumes of the studied compounds, V^{o}_{ϕ}, used for calculations of the $C_{p,\phi}$ values have been reported in ref. 5.

Within the range of concentrations employed (0.3–1.5%), no dependence of the apparent molar heat capacity $C_{p,\phi}$ on the concentration was found. This permits one to consider the values determined for $C_{p,\phi}$ as corresponding to infinite dilution, i.e., $C_{p,\phi} = C^{o}_{p,\phi}$.

3. Results

The partial molar heat capacities of the peptides studied at different temperatures are listed in table 1. At 25 °C the partial molar heat capacity values of the cyclodipeptides c(Gly-Gly) (138.0 J K^{-1} mol^{-1}) and c(Ala-Ala) (321.7 J K^{-1} mol^{-1}) are in reasonable agreement with those reported earlier by Cabani et al. [7] (149 J K^{-1} mol^{-1} and 335 J K^{-1} mol^{-1}, respectively).

A model compound, which can be used for calculation of the partial heat capacity of the peptide unit (the -CHCONH- group), is c(Gly-Gly)

Table 1

Partial molar heat capacities, $C^{o}_{p,\phi}$ (J K^{-1} mol^{-1}), of some peptides in aqueous solution at various temperatures

Peptide	Temperature (°C)						σ (standard deviation)
	5	25	50	75	100	125	
Gly-Gly-Gly [a,c]	93.1	194.6	275.7	348.9	413.1	474.2	11.0
Gly-Ala-Gly [a]	215.1	252.4	343.0	405.7	471.0	530.6	13.4
Gly-Val-Gly [a]	334.5	413.0	484.2	554.9	622.9	685.6	15.1
Gly-Ile-Gly [a]	406.5	487.8	558.1	618.0	681.4	726.8	16.0
Gly-Leu-Gly [a]	424.2	502.8	566.6	628.3	694.7	758.7	15.6
Gly-Ser-Gly [a]	86.4	197.8	289.7	373.9	450.7	522.4	14.2
c(Gly-Gly) [b]	88.4	138.0	184.3	220.0	245.8	270.8	5.0
c(Gly-Ala) [b]	186.3	235.3	273.6	307.8	330.9	334.6	6.2
c(Ala-Ala) [b]	280.7	321.7	360.7	388.2	407.6	416.9	7.4

[a] In 0.5 M sodium acetate (pH 4.0) buffer as solvent.
[b] In pure water as solvent.
[c] From ref. 4.

Table 2

Temperature dependences of the partial heat capacities of a number of amino acid side chains in aqueous solution

All heat capacity values are given in J K^{-1} mol^{-1}.

Side chain of amino acid residue	Model	Temperature (°C)					
		5	25	50	75	100	125
Gly [a]	additive	82.3	78.0	71.7	66.4	59.7	53.9
Ala [a]	analog	175.7	166.7	156.2	144.7	134.6	124.1
Ala	tripeptide	204.3	165.8	139.0	123.2	117.6	110.3
Ala [a]	additive	178.5	169.2	155.6	144.1	129.5	116.9
Ala	cyclic dipeptide	175.7	166.7	156.2	144.7	134.6	124.1
Val [a]	analog	324.6	314.4	305.0	294.7	285.7	269.6
Val	tripeptide	323.7	296.4	280.2	272.4	269.5	265.3
Leu [a]	analog	385.9	381.7	377.8	372.9	369.4	365.5
Leu	tripeptide	413.4	386.2	362.6	345.8	341.3	338.4
Ile [a]	analog	406.8	402.3	397.1	390.8	386.0	380.8
Ile	tripeptide	395.7	371.2	354.1	335.5	328.0	306.5
Ser [a]	analog	72.9	80.3	88.6	94.0	103.2	112.2
Ser	tripeptide	75.6	81.2	85.7	91.4	97.3	102.1
-CHCONH- [a]	Gly_n ($n = 3$–5)	3.7	15.2	26.2	29.8	33.7	33.7
-CHCONH-	c(Gly-Gly)	−38.1	−9.0	20.5	43.6	63.2	81.5

[a] From ref. 4.

which contains two glycyl residues (-CH_2CONH-):

$$C^{o}_{p,\phi}(\text{-CHCONH-}) = \tfrac{1}{2} \cdot C^{o}_{p,\phi}(\text{c(GlyGly)} - C^{o}_{p,\phi}(\text{H}) \quad (2)$$

where $C^{o}_{p,\phi}$(H) denotes the heat capacity for a hydrogen atom and its value is given as a function of temperature in ref. 4. The temperature dependences of the partial heat capacity of the -CHCONH- group, calculated in this manner, are presented in table 2.

From the partial molar heat capacities of the cyclic dipeptides one can calculate the heat capacity contribution of the Ala amino acid side chain, i.e., the -CH_3 group. Fig. 1 displays plots of the $C^{o}_{p,\phi}$ values for the studied cyclic dipeptides vs the number of carbon atoms in the corresponding molecules at various temperatures. As one observes, there is a linear dependence of the partial heat capacity on the number of carbon atoms over the temperature range studied. The slope of this plot at a given temperature will correspond to the heat capacity of the alanine side chain relative to that of the glycine side chain (the hydrogen atom) $C^{o}_{p,\phi}$ (-CH_3-H). The partial molar heat capacity of the -CH_3 group can then be readily obtained by addition of the partial molar heat capacity of the hydrogen atom, derived in our earlier article [4]:

$$C^{o}_{p,\phi}(\text{-CH}_3) = C^{o}_{p,\phi}(\text{-CH}_3\text{-H}) + C^{o}_{p,\phi}(\text{H}) \quad (3)$$

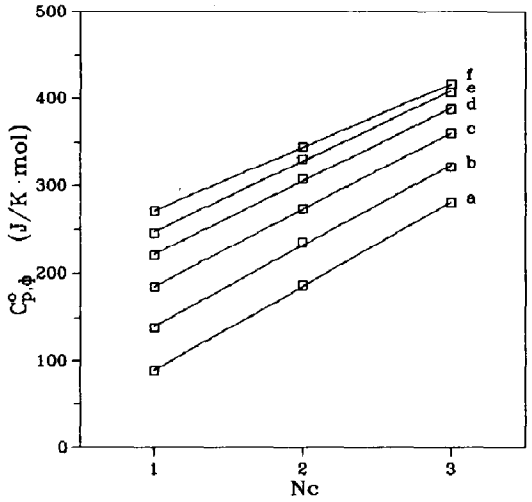

Fig. 1. Dependence of the partial heat capacity of cyclic dipeptides on the number of carbon atoms in the molecule, N_c, at various temperatures: (a) 5, (b) 25, (c) 50, (d) 75, (e) 100, (f) 125 °C.

The values of the partial heat capacity of the $-CH_3$ group, i.e., alanyl side chain, thus obtained, are listed in table 2 as a function of temperature. From the data obtained on the partial molar heat capacity of tripeptides one can calculate the partial heat capacity of the side chain of internal amino acid residues, $C^{\circ}_{p,\phi}$(-R), as:

$$C^{\circ}_{p,\phi}(\text{-R}) = C^{\circ}_{p,\phi}(\text{Gly-X-Gly}) - C^{\circ}_{p,\phi}(\text{Gly-Gly-Gly}) + C^{\circ}_{p,\phi}(\text{-H}) \quad (4)$$

The heat capacity contributions of the amino acid side chains of Ala, Val, Leu, Ile and Ser, calculated according to this procedure, are also listed in table 2 together with data we obtained in a previous study of analog compounds [4].

4. Discussion

Fig. 2 represents the partial molar heat capacity values for the -CHCONH- group determined from investigation of c(Gly-Gly) together with those obtained in our previous study of glycine homopeptides in aqueous solution [4]. As may be seen from fig. 2, there is a significant difference between the two heat capacity functions evaluated from the study of two different model compounds. At least two explanations may be suggested to account for the observed difference. First, in the case of c(Gly-Gly), the -CHCONH- group has a smaller water-accessible surface area (ASA) than that in the case of glycine homopeptides and, according to our previous study [4], this could lead to an increase in the heat capacity.

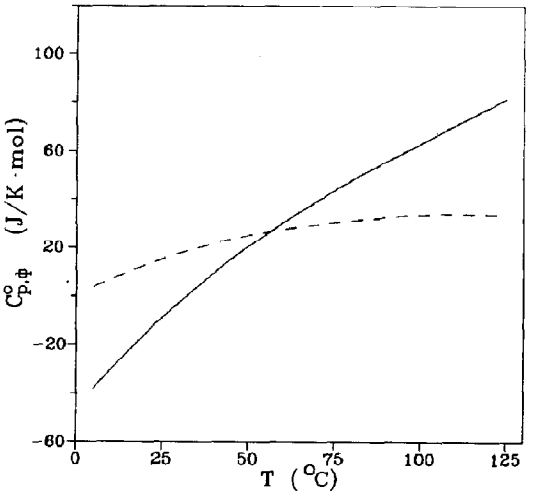

Fig. 2. Temperature dependences of the heat capacity of the -CHCONH- group obtained from studies of glycine homopeptides (- - - - -) [4] and c(Gly-Gly) (———).

Table 3 gives a comparison of the data on the partial molar heat capacities of amino acid side chains at 25 °C obtained from the calorimetric study of their analogs [4], amino acids [2] and tripeptides in pure water [8] and in buffered solution. The values resulting from the study of amino acids [2] differ significantly from those measured in the investigation of amino acid side chain analogs. This difference is probably due to the presence of charges at the end groups of amino acids, and it is likely that this charge-related effect is also manifested in tripeptides when they are examined in pure water [8]. At the same time, the

Table 3

Comparison of the heat capacities of amino acid side chains at 25 °C derived from the study of various model compounds

Side chain of amino acid residue	Heat capacity ($J\,K^{-1}\,mol^{-1}$) derived from the study of			
	Analogs [4]	Amino acids [2]	Tripeptides [8]	Tripeptides (this work)
Ala	166.7	180.2	180.0	165.8
Val	314.4	340.7	333.0	296.4
Leu	381.7	436.5	416.0	386.2
Ile	402.3	422.1	–	371.2
Ser	80.3	156.2	153.0	81.2

correspondence between the results obtained on the analogs and on tripeptides in buffered solutions is much better. This shows that in the presence of a buffer, the influence of charges on the side chain of the central amino acid residue is considerably diminished. Nevertheless, even in a buffered solution, the heat capacity measured in the study of tripeptides differs slightly from those determined for other types of model compounds. This becomes especially clear if one considers the example of an Ala side chain (see table 2) the heat capacity of which has been obtained in four different ways: using the additivity scheme [4], analogs [4], cyclic dipeptides and tripeptides in buffered solutions. As is evident three sets of data are in reasonable agreement with each other while the fourth set, corresponding to tripeptides in a buffered solution, has a somewhat different temperature dependence. This difference can only arise from the residual influence of the charges in tripeptides in buffered solutions. However, it is not caused by the difference in solvent composition, since the values of the partial molar heat capacities of cyclodipeptides in the same buffer (0.5 M sodium acetate (pH 4.0), data not shown) are indistinguishable from those obtained in pure water.

This study of the temperature-dependent behavior of the partial heat capacity of side chains of amino acid residues in aqueous solution shows that simple organic analogs of the amino acid side chains and cyclic dipeptides are most suitable models for investigation of the heat capacity of individual and non-neighbour-group-interactive amino acid side chains over a broad range of temperatures. Tripeptides can also presumably fulfil this role, but only at 25 °C where the influence of the end charged groups on the partial molar heat capacity of the side chains is apparently insignificant in a buffered solution. However, this may also depend upon the particular tripeptide employed.

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