



## Original articles

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## Thermochemical characteristics of the formation of aqueous solutions of imino acids

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### Abstract

The calorimetry methods are an important source of thermodynamic information in the physicochemistry of solutions of biologically active substances, including amino acids. The goal of the work was to prepare a thermodynamic description of the formation of an aqueous solution of heterocyclic imino acids, proline and hydroxyproline, that have different structures and sizes of the side radical, in a wide range of concentrations, which can be used for a qualitative analysis of changes occurring in their solutions.

Thermochemical measurements of the formation of an aqueous solution of imino acids in the concentration range  $1.0 \cdot 10^{-3}$  –  $40.0 \cdot 10^{-3}$  mol/kg was performed on a MID-200 differential heat-conducting microcalorimeter at 293 K. The equilibrium moment in the solution formation was determined by the output of the thermokinetic curve to the zero line. The enthalpy of the formation of an aqueous solution of imino acids was calculated by the integration of the time dependence of thermal power.

It is shown that the increase in the equilibrium time, the increase in the maximum heat flow, and the decrease in the rate of change of the heat flow during the dissolution of hydroxyproline is due to the formation of intra and intermolecular bonds in the Hypro structure with the participation of the OH group. The difference in the structure of imino acids is reflected in the sign of the thermal effect and the form of the concentration dependence of the enthalpy of formation of aqueous solutions. The exoeffect of proline dissolution is due to the stabilisation of the water structure influenced by imino acid.

**Keywords:** Imino acids, Aqueous solutions, Thermochemical characteristics

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## 1. Introduction

Among all the diversity of thermodynamic properties in chemistry of solutions of biologically active substances, much attention is paid to the study of thermochemical characteristics [1–4]. Calorimetry methods are often used as a source of thermodynamic information in the physicochemistry of solutions. Amino acids are among the most important biologically active substances. Thermodynamic description of the process of formation of an aqueous solution of amino acids that have different structures and sizes of the side radical in a wide range of concentrations are also of great interest. The course of the dependence isotherm of the integral heat of dissolution on the concentration of the dissolved substance can be used for a qualitative analysis of changes occurring in the solution. Heterocyclic amino acid (proline) and its derivative (4-hydroxyproline) are contained in the connective tissue protein called collagen. They stabilise the secondary structure of the polyproline helix and play an important metabolic role in the body. In this work we present the results of the study of thermochemical properties of the formation of aqueous solutions of imino acids, specifically proline and hydroxyproline.

## 2. Experimental

Heterocyclic amino acids proline (pyrrolidine-2-carboxylic acid) (Pro) and hydroxyproline (L-4-hydroxypyrrolidine- $\alpha$ -carboxylic acid) (Hyp), which belong to imino acids, were chosen as the objects of study. Their carbon skeleton forms a cyclic compound that includes an amino group. Imino acids can be found in an aqueous solution as a bipolar ion (Fig. 1) [8].

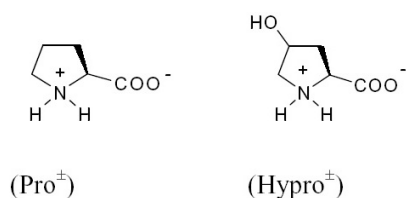


Fig. 1. Structure of amino acids

The samples of amino acids produced by Reanal (Hungary) and bidistilled water with specific electrical conductivity of not more than  $1.4 \cdot 10^{-6}$  (Ohm/cm)<sup>-1</sup> were used for the study.

Solubility in water at 298 K was 162.3 g/100 ml for Pro and 36.1 g/100 ml for Hyp. Thermochemical measurements of the formation of an aqueous solution of imino acids in the concentration range  $1.0 \cdot 10^{-3}$ – $40.0 \cdot 10^{-3}$  mol/kg were conducted on a MID-200 differential heat-conducting microcalorimeter at  $293 \pm 1$  K [9,10]. The sensitivity of the microcalorimeter was  $5 \cdot 10^{-3}$  W. An amino acid sample weighed with an accuracy of  $\pm 0.0002$  g was placed in a thin-walled ampoule which was immersed in  $50.0 \text{ cm}^3$  of water and thermostated in an adiabatic calorimeter for 24 hours. Then the ampoule was broken with a special stock, and a weighed portion of amino acid was poured into the solvent. The equilibrium moment in the solution formation was determined by the output of the thermokinetic curve to the zero line. The enthalpy of the formations of Pro and Hyp aqueous solutions were calculated by the integration of the time dependence of thermal power. The values of standard deviation and the confidence interval were  $4.43 \cdot 10^{-3}$  and  $5.12 \cdot 10^{-3}$ , respectively, for five independent measurements with the confidence interval 0.95 and Student's coefficient 2.78 [11].

A series of measurements of the thermal effect of potassium chloride dissolution in water at 298 K, which is used as a standard substance in calorimetry for calibration of devices, was performed in order to assess the accuracy and reliability of the obtained calorimetric measurements [12]. We used a weighed portion of potassium chloride weighing  $1.0642 \pm 0.0002$  g based on the final concentration of the solution which was 0.278 mol/1000 g of water. As a result of five independent measurements, the obtained value of enthalpy of the formation of an aqueous solution KCl upon infinite dilution was  $17.34 \pm 0.28$  kJ/mol, and it corresponds well with the data provided in [13].

## 3. Results and discussion

The thermodynamic parameter of the formation of an aqueous solution of amino acids in the whole concentration region is determined by the sum of the main effects, including endothermic effect, caused by the displacement of amino acid molecules from their distance in the molecular crystal lattice to the average distance between them in the solution

of this concentration, and exothermic effect of hydration, which is presented as a sum of a number of endo and exoeffects [1]. The formation of an aqueous solution of proline in the selected region of concentration, unlike hydroxyproline and such previously studied amino acids as phenylalanin, histidine, and cystine [6, 7, 14], is accompanied by heat production (Fig. 2).

The exothermic effect of the hydration process caused by a number of endo and exoeffects prevails over the energy consumption for the destruction of the proline crystal lattice and the structure of water [12]. The introduction of a hydroxyl group into the structure of the hydroxyproline molecule is reflected in a change of the thermal effect sign and in the nature of the concentration dependence of the enthalpy of dissolution of imino acids.

Thermokinetic curves of the formation of an aqueous solution of proline and hydroxyproline representing the energy of the process as a function of time are presented in Figs. 3 and 4. Maximum intensity of the thermal effect ( $W_{\max.}$ ) for imino acids in the studied concentration region was observed during the initial period of dissolution (2-4 min) and increased linearly with the growth of concentration. The dissolution of hydroxyproline is characterised by great values of  $W_{\max.}$  (Fig. 5). However, in case of hydroxyproline, unlike proline, we observed a smaller value of the rate of change in the heat flow and its linear dependence on concentration (Fig. 6). The differences in the rates of change in the heat

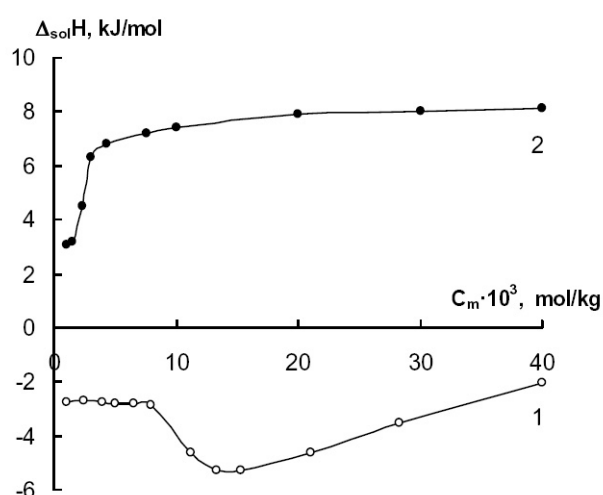


Fig. 2. Concentration dependence of enthalpy of formation of aqueous solutions of proline (1) and hydroxyproline (2)

flow of the dissolution process ( $v_{\text{Pro}}$  and  $v_{\text{Hypro}}$ ) increased with an increase in the concentration of the obtained solution of imino acids.

The isotherm of proline dissolution (Fig. 2) has an extreme nature. The presence of a maximum on the isotherm was apparently caused by the competition between the effects of hydrophobic stabilisation of the water structure influenced by a non-polar radical and the destruction of its structure influenced by the zwitterionic group of the imino acid. The presence of absorption bands at 1609, 1643, and 3174  $\text{cm}^{-1}$ , as well as at 1625 and 3152  $\text{cm}^{-1}$  on an IR spectrum of the initial samples confirms the previous data [13] on the formation of  $\text{COO}^- \dots \text{NH}_3^+$  bonds in the structure of amino acids.

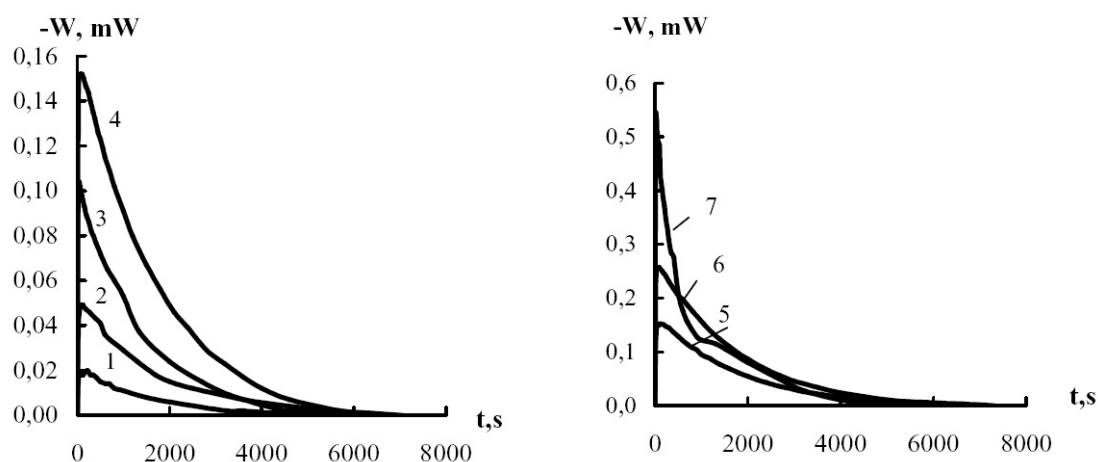
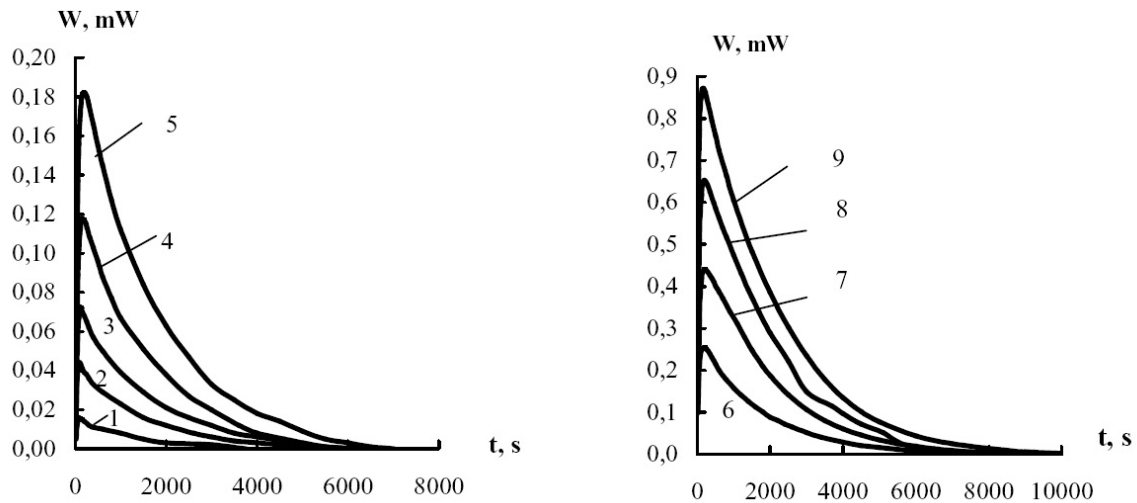
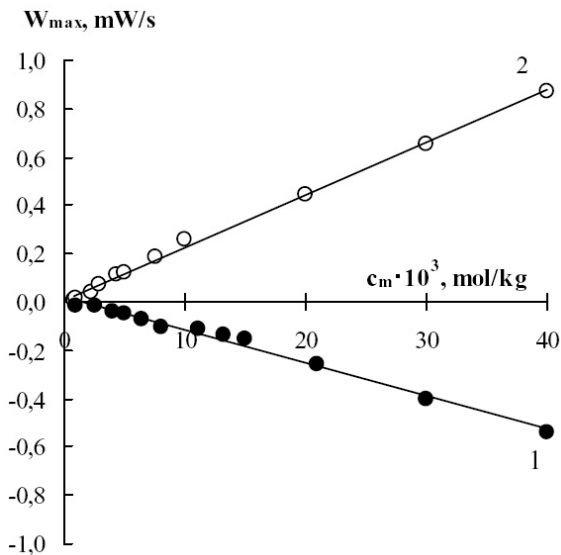


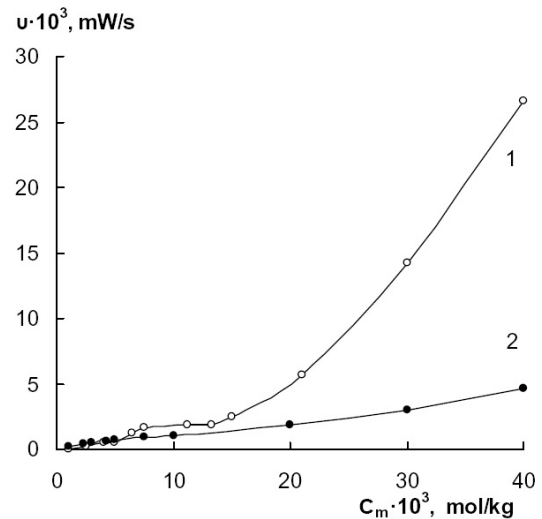
Fig. 3. Thermokinetic curves of formations of aqueous solutions of proline with concentrations: 1 –  $2.5 \cdot 10^{-5}$ , 2 –  $5 \cdot 10^{-5}$ , 3 –  $8 \cdot 10^{-5}$ , 4 –  $10 \cdot 10^{-5}$ , 5 –  $15 \cdot 10^{-5}$ , 6 –  $20 \cdot 10^{-5}$ , 7 –  $40 \cdot 10^{-5}$  mol/kg



**Fig. 4.** Thermokinetic curves of formations of aqueous solutions of hydroxyproline with concentrations: 1 –  $1 \cdot 10^{-3}$ , 2 –  $2.5 \cdot 10^{-3}$ , 3 –  $3 \cdot 10^{-3}$ , 4 –  $5 \cdot 10^{-3}$ , 5 –  $8 \cdot 10^{-3}$ , 6 –  $10 \cdot 10^{-3}$ , 7 –  $20 \cdot 10^{-3}$ , 8 –  $30 \cdot 10^{-3}$ , 9 –  $40 \cdot 10^{-3}$  mol/kg



**Fig. 5.** Concentration dependence of the maximum value of the heat flux during the formation of a solution of hydroxyproline (1) and (2) proline



**Fig. 6.** Concentration dependence of the rate of change in the heat flow of the process of dissolution of proline (1) and hydroxyproline (2)

In the region of concentrations  $\leq 8.0 \cdot 10^{-3}$  mol/kg, the independence of the value of the integral heat of dissolution from the concentration was observed. It can be assumed that in this region of concentrations the influence of proline on the water structure showed to a smaller degree [13]. The growth of exothermicity of the formation of solution in the region of concentrations ( $8.0 \cdot 10^{-3}$ – $15.2 \cdot 10^{-3}$  mol/kg) was caused by an increase in the hydrophobic component of hydration, meaning an increase in the stabilisation of the water structure under the influence of a non-polar radical of an amino acid [1, 15, 16]. Authors of [15]

considered this phenomenon as a violation of the dynamic structure of water. The process of amino acid dissolution is characterised by a change in the nature of the concentration dependence of the enthalpy of dissolution with an increase in the concentration of the obtained solution by more than  $15.2 \cdot 10^{-3}$  mol/kg. Upon reaching the maximum stabilisation of the water structure influenced by the hydrocarbon radical of proline, the solvent molecules were reoriented under the influence of the formed imino acid associates due to the hydrophobic interaction of side radicals as well as to the direct pair interaction of zwitterionic

groups [1, 17]. It should be taken into account that proline's hydrophobic hydration ability decreased with an increase in concentration.

The isotherm of hydroxyproline dissolution (Fig. 2) belongs to the type with a continuously increasing endothermicity with a growth in concentration [12]. The solvent's structure is damaged due to the participation of an OH group in the formation of H-bonds with water molecules. The endoeffect of the formation of an aqueous solution of hydroxyproline was caused by the participation of the OH group in the formation of H-bonds with water molecules. The participation of the OH group in the formation of hydrogen bonds with water molecules was confirmed by the absorption bands at 3194 and 2495  $\text{cm}^{-1}$  [18]. The positive contribution of the OH group to the enthalpy value increased in proportion to the concentration in the region of solution concentrations  $1.5 \cdot 10^{-3}$ – $5.0 \cdot 10^{-3}$  mol/kg, The isotherm is characterised by a slight change in the enthalpy value in case of a concentration of more than  $5.0 \cdot 10^{-3}$  mol/kg, The ability of hydroxyproline to form associates increases with an increase in the concentration of the solution, which suggests an increased role of hydrophobic hydration.

The stability of the values of enthalpies of dissolution for imino acids in the region of proline concentrations of  $1.0 \cdot 10^{-3}$ – $8.0 \cdot 10^{-3}$  mol/kg and  $1.0 \cdot 10^{-3}$ – $1.5 \cdot 10^{-3}$  mol/kg of hydroxyproline allowed attributing the obtained enthalpy value to the maximum dilution condition. In this region of concentrations the state of amino acids probably corresponds to the smallest change in the water structure. The value of the enthalpy of dissolution upon infinite dilution was calculated using an extrapolation of thermodynamic functions to zero solution concentration [12, 19]. The values of maximum enthalpy of dissolution were  $2.85 \pm 0.04$  kJ/mol for proline and  $3.20 \pm 0.12$  kJ/mol for hydroxyproline.

#### 4. Conclusions

Therefore, the established differences in the nature of changes in thermokinetic characteristics, the sign of the thermal effect, and the form of dissolution isotherms for proline and hydroxyproline can be explained by the differences in the structural organisation of the solvent in different concentration zones.

#### Contribution of the authors

The authors contributed equally to this article.

#### Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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