ВЗАИМОДЕЙСТВИЕ L-КАРНОЗИНА С НИКОТИНОВОЙ И ИЗОНИКОТИНОВОЙ КИСЛОТАМИ В ВОДНЫХ РАСТВОРАХ ПРИ 298.15 К

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Понимание механизма взаимодействия лекарственных веществ с белками привлекает внимание многих исследователей. Термодинамические исследования жидкокристаллических систем, содержащих пептид и лекарственное средство, позволяют определить природу и движущие силы взаимодействия между ними. В данной статье представлены результаты исследования взаимодействия пептида L-карнозина (Car) как модели полипептидных цепей в белке, с изоникотиновой и никотиновой кислотами, как моделями лекарственных средств, в водных растворах с использованием калориметрического метода. Калориметрические измерения энтальпии растворения L-карнозина в воде и водном растворе с добавками изомеров пиридинмонокарбоновой кислоты проводили на изопериболиче-ском калориметре раствора ампульного типа при 298.15 К. Погрешность измерения одиночных тепловых эффектов не превышала 0,2%. Относительная комбинированная погрешность измерения энтальпии растворения составляла не более 0,7%. На основании полученных экспериментальных данных и с помощью компьютерной программы HEAT рассчитаны термодинамические параметры (lgKc, ΔcG, ΔcH, ΔcS) комплексообразования между реагентами. Проведено сравнение способности пептида к взаимодействию с никотиновой и изоникотиновой кислотами. Показано, что взаимодействие L-карнозина с изоникотиновой кислотой приводит к образованию более стабильного комплекса, чем с никотиновой кислотой. Этот факт можно объяснить, прежде всего, изменением ионного состояния реагентов в растворе и преобладанием цвиттер-ионных форм для изоникоти-новой кислоты по сравнению с никотиновой кислотой. Полученные данные свидетельствуют о наличии в водных растворах молекулярных комплексов карнозина с изомерами пиридинкарбоновой кислоты со стехиометрией 1:2. Комплексы карнозина с изоникотиновой кислотой в основном стабилизированы энтальпийным вкладом, а комплексы карнозина с никотиновой кислотой стабилизированы как энтальпийным, так и энтропийным вкладами в свободную энергию Гиббса. Предполагается, что основными типами взаимодействий при образовании комплексов являются электростатические силы и образование водородных связей между пептидом и пиридинкарбоновыми кислотами.

Ключевые слова: термодинамика, растворы, калориметрия, L-карнозин, никотиновая кислота, изоникотиновая кислота

INTERACTION OF L-CARNOSINE WITH NICOTINIC AND ISONICOTINIC ACIDS IN AQUEOUS SOLUTIONS AT 298.15 K

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Understanding the mechanism of interaction of drugs with proteins has drawn attention of numerous researchers. The thermodynamic investigations of liquid-phase systems containing peptide and drug make it possible to determine the nature and driving forces of the interaction between them. In this work, we present results of interaction study of peptide L-carnosine (Car), as model of polypeptide chains in protein, with nicotinic and isonicotinic acids, as the drug models, in aqueous solution using calorimetric method. Calorimetric measurements of the enthalpy of L-carnosine dissolution in water and aqueous solutions with two isomers of pyridine carboxylic acid additives were performed on an ampoule-type isoperibolic dissolution calorimeter at 298.15 K. The error of measuring single heat effects was below 0.2%. The relative combined uncertainty in the measurements of the enthalpies of dissolution was not more than 0.7%. Based on the obtained experimental data and the using the HEAT computer program, the thermodynamic parameters ($\Delta G$, $\Delta H$, $\Delta S$) of the complex formation between the reagents were calculated. A comparison of the affinity of peptide to interaction with nicotinic acid and isonicotinic acid was carried out. It is shown that the interaction of L-carnosine with isonicotinic acid leads to the formation of a more stable complex than with nicotinic acid. This fact may be mainly explained by the changing of ionic state of the reagents in solution and the predominance of the zwitterionic forms of isonicotinic acid in compared with nicotinic acid. The data obtained reveal the presence of molecular complexes between Car and isomers of pyridine carboxylic acid with 1:2 stoichiometry in aqueous solutions. The complexes of Car with the isonicotinic acid are mainly enthalpically stabilized, while those of Car with nicotinic acid are stabilized by both enthalpic and entropic contributions to the free Gibbs energy. The main interactions in complexes stabilization are believed to be electrostatic forces and hydrogen bonds formation between peptide and pyridine monocarboxylic acids.

**Key words:** thermodynamics, solutions, calorimetry, L-carnosine, nicotinic acid, isonicotinic acid

**INTRODUCTION**

Molecular interactions have received extensive attention in a wide range of disciplines, from chemistry and biomedical to biotechnology [1, 2]. In recent decades, the research and development of drug delivery systems have progressed, including in areas such as delivery routes, transport vehicles and targeting strategies [2, 3]. The human serum albumin (HSA) is the model protein lacking toxicity and immunogenicity that makes it an ideal aspirant as drug carrier [4, 5]. Investigation on the nature and driving forces of the interaction between drugs and proteins, determination of their selectivity and preferred types of binding are still relevant. Due to the experimental difficulties often associated with the direct study of protein thermodynamics in aqueous solution of drugs, there have been studies of drugs interactions with amino acids and peptides which are the basic blocks of proteins. The thermodynamic properties of model compounds in aqueous media provide information on solute-solvent and solute-solute interactions which in turn enhance our understanding of more complex biochemical processes [4-9].

Pyridine derivatives (including pyridine carboxylic acids) are important compounds in pharmaceutical, cosmetic and food products. These organic molecules exist in different isomeric forms (nicotinic acid, isonicotinic acid, picolinic acid), having biological importance such as antibacterial, antitubercular, antioxidant, pellagra-preventing, taking physiological and metabolic effects, etc. Only few studies on interaction between pyridine derivatives and biological active compounds (amino acids, cyclodextrins, biometals) have been published [10-13].

The dipeptide β-alanyl-L-histidine, also known as L-carnosine (Car), is a naturally occurring substance synthesized by endogenous carnosine synthetase. In carnosine molecule the two amino acids (β-alanine and L-histidine) is bound together by means of a peptide...
linkage. The L-carnosine and its derivatives have several biomedical applications. Carnosine has a protective effect in inhibiting the formation of cataracts [14]. Due to its antioxidant, protective, chelating, anti-glycation activity, this dipeptide can be used to prevent and treat diseases such as diabetes, neurodegenerative diseases, diseases of the sense organs and cancers [15]. L-Carnosine was found to be form a 1:2 complex with nicotinic acid in aqueous and buffer solutions [16]. Isonicotinic acid is a structural isomer of nicotinic acid, with the carboxylic group in para-position of pyridine ring. In this paper, we report the interaction of peptide L-carnosine, as model of polypeptide chains in protein (albumin), with isonicotinic acid (INA), as the drug model, in aqueous solution using calorimetric method. For the first time, the experimental values of the enthality of dissolution of Car in an aqueous solution of isonicotinic acid have been measured. Under the experimental conditions chosen, the stability constants (lgKs) and thermodynamic parameters of complex formation (ΔcG, ΔcH, ΔcS) were determined for the reaction of Car with INA at 298.15 K. The obtained results were compared with the data for Car – NA – H2O system [16]. The chemical structures of above solutes are shown in the scheme.

**EXPERIMENTAL PART**

All chemicals were used as received from the suppliers. The specifications, molecular mass and structures of the chemicals used are given in Table 1. L-Carnosine (Car) and isonicotinic acid (INA) were used without further purification after drying for 48 h at 356.15 K in a vacuum until reaching constant weight. They were kept in desiccators at least for 48 h before use. Bidistilled water (with specific conductivity 3.1 μS·cm⁻¹, pH 5.4) was used to prepare the aqueous solutions by mass (accurate to 1×10⁻⁵ g) using a Sartorius-ME215S balance. The standard uncertainty in the molality of the solutions was estimated within ±2×10⁻⁴ mol·kg⁻¹.

The measurement of heat effects of dissolution of crystalline samples of Car in in pure water and in aqueous solution with INA were performed at 298.15 K, using a precise hermetic isoperibol ampoule-type calorimeter fitted with a 110 mL reaction vessel. The description of the construction of calorimeter and the measuring procedure has been described in depth previously [17]. The calorimetric measurements for sample were performed at least twice and average values are reported. The error of measuring single heat effects was below 0.2%. The relative combined uncertainty in the measurements of the enthalpies of dissolution was not more than 0.7%.

The enthalpies of dissolution of the solid Car were measured in water and in water containing INA (0.0014±0.0002 mol·kg⁻¹) at T = 298.15 K. The obtained values are presented in Table 1 as functions of peptide molality.

### Table 1

<table>
<thead>
<tr>
<th>m_Car /mol·kg⁻¹</th>
<th>Car – H2O</th>
<th>Car – H2O + INA*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ_H_d(w) /kJ·mol⁻¹</td>
<td>Δ_H_d(w+L) /kJ·mol⁻¹</td>
</tr>
<tr>
<td>0.000</td>
<td>11.19</td>
<td>11.19</td>
</tr>
<tr>
<td>0.0019</td>
<td>11.28</td>
<td>-11.61</td>
</tr>
<tr>
<td>0.0021</td>
<td>11.30</td>
<td>-11.75</td>
</tr>
<tr>
<td>0.0034</td>
<td>11.36</td>
<td>-11.99</td>
</tr>
<tr>
<td>0.0037</td>
<td>11.38</td>
<td>-12.11</td>
</tr>
<tr>
<td>0.0048</td>
<td>11.44</td>
<td>-12.35</td>
</tr>
<tr>
<td>0.0049</td>
<td>11.45</td>
<td>-12.46</td>
</tr>
<tr>
<td>0.0059</td>
<td>11.49</td>
<td>-12.59</td>
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<tr>
<td>0.0086</td>
<td>11.64</td>
<td>-12.89</td>
</tr>
<tr>
<td>0.0112</td>
<td>11.77</td>
<td>-13.02</td>
</tr>
<tr>
<td>0.0120</td>
<td>11.82</td>
<td>-13.22</td>
</tr>
<tr>
<td>0.0148</td>
<td>11.96</td>
<td>-13.25</td>
</tr>
<tr>
<td>0.0167</td>
<td>12.06</td>
<td>-13.46</td>
</tr>
<tr>
<td>0.0182</td>
<td>12.14</td>
<td>-13.56</td>
</tr>
<tr>
<td>0.0207</td>
<td>12.27</td>
<td>-13.68</td>
</tr>
<tr>
<td>0.0252</td>
<td>12.51</td>
<td>-13.69</td>
</tr>
<tr>
<td>0.0281</td>
<td>12.66</td>
<td>-13.58</td>
</tr>
</tbody>
</table>

Note: *Concentration of INA is fixed as (1.4±0.2)·10⁻³ mol·kg⁻¹.

Примечание: *Концентрация ИНК фиксирована и составляет (1,4±0.2)·10⁻³ моль кг⁻¹
RESULTS AND DISCUSSION

L-Carnosine (Car) and pyridine monocarboxylic acids (PyCOOH) can exist in different ionic forms in aqueous solutions depending on the pH. The molecule of PyCOOH has two main ionizable sites (COOH and N atom in pyridine ring) whereas the molecule of Car has three active sites (amino- and carboxyl groups, imidazole ring with two nitrogen atoms), subject to ionization when pH changes. The diagrams of the fraction of the ionic forms of the peptide and the pyridine derivatives depending on the pH of the medium were earlier presented in our works [16, 18, 19]. When PyCOOH dissolved in pure water (with pH 5.4), their aqueous solution becomes acidic with pH ~ 3.6. Carnosine, when dissolved in this acidic solution, get protonated on the N terminal group (-NH$_3^+$) and the imidazole side chain and also ionized on carboxyl group (COO$^-$). The addition of Car into the PyCOOH aqueous solution leads to small shift of the pH of the solution. In the concentration range studied, the pH value in the experimental aqueous solutions was found to be ~ 4.1. At this pH value, the presence of predominantly zwitterionic forms [HL]$^+$ and partially negatively charged form [A] of PyCOOH was observed. The peptide takes the form of cation [H$_2$L]$^+$, i.e., tricharged ion (+++. Thus, the solute-co-solute interactions can be interpreted by considering the dominant ionic forms of the peptide and PyCOOH in water.

As can be seen (Table 1), the values of dissolution enthalpy, $\Delta_{\text{sol}}H_{\text{m}}$, obtained for Car are positive in the case of pure water solvent and negative in the case of an aqueous solution of INA. The dissolution enthalpies become more negative with increasing the concentration of peptide in the (Car – INA – water) system. Thus, the interactions of Car with components of solvents cause the heat evolution (a negative contribution to the enthalpy of solution). Calorimetry is a direct method to obtain the thermodynamic parameters of complex formation (lg$K_c$, $\Delta_cG$, $\Delta_cH$, $\Delta_cS$) and to designate the driving forces of binding. The enthalpies of transfer, $\Delta_{\text{d}}H_{\text{m}}$, of Car from solvent (water) to the PyCOOH (L) solutions were calculated from the experimental enthalpies for Car dissolution in a pure solvent (w), $\Delta_{\text{sol}}H_{\text{m}}(w)$, and in an aqueous solution containing co-solute (L), $\Delta_{\text{sol}}H_{\text{m}}(w+L)$:

$$\Delta_{\text{d}}H_{\text{m}} = \Delta_{\text{sol}}H_{\text{m}}(w+L) - \Delta_{\text{sol}}H_{\text{m}}(w)$$

(1)

The $\Delta_{\text{sol}}H_{\text{m}}(w+L)$ and $\Delta_{\text{d}}H_{\text{m}}$ values formed a basis for calculation of the thermodynamic functions of complex formation. Enthalpy of dissolution of Car in pure water was found to be (11.19±0.08) kJ·mol$^{-1}$, which agrees with the available literature value being (11.09±0.09) kJ·mol$^{-1}$ [17]. The concentration dependences of the enthalpies of transfer of the peptide, $\Delta_{\text{d}}H_{\text{m}}$, from pure water to aqueous PyCOOH solutions are represented in Fig.

![Fig. Isotherms of L-carnosine binding with nicotinic acid (1) and isonicotinic acid (2) in aqueous solutions at T=298.15 K](image)

It was found that the heat effects of the interaction of Car with PyCOOH in an aqueous solution after some decrease become practically constant. The $\Delta_{\text{d}}H_{\text{m}}$ values demonstrate non-linear dependence on peptide concentration indicating the complex formation between Car and INA (or NA). The thermodynamic functions of complex formation were calculated using the initial concentrations of reagents and experimental values of the enthalpies of transfer by means of the computer program HEAT [20, 21], in which the search for the unknown parameters (lg$K_c$, $\Delta_cH$) are reduced to the numerical minimization of the F functional given by

$$F = \sum_{i=1}^N w_i(\Delta_{\text{d}}H_{\text{i,exp}} - \Delta_{\text{d}}H_{\text{i,calc}})^2$$

(2)

where $\Delta_{\text{d}}H_{\text{i}}$ is the enthalpy effect from the i-th reaction, $N$ is the number of experiments and $w_i$ is a weighted factor. Thus, the mathematical treatment of the $\Delta_{\text{d}}H_{\text{m}} = f(m)$ dependences allows to simultaneously estimate the stability constant and the enthalpy of complex formation. The binding stoichiometry was also given as parameters when fitting the binding isotherm. To remove the contributions of protolytic equilibriums from thermodynamic parameters of complex formation, the values of the equilibrium constants and heat effects of the possible secondary reactions of Car and INA dissociation were introduced into the calculation program [16, 19]. It was found out that the best matching of experimental points and calculated data occurs when complex with 1:2 stoichiometry is assumed to form for the Car with INA in water. The thermodynamic parameters obtained for complexation of Car with NA and INA are presented in Table 2.
Table 2

Table 2. Кажущиеся термодинамические параметры комплексообразования L-карнозина с нитридинкарбоновыми кислотами в воде при Т=298,15К

<table>
<thead>
<tr>
<th>Complex</th>
<th>Car:2INA</th>
<th>Car:2NA [*]</th>
</tr>
</thead>
<tbody>
<tr>
<td>lgKc</td>
<td>6.832±0.005</td>
<td></td>
</tr>
<tr>
<td>ΔG (kJ mol⁻¹)</td>
<td>-38.98±0.10</td>
<td>-31.15±0.05</td>
</tr>
<tr>
<td>ΔH (kJ mol⁻¹)</td>
<td>-53.45±0.20</td>
<td>-24.32±0.19</td>
</tr>
<tr>
<td>ΔTΔS (kJ mol⁻¹ K⁻¹)</td>
<td>-14.47±0.74</td>
<td>6.83±0.71</td>
</tr>
</tbody>
</table>

*Data from [16]
*Данные из [16]

Free energy and entropy of complex formation were calculated in term of the well-known thermodynamic equations:

\[ ΔG = -RT \ln K_c \]  
\[ ΔG = ΔH - TΔS \]

The results of calorimetric study showed that the ΔG values are more negative for aqueous solution of Car and INA, thus indicating that the process of complex formation is more spontaneous in comparison to Car and NA in water. The influence of position of N atom and COOH group in the pyridine ring of PyCOOH on the complex formation with Car was shown. The binding constants of the complexes of Car with INA are greater than with NA. The study indicate that the Car:2INA complex is more stable than Car:2NA complex.

Thermodynamic parameters of complex formation reflect the contributions from different processes: destruction of solvation shells of the solutes; amino acid – PyCOOH interactions (hydrogen bonding, electrostatic, hydrophobic, van der Waals interactions, staking effects), and hydration of the complex [22]. Various types of interactions that can give positive or negative contributions to the enthalpy of complexation are as follows: (i) electrostatic interaction and hydrogen bond formation (negative contribution); (ii) hydrophobic – hydrophobic interaction (positive contribution); (iii) hydrophobic – hydrophilic interaction (positive contribution); (iv) partial desolvation of hydrated shells of the molecules of solutes (positive contribution); (v) hydration of the solute – solute complexes obtained (negative contribution). The competitive balance of the above interactions leads to the difference in thermodynamic parameters of complex formation of the solutions studied.

In general, the complexation between Car and PyCOOH (INA, NA) is an exothermic process. The complexation of Car with INA was found to be highly exothermic, the exothermicity decreasing for complex Car with NA. The negative ΔH values observed for Car in solutions of INA and NA can be mainly attributed to hydrogen bonding and electrostatic interactions [22]. Total entropy change upon binding is the sum of a change in configuration entropy and change in solvation entropy [21, 23]. The TΔS value is negative for complex of Car with INA whereas it becomes positive for complex of Car with NA. In case of Car – NA – water, positive ΔS values can be explained by the prevalence of dehydration of the solutes upon the complex formation and by an existence of various configurations of complex [24]. It should be also mentioned that nicotinic acid can exist in two conformations which differ in the orientation of the carboxyl group, whereas only one stable conformer has been described for isonicotinic acid [25]. In case of Car – INA – water, it is the predominance of the processes of peptide – PyCOOH interactions and hydration of the complex that determines the negative changes for enthalpy and entropy of complexation. Negative entropy value obtained for this system confirms that INA contributes to more strengthening of the structure of the Car – water system than NA.

In the aqueous solution, the formation of complex between Car and isomers of PyCOOH probably involves the interactions of a positively charge imidazole ring and an end amino group (NH₃⁺) as well as a negative charge COO⁻ group of peptide with an ionized carboxyl group (COO⁻) and with a protonated pyridine nitrogen atom of pyridine carboxylic acids. The main interactions in complexes stabilization are believed to be electrostatic forces and hydrogen bonds formation. In addition, hydrophobic forces owing to the presence of non-polar fragments in the molecules of the compounds involved can also affect the stability of complexes formed. The more efficient stabilization of complex between Car and INA as compared to NA in aqueous solution may be mainly explained by the changing of ionic state of the reagents. Since the molar fraction of zwitterion for NA (0.82) is some less than for INA (0.86) at pH 4.1, it can be expected that the exothermic contribution of electrostatic interactions with Car in the case of NA will be less than in the case of INA. The adding of INA into Car – water system is apparently accompanied with a increase in the solution structuring upon the formation of the solvate complex of Car:2INA. Thus, all above factors determine the same different ability of nicotinic and isonicotinic acids to form complexes with Car and to structure their aqueous solutions.

CONCLUSION

The present study shows that Car forms complexes of 1:2 stoichiometry with INA and NA in aqueous medium. The stability of Car complexes obtained is higher for INA than that for NA. The formation of complexes between Car and NA is accompanied by the negative enthalpy and positive entropy changes in water, so one can say that the complex of Car with NA is
stabilized by both enthalpic and entropic contributions to the Gibbs energy. The complex formation between Car and INA is characterized by large negative values of enthalpy and entropy changes, so one can say that it is enthalpy driven. The various stability of above complexes is mainly governed by the same difference in ionic states of 3- and 4- structural isomers of PyCOOH as well as their conformational and energetic complementarity under binding with the peptide Car.

The work was performed at financial support from the Russian Science Foundation, grant No. 22-23-01118.

The authors express their acknowledgment to O.N. Krutova, Ph.D, docent of the department of analytical chemistry at ISUCT for her assistance in carrying out calorimetric measurements.

The authors declare the absence a conflict of interest warranting disclosure in this article.

Literature


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