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Complexation studies of zwitterionic amino acids with crown and guanidinium compounds using titration calorimetry

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Abstract

Log K, ΔG, ΔH, and $T\Delta S$ were determined by titration calorimetry for the reaction of amino acids in their zwitterionic form with bicyclic guanidinium salts as anchor groups for the carboxylate and crown compounds as ammonium binding moiety in methanol at 25°C. Under the experimental conditions chosen, bicyclic guanidines show very low enthalpic contribution (<-2 kJ mol^{-1}) due to their strong solvation by methanol. The complexation of the ammonium form of amino acids is strongly influenced by the ring size, nature and position of donor atoms of the macrocyclic crown compound investigated. The highest enthalpic contribution accompanied by a negative entropic term was obtained with 18-crown-6. The substitution of ether oxygen by both amine and pyridine nitrogen leads to a decrease of enthalpic contribution. The introduction of bulky substituents into the macrocyclic ring system increases the reaction entropy. Yielding a medium value of complexation enthalpy and a slightly positive value of $T\Delta S$ the highest stability constant was observed with pyridino 18-crown-6. © 1998 Elsevier Science B.V.

Keywords: Amino acid; Complex formation; Titration calorimetry; Crown compounds; Bicyclic guanidines

1. Introduction

Due to the importance of amino acids in biology and technique, their investigation is a steady growing field. The studies are focused on separation, transport and analytical sensing of amino acids. A lot of different types of complexing agents have been tested for different kinds of interest, but especially the binding of small hydrophilic derivatives is very complicated [1]. The most promising way to obtain selective hosts for amino acids should be the use of polytopic receptors [2–7] with binding sites for the ammonium and carboxylate function of zwitterionic amino acids (Fig. 1).

Recently, we reported on a ditopic host using a chiral bicyclic guanidinium salt as an anchor function for the carboxylate and a triaza-crown ether as an ammonium binding moiety [8,9]. Molecular modelling calculations comparing the chain length of guest molecules predicted a preferred complex formation with γ-amino butyric acid (GABA) over β-alanine (β-ala) and glycine (gly). In contrast to this in liquid–liquid-extraction experiments this host favours the complexation of glycine over β-ala and GABA [9].

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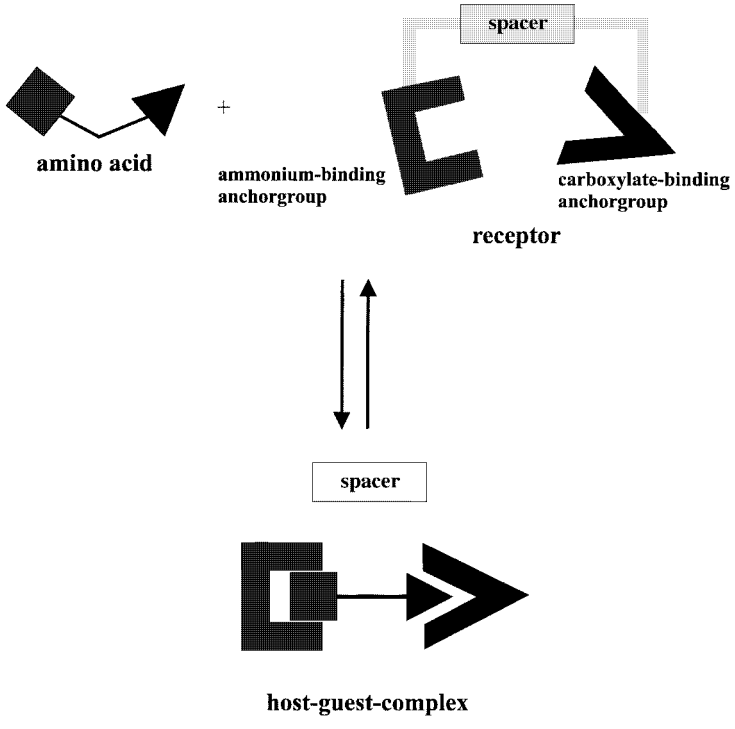


Fig. 1. Conception of a ditopic receptor for the complexation of zwitterionic amino acids.

Our experimental results show that molecular modelling calculations are a very useful tool to visualise the complex formation of coordination compounds. In the special case of phase transfer reactions, there are additional factors (e.g. hydrophobicity of the host–guest-complex) which could not yet be considered in calculations.

Some structural optimisation is necessary in order to improve the separation selectivity of this ditopic host. In this context, thermodynamic data of the interaction of amino acids with different ligands are needed. There are only few results concerning the thermodynamics of complexation of amino acids with macrocyclic compounds [10–14].

In this paper, we report the host–guest interaction of amino acids (gly, ala, β-ala and GABA) with several bicyclic guanidinium chlorides **1**–**4** and crown compounds **5**–**17** using titration calorimetry (Fig. 2). Ligand structure-complexation relationships are discussed and used for the prediction of an optimum host structure.

2. Experimental

2.1. Materials

Amino acids, 18-crown-6 (**5**), 15-crown-5 (**6**) and 12-crown-4 (**7**) were supplied by Aldrich, the macrocyclic hosts aza-18-crown-6 (**8a**) and diaza-18-crown-6 (**9a**) by Merck and 1,4,7,10,13,16-hexamethyl-1,4,7,10,13,16-hexaazacyclooctadecane (**11**) by Fluka. The bicyclic guanidinium compounds **1**-**4** [15], the aza crowns **8b**, **9b**, **10** [16], **9c**, **9d** [17], **12**–**14** [18], and the pyridino crowns **15**–**17** [19–21] were prepared according to known procedures. All materials were carefully dried before use. Methanol (Merck) containing <0.05% water was used without further treatment.

2.2. Calorimetric measurements

The determination of the thermodynamic data was carried out in a TRONAC 458 titration calorimeter.

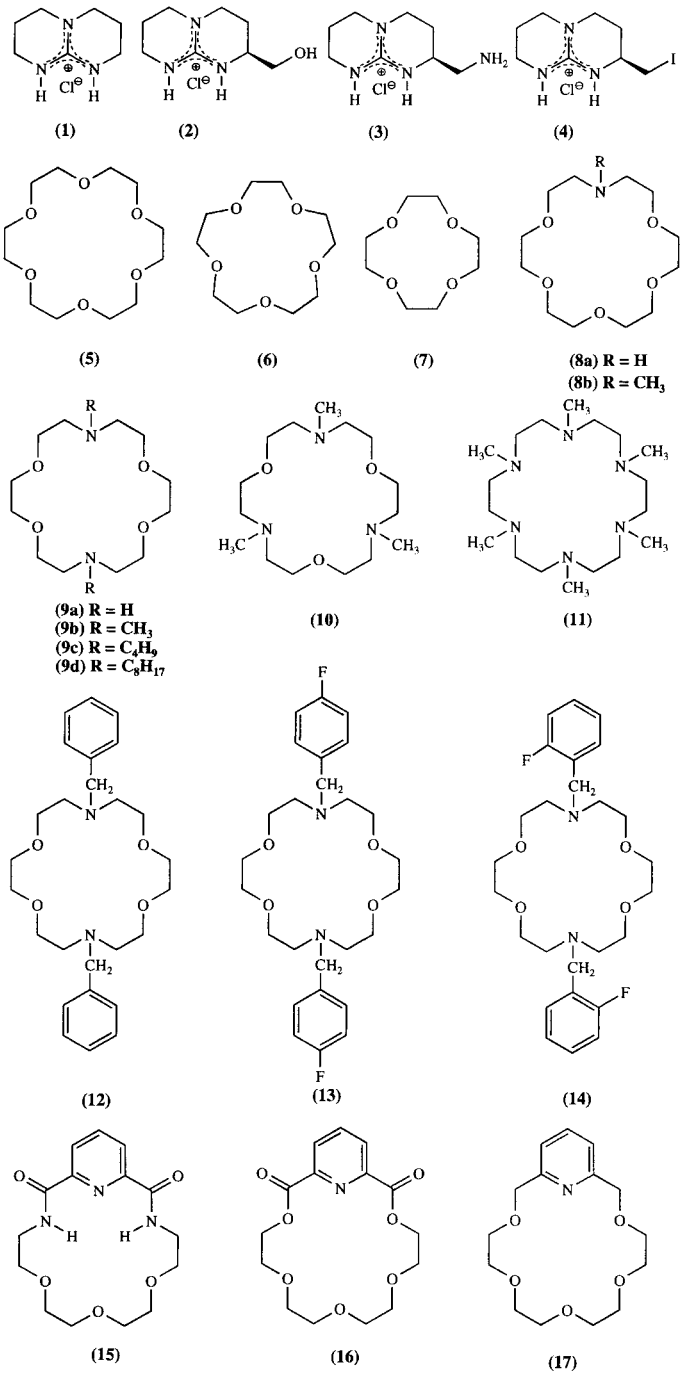


Fig. 2. Investigated compounds.

The reproducibility of the calorimeter was checked using the complexation of Ba^{2+} by 18-crown-6. During the titration a solution of the host in methanol (0.04 M) was added continuously to 40 ml of a 0.003 M amino acid solution in methanol. The data obtained were analysed using a modified least-squares method [22] based on the procedure described by Christensen et al. [23–25]. All non-chemical heat effects were corrected (e.g. heat of dilution, heat produced by stirrer). To obtain the stability constant ($\log K$) and the reaction enthalpy (ΔH), $\log K$ and ΔH were simultaneously varied and the minimum of the error square sum was determined. Thus, both unknown parameters can be fitted to the experimental data.

3. Results and discussion

In dependence on the pH amino acids exist in cationic, zwitterionic or anionic form (Fig. 3). Under the experimental conditions chosen in this study, the zwitterionic form is favoured [12]. For binding the carboxylate unit of amino acids guanidinium salts are suitable [26]. The X-ray structure of an acetate complex shows that the carboxylic group aligns in a coplanar fashion with both the guanidinium fragment and the hydrogen bonding system giving a strong complex formation [27].

In the same way, the zwitterionic form of amino acids should be complexed. Unfortunately, the enthalpic contribution of amino acids with the bicyclic guanidinium salts **1–4** investigated was very low

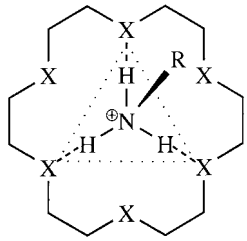


Fig. 4. Formation of hydrogen bonds between crown compounds and glycine in its zwitterionic form.

(<-2 kJ mol^{-1}). Obviously, the host compounds are strongly solvated by methanol. The solvation might be diminished by introduction of bulky hydrophobic substituents in α,α'-position of the bicyclic system.

As presented in Fig. 4, crown compounds can interact with the ammonium group of amino acids forming three hydrogen bonds. Besides the formation of three hydrogen bonds an electrostatic interaction between the positive charged nitrogen atom of the ammonium group and the oxygen atoms of 18-crown-6 is discussed [13,28]. This coordination pattern was confirmed by X-ray structures of both the dipeptide gly–gly [29] and ammonium complexes [30,31] with 18-crown-6 (NH···O distances between 2.86 and 2.98 Å).

In order to guarantee that the ammonium group is available for complexation under the experimental conditions chosen titration calorimetry experiments

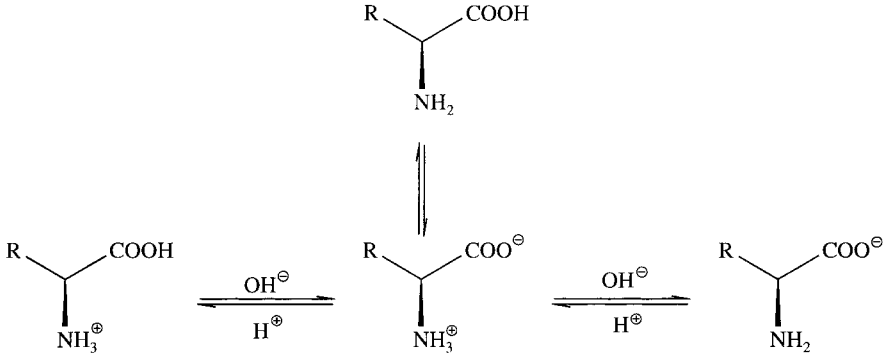


Fig. 3. Formation of amino acid species depending on pH.

Table 1
Influence of pH on stability constant (log K, K in M^{-1}) and thermodynamic parameters ΔG, ΔH and $T\Delta S$ (kJ mol^{-1}) on the complexation of glycine with 18-crown-6 in methanol at 25°C

Conditions	pH [a]	log K	ΔG	ΔH	$T\Delta S$
Acidic [b]	4.6	3.4	−19.4	−47.0	−28.4
Neutral	7.5	3.5	−20.0	−49.8	−29.8
Alkaline [c]	9.6	2.8	−16.2	−17.1	−1.1

[a] pH determined in methanol using a glass electrode.
[b] Mixture of methanol with acetic acid (v/v=99.9/0.1).
[c] Mixture of methanol with tetrabutylammonium hydroxide (v/v= 99.9/0.1).

were performed in the system 18-crown-6/ glycine/ methanol at different pH. The results obtained are summarised in Table 1. As expected ΔG, ΔH and $T\Delta S$ of the complex formation at the acidic and neutral environment are almost the same, because the amino group is protonated. In contrast to this, the interaction between glycine and 18-crown-16 is drastically decreased in alkaline solution caused by the deprotonation of the amino group. For our experiments, the pH was <7.5 after the addition of the compounds investigated indicating the availability of the ammonium moiety for the complexation. The only exception was compound **11** causing a pH of 8.4. We performed the same experiment with a solution of the host compound containing 0.01% acetic acid to decrease the final pH to 7.5. Thus, the enthalpic contribution was slightly increased (2 kJ mol^{-1}), while log K changed from 2.8 to 3.1. Our results indicate the importance of controlling the pH value in complexation reactions with amino acids.

At first, we investigated the influence of the amino acid structure on the complex formation with 18-crown-6 (**5**). As shown in Table 2 gly, ala, β-ala and GABA have only slightly different complexing behaviour towards 18-crown-6 (**5**). The highest enthalpic contribution was found for β-ala and GABA. In this case, there is no steric hindrance for the complex formation with the ammonium unit. If the carboxylic (gly) and both carboxylic group and methyl group (ala) are introduced at the α-carbon atom of the amino acid ΔH is significantly decreased. It is interesting that the different values for ΔH were compensated by ΔS resulting in almost indentically values for ΔG and log K for the complex formation of different amino acids with 18-crown-6 [11].

Table 2
Stability constants (log K, K (M^{-1})) and thermodynamic parameters ΔG, ΔH and $T\Delta S$ (kJ mol^{-1}) for the complexation of amino acids with 18-crown-6 in methanol at 25°C

Host	Guest	log K	ΔG	ΔH	$T\Delta S$
18-crown-6 (**5**)	Glycine	3.5	−20.0	−49.8	−29.8
	Alanine	3.4	−19.3	−43.0	−23.7
	β-Alanine	3.5	−19.9	−56.4	−36.5
	GABA	3.5	−19.9	−55.0	−35.1

The influence of ring size, nature and position of donor atoms of the macrocyclic crown compounds on the complex formation with gly was investigated. The values of the stability constants and the thermodynamic parameters ΔG, ΔH and $T\Delta S$ are summarised in Table 3. Ligand structure–complexation relationships can be discussed. The highest enthalpic contribution for the complexation of glycine was observed for 18-crown-6. The complexation reaction is controlled by enthalpic and disfavoured by entropic contributions indicating a high flexibility of the free host

Table 3
Stability constants (log K, K (M^{-1})) and thermodynamic parameters ΔG, ΔH and $T\Delta S$ (kJ mol^{-1}) for the complexation of glycine by different bicylic guanidinium and crown compounds in methanol at 25°C

Host	log K	ΔG	ΔH	$T\Delta S$
1–4	a	a	<−2.0	a
5	3.5	−20.0	−49.8	−29.8
6	a	a	−3.0	a
7	a	a	a	a
8a	3.4	−19.2	16.3	+2.9
8b	3.6	−20.7	−17.5	+3.2
9a	2.9	−16.4	−1.9	+14.5
9b	2.9	−16.7	+6.6	+23.3
9c	3.6	−20.5	−11.3	+9.2
9d	3.7	−21.1	−11.1	+10.0
10	3.4	−19.7	−21.1	−0.5
11	2.8	−15.8	−8.8	+7.0
12	3.3	−18.6	−13.4	+5.2
13	3.8	−21.8	−8.2	+13.6
14	3.4	−19.2	−17.3	+1.9
15	a	a	−1.5	a
16	2.9	−16.8	−5.4	+11.4
17	4.2	−24.1	−20.8	+3.3

[a] Heat produced in these reactions is too small so that log K and ΔH can not be calculated.

and almost the same solvation stage of the complexed and uncomplexed form. Our results are in good agreement with the earlier published data [13]. Slight differences can be explained by the influence of water on the complex formation [32].

The ability to complex glycine is drastically reduced if the ring size of the host compounds is decreased (18-crown-6≫15-crown-5>12-crown-4). This finding is also confirmed by potentiometric studies of complex formation of ammonium compounds with crown ethers in methanol. Gokel et al. [33] reported that the complexation of the ammonium ion is remarkably diminished by both the increase and decrease of the ring size comparing to 18-crown-6. Generally, 18-crown-6 (**5**) seems to have the best ring size for the complexation of ammonium.

Knowing that the ability to form hydrogen bonds increases in the order ether oxygen<amine nitrogen< pyridine nitrogen [34] also, aza and pyridino crowns were included in our investigations. For all nitrogen containing crown compounds ΔH is significantly lower comparing to 18-crown-6. This could be caused by an increase of the ring size if ether oxygen is substituted by nitrogen atoms and consequently the hydrogen bonds are weaker. This fact is proven by X-ray structures [35,36] as well as molecular modelling calculations using PM3 [37]. If we compare the dimensions of the triangles built by the donor atoms of the host compound involved in the complexation (Fig. 4), a remarkable increase is detectable. For the lowest conformation found by PM3 calculations of the 18-crown-6 complex with glycine, the side length of this triangle is 4.85 Å. If we introduce two methylated nitrogen in the macrocyclic ring the dimensions are increased to 5.43, 4.94 and 4.94 Å. In the host compound with six methylated nitrogen atoms the triangle is even bigger (4.92, 5.49 and 5.49 Å).

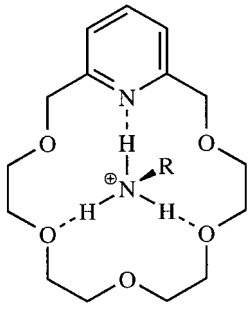
In case of **8a** and **9a**, the formation of intramolecular hydrogen bonds [28,38] decreases the enthalpic contribution. In addition to this, the host molecule has to undergo a change in its conformation. In the free host, the lone electron pair of the nitrogen atom is located on the outside of the macrocyclic ring. The nitrogen atom has to be inverted in order to realize the complexation [38].

After methylation of the amine nitrogen donor atoms, the thermodynamic parameters are only slightly changed (**8a** and **8b**; **9a** and **9b**). The reason for this behaviour is probably a strong solvation of the methylated nitrogen atom by methanol. This finding is supported by the fact that the stability constant is significantly lower for aza crown compounds forming NH···N hydrogen bonds (**9a**, **9b**, **11**) relating to **8a**, **8b** and **10** which are able to form three NH···O hydrogen bonds (Fig. 4).

Derivatives of compound **9** with substituents of a chain length of 4 or 8 carbon atoms (**9c**, **9d**) show more exothermic complexation enthalpies in the reaction with glycine and the complex stability increases to values greater than for 18-crown-6. This fact indicates that long alkyl groups stabilise the endo–endo-conformation of the free host compound [39]. Furthermore, bulky substituents at the macrocyclic ring might shield the host from solvent molecules and increase the complex stability in both the enthalpic and entropic contribution.

An other way to reduce the solvation of the amine nitrogen atom is to introduce benzyl groups into diaza-18-crown-6 compounds. Thus, the basicity of the nitrogen atom is decreased. The benzyl unit should also stabilise the endo–endo conformation of the lone electron pair which is suitable for complex formation. As expected, the compounds investigated **12**–**14** show significantly higher stability constants and ΔH values comparing to **9b**. The para fluorinated derivative (**13**) reveals even a higher stability constant as 18-crown-6.

Forming strong hydrogen bonds connected with a low Brønsted basicity pyridine nitrogen is a suitable anchor group for binding the ammonium ion [28,40]. In dependence on both flexibility and functionality of the pyridino crown compound **15**–**17** graduated complex formation with glycine was observed. The diamido pyridino crown **15** as the most rigid compound shows only very weak interactions with glycine (ΔH<−1.5 kJ mol^{-1}) in methanol due to an unfavourable conformation for complexation. Two possible intramolecular hydrogen bonds (as mentioned for compound **9a**) might be a possible explanation for the very low enthalpic contribution. Also in the case of the more flexible diester pyridino ligand **16**, the enthalpic contribution with glycine is low. Our investigations uncovered a positive entropic term of complexation and a stability constant in the medium range for **16**. The higher number of donor atoms in compound **15** and **16** comparing to the other compounds investigated should lead to an enhanced degree of



R = tert.-butyl

Fig. 5. Coordination pattern of an ammonium complex with pyridino 18-crown-6.

solvation of these host compounds resulting a lower enthalpic contribution.

The most promising results were obtained with the pyridino crown **17**. Thus, highest stability constant was determined resulting from both a medium value of ΔH and a weakly positive term of $T\Delta S$. The X-ray structure of the *tert*-butylammonium complex with **17** [41] prove the formation of one hydrogen bond to the pyridine nitrogen and two hydrogen bonds to the ether oxygen atoms (Fig. 5).

In the case of **17,** the side length of the triangle shaped by the donor atoms which are involved in the complexation is 4.89, 4.91 and 4.92 Å. This is slightly larger than obtained for the ammonium complex with 18-crown-6 (4.82–4.86 Å) [31] and consequently, a lower enthalpic contribution for **17** results. But the reaction entropy for **17** is remarkably increased comparing to 18-crown-6. This could be explained by the higher degree of preorganisation of **17** for the complex formation with the ammonium ion.

Our future investigations will be focused to synthesise new polytopic host compounds with optimised building blocks. The guanidinium compounds with bulky hydrophobic substituents in α,α'-position of the bicyclic system having a low solvation degree shall be used as carboxylate anchor group. Pyridine containing macrocycles seem to be the most favourite binding moieties for ammonium groups. Finally, the variation of the spacer distance between, thus, both binding sites might offer the possibility to discriminate guest molecules of different chain length. These modifications should result a host compound with a higher selectivity and increased extraction yield for zwitterionic amino acids.

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