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The Complexation of Amino Acids by Crown Ethers and Cryptands in Methanol

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Abstract. Complex formation between several crown ethers and the cryptand (222) and α-amino acids in methanol was studied by calorimetric titration. The ligand structure and the donor atoms of the ligands play an important role in determining the measured values of the reaction enthalpies and entropies. However, with the exception of the diaza crown ether (22) all stability constants are of the same order of magnitude. The enthalpic and entropic contributions to the stabilities of the complexes formed compensate each other. In methanolic solution the amino acids exist in their zwitterionic form. This equilibrium can be influenced. Under acidic, neutral or basic conditions different values of the reaction enthalpies are measured for the complexation of some amino acids with 18-crown-6. These results demonstrate that the concentration of the zwitterionic form of the α-amino acids can be influenced. Thus the reaction between macrocyclic and macrobicyclic ligands and amino acids should be described by at least two different reaction schemes.

Key words: Amino acids, crown ethers, cryptands, complexes.

1. Introduction

During recent years the development of host–guest chemistry has attracted interest in the energetics of the processes involving amines and amino acids being complexed by macrocyclic polyethers such as crown ethers and cryptands [1–9].

Thermodynamic studies allow a better understanding to be gained of the driving forces underlying host–guest interactions. Generally, a cation–ligand complex is characterized by its geometric properties, e.g. structure and conformation, and also by such thermodynamic properties as stability, enthalpy and entropy of complex formation. For example the stability and selectivity of crown ether complexes depend on the size of the crown ether and the number of donor atoms. The solvation of ligand and cation also plays an important role. Many computational studies have confirmed that solvent effects play an important role in the conformational flexibility of the crown ethers and for the selectivities and stabilities of crown ether-cation complexes [10–14].

The amino acids are one of the most important classes of natural chemical compounds, biologically interesting and widely implicated both in biochemical

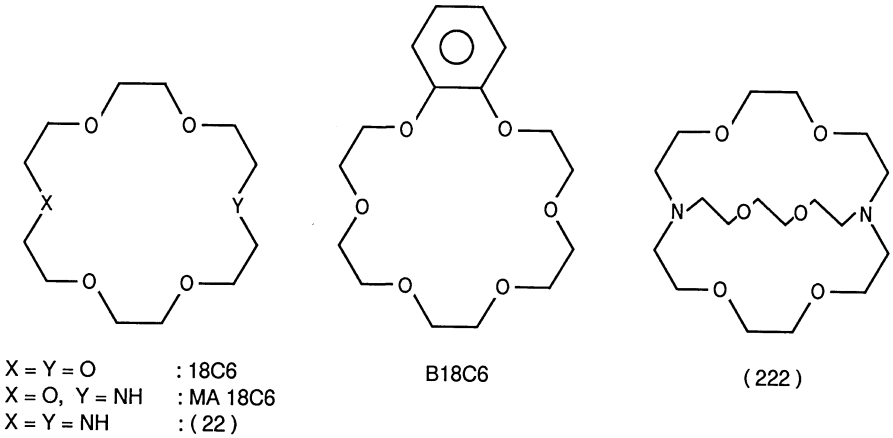

Figure 1. Chemical structures of the ligands used.

processes as well as in medicine, the drug industry and cosmetics. The structures of complexes formed between ammonium salts or amino acids and macrocyclic ligands have been reported [2, 15–19]. In the presence of macrocyclic ligands an increase of the solubility of amino acids in organic solvents together with thermodynamic data for the complex formation have been reported [20, 21].

In some previous reports we have presented [22–24] the factors which possibly influence both the transfer of amino acids from aqueous solution to an organic solvent and also the active transport through liquid membranes using macrocyclic ligands.

In the present study the stability constants, the enthalpies and the entropies of the complexes formed between several α-amino acids and macrocyclic and macrobicyclic ligands in methanol as solvent have been determined.

2. Experimental

The following ligands were used: 18-crown-6 (18C6), benzo-18-crown-6 (B18C6), monoaza-18-crown-6 (MA18C6), diaza-18-crown-6 (22) and cryptand (222) (all Merck). The chemical structures of the ligands are given in Figure 1. The macrocyclic ligands and macrobicyclic ligands were used without further purification. The following amino acids were used: L-α-alanine (L-Ala, Fluka), L-valine (L-Val, Fluka), L-phenylalanine (L-Phe, Fluka), glycine (Gly, Fluka), L-methionine (L-Met, Fluka), L-serine (L-ser, Fluka), L-tryptophan (L-Trp, Fluka), L-leucine (L-Leu, Fluka), L-isoleucine (L-Iso, Fluka) and L-cysteine (L-Cys, Fluka). They were of the highest purity commercially available. Anhydrous methanol (Merck; H_2O content less than 0.01%) was used as solvent.

The following reaction between an amino acid A and a ligand molecule L takes place:

$$\mathrm{A} + \mathrm{L} \rightleftharpoons \mathrm{AL}.$$

The stability constant, K, is given by:

$$K = \frac{[\mathrm{AL}]}{[\mathrm{A}][\mathrm{L}]}.$$

The stability constants and reaction enthalpies were determined using calorimetric titrations (Tronac Model 450). A solution of a ligand (1.6×10^{-2} M) was titrated into a solution of an amino acid (1×10^{-3} M). The heat Q produced during the titration is related to the number of moles of the complex formed Δn and the reaction enthalpy ΔH° by the following equation:

$$Q = \Delta n \cdot \Delta H^{\circ}.$$

Δn depends on the stability of the complex formed. The calculation of the stability constant and reaction enthalpy from the measured thermogram has already been described [25].

Insufficient heat was produced during the titrations of the amino acids with the diaza crown ether (22). Therefore, competitive calorimetric titrations were performed using 18C6:

$$18\mathrm{C6} + \mathrm{A} \cdot (22) \rightleftharpoons \mathrm{A} \cdot 18\mathrm{C6} + (22).$$

The ligand 18C6 (1.6×10^{-2} M) was titrated into solutions containing the amino acid A (1×10^{-3} M) and the ligand (22) ($3–5 \times 10^{-3}$ M). Using the known stability constant and reaction enthalpy for the complexation of the amino acid by 18C6 one can calculate the stability constant and the reaction enthalpy [26].

To perform calorimetric titrations in acidic methanolic solutions trifluoromethane sulfonic acid (Fluka) was added to the solutions of the α-amino acids. To obtain basic solutions of the amino acids tetramethylammonium hydroxide (Fluka) was added. The resulting solutions were dried over molecular sieve (3 and 4 Å) to remove water molecules.

The reliability of the results obtained from calorimetric and competitive calorimetric titrations compared with results from conductometric and potentiometric titrations has already been demonstrated in detail [27].

3. Results and Discussion

It is well known that α-amino acids R—CH(NH_2)COOH have the structure of dipolar ions with one basic —NH_2 and one acidic —COOH group; the properties of these compounds are equally influenced by both the nature of the side chain,

R, and the pH value of the amino acid solution. The exponent of acidity is about 2 for the —COOH group and about 9 for the —NH_2 group so that at a certain pH both groups may be ionized, the amino acid appearing as a zwitterionic R—$CH(NH_3^+)COO^-$ form. It was suggested that in methanol the amino acids are present in their zwitterionic ($^+H_3NCH(R)CO_2^-$) form [20]. Several authors have indirectly measured the lipophilic character of amino acids [28, 29]. It was shown that the hydrophobicity of the amino acids is linearly correlated with their van der Waals volume in solution [21].

The values of the stability constants and the thermodynamic parameters for the complexation of some α-amino acids with different macrocyclic and macrobicyclic ligands in methanol as solvent are given in Table I. In most cases the agreement between the data from the literature for the complex formation of 18C6 and the ligand (222) with α-amino acids is very good. No literature data are available for the reactions of the other ligands examined.

Comparing the results for the complexation of amino acids with the results for the ammonium ion one finds that the stability of all ammonium complexes is higher than that of the amino acid complexes. In contrast the values of the reaction enthalpies for the formation of amino acid complexes are higher than the values for the ammonium ion complex formation. The basicity of the NH_2— groups of the α-amino acids increases due to inductive effects of the substituents. These substituents increase the spherical requirements during complex formation. As a result large negative values of the reaction entropies are observed in the case of the amino acids. The high values of the reaction enthalpies are compensated by entropic contributions.

The different substituents on the CH_2-group of glycine obviously have no influence upon the stability of the complexes formed with the ligand 18C6. However, the highest value of the reaction enthalpy is found for the reaction of glycine with 18C6. Any substituent at the CH_2-group of glycine reduces the value of the reaction enthalpy by at least 10 kJ mol^{-1}. The reduction of the values of the reaction enthalpy is compensated by the reaction entropy. As a result the stabilities of all α-amino acids complexes formed with the ligand 18C6 are nearly identical.

The stability constants for the reaction of the amino acids with the crown ether B18C6 are nearly identical or only a little smaller when compared with the ligand 18C6. The values of the reaction enthalpies are much smaller than with 18C6. This decrease of the values of the reaction enthalpies is again compensated by the reaction entropy. Compared with 18C6 the cavity size of the crown ether B18C6 is not influenced by the benzene group. The basicity of the ether donor atoms attached to the benzene group and the flexibility of this ligand are reduced. Both effects may be responsible for the observed changes of the reaction enthalpy and entropy.

The substitution of one oxygen by one nitrogen donor atom in the crown ether molecule again has no influence upon the measured stability constants. The values of the reaction enthalpies are smaller compared with the ligands 18C6 or B18C6. The complex formation is however favoured by entropic contributions. The substi-

Table I. Stability constants (log K, K in M^{-1}) and thermodynamic parameters ΔH° and $T\Delta S^\circ$ (in kJ mol^{-1}) for the complexation of the ammonium ion and α-amino acids by macrocyclic and macrobicyclic ligands in methanol at 25 °C.

Amino acid	Value	18C6	B18C6	MA18C6	(22)	(222)
NH_4^+	log K	4.32[a]	3.24[a]	–	2.72[a]	8.19[a]
	$-\Delta H^\circ$	39.6[a]	30.0[a]	–	−23.7[a]	−68.0[a]
	$T\Delta S^\circ$	−15.1[a]	−11.6[a]	–	39.2[a]	−21.5[a]
L-Ala	log K	3.24 ± 0.01 3.59[b] 3.34[c]	3.01 ± 0.01	3.31 ± 0.8	0.7 ± 0.08	3.11 ± 0.09 3.22[b]
	$-\Delta H^\circ$	46.2 ± 2.6 45.9[b] 43.0[c]	22.2 ± 0.6	7.5 ± 1.2	16.0 ± 3.5	16.0 ± 0.8 15.4[b]
	$T\Delta S^\circ$	−27.8 ± 2.7 −25.5[b] −23.3[c]	−5.1 ± 0.7	11.3 ± 1.6	−12.0 ± 4.0	1.6 ± 1.2 2.9[b]
L-Cyst	log K	3.28 ± 0.02 3.28[b]	3.12 ± 0.03	3.21 ± 0.05	1.99 ± 0.06	3.09 ± 0.12 3.36[b]
	$-\Delta H^\circ$	44.1 ± 4.6 30.7[b]	−17.5 ± 1.7	12.6 ± 1.9	14.1 ±5.1	25.2 ± 3.1 15.5[b]
	$T\Delta S^\circ$	−25.5 ± 4.7 −12.1[b]	0.2 ± 1.8	5.7 ± 2.2	−2.8 ± 5.4	−7.7 ± 3.7 3.6[b]
Gly	log K	3.68 ± 0.09 3.98[b] 3.50[c]	2.77 ± 0.06	3.07 ± 0.10 3.16[c]	1.02 ± 0.14	3.20 ± 0.08 3.48[b]
	$-\Delta H^\circ$	49.8 ± 2.1 53.8[b] 49.8[c]	41.8 ± 2.5	12.4 ± 2.2 18.9[c]	26.6 ± 5.5	51.0 ± 2.9 41.8[b]
	$T\Delta S^\circ$	−28.6 ± 2.7 −31.2[b] 29.8[c]	−26.1 ± 2.8	5.1 ± 2.8 −0.9[c]	−20.8 ± 6.3	−32.8 ± 3.4 −22.0[b]
L-Iso	log K	2.98 ± 0.05 3.17[b]	2.73 ± 0.02	3.03 ± 0.06	1.04 ± 0.09	3.22 ± 0.05 3.81[b]
	$-\Delta H^\circ$	53.6 ± 0.5 36.4[b]	32.7 ± 0.9	19.5 ± 1.4	18.4 ± 1.8	16.6 ± 0.7 6.4[b]
	$T\Delta S^\circ$	−36.7 ± 0.8 −18.4[b]	−17.2 ± 1.0	−2.2 ± 1.8	−12.5 ± 2.4	1.7 ± 1.0 15.3[b]
L-Leu	log K	3.14 ± 0.02 3.35[b]	3.14 ± 0.04	3.05 ± 0.05	1.47 ± 0.05	3.28 ± 0.03 3.38[b]
	$-\Delta H^\circ$	49.0 ± 0.3 42.5[b]	18.1 ± 1.1	20.1 ± 0.9	8.1 ± 0.7	14.6 ± 0.9 9.1[b]
	$T\Delta S^\circ$	−31.1 ± 0.5 −23.5[b]	−0.3 ± 1.3	−2.8 ± 1.2	0.3 ± 1.0	4.0 ± 1.0 10.1[b]

Table I. (*Continued*)

Amino acid	Value	18C6	B18C6	MA18C6	(22)	(222)
L-Met	$\log K$	3.23 ± 0.01 3.66[b]	3.20 ± 0.08	3.22 ± 0.10	0.97 ± 0.04	3.21 ± 0.04 3.58[b]
	$-\Delta H^\circ$	45.4 ± 1.9 37.9[b]	15.8 ± 2.2	13.7 ± 1.8	11.6 ± 2.6	16.8 ± 1.1 4.4[b]
	$T\Delta S^\circ$	-27.0 ± 2.0 -17.1[b]	2.4 ± 2.7	24.6 ± 2.4	$-\ 6.1 \pm 2.8$	1.5 ± 1.4 15.9[b]
L-Phe	$\log K$	3.18 ± 0.01 3.15[b]	2.74 ± 0.06	3.10 ± 0.12	0.25 ± 0.03	3.26 ± 0.02 3.48[b]
	$-\Delta H^\circ$	31.3 ± 0.8 39.2[b]	17.4 ± 1.4	9.6 ± 1.1	<1	12.0 ± 0.9 10.2[b]
	$T\Delta S^\circ$	-13.2 ± 0.9 -21.3[b]	-1.8 ± 1.8	8.0 ± 1.8		$-\ 6.5 \pm 1.0$ 9.6[b]
L-Ser	$\log K$	3.32 ± 0.15 3.37[b]	2.78 ± 0.09	3.12 ± 0.07	1.33 ± 0.21	3.24 ± 0.08 3.64[b]
	$-\Delta H^\circ$	41.1 ± 2.5 38.8[b]	20.5 ± 2.7	24.7 ± 1.8	6.1 ± 3.2	12.8 ± 1.6 15.7[b]
	$T\Delta S^\circ$	-22.2 ± 3.4 -19.7[b]	-4.7 ± 3.2	-6.9 ± 2.3	1.5 ± 4.4	5.7 ± 2.1 5.0[b]
L-Trp	$\log K$	3.19 ± 0.09 3.06[b]	2.58 ± 0.07	3.18 ± 0.06	0.98 ± 0.14	3.22 ± 0.04 3.72[b]
	$-\Delta H^\circ$	41.1 ± 3.7 41.6[b]	26.1 ± 2.2	14.5 ± 1.8	9.1 ± 4.5	11.8 ± 1.1 7.9[b]
	$T\Delta S^\circ$	-22.9 ± 4.3 -24.2[b]	-11.4 ± 2.6	3.6 ± 2.2	-3.5 ± 5.3	6.5 ± 1.3 13.2[b]
L-Val	$\log K$	2.99 ± 0.09 3.19[b]	2.98 ± 0.01	3.32 ± 0.05	1.63 ± 0.13	3.26 ± 0.02 3.16[b]
	$-\Delta H^\circ$	32.2 ± 0.9 34.5[b]	18.0 ± 0.2	23.9 ± 1.0	12.2 ± 1.5	11.3 ± 1.3 4.6[b]
	$T\Delta S^\circ$	-15.2 ± 1.4 -16.4[b]	-1.1 ± 0.2	5.0 ± 1.3	-2.9 ± 2.3	7.2 ± 1.4 13.4[b]

[a] From Ref. 31.
[b] From Ref. 21.
[c] From Ref. 30.

tution of two oxygen donor atoms by two nitrogen atoms causes a decrease of the stability constants caused only by entropic contributions.

The values of the stability constants of the amino acid complexes with the macrobicyclic cryptand (222) and with the crown ether 18C6 are nearly identical. However, the values of the reaction enthalpies with the cryptand are much smaller

Table II. Values of the reaction enthalpy ΔH° [kJ mol^{-1}] for the complex formation of 18C6 with some amino acids in acidic, neutral and basic methanolic solution at 25 °C.

	$^+NH_3$—CHR—COOH	$^+NH_3$—CHR—COO^-	NH_2—CHR—COO^-
L-Ala	-47.9 ± 1.8	-46.2 ± 2.6	-34.1 ± 2.0
Gly	-59.6 ± 2.4	-49.8 ± 2.1	-40.2 ± 1.7
L-Ser	-47.9 ± 1.8	-41.1 ± 2.5	-40.5 ± 1.9
L-Val	-35.1 ± 1.6	-32.2 ± 0.9	-32.2 ± 1.3

than those with 18C6. The complexation with the ligand (222) is favoured by the reaction entropy.

The concentration of the zwitterionic form of the amino acids is not known in methanol. Some complexation reactions of the ligand 18C6 with α-amino acids in acidic, neutral and basic methanolic solutions were therefore carried out. The results are summarized in Table II.

In acidic solution one expects the complete protonation of the amino group. As a result the highest values of the reaction enthalpies are observed. In basic solution the measured values of the reaction enthalpies are lower than in acidic solution. In acidic solution the interactions between a $^+NH_3$-group and the crown ether are observed whilst in basic solution the interactions occur between a neutral NH_2-group with the ligand 18C6. Due to ion–dipole interactions the strongest interactions are observed in the case of the charged $^+NH_3$-group. In basic solution only interactions between the uncharged NH_2-group and the ligand are possible. The reaction enthalpies observed in pure methanol are between the values found in acidic or basic methanolic solution. The values of the reaction enthalpies clearly demonstrate, that beside the zwitterionic form, the neutral form of α-amino acids is present in methanolic solution. As a result the complex formation between macrocyclic and macrobicyclic ligands and amino acids should be described by at least two different reaction schemes. With one exception [30] this has been neglected up to now in the literature [21]. Further studies are necessary to measure the stability constants and the thermodynamic data for the reaction of macrocyclic and macrobicyclic ligands with the completely protonated and unprotonated forms of the amino acids.

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References

1. D.J. Cram, R.C. Helgeson, L.R. Sousa, J.M. Timko, M.L.A. Domeier, S.C. Peacock, K. Modan, and L. Kaplan: *Pure Appl. Chem.* **43**, 327 (1975).

2. R.M. Izatt, J.D. Lamb, N.E. Izatt, B.E. Rossiter, Jr., J.J. Christensen, and B.L. Haymore: *J. Am. Chem. Soc.* **101**, 6273 (1979).
3. J.D. Lamb, J.E. King, J.J. Christensen, and R.M. Izatt: *Anal. Chem.* **53**, 2127 (1981).
4. F. Vögtle, H. Sieger, and W.M. Müller: *Top. Curr. Chem.* **98**, 143 (1981).
5. C. Seel and F. Vögtle: *Angew. Chem. Int. Ed. Engl.* **4**, 442 (1991).
6. J.M. Timko, R.C. Helgeson, M. Newcomb, G.W. Gokel, and D.J. Cram: *J. Am. Chem. Soc.* **96**, 7097 (1974).
7. J.-M.Lehn: *Supramolecular Chemistry*, VCH, Weinheim (1995).
8. H.-J. Buschmann: *Polyhedron*, **11**, 559 (1992).
9. H.-J. Buschmann and L. Mutihac: *Thermochim. Acta* **237**, 203 (1994).
10. J.A.A. de Boer and D.N. Reinhoudt: *J. Am. Chem. Soc.* **107**, 5347 (1985).
11. G. Wipff, P. Weiner, and P. Kollman: *J. Am. Chem. Soc.* **104**, 3249 (1982).
12. M.G.B. Drew and P.C. Yates: *Pure Appl. Chem.* **61**, 835 (1989).
13. M.H. Mazor, J.A. McCammon, and T.P. Lybrand: *J. Am. Chem. Soc.* **112**, 4411 (1990).
14. H.-J. Buschmann, G. Wenz, E. Schollmeyer, and L. Mutihac: *Thermochim. Acta* **261**, 1 (1995).
15. J.-M. Lehn and P. Vierling: *Tetrahedron Lett.* **21**, 1323 (1980).
16. M. Newcombe, J.M. Timko, D.H. Walba, and D.J. Cram: *J. Am. Chem. Soc.* **99**, 6392 (1977).
17. H.W. Colquhoun, D.F. Lewis, J.F. Stoddart, and D.J. Williams: *J. Chem. Soc., Dalton Trans.* 607 (1983).
18. M.R. Johnson and I.O. Sutherland: *J. Chem. Soc. Perkin Trans. I* 357 (1979).
19. J.P. Behr, J.-M. Lehn, and P. Vierling: *Helv. Chim. Acta* **65**, 1853 (1982).
20. A.F. Danil de Namor: *Pure Appl. Chem.* **62**, 2121 (1990).
21. A.F. Danil de Namor, M.C. Ritt, M.-J. Schwing-Weill, F. Arnaud-Neu, and D.F.V. Lewis: *J. Chem. Soc., Faraday Trans.* **87**, 3231 (1991).
22. H.-J. Buschmann and L. Mutihac: *Colloids Surfaces A: Physicochem. Eng. Aspects* (1997) in press.
23. L. Mutihac, D.O. Popescu, and R.-I. Stefan: *Anal. Lett.* **28**, 835 (1995).
24. L. Mutihac, R. Mutihac, and H.-J. Buschmann: *J. Incl. Phenom.* **23**, 167 (1995).
25. D.J. Eatough, J.J. Christensen, and R.M. Izatt: *Experiments in Thermometric Titrimetry and Titration Calorimetry*, Brigham Young University Publications, Provo (1973).
26. H.-J. Buschmann: *Thermochim. Acta* **102**, 179 (1986).
27. H.-J. Buschmann: *Inorg. Chim. Acta* **195**, 51 (1992).
28. N.E. Tayar, R.S. Tsai, P.A. Carrupt, and B.J. Testa: *J. Chem. Soc., Perkin Trans. 2*, 79 (1992).
29. W.P. Jencks and J. Regenstein: in G.D. Fasman (ed.), *CRC Handbook of Biochemistryand Molecular Biology: Physical and Chemical Data*, CRC Press, Boca Raton, Vol. 1, p. 305 (1976).
30. M. Czekalla, K. Goe, B. Habermann, T. Krüger, H. Stephan, F.P. Schmidtchen, and R. Trültzsch: *Poster PB37*, XXI International Symposium on Macrocyclic Chemistry, Montecatini Terme (Italy) (1996).
31. H.-J. Buschmann, E. Schollmeyer, and L. Mutihac: *Supramol. Sci.*, in print.