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# Complexation of Amino Acids with 18-Crown-6

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**Abstract** — In complexation of 18-crown-6 with glycine, *L*-alanine, *L*-serine, *L*-threonine, *L*-asparagine, *L*-glutamine, *L*-histidine, *L*-phenylalanine, and diglycine in $D_2O$ at 298 K, side groups of the amino acids are not involved in additional interactions with the macrocycle, except the OH group of threonine.

All the known applications of crown ethers are based on their unique capability for selective complexation with cations and neutral molecules. The choice of the guest molecule by host macromolecules is determined by the complementarity principle and is the basis for many biochemical processes such as enzymatic catalysis, membrane transport, antigen–antibody interactions, etc. [1, 2]. The principle of molecular recognition determines the use of crown compounds and their derivatives for separation of biomolecules (e.g., amines and amino acids) [3–5].

Published data [6–8] show that amino acids and peptides form with 18-crown-6 1 : 1 complexes. A $^{19}F$ NMR study shows that complexation of 18-crown-6 with β-fluoro-α-amino acids in acid solutions is due to H bonding of the $NH_3^+$ group of the amino acid with alternating oxygen atoms of the macroring [7]. The potentiometric titration data and results of computer simulation, given in [8], also support this complexation mechanism. First, the curves of titration of *D,L*-phenylalanine in methanol in the presence of 18-crown-6 and without it confirm that the $NH_3^+$ group is the center of the complexation with the crown ether. Second, in the case of blocking of the $NH_3^+$ group (e.g., with acetate ion), no complexation occurs. Third, the results of computer simulation show that the hydrogen atoms of the amino group are in short contact with the macrocycle: three hydrogen bonds are formed, and three oxygen atoms of 18-crown-6 are involved in the electrostatic interaction with the $NH_3^+$ group. However, participation of side groups of amino acids in the complexation with 18-crown-6 was not discussed, and data on this problem are lacking.

Previously we made a thermodynamic study of complexation of 18-crown-6 with *L*-amino acids and oligopeptides in aqueous solution at 298.15 K [9, 10]. The results were interpreted assuming participation of side groups of some amino acids in additional interactions with the macrocyclic ring of the ether. To confirm this assumption, we studied in this work the mechanism of complexation of 18-crown-6 with a series of amino acids (glycine, *L*-alanine, *L*-serine, *L*-threonine, *L*-asparagine, *L*-glutamine, *L*-histidine, *L*-phenylalanine) in water by $^{13}C$ NMR spectroscopy and correlated the coordination shifts with the previously obtained thermodynamic parameters of the complexation.

The shifts $\Delta\delta_C$ of the signals of the carbon nuclei in the amino acids incorporated in the complex with 18-crown-6 relative to the free amino acids are listed in the table. According to [11], at these concentrations the amino acids do not form associates and the interactions of species are long-range and are accompanied by destruction of the solvation shells and decrease in the hydration numbers. The pH measured with an I-130 pH meter did not change on adding 18-crown-6 and corresponded to the isoelectric point of the amino acid, which confirmed the existence of the amino acids in the form of zwitter-ions. Therefore, we assumed that association, dissociation, and protonation of the amino acids did not occur and that the changes in the chemical shifts were exclusively due to the complexation with the crown ether.

For all the amino acids studied, the shift of the $^{13}C$ signal of the $C^1$ atom ($COO^-$ group) is the most pronounced. If the $COO^-$ group were involved in H bonding with the macrocyclic ring, the $C^1$ signal would be shifted downfield, as it was observed in the complexation of *D*-phenylglycine with 18-crown-6-tetracarboxylic acid ($^1H$ and $^{13}C$ NMR data [5]). With our system, however, this is not the case: The $C^1$ signal is shifted upfield, which is most likely due to the desolvation of the $COO^-$ group. To confirm this assumption, we studied the system 18-crown-6 + diglycine. In the diglycine molecule, the carboxylate anion is more remote from the macrocyclic ring and hence its possible interaction with 18-crown-6 is less probable. The $\Delta\delta_C$ value for $C^1$ is –0.2 ppm, being within

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Changes in the $^{13}C$ chemical shifts ($\Delta\delta_C$, ppm[a]) of *L*-amino acid molecules upon complexation with 18-crown-6 (298 K)

| Amino acid | $C^1$ | $C^2$ | $C^3$ | $C^4$ |
|---|---|---|---|---|
| Glycine | –1.26 | –0.29 | – | – |
| Alanine | –0.34 | 0.09 | 0.03 | – |
| Serine | –0.30 | 0.04 | –0.12 | – |
| Threonine | –0.24 | 0.24 | 0.03 | 0.02 |
| Asparagine | –0.19 | –0.01 | –0.11 | –0.31 |
| Glutamine | –0.11 | 0.10 | 0.14 | –0.02 |
| Histidine | –0.20 | 0.03 | 0.06 | –0.04 |
| Phenylalanine | 0.07 | 0.08 | 0.15 | 0.07 |
| Glycylglycine | –0.20 | 0.04 | –0.36 | 0.01 |

[a] ($\Delta\delta_C$) Difference between the $^{13}C$ chemical shifts of the amino acids incorporated in the complex and free amino acids.

the range of shifts observed with the other amino acids (see table). Therefore, we can conclude that the $COO^-$ group does not interact with the crown ether in the complex and undergoes desolvation accompanied by partial destruction of its hydration shell.

The $C^2$ signal in the majority of systems undergoes a small downfield shift. This is due to the fact that the complexes are weak ($\log K$ 0.4–1.5 [9, 10]) and their formation is due to desolvation and subsequent penetration of the complexation center of the amino acid ($NH_3^+$ group) into the 18-crown-6 cavity, with the formation of H bonds between the hydrogen atoms of the amino acid and oxygen atoms of the ligand.

Our results show that, in threonine, $\Delta\delta_C$ of the $C^2$ atom is the largest and the signal of the $C^3$ atom bearing the OH substituent is shifted downfield, whereas in structurally related serine $\Delta\delta_C$ of the $C^2$ atom is small and the $C^3$ signal is shifted upfield. Some amino acids contain in the vicinity of the complexation center a polar OH group capable of forming an additional H bond with the oxygen atoms of the ligand. Apparently, this bond is actually formed only in the system threonine–crown ether, which makes the complex more structured; this is confirmed by the thermodynamic parameters of the complexaton. The entropy of complexation of 18-crown-6 with threonine is more negative ($\Delta S$ –9.4 J mol$^{-1}$ K$^{-1}$) compared to the complexation with serine ($\Delta S$ –1.9 J mol$^{-1}$ K$^{-1}$). Furthermore, the complexation with threonine is more exothermic [9, 10].

The side amide group of asparagine and glutamine can also participate in hydrogen bonding with the macroring. As seen from our results (see table), the coordination shifts of the carbon atom of this group are insignificant, suggesting that this group is remote from the macroring and is not involved in the complexation.

Note that the $\Delta\delta_C$ values obtained for the complexes of 18-crown-6 with aromatic and heteroaromatic amino acids (phenylalanine, histidine) are insignificant, which is due to the effects of the benzene and imidazole rings, respectively. A study of the interaction of 18-crown-6-tetracarboxylic acid (2.2 mM) with phenylglycine and its methyl ester (2 mM) revealed the interaction of the side phenyl substituent in these compounds with the $CH_2CH_2$ groups of the ligand ($\Delta\delta$ of the carbon atoms of the aromatic ring varied from –0.57 to 0.77 ppm) [5]. In our case, the $\Delta\delta_C$ values are considerably lower (from –0.06 to 0.04 ppm), and we can conclude that the side groups of aromatic and heteroaromatic amino acids are not involved in hydrophobic interactions with 18-crown-6.

The crown ether carbon atoms give a single signal in the $^{13}C$ NMR spectrum, and its coordination shift ranges from –0.12 to 0.03 ppm. We believe that small negative values of $\Delta\delta_C$ are associated with the symmetric structure of the macrocycle molecule. In the interaction of 18-crown-6 with amino acids, the electron density from the $NH_3^+$ group shifts toward oxygen atoms of the ligand; hence, the carbon nuclei become uniformly shielded.

Thus, the side groups of the majority of amino acids are not involved in additional interactions with the macroring, except threonine whose OH group is located in the vicinity of the macroring and can form hydrogen bonds with the ether oxygen atoms.

## EXPERIMENTAL

The $^{13}C$ NMR spectra were recorded on a Bruker AC-200 spectrometer (200 MHz) at 298 K using a Bruker BVT-3000 temperature unit (temperature control accuracy ±0.1 K). The $^{13}C$ chemical shifts of the amino acids, 18-crown-6, and their mixtures in $D_2O$ were determined relative to external cyclohexane.

*L*-Amino acids, glycine, and glycylglycine (Reanal, Hungary) were recrystallized from aqueous ethanol and vacuum-dried at 343 K for 3 days to constant weight. 18-Crown-6 (ICN Pharmaceuticals) was used without additional purification (main substance content >99.1%).

All the solutions were prepared gravimetrically, by dissolving equimolar amounts of the amino acid and

18-crown-6 in $D_2O$. The reactant concentrations were 0.2 mol $kg^{-1}$.

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