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RESEARCH PAPER

Investigation of Non-covalent Interactions of 18-Crown-6 with Amino Acids in Gas Phase by Mass Spectrometry

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Abstract: The non-covalent interactions between 18-Crown-6 (18c6) and 20 common types of protonated amino acids were explored by electrospray ionization mass spectrometry. The mass spectra showed that 18c6 could react with amino acids to form a non-covalent complexe in a stoichiometric ratio of 1:1. The calibration curves and linear equations for the complexes of *L*-Phe, *L*-Tyr, *L*-Lys and *L*-Asp with 18c6 were established by mass spectrometric titration and used as reference values for competitive ESI-MS. Through competitive equilibria, the binding constants for the complexes of 18c6 with other *L*-amino acids and their *D*-isomers were derived. It was found that, as a general trend, lgK_a for the complexes of 18c6 with the basic amino acid and the amino acid with alkyl side chain were larger than other complexes, and among the amino acid with alkyl side chain, Gly and Ala exhibited greater 18c6 binding affinities. As for Ser and Thr, the intramolecular hydrogen bond between the nitrogen atom from terminal $-NH_2$ and the oxygen atom from carboxyl might impede their protonated amino-group to attack the 18c6. Furthermore, Gln and Asn exhibited lower binding affinities to 18c6, probably due to effects of electron-withdrawing group of acylamide. Finally, the chiral selectivity of 18c6 for *L*-amino or *D*-amino acids were measured by ESI-MS, and the result showed that 18c6 could only recognize some neutral amino acid isomers.

Key Words: 18-Crown-6; Amino acid; Electrospray ionization mass spectrometry; Non-covalent binding affinity

1 Introduction

Crown ethers, a kind of macrocyclic polyether compounds with repeating units of $-CH_2CH_2O-$, were first actually discovered by Pedersen at du Pont in 1967, for the time[1,2]. Among them, 18-crown-6 (18c6, which is also called 1,4,7,10,13,16-hexaoxacycloocatdecane or $C_{12}H_{24}O_6$) are the most important and typical ones (Fig.1a).

Although their structures are simple, the performances of them are as excellent as that of natural ionophores[3]. Crown ethers have the ability to form complexes with cations and small molecules such as hydrated proton ions and pronated amines due to their regulatable holes formed by structures and various solubility capability[4]. Besides, it is considered that crown ethers can even be used in chiral recognition and enantiomers separation[5,6].

Therefore, there are many practical applications within crown ethers due to their unique binding properties. The verified applications of crown ethers involve ion-selective electrodes, ion transport, anion activation, cation inhibition, stationary phase in gas chromatography, and macrocyclic liquid crystals[7–10].

For biological processes, the interactions between 18c6 and proteins or peptides may exist. For example, the anti-coccidia activity against Eimeria tenella in vitro was found when applying synthetic crown ether compounds[8]. Furthermore, Mistarz *et al*[10] investigated the non-covalent interactions between 18c6 and Leu-enkephalin, which prevented the deuteration of hydrogen atom connected to heteroatoms.

Moreover, protein-protein interactions were explored from protein structure elucidation and protein surface recognition[11]. Liu *et al*[12] reported the experiments of crown ethers as structural probe by the selective noncovalent adduct protein probing (SNAPP). There crown ethers provided the high binding affinity between 18c6 and lysine side chains. On the basis of those previous study, Frański *et al*[13] conducted

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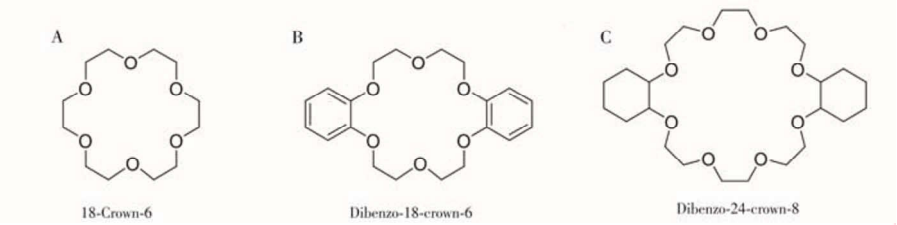

Fig.1 Diagram of some classical crown ethers

experiments on the compounds formed by 12c4, 15c5 or 18c6 and several peptides with lysine residues. The results showed that the compound remained the steadiest in methanol.

In addition, Stedwell *et al*[14] explored crown-adducted protonated AAs by infrared multiple photon dissociation (IRMPD), and found that there was a considerable red shift of the NH_3^+ stretch modes, which was partly due to the tight chelation of 18c6 with protonated NH_3^+. Moreover, Chen *et al*[15] used guided ion beam tandem mass spectrometry (MS) to determine the absolute affinity of 18c6 to five amino acids. According to the theoretical electronic structure calculations, a conclusion was drawn that there was inverse correlation between the polarizability (or proton affinity) and the binding affinities of 18c6 with those five amino acids.

But so far, noncovalent interactions between 18c6 and amino acids have not been investigated systematically by ESI-MS. Therefore, it is necessary to measure the binding constants and give a quantitative comparison on the binding affinities betweenof 18c6 and to amino acids.

In this study, we extended the research to on 20 common *L*-amino acids and their *D*-isomers. All the experiments were conducted by ESI-MS. The calibration curves and binding constants for the complexes of 18c6 with *L*-phenylalanine, tyrosine, lysine or Asparaginic acid were established by the mass spectrometric titration, and on the basis of this, according to which, the lgK_a values for the complexes of 18c6 with other *L*-amino acids and their *D*-isomers in the competitive ESI-MS spectra were derived. Finally, the chiral selectivity (R_{chiral}) of 18c6 for to amino acids' enantiomers was also determined.

2 Experimental

2.1 Reagents and chemicals

The 18-crown-6 (M_W = 264.32) was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). The *L*- and *D*-amino acids were purchased from Aladdin (Shanghai, China). The methanol (HPLC grade) and all of the amino acids including phenylalanine (Phe), tryptophan (Trp), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), methionine (Met), tyrosine (Tyr), serine (Ser), threonine (Thr), glycine (Gly), cysteine (Cys), asparagine (Asn), glutamine (Gln), lysine (Lys), arginine (Arg), His, asparaginic acid (Asp) and glutamic acid (Glu) were purchased from Merck (Darmstadt, Germany).

2.2 Experimental methods

2.2.1 Instruments and its parameters

A triple-quadrupole mass spectrometer (MS) with electrospray ionization source (API 3000, SCIEX, U.S., *m/z* 3000) was used. The polypropylene glycol (PPG) was used as the calibration standard. The purity of nitrogen (99.999%) was used as curtain gas and nebulizer gas.

The MS experiments were operated in a positive mode. The spray voltage was set at 4.5 kV. The rate of spraying the sample solution was 3 μL min^{-1}. The solvent composition in this research was all 1:1 methanol-water with additional 0.1% formic acid, and the pH value of the solutions was adjusted to 3.6.

2.2.2 Binding constants of 18c6 with Tyr, Phe, Lys or Asp by mass titration

A quantitative evaluation for the interactions between 18c6 and protonated amino acids was made firstly, because the accuracy of binding constants was affected greatly by the ionization efficiency as well as the signal suppression according to the achievement of Schmidt *et al* [16]. In addition, the structure and abundance of ions in the solution may differ from those generated in the gas phase by ESI-MS[17].

The equations of the binding constants should only be derived when the following hypotheses are valid[18]. First of all, there are no changes whenever in the liquid or in the gas phase as well as no dissociation of the complexes. Secondly, the concentration of the substance in the mixture and its corresponding peak intensity in the figure are in the direct ratio. Thirdly, substances in the mixture all have similar ionization efficiency.

The equations are as follows[18]:

$$a_m = \frac{[HG]}{[H]_0} = \frac{I_{HG}}{I_H + I_{HG}} \quad (1)$$

$$K_{d,m} = \frac{(1-\sum a_n)([G]_0 - [H]_0 \sum n a_n)^m}{a_m} \quad (2)$$

$$K_a = 1/K_d \quad (3)$$