ELECTROSPRAY IONIZATION MASS SPECTROMETRY OF CYCLODEXTRIN COMPLEXES OF AMINO ACIDS AND PEPTIDES

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ABSTRACT

Appropriate complex-forming side-chains of peptides and proteins can bind cyclodextrins. We have characterized interactions of amino acids with cyclodextrins by electrospray ionization mass spectrometry. The stoichiometry of various α-cyclodextrin/protonated tryptophan complexes sampled from solution to the gas phase by ESI was revealed by taking advantage of the extended mass range and high mass resolution of a Fourier-transform ion cyclotron resonance instrument. Binding of β-cyclodextrin to recombinant human insulin has been demonstrated.

1. INTRODUCTION

Large molecules such as peptides and proteins cannot be fully entrapped into inclusion complexes of cyclodextrins (CDs). Appropriate complex-forming side-chains can, however, bind cyclodextrins, resulting in modified solubility, stability and/or membrane transport properties [1]. The lack of adequate techniques has hindered the proper characterization of peptide - cyclodextrin interactions, because of the complexity of their association involving multiple binding sites for cyclodextrins on these large biomolecules.

Electrospray ionization (ESI) can be used effectively to study noncovalent complexes involving CDs. Our recent studies[2] on amino acids and CDs have revealed that complexation equilibria in solution are reflected by ESI mass spectra. Amino acid - CD complexes were stable for characterization by ESI mass spectrometry; thus, their relative abundances and the stoichiometry could be determined. The preferential formation of CD- aromatic over CD- aliphatic amino acid complexes have been confirmed, and the

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relative gas-phase stabilities were also determined by collisionally-induced dissociation (CID). Our preliminary results[3] for peptides, together with recent investigations by others on CD complexes of small [4] and large peptides[5], have shown the potential of the technique to characterize their formation and stability. However, the high molecular weight and complexity of the systems may benefit from the use of high-performance mass spectrometry, such as Fourier-transform ion cyclotron resonance (FTICR) [6], that provides an extended mass range and increased resolution, compared to the commonly used quadrupole mass analyzers [2-4]. We present results of a detailed study on complexes between amino acids and CDs detected and identified by ESI-FTICR, and report preliminary data on the binding of CDs to a therapeutic peptide, insulin.

2. MATERIALS AND METHODS

ESI - quadrupole mass spectrometry was done by using a Vestec 200 ES instrument (PerSeptive Biosystems/Vestec, Houston, TX). Ionization was achieved by applying 2.5-2.7 kV to a flat tipped (120 μm ID) stainless steel needle. The sample solution was delivered via a fused silica capillary by a syringe pump at 2-5 μL/min flow rate. A stainless steel plate with a 0.4-mm orifice functioned as the counterelectrode. The source was heated to 250 °C to reach 55-60 °C in the spray chamber. The collimator and repeller potentials were 10 V and 15 V, respectively, to avoid CID. Sample concentrations were 1×10^{-4} M for insulin, and 5×10^{-5} M in 45/45/10 (v/v/v) methanol/water/acetic acid for β-CD.

FTICR experiments were performed on a 4.7-tesla instrument (Bruker Instruments, Inc., Billerica, MA). Ions were produced via an ESI source equipped with a hexapole ion guide/trap and heated capillary (Analytica of Branford, Branford, CT). ESI was achieved at a flow rate of 2 ul/min with a needle voltage of 3 kV. The capillary was held at ground potential while the temperature was maintained at 100 °C. Sample concentrations in 45/45/10 (v/v/v) methanol/water/acetic acid were 1×10^{-6} M for the CDs and 5×10^{-5} M for the amino acids and peptides.

3. RESULTS AND DISCUSSION

Electrospray ionization (ESI) mass spectrometry offers some unique advantages for studying inclusion complexes. With this "soft" ionization technique, ions existing in solution can be transferred into the gas phase without breaking noncovalent interactions, allowing their subsequent investigation by various mass spectrometric techniques. The stoichiometry of an inclusion complex may, among others, be revealed unequivocally.

Fig. 1 shows the ESI mass spectrum of a solution containing α-CD and tryptophan obtained by the FTICR technique. Besides the already reported 1:1 α-CD/protonated

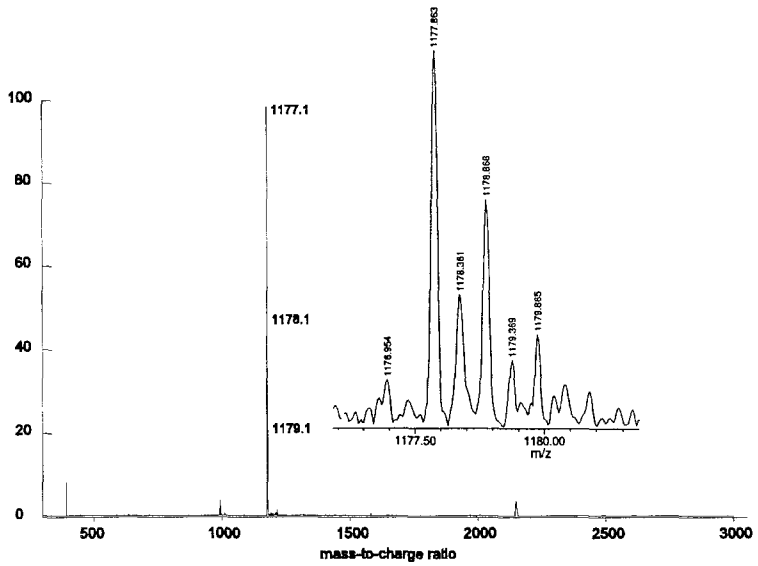

Figure 1. ESI-FTICR mass spectrometry of α-CD and tryptophan complexes. (The inset shows the expanded region between 1174 to 1183.)

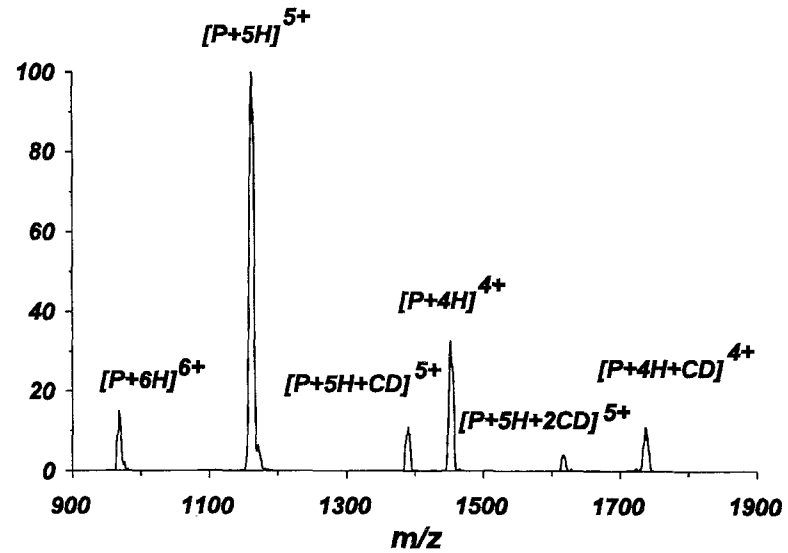

Figure 2. ESI-MS of human recombinant insulin solution containing excess β-CD.

tryptophan complex [2], the fully resolved isotope profiles made the identification of a 2:2 complex (a proton-bound dimer) possible. Note that mass spectrometry determines mass-to-charge (*m/z*) values; therefore, the doubly-charged ions show isotope ions with about 0.5 Da apart, as opposed to the about 1.0 Da difference for singly-charged ions. The 1:1 and 2:2 adducts that contain only ^{12}C, ^{1}H, ^{16}O, and ^{14}N isotopes have identical *m/z* values. The mass resolution ($M/\Delta M$) required to resolve this profile is >2,400, which is not met by most commercial quadrupole analyzers, but easily obtained on FTICR instruments. (The actual mass resolution exceeded 10,000.) In addition, the extended mass-range acquisition (up to m/z 3,000) has allowed us to detect a less abundant 2:1 α-CD/protonated tryptophan complex (*m/z* 2,149.3).

For the studies on binding of CDs to a large peptide such as recombinant human insulin (Mr 5807.6), a large molar excess of CD was necessary to detect the formation of intense adduct ions. We used the routine ESI - quadrupole mass spectrometer system for a preliminary study. The β-CD adducts, together with the multiply-charged insulin molecular ions (due to acid-base equilibria involving the basic amino acid residues of the peptide), were indicated in Fig. 2. (P represents the peptide, CD abbreviates β-CD, and H indicates the number of protons). Under this solution conditions, the formation of isobaric ions is unlikely, but ESI-FTICR is necessary to confirm this hypothesis.

In conclusion, ESI mass spectrometry has been a powerful technique for the analysis of binding of CDs with amino acids and peptides. The technique is ideal for the characterization of binding CDs to amino acids and peptides, because they are present mostly as ions in aqueous solutions, which are the preferred solvents for ESI.

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