International Journal of Biological Macromolecules 64 (2014) 383–391

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Macromolecular ensembles of cyclodextrin crystallohydrates and clathrates – experimental and theoretical gas – and condense phase study

Bojidarka Ivanova *, Michael Spiteller

Lehrstuhl für Analytische Chemie, Institut für Umweltforschung, Fakultät für Chemie, Universität Dortmund, Otto-Hahn-Straße 6, 44227 Dortmund, North Rhine-Westphalia, Deutschland, Germany

ARTICLE INFO

Article history:
Received 14 December 2012
Received in revised form
15 December 2013
Accepted 18 December 2013
Available online 25 December 2013

Keywords:
Cyclodextrins
Crystallography
Mass spectrometry
Quantum chemistry

ABSTRACT

The paper reported a joined mass spectrometric, crystallographic and quantum chemical study of crystallohydrates, emphasizing on clathrates of native α-, and β-cyclodextrins as well as their randomly acetylated derivatives (**4** and **5**). The physical data are compared with 19 crystals of CDs, three of which new ones, differed by number of crystallohydrate (and/or clathrate) molecules (n)). The macroscopic complex CDs/n stability ($n \in [0.60–12.26]$) is evaluated theoretically, accounting the surface and clathrate self-assembly of non-covalent hydrogen bonding interactions. The variety of competitive condensation processes of randomly acetylated products and the non-specific phase transition adduct of CDs and aggregates, which complicated significantly the MS picture are discussed. The single crystal X-ray diffraction, enable to determine the non-covalent interactions in CDs crystals, which physical phenomena in the gas-phase and crystalline phase → liquid phase → GP and CP → GP transitions are evidenced mass spectrometrically. The quantum chemical method provided important thermodynamics and structural information at variety of states, allowing understanding comprehensively the complex GP phenomena. Special emphasis in the paper content is dedicated to the phenomenology of the GP mass spectrometric ionization processes and thermodynamics of fragmentation molecular ions of CDs and their supramolecular self-assembly which, strongly depends on the experimental factors.

1. Introduction

The extensive research efforts on CDs and their inclusion complexes are due to their importance and widely interdisciplinary applications such as pharmaceutical science, agriculture, food science and industry, cosmetic, chemical and many other industrial branches [1]. The frontiers of modern medicinal chemistry largely involved macromolecular host–guest systems in the molecular drugs design of novel effective therapeutics. It has been found that the incorporation of active substances *into* amphiphilic CDs cavity reduces significantly the drug's side affects [1]. That is why the CDs are among the basic host systems involved on the frontier discoveries in the field of medicinal chemistry and pharmacy. The molecular flexibility of 6–8D-glucose rings, linked by α-1,4-glucose bonds in CDs, is caused mainly by —CH_2OH moieties. They can be functionalized further to encapsulate various organic substrates, and optimization of the chemical, physical and physic-chemical properties of the functionalized macromolecules, in order to ensure the maximal biological activity of the biologically active ingredient under physiological conditions. Furthermore, more recent studies emerged cyclodextrins in the new complementary technologies of the classical approaches related the molecular logic operations, intelligent bionanomaterials, diagnostic devices, bioengineering nanomaterials and biomedical technology [2a–g]. The CDs have been found as excellent platforms for construction of the supramolecular soft materials and/or applied catalysis and organic synthesis in supramolecular systems as well [2a–h]. Therefore the studies on the correlation between the molecular CDs conformation and thermodynamic stability at variety of condense and gas-phase have significant both fundamental and applied impact in all these above mentioned interdisciplinary fields. In terms of hydrophobic CDs micro-environment cavity, the cooperative contribution has the non-covalent forces, including hydrophobic ones, π—π

Abbreviations: APCI, Atmospheric-pressure chemical ionization (mass spectrometric method); CDs, Cyclodextrins; CIS, Configuration-interaction singles method (quantum chemical method); CP, Crystalline phase; DFT, Density Functional Theory (quantum chemical method); ESI, Electrospray ionization (mass spectrometric method); GP, Gas-phase; MALDI, Matrix assisted laser desorption ionization (mass spectrometric method); MS, Mass spectrometry; MP2, Second-order Møller–Pleset perturbation theory (quantum chemical method); LP, Liquid phase; TD–HF, Time-dependent Hartree-Fock method (quantum chemical method).

* Corresponding author. Tel.: +49 231 755 4089; fax: +49 231 755 40 84.
E-mail address: B.Ivanova@infu.uni-dortmund.de (B. Ivanova).

0141-8130/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2013.12.026

stacking, van der Waals, and hydrogen bonding interactions. The latter ones are main factors for isolation of CDs clathrates [3]. The biological importance of CDs and the complexity of their structural and physical phenomena lead to increasing interest on CDs supramolecular ensembles, stimulating more and more research efforts. The application of advanced physical instrumental methods and theoretical approaches for a comprehensive understanding of factors contributing to the complex macromolecular CDs stability are of significant importance and emergency. The developments in this direction have both fundamental and applied interdisciplinary interest for above mentioned areas of modern science. The methods of mass spectrometry are amount the indispensable analytical approaches for achievement of important experimental analytical qualitative, quantitative and structural information. These advantages are based on the instrumental flexibility and capability within the large scale of: (*i*) phase analysis (solution, including liquid and semi-liquid state, solid amorphous and crystalline state, gas-phase); (*ii*) analyte concentrations encompassing from biomacromolecular screening to small molecular analyte determination; (*iii*) wide concentration diapason including ∈*fmol–attomol* levels; (*iv*) ultra-high resolving power enable to determine the analyte of interest in highly complex multicomponent systems such as the living cells and whole organs; (*v*) imaging capability for direct bioassay. Variety of MS methods has been already extensively utilized for analytical studies of CDs and their biologically important host–guest systems [4]. As has been comprehensively discussed host–guest interaction in GP may be described as much more limited, since hydrophobic affect, attributed to formation of cyclodextrin inclusion complexes in solution. The hydrophobic affect is negligible in GP, where the electrostatic interactions between CDs and the attaching molecules become dominant [4]. The electrostatic energy, however, contributing to total free energy of the macromolecular CDs ensembles in GP. It depends of the electrostatic interactions specificity. The latter ones are accurately evaluated by quantum chemical approaches. Thus, the complementary application of the experimental MS, singly crystal X-ray diffraction and quantum chemical methods provides unique opportunity to correlate the experimental structural conformations and thermodynamics encompassing gas- and condense (solution, solid-state and crystalline state) phase. The observed mass spectrometric complex picture of CDs systems and correlation study molecular conformation/thermodynamics stability enable to obtain crucially important information for the behaviour, related to the structural conformations, stability and biological activity of the CDs complexes direct in the living cells and organs utilizing the capability of MALDI method for *in vivo* assay and imaging. Since, the detailed mechanisms of ion formation and GP reactions depended on large scale of factors, causing the proton (resp. electron) accepting ability, including balance between the enthalpy and the entropy as factors defining the macro-system thermodynamics stability, it is difficult to assign directly in an unambiguous manner, the given specific molecular interactions causing the phenomena in condense phase to those defining the macro-system thermodynamics stability in the GP systems. Furthermore the GP "continuum" of MS experiments, particularly those of the electrospray ionization method originating from the mobile phase, is described as ensemble of neutral and ionized solvation molecules. While under MALDI ionization conditions a transition CP → GP occurred. Since the proton accepting ability depended both on molecular and environmental factors, the quantum chemical method provided opportunity to evaluate accurately the molecular fragments observed within the frame of MS experiments evaluating consequently the contributions of all above stated factors [5]. On the other site the single crystal X-ray diffraction is enable to provide absolute structural information in CP, clarifying the inclusion phenomena and non-covalent interactions between CD and guest-molecules, including the solvation clathrates, thus allowing an evaluating of the complex stability and the affect of the solvent water molecules.

Therefore, the paper deals with a joined mass spectrometric, crystallographic and quantum chemical study on 19 model crystallohydrate, including clathrate systems of α- and β-CD, with the number of solvent water molecules $n \in$ [0.60–12.26], aiming to understand comprehensively the factors, contributing to their thermodynamics stability. Three of the reported systems (**1**–**3**) are new for the literature crystals of CDs. Experimentally and theoretically are studied the phase transitions LP → CP → GP (under MALDI experiment), and LP → GP (under ESI– (or APCI–) experiments, at the large scale of experimental conditions involving variety of the solvent types, their proton accepting/donating ability and polarity, pH ∈ 0–13; $T \in$ 298–673 K and $P \in$ 100–500.84 kPa. Firstly in the literature we presented the study on the affect of these experimental parameters on the crystallohydrate and clathrate self-assembly in the CDs and their thermodynamic stability both in condense and in the gas-phase. Despite the significance of cyclodextrine hydrates for all above mentioned interdisciplinary areas, the comprehensive studies encompassing crystallohydrates and clathrates self-assembly forces and their impact on the thermodynamic of the cyclodextrine systems under variety of phases and experimental factors have been limited to scarce mainly more recent reports [6].

2. Experimental

2.1. Physical methods

The X-ray diffraction intensities of **1**–**3** were measured on a Bruker Smart X2S diffractometer, using micro-source Mo-Kα radiation and employing the ω scan mode. The data were corrected for Lorentz and Polarization affects. An absorption correction based on multiple scanned reflections. The crystal structures were solved by direct methods using SHELXS-97 [7]. The crystal structures were refined by full-matrix least-squares refinement against F^2 [7]. Anisotropic displacement parameters were introduced for all non-hydrogen atoms. The hydrogen atoms attached to carbon were placed at calculated positions and refined allowing them to ride on the parent carbon atom. The hydrogen atoms bound to oxygen were constrained to positions which were confirmed from difference map and refined with appropriate riding model, with the exception of water hydrogen atoms. Structures were processed by PLATON [7]. The crystallographic refinement parameters are summarized in Table S1.

HPLC ESI– and APCI–MS/MS measurements were performed on TSQ 7000 instrument (Thermo Fisher Inc., Rockville, MD, USA). The mobile phase compositions were 0.1% v/v aqueous HCOOH, 0.1% v/v HCOOH in CH_3CN; or 20.0–0.02% v/v HCOOH in CH_3CN:CH_3OH solvent mixture 1:1. A triple quadrupole mass spectrometer (TSQ 7000 Thermo Electron, Dreieich, Germany) equipped with an ESI 2 source was utilized for ESI–MS measurements. The experimental conditions were conditions: capillary temperature 180 °C; sheath gas 60 psi, corona 4.5 μA and spray voltage 4.5 kV. Sample was dissolved in CH_3CN (1 mg mL^{-1}) and is injected in the ion source by auto-sampler (Surveyor) with a flow of pure CH_3CN (0.2 mL min^{-1}). Data processing was performed by Excalibur 1.4 software. LTQ Orbitrap XL (Thermo Fisher Inc.) instrument was used for MALDI–MS measurements, using the UV laser source at 337.2 nm. An overall mass range of *m/z* 100–1000 was scanned in Orbitrap analyser. The ImageQuest 1.0.1 program package was used. The laser energy values were ∈11.7–21.6 μJ. The numbers of averaged laser shots lies ∈22–134, the MALDI flow rate values were ∈21.7–26.4; the acquisition time was ∈29.3–131.0 min, the elapsed scan time range lies ∈20.0–2.11 s, respectively. The chromatographic purification was performed with a Gynkotek (Germering, Germany) HPLC