Structures and reactions of hydrated biomolecular cluster ions

S. Nonose¹,a, S. Iwaoa², K. Mori², Y. Shibata², and K. Fuke²

¹ Faculty of Science, Yokohama City University, Yokohama 236-0027, Japan
² Department of Chemistry, Faculty of Science, Kobe University, Kobe 657-8501, Japan

Received 6 September 2004
Published online 13 July 2005 – © EDP Sciences, Società Italiana di Fisica, Springer-Verlag 2005

Abstract. Photo-induced reactions and metastable decompositions of cluster ions containing glycine, tryptophan, tryptophanylglicine and [Fe(III)-protoporphyrin]+ (hemin⁴⁺) ions solvated with water molecules are studied with electrospray ionization (ESI). The ESI ion source is improved to produce hydrated biomolecular cluster ions. Metastable decompositions of the hydrated clusters following primary mass selection are measured to determine the incremental solvent binding energies for the clusters by using evaporative ensemble model. From these experimental findings, stability of the cluster ions is discussed in terms of delocalization of ionic charges. We also measure the photodissociation yields of mass-selected water clusters containing hemin⁴⁺ ions at 355 and 532 nm. The mass spectra of photofragments show the β-cleavage of carboxymethyl groups in addition to the evaporation of solvent molecules.

PACS. 36.40.-c Atomic and molecular clusters – 73.22.-f Electronic structure of nanoscale materials: clusters, nanoparticles, nanotubes, and nanocrystals

1 Introduction

The characteristic properties of water molecules lead to strong preference of water to interact with functional groups of biomolecules. As a consequence of strong water — solute interaction in the aqueous environment has a significant influence on the solute charge distribution. As shape and charge distribution are intimately related to biological activity of biomolecules, the solvent water is an integral part of a functioning biological system. NMR and X-ray diffraction methods have addressed the issue of hydration of biopolymers in condensed phases [1,2]. However, little microscopic information has been obtained from these methods about the contribution of the water molecules to the system stabilities. Gas-phase spectroscopic methods along with theoretical calculations provide detailed information on molecular structures of biomolecules [3,4]. However, these methods are limited to smaller model systems with typically one or two water molecules attached. Certain important processes that occur during stepwise hydration such as changes in the charge distribution are not explained by these simple modeling systems. In order to investigate the influence of an individual water molecule on the contribution of solvation to the system stabilities, the biomolecules with solvent waters should be introduced into vacuum as clusters. The gas phase is an unusual environment for the investigation of these biological molecules. However, the gas-phase studies are expected to provide a deeper understanding of hydration interactions that determine the structures and reactions. Therefore, investigations of clusters are expected to bridge the gap between the gas-phase reactions and the biological activities on condensed phase. Structures and reactions of gas-phase clusters have been studied extensively using various spectroscopic methods [5–9]. Recent advances in electrospray ionization (ESI) allow us to produce various kinds of nonvolatile molecules in the gas phase without destruction [10]. Fenn and coworkers have developed the method to produce hydrated cluster ions by using ESI [11,12]. Several groups have applied ESI to mass spectrometric studies of the gas-phase cluster ions [13–21]. In the present study, water clusters containing biomolecules such as glycine (Gly), tryptophan (Trp), tryptophanylglicine (TrpGly) and [Fe(III)-protoporphyrin]+ (hemin⁴⁺) ions are produced by using ESI method. We measure metastable decomposition yields of the clusters to estimate incremental binding energies of solvent waters. We also investigate photo-induced reactions of water clusters containing hemin⁴⁺ ions in order to clarify the mechanism of the β-cleavage reaction. Because of characteristic properties of water, extensive solvation effects are observed for the reactions of water clusters containing biomolecular ions.

2 Experimental

Figure 1 shows a schematic diagram of the apparatus consisting of an ESI source and a tandem mass spectrometer with octapole ion beam guides. Ions of amino acids and peptides are produced by ESI of a dilute solution.
of amino acids and a peptide, Gly, Trp and TrpGly (Sigma-Aldrich), in methanol including acetic acid (1.0%). Hemin$^+$ ions are produced by ESI of a dilute solution of Fe(III)-protoporphyrin chloride (hemin chloride, Wako Chemicals) in methanol-dichloromethane (1:1 v/v) mixture. Optimum intensity and stability of ion signals are obtained with a 5 × 10$^{-4}$ M solution of the samples, which is delivered to the needle tip at a rate of 0.01 mL/min. The electrospray needle (3) is biased at 4.5−5.0 kV with respect to the drying chamber (4). The contemplated experiments require a continuous flow of bath gas; the mixture of inert carrier and solvent vapor with which the ions are solvated under well-defined conditions [11,12]. Temperature and composition must be independently variable over a wide range. Carrier gas from a high pressure source (nitrogen in these experiments) is throttled by a stop valve passes via a mass flow meter (Brooks, 5850TR) through a heated vaporizing chamber (1) into which solvating species are admitted into the first vacuum chamber through stainless capillary (6). The first vacuum chamber is evacuated by a 290 m$^3$/h roots pump (ULVAC, YM-VG-300C). The ion beam is then passing through a stainless-steel skimmer (7), and is focused by electrostatic lenses into the first octapole-ion-beam guide (QDF1) (10), and is admitted into the second octapole-ion-beam guide (QDF2) (11). High transmission efficiency for slow ions is accomplished by applying a radio frequency (RF) field to the QDFs. The QDF2 in the fourth vacuum chamber is joined to the first and second quadrupole ion deflectors. The fourth chamber is evacuated with a 400 L/s turbo molecular pump (SEIKO SEIKI, STP-400). The difference of dc voltages between the skimmer and the QDF2 corresponds to the kinetic energy of ions passing through QDF2. After passing through QDF2, the parent and product ions are deflected 90° by the second quadrupole ion deflector (QDF2) (12), and are admitted into the second quadrupole mass spectrometer (QMASS2) (ABB EXTEL, 652601) (13). The ions are detected by a channeltron electron multiplier equipped with a conversion dynode (14). By adjusting dc voltages of QDF2 and QDF2, the collection efficiency of product ions having a sizable kinetic energy spread is maximized. Signals from the detector are fed into a preamplifier (Stanford Research Systems SR445). Single ion pulses are subsequently scored by a two-channel gated photon counter (Stanford Research Systems SR400), which is synchronized with the laser beam. Data acquisition and instrument control are managed by a personal computer through GPIB interfaces. Mass spectra showing the distribution of ions produced with ESI are obtained by switching off the DC voltage of QMASS1 and scanning QMASS2.

Mass spectra of photofragments are recorded by measuring production yields of the fragment ions. The laser beam is introduced into QDF2 collinearly and counterpropagatedly with the ion beam. We use the 2nd or the 3rd harmonic of a YAG laser as light sources of the photolysis. In order to improve the intensities of the photodissociation spectra, the parent ions are trapped in QDF1 (8). The gas cell is filled with helium and kinetic energies of the parent ions are reduced by multiple collisions with helium atoms. By getting up DC voltage of QDF1, the trapped ions are extracted from QDF1 to synchronize with the photolysis laser. Typical pulse width of the ions is 0.5 ms. Intensities of the photodissociation spectra are

---

**Fig. 1.** Schematic diagram of the tandem mass spectrometer with ESI source; (1) vaporizing chamber, (2) syringe pump, (3) electrospray needle, (4) drying chamber, (5) solvation chamber, (6) stainless steel capillary, (7) skimmer and electrostatic lenses, (8) first octapole ion-beam guide with gas cell, (9) first quadrupole mass filter, (10) first quadrupole ion deflector, (11) second octapole ion beam guide, (12) second quadrupole ion deflector, (13) second quadrupole mass filter, (14) channeltron with conversion dynode, (15) quartz window.