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International Journal of Mass Spectrometry 241 (2005) 11–23
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Using electrospray ionization-mass spectrometry/tandem mass spectrometry and small molecules to study guanidinium–anion interactions

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Received 13 September 2004; accepted 21 October 2004
Available online 26 November 2004

Abstract

A previous study highlighting the interaction between guanidinium- and phosphonate-functionalized molecules and the development of a screening protocol for noncovalent interactions using ESI-MS and MS/MS methodologies is extended here to incorporate sulfonate- and carboxylate-functionalized binding partners for guanidinium. Multiple high order homomeric and heteromeric adduct ions are observed in the mass spectra when mixtures of complementary analytes are ionized. Comparison of relative binding and ionization efficiencies are made using the solution-phase competition methods and gas-phase collision threshold dissociation ($E_{1/2}$) measurements. Transmission factors are determined to compare the effect of structural variation of the analytes on their relative ionization efficiencies. Results indicate that while phosphonate- and sulfonate-functionalized analytes form more and higher order adduct ion complexes with guanidinium-containing molecules (represented here by free and modified arginines) as a result of the ESI process, when solvent is removed and collisional dissociation is employed, the trend is reversed, and the carboxylate group yields a stronger interaction with guanidinium, relative to the other oxoanions. Ionization differences reflected in the mass spectra are attributed to pH effects present in the condensed phase, whereas differences in stability measured in the gas-phase are attributed to the gas-phase acidities of the oxoanions and their geometric complementarity when forming noncovalent interactions with guanidinium. This work highlights the interaction of guanidinium with oxoanion binding partners using various ESI-MS and MS/MS methods, but also addresses explicitly the advantages and disadvantages of using small molecule analytes for routine analysis of noncovalent interactions.

Keywords: Guanidinium; Oxoanion; Small molecule; Noncovalent interaction; Adduct ion; Electrospray

1. Introduction

The propensity of noncovalent interactions in biological systems and the interest in studying novel structures and functions of molecules in this setting has created an impetus for the development of efficient, effective, and information-rich methods and techniques of analysis. Of the more common techniques used for studying these interactions, soft ionization mass spectrometry, specifically electrospray ionization-mass spectrometry (ESI-MS), has shown the greatest development in the last several years. Several comprehensive reviews have been published which cover this topic [1–8]. These reviews detail a plethora of methodologies which have become commonplace in the analysis of noncovalent complexes between a large variety of different molecule types. Still, in light of the wealth of information which exists, the study of small molecule interactions, specifically designed to highlight complementarity between different functional groups, has been investigated to a lesser extent. Analysis of amino acid and peptide clustering [9–11] and the use of transition-metal mediated systems for conformational and configurational determinations [12–16] are the most

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doi:10.1016/j.ijms.2004.10.019

prevalent small molecule investigations of noncovalent interactions encountered in the literature. What has become apparent is that through careful consideration of the electrospray ionization process and the choice of suitable interaction systems of interest, useful information can be extracted. Current work in our laboratory is focused upon understanding the interactions between the basic guanidinium functional unit and complementary acidic functional groups and development of the methodology to do so.

The proton-loving guanidinium group is present in a vast number of naturally occurring and synthetic biologically and pharmacologically relevant interaction systems [17–27]. This functional unit, composed of a forked, Y-shaped, planar geometry is known to be capable of both directed hydrogen-bonding, as well as nondirected Coulombic interactions with complementary groups [17,20]. In biological environments, referring to amino acids, peptides, and proteins as the most dominant species, guanidinium is most commonly encountered in the side chain of arginine and arginine residues. Here, interacting partners are composed mainly of acidic carboxylate, phosphate and sulfate groups. These anionic groups can be present as the side chains of aspartic and glutamic acid residues (carboxylate) or as a result of post-translational modification (phosphorylation and sulfation). Together, interactions between these units in biological systems are important for processes such as protein stabilization, RNA messaging, membrane transport of small and large biomolecules, and enzymatic catalysis, to name a few [20,24,25]. In synthetic systems, other variations of both guanidinium (free or cyclized) and anionic interacting partners (phosphonate, sulfonate, acid esters, etc.) may be encountered. These systems are designed for a variety of purposes, including pharmaceutical and bio-pharmaceutical (drugs, synthetic peptides, etc.) utility [22], as well as selective recognition (receptor–ligand, host–guest, etc.) and sensing [26,27], often mimicking biological schemes. Overall, studies of the interaction between guanidinium-based units and complementary anionic groups, particularly those resulting from phosphorylation events, currently comprise a relevant and analytically interesting topic in biochemical, pharmaceutical, and other related fields.

In this regard, we have recently published a study of the interaction between guanidinium and the phosphonate group using amino acids and various ESI-MS and tandem mass spectrometry (MS/MS) techniques [28]. The use of small molecules (previously, free and blocked arginine and aminophosphonic acid analytes) and mass spectrometry to assess noncovalent interactions between specific functional units has several advantages and disadvantages. In contrast to large molecules, where the cooperativity of multiple interaction sites precludes the determination of the role of each specific functional unit by ESI-MS, small molecules allow a more simplified and direct approach to isolating connectivity between two interacting partners. Analytes, such as amino acids, are useful because ionizable sites can be easily modified (e.g., C- and N-terminal blocking) to: (a) study that group's effect on the binding of a partner analyte; and (b) isolate functional units (in our previous work, the guanidinium and phosphonate groups [28]), making them the dominant interaction sites in a system under study. By systematically varying the analytes of interest, MS-based analysis techniques can be applied to screen specific and nonspecific interactions in a large number of complementary systems for comparison of different functional group interactions. In addition, the established mass spectrometric techniques are widely varied in their approach and the information which they provide; offering versatility to the experimentalist focused upon new systems of interest. Although this is appealing, disadvantages to performing experiments based upon small molecule interaction analysis by ESI-MS do exist. Inherently, the structure of each analyte greatly affects the efficiency by which it can be transferred from solution to the gas-phase during the electrospray process [29]. These changes in ionization efficiency are also apparent when comparing ionic complexes formed ("adduct ions") which incorporate different analytes. To minimize this effect, analytes with similar structure must be used and careful consideration of the affect of each of their ionization efficiencies must be made. Also, in cases where several ionizable sites on small molecules exist, the multiple interaction equilibria present, both in solution and during ESI and gas-phase processes, can result in complex mass spectra. This can hamper interpretation of the spectra as well as application of simple models or assumptions, useful when applying many of the established MS-based techniques for analysis. For example, the application of the equilibrium partition model [30] for predicting ionization response in a simple host–guest scheme, such as reported by Sherman and Brodbelt, becomes extremely difficult [31]. Still, concepts based on assessing the molecular and complex activities and relative partition factors inside the droplet are valid, even if they cannot be quantitatively elucidated. In general, useful information can be extracted from such systems through careful choice of experimental procedures, as well as explicit consideration of the effect of the ionization process on what is observed in the mass spectra.

The common methods for qualitative and quantitative analysis by ESI-MS and MS/MS can be separated into solution and gas-phase methods [7]. Solution-phase methods are designed for probing information about preformed complexes in solution by measuring ion abundances observed in the mass spectra. These include competition [3,7,32–34], titration [35–37], and temperature-dependent methods [38,39]. In these approaches, where specific information about interaction equilibria in solution is not known, assumptions must be made which state that the solution-to gas-phase transfer of a bound ionic complex is equal to that of the free, unbound host. This is often valid for large molecules, but is problematic for small molecules where the host–guest complex is often twice the size of either the host or the guest by itself. In such cases, gas-phase methods may offer a better approach for quantitatively evaluating interaction