Presented at the 51st ASMS Conference, Montreal, Quebec, Canada, June 8 - 12, 2003.

Mass Spectrometric and Molecular Modeling Investigation of Alkali Metal Ion Cationization from the Liquid Phase

James A. Kelley[1], Lawrence R. Phillips[2], Christopher C. Lai[1], Tracy L. Wolfe[3], Guangyu Sun[1] and Marc C. Nicklaus[1]

[1]Laboratory of Medicinal Chemistry, CCR, National Cancer Institute, NCI-Frederick, Frederick, MD 21702
[2]Biological Testing Branch, DTP, National Cancer Institute, NCI-Frederick, Frederick, MD 21702
[3]SAIC-Frederick, Frederick, MD 21702

Introduction

Alkali metal ion cationization can be observed to varying degrees for all methods which involve ionization from a condensed phase. Under normal circumstances, cationization of this type is not desirable because it can decrease sensitivity and/or complicate spectral interpretation. However, for certain circumstances and classes of analytes, such as oligosaccharides and sesquiterpenoids, selective and controlled cationization can confer an analytical advantage. Exploratory studies on potential antitumor agents, which were trioxane dimers derived from the antimalarial natural product artemisinin (qinghaosu), required alkali metal ion cationization before useful ESI or FAB mass spectra could be obtained. This led us to ask what factors control cationization during ionization from the liquid phase and what is the extent to which this process can be manipulated to provide desired analytical information.

Methods

Artemisinin (M_r = 282), dihydroartemisinin (M_r = 284), arteether (M_r = 312), an artemisinin-derived 1,2,4-trioxane dimer (M_r = 594) and a tetra-O-acetyl disaccharide derivative (M_r = 649) were employed as model compounds. For FAB/MS studies, 1.0 µl of a 0.05 M methanol/H_2O (9:1) solution of the alkali metal (Li^+, Na^+, K^+, Rb^+, Cs^+) chloride or acetate was added to a partially evaporated solution of the analyte before addition of the 3-nitrobenzyl alcohol (NBA) matrix. Cation competition for analyte was evaluated by using a solution that was 0.017 M in each of three alkali metal acetates. Spectral scans were averaged and the NBA background was subtracted before comparison. For ESI/MS studies, 0.1 mM aqueous solutions of the alkali metal acetates were mixed with 65% CH_3CN for isocratic elution of the analytes from a 3.2 X 15 mm C_{18} column at 0.7 ml/min. Cation competition for analyte during ESI/MS was evaluated by using a solution 0.033 mM in each alkali metal acetate. Positive ion ESI mass spectra were obtained on an Agilent ion trap. Initial quantum chemical calculations were carried out on dihydroartemisinin using density functional theory (DFT) and comparisons were made on the basis of relative energy. The geometries of dihydroartemisinin, both protonated and cationized (Li^+, Na^+, K^+) dihydroartemisinin, and hydrated cation complexes (H_2O = 0 - 6) were optimized at the B3LYP level of theory. The medium basis set 6-31G* was used for all atoms except the alkali metal cations for which the 6-311+G** basis set was employed. All calculations were done using the program Gaussian 98.

Results

Formation of MH^+ by FAB/MS was quite pronounced for artemisinin and arteether, but was lost in the chemical noise for dihydroartemisinin, the trioxane dimer and the disaccharide derivative. In contrast, ESI/MS showed no evidence of MH^+ for artemisinin or its derivatives, although some cationization was observed from residual Na^+ and K^+ . Artemisinin cationization with K^+ exceeded that of the other alkali metal ions in FAB/MS when added singly but was only slightly favored over Cs^+ when equimolar solutions of all the ions were used. For dihydroartemisinin, cationization with Rb^+ was slightly favored when employed individually, but cationization with K+ strongly predominated when using a “cation soup” of Li, Na and K acetates (Figure 1A). An interesting feature of the artemisinin FAB spectrum was the prominence of the dimer $(M_2+H)^+$, which persisted in the presence of all the alkali metal cations and which itself underwent cationization with a preference for Li^+ and Na^+. This same preference for dimer formation with Li^+ and Na^+ was also noted in ESI/MS, where these adducts were the most abundant species; K^+ and Cs^+ tended to favor monomer adducts. For ESI/MS analysis of dihydroartemisinin, the preference for cationization of the dimer included K^+ as well as Li^+ and Na^+, while cationization with Na^+ predominated in the “cation soup” (Figure 1B). The 1,2,4-trioxane dimer, which results from the tethering of two dihydroartemisinin molecules via an ethylene bridge, showed a slight preference for Cs^+ over K^+ in FAB/MS and an overwhelming preference (>6:1) in ESI/MS.

We focused our molecular modeling analysis on dihydroartemisinin since it is the simplest reduced artemisinin derivative. Gas-phase protonation and energy minimization predicted facile loss of water (Figure 2), exactly what is observed in FAB/MS. For gas phase cationization, binding energies followed the order $Li^+ > Na^+ > K^+$, the opposite of what was observed in the mass spectra of dihydroartemisinin. Since the mass spectra most likely represent, either wholly or partially, the state of the compounds in the condensed phase, we undertook a consideration of cation solvation in our calculations. A simple cation exchange scheme was employed to evaluate the stability of the solvated cation-dihydroartemisinin complex. The predicted energy change of this reaction favors K^+ complex formation (Figure 3) with a single dihydroartemisinin when the cations are solvated by 3 or 4 water molecules (Figure 4). Further studies are underway to refine this model and extend it to cationization of "dimers".

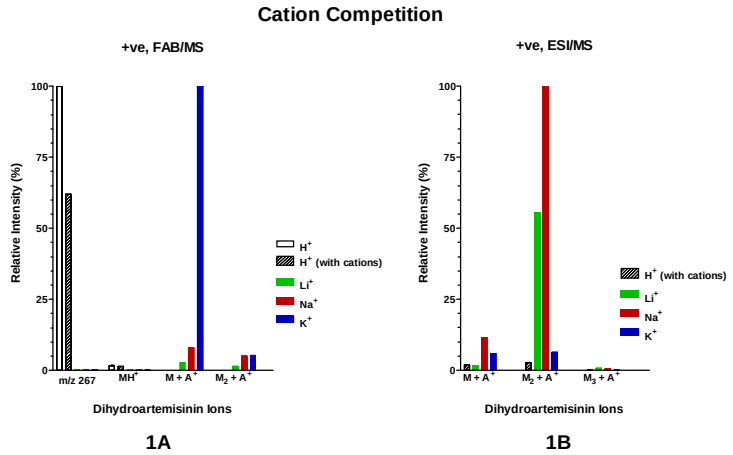

Figure 1. A solution equimolar in Li^+, Na^+ and K^+ was added to the matrix (FAB) or mobile phase (ESI).

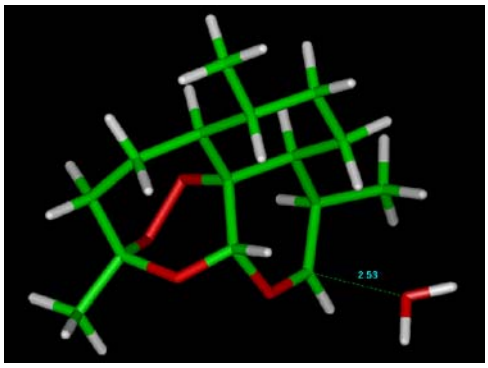

Figure 2. DFT-optimized structure of protonated dihydroartemisinin.

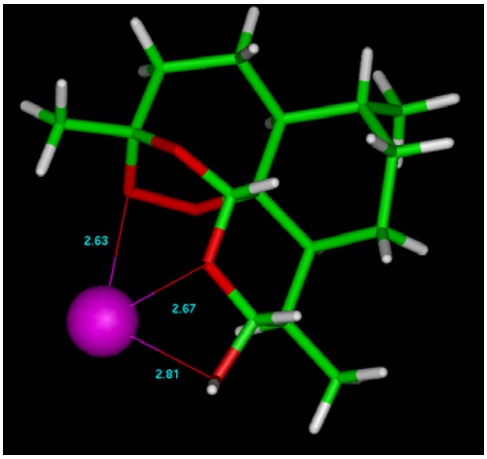

Figure 3. DFT-optimized complex of K^+-dihydroartemisinin.

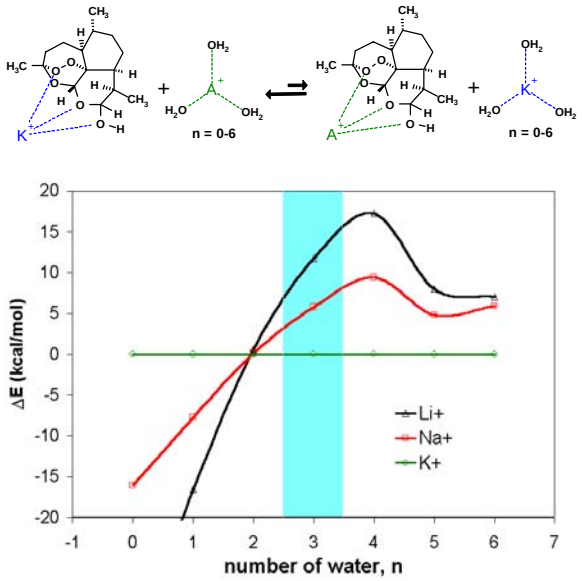

Figure 4. DFT-predicted energy change for the above cation exchange reaction.

Conclusion

Cation adducts formed during ionization in the liquid phase represent at least partially the state of the analyte in the matrix.