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RESEARCH ARTICLE

Studying Chemistry in Micro-compartments by Separating Droplet Generation from Ionization

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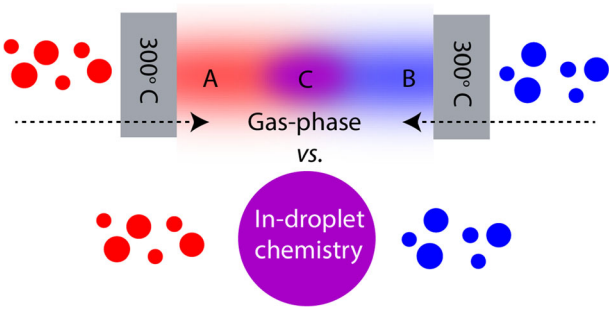

Abstract. Recent studies show that reactions inside micron-sized compartments (e.g., droplets, emulsions) can proceed at significantly accelerated rates and with different mechanisms compared to the same reactions in a macroscopic container. Many of these studies use electrospray ionization (ESI) to both generate droplets and to quantify, via mass spectrometry (MS), droplet reaction kinetics. The highly charged and rapidly evaporating droplets produced in ESI make it difficult to examine precisely the underlying cause for droplet-induced rate enhancements. Additionally, interpretation of the spectra from ESI-MS can be complicated by gas-phase ion-molecule and clustering reactions. Here, we use an approach where droplet generation is separated from ionization, in order to decouple the multiple possible sources of acceleration and to examine more closely the potential role of gas-phase chemistry. The production of sugar phosphates from the reaction of phosphoric acid with simple sugars (a reaction that does not occur in bulk solution but has recently been reported to occur in droplets) is measured using this approach to compare reactivity in droplets (i.e., with compartments) with that in the gas phase (i.e., without compartments). The same product ions that have been previously assigned to in droplet reactions are observed with and without compartmentalization. These results suggest that in some cases, gas-phase processes in the ionization region can potentially complicate the quantification and interpretation of accelerated reactions in droplets using ESI-MS (or one of its variants). In such cases, contributions from in-droplet chemistry cannot be ruled out, but we demonstrate that gas-phase processes can be a significant (and possibly dominant) reaction pathway. We suggest that future studies of rate acceleration in droplets be modified to better assess the potential for non-droplet-related processes.

Keywords: Electrospray ionization, Direct analysis in real time (DART), Rate acceleration, Droplet reactions

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Introduction

An increasing number of studies suggest that chemical reactions in micrometer-sized compartments occur at significantly enhanced rates compared to those in bulk solution [1–19]. The mechanism for these rate enhancements is unclear. For simplicity, many of these studies [3–13] use electrospray sources to produce highly charged, rapidly evaporating droplets, thereby accessing concentration and pH extremes far beyond what can be obtained in bulk solutions [1]. Extremes in concentration and pH produced by evaporation could explain many of the observed in-droplet rate enhancements since many of these reactions proceed via acid-catalyzed reaction mechanisms. In contrast, rate enhancements are also observed in studies where droplets are uncharged and evaporation does not occur, for example, in oil-water emulsions [14], droplets levitated by the Leidenfrost effect, [15, 16] and droplets levitated in an acoustic trap [17] or electrodynamic balance [18].

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These experiments, in particular, suggest that the droplet interface could also play a role in changing reaction energetics and enhancing reaction rate constants.

Experiments using electrospray to generate droplets and to study reaction rate enhancements assume that in-droplet reactions stop when they enter the mass spectrometer [3–10], or when they are deposited onto a surface [2, 12]. Thus, new ions that appear in mass spectra are attributed to in-droplet chemistry. This interpretation can be complicated in electrospray ionization mass spectrometry (ESI-MS) by unwanted gas-phase ion-molecule reactions [20], ion clustering [7, 8, 21], and thermally activated reactions on the heated MS inlet [11]. While these processes have been eliminated in some studies where the reactants had an appreciable vapor pressure and were easily introduced to the gas phase [13], it is often difficult to distinguish potential gas-phase processes from in-droplet reactions using mass spectrometry alone, especially in cases where reactants have low vapor pressures.

To better elucidate the possible mechanism of rate enhancements in compartments, we have developed an approach whereby uncharged droplets are generated and decoupled from ionization. Uncharged droplets are generated using an aerosol atomizer and then separately ionized using a direct analysis in real time (DART) ionization source. Unlike ESI, DART directly ionizes species in the gas phase and formally ensures that all uncharged, in-droplet chemistry is stopped prior to entering the mass spectrometer. Furthermore, if two aerosol atomizers are used, it is possible to separate reactants into different compartments and only allow them to mix in the gas phase within the ionization source, allowing potential gas-phase chemistry to be isolated from in-droplet reactions using this approach. With this technique, it is therefore possible to unambiguously determine whether gas-phase processes may be contributing to observed rate enhancements. Additionally, by separating droplet formation from ionization, this approach, in principle, allows for the independent investigation of the many factors, such as the interface, charge, and evaporation, which may underlie the observed rate enhancements in micron-sized compartments.

We have used our setup to study the phosphorylation of sugars by phosphoric acid (Figure 1). In bulk solution, the production of sugar phosphates (via reaction with phosphoric acid) is both thermodynamically unfavorable and kinetically slow. However, it was recently reported that reaction proceeded rapidly in ESI droplets [7]. The nature of this observed rate enhancement is further examined here.

Methods

A schematic of the experimental setup is shown in Figure 2. In contrast to the highly charged droplets from an ESI source (Figure S1a), uncharged streams of droplets (average diameter, $D \sim 1.1$ μm, Figure S2) are generated using an atomizer (Model 3076, TSI, Inc.). The experimental setup can be run in two different configurations. The first configuration (termed "reactants in the same compartment" and shown in Figure 2a) is used to study in-droplet reactions. In this mode, 12.5 mM phosphoric acid and 12.5 mM sugar (glucose, ribose, or fructose) are mixed, atomized, and vaporized together (i.e., reagents in the same compartment). In the second configuration (termed "reactants in different compartments" as shown in Figure 2b), the sugar (12.5 mM) and phosphoric acid (12.5 mM) are atomized separately and never reside in the same droplet, in order to determine if peaks observed in the mass spectra arise from purely gas-phase chemistry in the ionization source. This is accomplished using two different atomizers and two different heaters, so that the reactants are only allowed to mix as gases within the ionization source. The outlets of the two heaters are directed toward one another (separated by ~2 cm), and the vaporized particle flows (700 sccm each) mix in the ionization source. Because particles are generated at low concentrations from the atomizer (~1000 μm/m^3), the vaporized reactants exist at very low concentrations within the ionization source (~20 ppb) and there are no expected solvent effects in this configuration. To confirm no condensed phase chemistry is possible in this configuration, a reaction which can only occur in solution (2,6-dichlorophenolindophenol (DCIP) oxidation by ascorbic acid) was studied [4]. No reaction was observed when DCIP and ascorbic acid were mixed in the gas phase (details in Supporting Information).

In both configurations, the vaporized gas-phase species are introduced to the DART ionization source of a mass spectrometer (Q-Exactive Orbitrap, Thermo Fisher, Inc.). This decoupling of ionization from droplet formation allows for a wider array of ionization sources to be used to probe in-droplet reactions. Unlike ESI, DART uses metastable helium atoms to

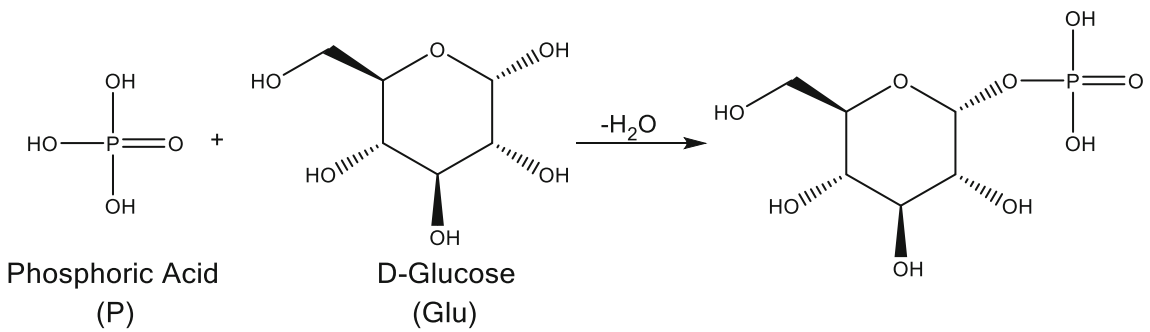

Figure 1. Droplet-induced glucose and phosphoric acid reaction scheme reported by Nam et al. [7]

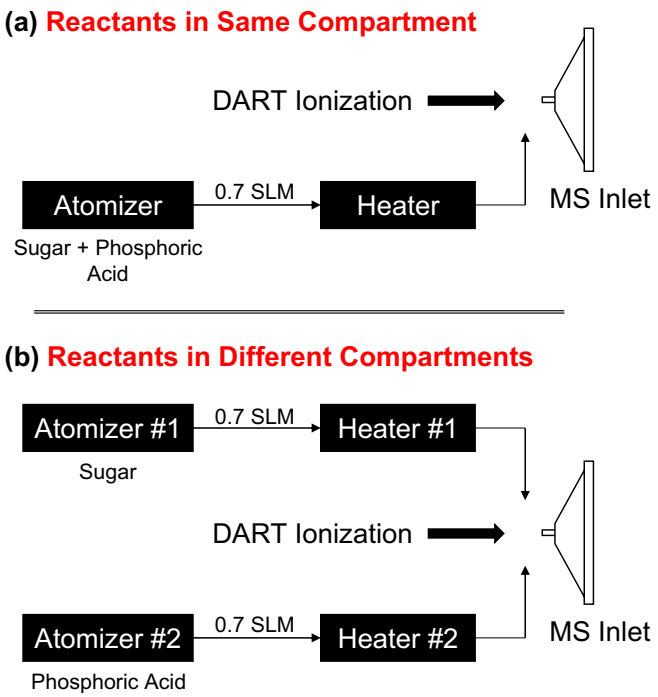

Figure 2. (**a**) To study in-droplet chemistry, reactants can be atomized together and put in the same compartment. (**b**) To study potential gas-phase processes, reactants can be atomized separately into different compartments and are only mixed in the gas phase within the ionization source

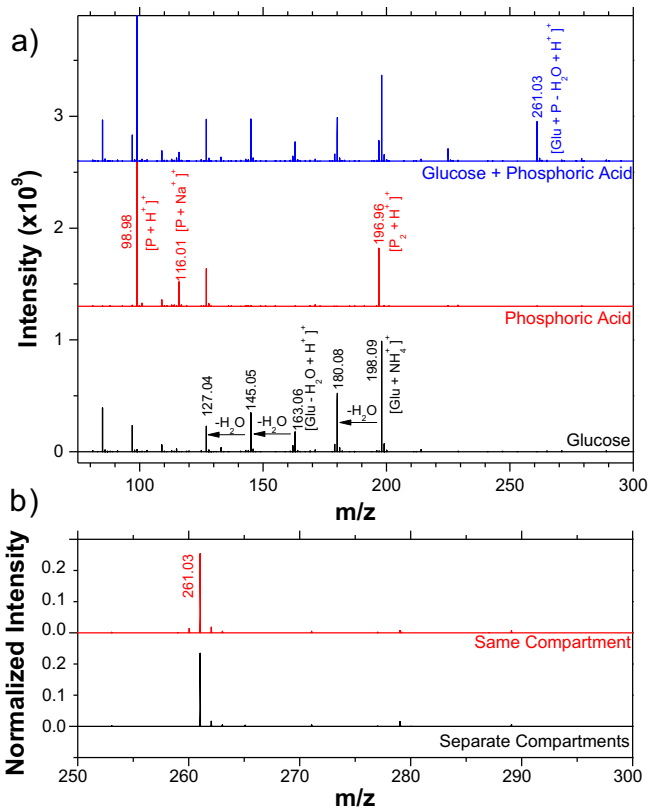

Figure 3. (**a**) DART mass spectra of 12.5 mM glucose solution (black line), 12.5 mM phosphoric acid solution (red line), and 12.5 mM glucose and phosphoric acid mixed within the gas phase (separate compartments, Figure 2b). (**b**) Comparison of product ion formation (*m/z* 261.03) when glucose and phosphoric acid are mixed in the same compartment (red line) and when kept in separate compartments but mixed in the gas phase (black line). Mass spectra are normalized to phosphoric acid peak intensity (*m/z* 98.98)

ionize molecules and is purely a gas-phase ionization source [22]. Thus, all potential in-droplet reactions in the ionization source are removed so that gas-phase processes can be observed directly.

For comparison and to reproduce previous results, the phosphorylation reaction was also conducted in droplets generated from an ESI source (Figure S1a). Details and results from the ESI source are given in the Supporting Information (Figure S1b).

Results and Discussion

To provide a point of comparison, we replicated the experiments [7] reporting in-droplet reaction between glucose and phosphoric acid using ESI (see Supporting Information and Figure. S1a for details) and observed the formation of a new ion at *m/z* 261.03 when the two reactants are contained in the same droplet (Figure S1b). This new ion matches the molecular weight of the expected glucose phosphate product and matches what has been previously observed [7]. Further details of this experiment are given in the Supporting Information.

The atomizer setup described in the "Methods" section was used both to study the phosphorylation reaction of glucose in uncharged droplets and to control for gas-phase processes. Reference mass spectra of each reactant (glucose and phosphoric acid) are shown in Figure 3a. The mass spectrum of glucose is consistent with what has been previously observed using DART ionization [23]. The largest ion (*m/z* 198.09) is a cluster between glucose and an ammonium ion, and the smaller fragments originate from successive H_2O loss. The main peaks in the phosphoric acid mass spectrum are assigned to the protonated monomer (*m/z* 98.98) and dimer (*m/z* 196.96) of phosphoric acid. No product ion at *m/z* = 261.03 is observed when each of the reactants are introduced separately into the ionization source.

To study the reaction in uncharged droplets, we used the setup shown in Figure 2a and atomized glucose and phosphoric acid together. When the reactants were in the same compartment, a new ion is observed at *m/z* 261.03 (Figure 3b). This observation is consistent with ESI experiments and previously reported glucose phosphate production [7]. Although the mass spectra suggest the phosphorylation reaction proceeds similarly in uncharged droplets as charged droplets produced by ESI, the "reactants in the same compartment" configuration alone cannot clarify whether the new ion at *m/z* 261.03 originates from in-droplet chemistry or gas-phase processes.

To examine potential gas-phase processes, we used the setup shown in Figure 2b and mixed the sugar and phosphoric acid as gas-phase species within the ionization source. In this arrangement, the reactants never reside together in same

compartment. In this configuration, the same new ion (m/z 261.03) is observed with a similar normalized intensity (Figure 3 with additional details shown in Figure S5), suggesting that in-droplet chemistry or compartmentalization is not necessary for its formation. To assess whether metastable helium atoms or other ions (e.g., hydronium ion) produced by the DART ionization source could be driving the gas-phase reaction, a second ionization source (extractive ESI) was used to probe potential gas-phase processes (experimental details in Supporting Information). The same new ion (m/z 261.03) was observed with the extractive ESI source (Figure S6), suggesting its formation in the gas phase is not driven by heated DART stream.

Collision-induced dissociation (CID), which can give structural insight into the nature of ions, shows that the ion of m/z = 261.03 that is formed in all three experimental configurations (i.e., ESI, reactants in the same compartment, and reactants in different compartments) is similar in structure (details in Supporting Information). Thus, we conclude that it is not possible to unambiguously attribute the formation of the peak at m/z = 261.03 to in-droplet reactions—as the ion can clearly be formed by gas-phase chemistry alone. A similar series of experiments were conducted for the phosphorylation of ribose and fructose by phosphoric acid (Figures S8 and S9) yielding similar results as the glucose reaction.

Conclusion

We have developed a new approach to study the compartmentalization of chemical reactions whereby droplet generation is separated from ionization. This setup has at least two distinct advantages over previous ESI droplet sources: (1) reactants in separate compartments can be introduced to the ionization region simultaneously, and (2) the uncharged droplets can be treated before ionization to distinguish between the multiple sources of rate enhancement. Introducing reactants to the ionization source in separate compartments serves as a control to ensure that new peaks attributed to in-droplet reaction products are derived solely from in-droplet reactions rather than gas-phase ion clustering, ion-molecule reactions, or reactions in the heated inlet to the mass spectrometer. With this additional control, we have demonstrated that ions previously attributed to in-droplet products from reaction between sugars and phosphoric acid can also originate from gas-phase chemistry. We have also demonstrated that other reactions which have shown rate enhancements in droplets (such as the oxidation of DCIP by ascorbic acid) can only occur in droplets, suggesting some reactions stop when reactants enter the gas phase (Figures S3 and S4), further demonstrating the utility of this technique as a method to evaluate the role of in-droplet and gas-phase chemistry in observed accelerations [4].

Additionally, because the method reported here uses uncharged, atomized droplets, this new approach could be used to independently study rate enhancement from interfacial and concentration effects for systems that show unambiguous rate enhancement in droplets. Interfacial effects could be studied by changing the surface area to volume ratio of the droplets via size selection (e.g., through use of a differential mobility analyzer), and concentration effects could be studied by controlling for evaporation with the addition of an inert substance to change solvent activity (e.g., addition of an inert salt as previously demonstrated) [18]. Going forward, the underlying mechanisms explaining enhanced rates in microcompartments can only be elucidated with more controlled experimentation.

Acknowledgments

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