



Peptide synthesis in aqueous microdroplets

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Organic chemistry on water has been shown to occur with large changes in reaction rates, products, and mechanisms as compared with the associated bulk aqueous chemistry (1–8). Chemistry “on” water refers to chemistry occurring between water and any nonpolar media such as oil or air. While detailed mechanisms of chemistry on water are still controversial (1, 9–15), investigations of the structure, charges, and hydrogen bonding at the water surface attempt to elucidate the unique nature of water–air interfaces, which have been proposed to be auspicious environments for organic reactions (1, 16–19). Holden et al. (2) make a significant contribution in this field, with the synthesis of peptides at the surface of microdroplets.

While reaction acceleration at water surfaces has been studied using experimental models in the laboratory, the implications are especially impactful for environmental chemistry, biology, and synthetic chemistry. Water–air interfaces are prevalent in natural environments (Fig. 1), including the surfaces of lakes, oceans, sea sprays, atmospheric aerosol particles, cloud drops, etc. (18, 19). In contemporary Earth's environment, a major source of complex organic molecules, both in the atmosphere and in living systems, stems from biology. However, abiotic processes, such as those that would have been present on prebiotic Earth, would have had to find a way to overcome thermodynamic and kinetic obstacles to biomolecular synthesis in the absence of enzymes. It is commonly agreed that the simple monomers required for life (nucleosides, amino acids, etc.) would have been present in liquid water on prebiotic Earth, but the synthetic chemistry required to make larger biopolymers (nucleotides, peptides, lipids, etc.) would have been difficult to achieve because of the thermodynamic and kinetic constraints in bulk aqueous environments. To this end, environmental water–air interfaces have been proposed to overcome the constraints faced by biomolecular synthesis in bulk water (18–20). Specifically, condensation reactions which occur with concurrent water elimination, such as peptide bond formation (2, 3, 5) or the phosphorylation of sugars (6, 21), have low equilibrium constants and positive free energy changes ($+\Delta G$) in bulk water as shown in Fig. 1A. However, recent studies using microdroplets have indicated that condensation reactions can occur with much higher rates at water surfaces (2, 5, 6, 19, 21).

Holden et al. (2) add to this field with an elegant study of the abiotic synthesis of peptides, an essential reaction for prebiotic chemistry, at the water–air interface of micrometer-sized water droplets. Peptide synthesis from free amino acids involves the simultaneous release of a water molecule and thus does not occur in bulk aqueous solution where $\Delta G > 0$. In this study (2), however, the microdroplet surface overcomes low bulk concentrations of precursors and low equilibrium constants. The size of the microdroplets used in this laboratory study are analogous to the expected average size of atmospheric aerosol

particles that would have been ubiquitous on a rotating planet with a liquid ocean, such as ancient Earth (18–20). Consequently, results of this study have important implications for prebiotic chemistry. In the modern Earth atmosphere, the total surface area of aqueous aerosols is estimated to be two orders of magnitude larger than all other available water surfaces. Given that this scale was likely similar on prebiotic Earth, microdroplet studies from Holden et al. and others point to the abundant water–air interfaces of atmospheric aerosols as auspicious reaction environments on prebiotic Earth (2, 18, 20).

Using nanoelectrospray ionization (nESI) with products monitored by mass spectrometry (MS), Holden et al. (2) observed formation of peptide isomers from neat amino acids (glycine [Gly] and alanine [Ala]) and investigated the effect of external charge, spray distance, and temperature on the product yields. In contrast with previous studies in the literature, the experiments reported here did not use activated reagents or external energy sources in the synthesis of peptides from neat amino acids. As a control experiment, Holden et al. (2) used bulk aged solutions of the neat amino acids and analyzed the results via ESI-MS. The ESI-MS did not identify dipeptide formation, showing that peptides are not formed in bulk solution and that the larger droplets generated in ESI-MS are not capable of promoting peptide formation. Holden et al. (2) therefore surmise that the higher surface-to-volume ratio in the nESI droplets is an important factor in peptide synthesis, thereby indicating the water–air interface as the key reaction environment.

In the natural environment, atmospheric aerosols can collide (Fig. 1C), forming larger coagulates. On modern Earth, atmospheric aerosols coagulate until they reach a diameter of a few microns, whereupon the gravitational pull of the planet causes them to settle back onto the surface, likely returning to the ocean. The development of droplet fusion mass spectrometric techniques (22) allows high-sensitivity, high-resolution experiments capable of capturing kinetic information for reactions that may occur due to such droplet collisions. The droplet fusion study performed by Holden et al. (2) investigates colliding

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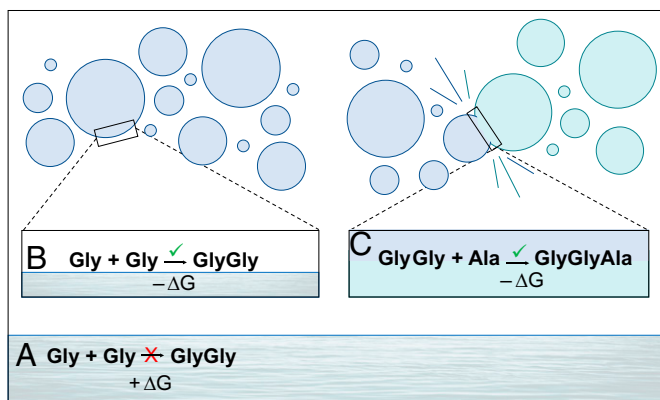


Fig. 1. Synthesis of peptides in microdroplets containing free amino acids (2) may inform on atmospheric aerosols enabling similar reactions in natural environments. GlyGly, a Gly dipeptide; GlyGlyAla, a tripeptide with two Gly monomers and one Ala monomer. (A) As shown in the reaction, aqueous systems hinder amide bond formation due to thermodynamic constraints which lead to a positive free energy change (+ΔG). (B) Dipeptides can form at the water-air interface of microdroplets (2), shown here in analogy with atmospheric aerosols, containing free amino acids. (C) Droplet fusion results in peptide extension reactions. Shown here, two independent streams of aerosols, one containing Gly and one containing Ala, form a tripeptide from both free Gly and free Ala (2). Chemistry in both (B and C) has a negative free energy change (-ΔG).

streams of Gly and/or Ala monomers in aqueous solution which produced protonated tripeptides and tetrapeptides with a lower abundance of longer peptides (2). When a plume containing aqueous Ala was collided with a separate plume containing aqueous Gly, heteropeptides were synthesized in these droplet collisions. To assess the relative importance of the water-air interface and droplet collision in forming these more complex peptides, Holden et al. (2) performed control experiments where heterogeneous mixtures (e.g., Gly and GlyGly or Gly and Ala) were studied in a single microdroplet plume. Very few longer peptides or heteropeptides were observed when only one plume was used, indicating collisions as an important source of reactivity leading to longer or more complex peptides. This study provides unprecedented detail about the products obtained in the condensation of neat amino acids on microdroplets and the reactions that may occur due to microdroplet collisions.

Ion mobility spectrometry with MS and MS/MS experiments were used (2) to compare the structure of the microdroplet synthesized peptides to the structure of

native peptides. The microdroplet synthesized dipeptides fragmented more readily and isomerized during collisional heating, indicating that the synthesized peptides were an isomer of native peptides, likely in a more condensed configuration that may be favorable at the water surface. These elegant experiments concluded not only that the condensation of neat amino acids occurs at the microdroplet surface, but also that the dipeptides generated are distinct from authentic dipeptides used as controls.

To investigate the mechanism for the microdroplet synthesis of peptide isomers from free amino acids, nESI-MS/MS was performed using low concentrations of GlyGly and AlaAla to more effectively probe molecules at the droplet surface (2). The empirical observations led Holden et al. to propose a mechanism involving five-membered oxazoliodinones and suggest that the condensation reaction is driven by the low interfacial pH and high surface electric field (11, 12) on the microdroplet. These peptide isomers are also more reactive than the native peptides, which was demonstrated by the droplet fusion experiments. Furthermore, native peptides studied using nESI-MS/MS also showed evidence of isomerization to this more compact and reactive form at the water-air interface. This demonstrates that partial solvation at the water-air interface can alter the structure of peptides, a process that could also occur on atmospheric aerosol particles in the natural environment, regardless of the initial sources of dipeptides.

The study of Holden et al. (2) provides insight into the products and mechanism of abiotic peptide bond formation at the water-air interface, a key reaction in prebiotic chemistry not possible in the bulk aqueous phase. Their results explore the unique features of the water-air interface, including partial hydration of organic reagents, which provide a favorable reaction environment for condensation reactions necessary for the abiotic synthesis of biomolecules necessary for life. By extrapolating the findings from this fundamental model study (2) to processes feasible in the natural environment, this study implies synthesis of peptides could have occurred at the water-air interface of atmospheric aerosols in prebiotic chemistry.

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