Prebiotic phosphorylation enabled by microdroplets

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The significant acceleration of reactions in confined environments has recently been reported in the literature (1–7). The reaction environments investigated span a diverse range, including charged microdroplets (1–3), microdiameter emulsions (5), inverted micelles (8), and the surfaces of aerosol particles (9). These experimental results have made the case that the surface of aqueous drops provides a special and unique reaction environment with qualitatively different thermodynamic and kinetic properties from bulk aqueous solutions. In PNAS, Nam et al. (7) report on a compelling application of accelerated reactions in aqueous microdroplets to chemistry that may have occurred prebiotically, in the absence of enzymes. They specifically address the abiotic production of sugar phosphates and uridine ribonucleoside in microdroplets, reactions that do not occur in bulk aqueous solutions. In biology, these reactions are enzyme-catalyzed. Investigations of reactions in confined aqueous environments are especially compelling in prebiotic chemistry, where nonenzymatic pathways must be found to explain the abiotic formation of biopolymers that can be used as building blocks of life (10–12). It has long been appreciated that phosphorus is important in life (13), yet abiotic phosphorylation remains an area of intense recent activity (14). The contribution of Nam et al. (7) provides a possible route for the formation of prebiotically plausible sugar phosphate in aqueous microdrops.

The experiments reported in the literature have used laboratory model systems to show different chemistry at aqueous interfaces than in bulk solutions; however, these model systems have counterparts in the natural environment (10, 15). Therefore, the thermodynamic and kinetic arguments emerging from laboratory model studies can be generalized. On the contemporary and early Earth, aqueous microdroplets and water–air interfaces are ubiquitous, as illustrated in Fig. 1. On any rotating planet with a liquid ocean, wind action at the ocean surface generates sea spray and aqueous aerosol. The most probable size for aerosol particles is given by the competition between coagulation of small particles and fast settling out of larger particles under the force of gravity. Atmospheric aerosols on Earth are commonly particles of a few micrometers in diameter (15). Some of these aerosol particles nucleate clouds and fogs, which contain aqueous drops with diameters of a few to tens of microns. Water–air interfaces are found in a number of natural environments, including the surface of oceans, lakes, and atmospheric aerosols on both the modern and early Earth. The collective surface area of aqueous aerosol particles is several orders of magnitude larger than the surface area of oceans. More than 90% of the early Earth was covered by oceans; therefore, aqueous atmospheric particles would have been present in even greater numbers than today. Their aqueous surfaces concentrate and align reagents, providing a unique hydrophobic reaction environment. During their lifetime, atmospheric aerosols experience fluctuations in temperature, relative humidity, pH, salinity, and exposure to radiation and gas phase species, as well as undergoing coagulation, hydration, and dehydration. Water–air interfaces on oceans, lakes, aerosols, and cloud and fog droplets

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provide auspicious reaction environments for reactions in the nat-
ural prebiotic and contemporary environment. In prebiotic times,
the planet was rotating significantly faster than today, with a higher
incidence of wave-breaking and aerosol production.

“Can all bulk phase reactions be accelerated in microdroplets?”
In response to this question, Banerjee et al. (1) reviewed the
literature showing reactions with accelerated rates of several or-
ers of magnitude over their bulk counterparts, as well as pro-
viding the counterexample of the intramolecular Diels–Alder
reaction in a droplet that did not give the expected product.
Nevertheless, taken together, all experiments that report on in-
terfacial aqueous chemistry point to the different reaction envi-
nronment provided by such surfaces than is available in bulk
aqueous solutions. The factors affecting these reactions, and the
mechanisms by which they proceed, while not completely under-
stood, have been considered. Concentration is one such factor
that likely contributes to the difference in reaction rates in con-
fined aqueous environments. Molecular crowding and its poten-
tial role in early evolution have been discussed for protocols
and the environment of biological cells (16, 17). In the crowded interior
of a vesicle, protocell, or microdroplet, longer range attractive
forces are more effective at producing organization, and so de-
crease a reaction’s Gibbs energy, while the repulsive forces that
produce dissipation lead to a decrease in entropy, the net effect
of which is a more favorable change in free energy and an accel-
eration in reaction rate (18). While crowding is a factor in the
observed rate changes, other contributions have been considered
(1). The high surface-to-volume ratio seems to be important in
these reaction environments, as the presence of more surface area
harnesses the unique properties of water–air interfaces, including
altered pH, surface charge, and orientation of reagents at surfaces
(9, 15, 19–23). Fallah-Araghi et al. (5) investigated the bimolecular
imine synthesis reaction in micrometer diameter emulsion dro-
plets and found that both the equilibrium constant and the rate
constant for this reaction increased as the droplet diameter de-
creased. A reaction-adsorption mechanism shows that the free
energy of binding of the reactants to the interface modifies the
thermodynamic constraints in microdroplets compared with bulk
solutions. Understanding all factors that govern reactions in aque-
ous microdroplets remains a challenge, but the advantages of
such environments, especially for prebiotic polymerization reac-
tions, are clearly demonstrated by the example of the abiotic
production of sugar phosphates and synthesis of uridine ribonu-
cleoside (7).

On Earth and elsewhere, simple organic compounds can be
generated from inorganic precursors under prebiotic constraints.
However, understanding how these simple organic molecules
can be abiotically concentrated and undergo polymerization
to generate high-energy biopolymers remains a challenge in
research on the origin of life. Because water is crucial to life, and
aqueous solutions are the preferred reaction media for bio-
chemistry, these challenges are both kinetic and thermodynamic
in nature. For example, in aqueous solutions, both polymerization
of amino acids to form peptides and polymerization of nucleo-
tides to form nucleic acids are highly unfavorable, as the
condensation of reagents is accompanied by elimination of water
(7, 9). In an aqueous solution, this chemistry has a very low equi-
librium constant and a positive free-energy change (ΔG > 0); con-
sequently, the reverse process of hydrolysis of the biopolymer is
favored. Phosphorylation as a condensation process that is essen-
tial for life (13), and whose prebiotic reactions remain a challenge
for research on the origin of life (14), is a good case study. In this
case, the successful phosphorylation of sugars to sugar-1-
phosphates in aqueous microdroplets containing mixtures of
sugars and phosphoric acid provides an important abiotic path-
way by which biopolymerization might have occurred on early
Earth (7). In this study, the experimental reaction yield provides a
negative free-energy change (ΔG < 0), lower than the value for the
same reaction in bulk solution (where ΔG > 0). Using the
temperature dependence of the reaction yield, Nam et al. (7)
further conclude that at the surface of the microdroplet, the re-
action overcomes the entropic challenge, which would be in-
surmountable in bulk aqueous solution. These results establish
that a reaction between phosphoric acid and sugars occurs with-
out an external charge, condensing matter, or organic phosphates
as an energy source. The production of uridine ribonucleoside in
aqueous micropods containing D-ribose, phosphoric acid, and
uracil demonstrates the usefulness of these reaction environments
for the abiotic synthesis of biopolymers required for life. Taken in
the context of the literature in this emerging area, the advantages
for reactions provided by microdroplets are relevant to the gen-
eration of chemical complexity in prebiotic chemistry as well as
many other fields, including synthetic chemistry, atmospheric
aerosol chemistry, and synthetic biology (1, 15, 24).

The contribution of Nam et al. provides a possible
route for the formation of prebiotically plausible
sugar phosphate in aqueous microdrops.

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