Please cite this article in press as: Mihoubi et al., Nonenzymatic autocatalysis generates coacervates and controls their structure, Chem (2023), https://doi.org/10.1016/j.chempr.2023.11.014

Chem

CellPress

Preview

Nonenzymatic autocatalysis generates coacervates and controls their structure

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Coacervate droplets are promising microcompartments for mimicking complex, life-like behavior. Autocatalytic reactions are at the basis of nonlinear reactions, such as chemical oscillations and self-replication. In this issue of *Chem*, Hanopolskyi et al. use the autocatalytic nonenzymatic guanidation of a polyamine to control the formation and structure of coacervates.

Attempts at building biomimetic systems—in an origin-of-life or synthetic biology context—often rely on three distinct approaches: metabolism first, replication first, and compartment first. Each approach implies that small reactive molecules, self-replicating nucleic acids, and self-assembling molecules, respectively, give rise to synthetic life-forms. Interestingly, Hanopolskyi et al. combined these three features by coupling a small-molecule autocatalytic reaction to the formation of compartments through liquid-liquid phase separation (LLPS).[1] The system is valuable not only for biomimicry but also as a proof of concept of the complexity achieved through nonlinear kinetics.

Autocatalytic reactions are at the basis of self-replication and therefore could have preceded genetically encoded reproduction at early stages of life. In general terms, autocatalysis is the acceleration of a reaction during its course because the products start to assist the process, leading to concentration curves with a classic autocatalytic profile: a sigmoidal shape with a slow start (lag period), a steep acceleration (exponential period), and a final plateau (saturation). The kinetics of autocatalytic reactions can enable pattern formation, waves, and other unpredictable, complex behavior. Semenov et al. had previously developed a small, organic-molecule-based oscillator that contained an autocatalytic motif (thioester-to-thiol conversion)[2]; recently, Ter Harmsel et al. developed a small-molecule oscillator by using the autocatalytic deprotection of Fmoc groups.[3]

The complexity of autocatalysis can be translated from the molecular to the microscopic level if the products are involved in supramolecular structures. The coupling of reactivity and self-assembly has focused on the generation of amphiphiles that assemble into micelles or vesicles, driving the reaction further by what is referred to as "physical autocatalysis."[4] Another type of self-assembly has recently regained momentum as a promising candidate for both primitive compartments and dynamic materials: complex coacervation. Complex coacervation is a type of associative LLPS leading to the nucleation and growth of membraneless, densely concentrated droplets and eventually to a dense coacervate macrophase. Because they form through LLPS, coacervate droplets dynamically adjust to subtle changes in chemical composition and have been largely studied as micro-reactors for their ability to host, organize, and often accelerate chemical reactions.

Things become even more interesting when the backbone of the coacervate phase itself is formed *in situ* or undergoes reactions that influence its phase-separation behavior. In this case, these coacervates are often referred to as "active droplets." There are many examples of active coacervates regulated by enzymatic and nonenzymatic reactions involving small molecules or polymers. What has been more challenging, though, is to achieve complex behavior other than control with these droplets. It was evoked early on that, since coacervation depends exactly on multivalency, such as that exhibited by polynucleotides and polypeptides, coacervate droplets would be ideal hubs for replication reactions. And, because coacervates can localize and concentrate molecules with very high partitioning coefficients, it was anticipated that they would not only support autocatalytic reactions but also enhance catalysis and thus lead to proliferating coacervate droplets.[5] The idea of autocatalysis in coacervates has frequently been proposed, but it has been realized only in the form of physical autocatalysis—Matsuo and Kurihara showed that when native chemical ligation (NCL), a reaction that is not autocatalytic by nature, produces peptides able to self-assemble into coacervate droplets, it displays autocatalytic kinetics.[6] Other examples of complex behavior with coacervate droplets include growth and division.[7]

In their work in this issue of *Chem*,[1] Hanopolskyi et al. used an autocatalytic

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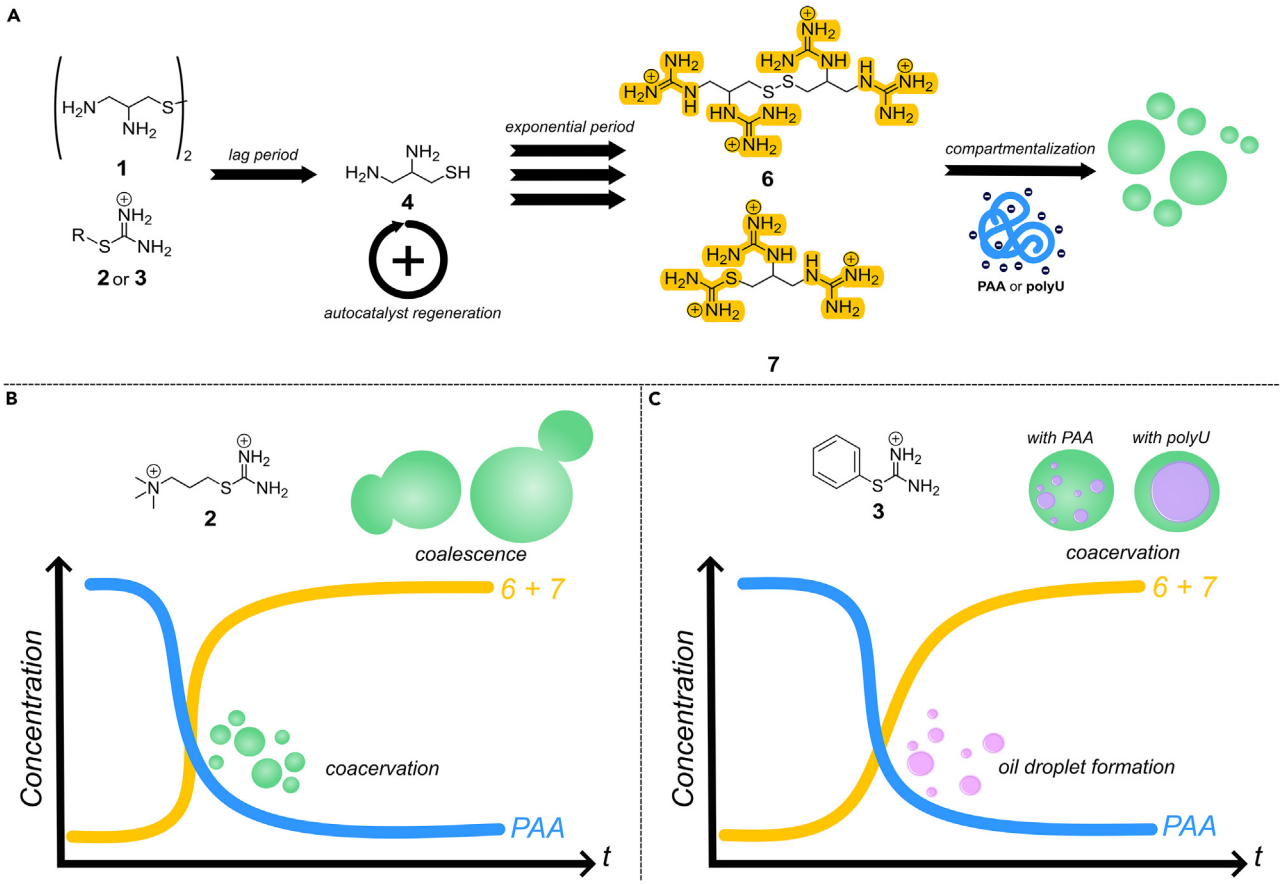

Figure 1. Autocatalysis drives the formation of multicompartment structures

(A) Reaction scheme of the tetra-guadination of **1** followed by the formation of compartments.

(B) Kinetics of the reaction between **1** and **2** and supramolecular structures generated in the presence of PAA or polyU.

(C) Kinetics of the reaction between **1** and **3** and hierarchical supramolecular structures generated in the presence of PAA or polyU.

reaction to control the formation and behavior of coacervate droplets. In order to affect complex coacervation, the charge density of the reactants must differ greatly from that of the products. The group previously developed an autocatalytic reaction that does just that: the thiol-assisted conversion of amines to guanidines (guanidation); the higher pK_{aH} of guanidium assures that it is positively charged at a wider pH range than amine moieties.[8] To work with high charge densities, they replaced the amino-disulfide in their previous work with a diamino-disulfide. With the goal of making guanidine disulfide **6** (charge +4), they started with diaminodisulfide **1** (charge +2). They also reported the formation of thiouronium ion **7** (charge +3). Compound **6**, but not **1**, phase separated with a negatively charged polyelectrolyte, such as polyacrylic acid (PAA) or polyuridylic acid (polyU) (Figure 1A).

In the presence of PAA and polyU, the guanidation reaction was still autocatalytic. The authors did not observe a significant effect of phase separation on rates or yields, even though some difference could be expected for a biphasic reaction. The formation of coacervate droplets, observed via microscopy and the decrease in the PAA concentration in the dilute phase, coincided fairly well with the formation of tetraguanidinium product **6** and side product **7** (Figure 1B). This observation confirms that complex coacervation is driven by the autocatalytic reaction. Given that the lag period of the autocatalytic reaction can be delayed with thiol "quenchers," such as maleimide, the assembly of the droplets can be timed, which had been achieved previously only with an enzymatic network.[9]

Next, the authors studied the role of the reactants' chemical structure over the course of the reaction. First, they replaced thiouronium salt **2** (derived from thiocholine) with one derived from thiophenol, compound **3**. One could imagine that the change might affect the hydrolysis rate, the reactivity of thiouronium toward aminothiols, and the partitioning of the resulting thiol in the coacervate phase (composed of **6** + **7** and PAA), which could together affect the rate of the

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autocatalytic reaction. This was indeed the case: a rate constant appeared one order of magnitude lower for **3** than for **2**. More interestingly, the authors observed the formation of diphenyl disulfide (PhSSPh), the oxidized form of thiophenol. PhSSPh partitioned poorly in both the coacervate and dilute phases, resulting in a third, oil-like phase insoluble in water. The oil-in-coacervate-in-water multiphase droplets are an interesting case study for distinguishing simple coacervates from oils. The oil droplets nucleate during the lag period and are stabilized by polyanions; possibly their interface becomes a reaction site for the initial formation of autocatalyst **4**. Coacervates composed of **6** + **7** and PAA grew around the oil cores (Figure 1C). It is remarkable that the now triphasic reaction retained the autocatalytic kinetics. In fact, the authors showed that the autocatalytic nature of the reaction was the driving force for the formation of hierarchical structures, whereas non-autocatalytic reactions formed amorphous, non-ordered aggregates.

The next point of interest is the effect of the polyanion over the reaction and the droplets formed: replacing PAA with polyU. Although the formation of RSH remained autocatalytic in both cases, PAA had a stronger effect over reaction rates than polyU. It is interesting that although one would expect the reaction to be accelerated by the formation of coacervates (because it occurs in micelle-mediated physical autocatalysis), PAA instead slowed down the reaction by 1.5×. The lower rate suggests that some reaction components are included, whereas others are excluded, from the coacervate phase or that other effects are at play, such as the polarity of the coacervate environment. What is more, in the experiments with the phenyl thiouronium salt, the nature of the polyanion determined the structure of the multiphase droplets: the oil droplets nucleated the same way, but only in the case of polyU did the droplets fuse into a single inner droplet by the end of the reaction. The difference in coalescence is a consequence of the viscosity of the coacervate phase generated, lower with polyU than with PAA, as well as less localization of polyU stabilizing the oil-coacervate interface than of PAA.

In summary, Hanopolskyi et al. have shown that a nonenzymatic autocatalytic reaction can control and time the formation of complex coacervates. The two stages of the autocatalytic reaction can produce unique multiphase colloids—a first example of oil-in-coacervate droplets. Their findings pave the way for new studies making use of autocatalysis to achieve complex behavior in coacervate droplets. The effect of autocatalysis over coacervation seems greater here than that of coacervation over autocatalytic reactions. Monitoring the dilute and coacervate phases separately would be insightful and could explain the lack of an overall effect. Moreover, such an effect would perhaps become more evident in a setting with two autocatalytic reactions—would a substrate that partitions more in the nascent coacervate phase outcompete a substrate that is excluded? Cross-catalysis between RNA replicators has been observed in spermine or PAA coacervates, and it would be interesting to explore it with small molecules too.[10] Finally, now that coacervation driven by autocatalysis has been achieved, it is natural to wonder what other motifs can be coupled to coacervation to produce even more complex behavior—transient compartmentalization and cycles of growth and division.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Hanopolskyi, A.I., Mikhnevich, T.A., Paikar, A., Nutkovich, B., Pinkas, I., Dadosh, T., Smith, B.S., Orekhov, N., Skorb, E.V., and Semenov, S.N. (2023). Interplay between autocatalysis and liquid-liquid phase separation produces hierarchical microcompartments. Chem *9*, ■–■.

2. Semenov, S.N., Kraft, L.J., Ainla, A., Zhao, M., Baghbanzadeh, M., Campbell, V.E., Kang, K., Fox, J.M., and Whitesides, G.M. (2016). Autocatalytic, bistable, oscillatory networks of biologically relevant organic reactions. Nature *537*, 656–660.

3. Ter Harmsel, M., Maguire, O.R., Runikhina, S.A., Wong, A.S.Y., Huck, W.T.S., and Harutyunyan, S.R. (2023). A catalytically active oscillator made from small organic molecules. Nature *621*, 87–93.

4. Morrow, S.M., Colomer, I., and Fletcher, S.P. (2019). A chemically fuelled self-replicator. Nat. Commun. *10*, 1011.

5. Oparin, A.A.I. (1969). Chemistry and the origin of life. R. Inst. Chem. Rev. *2*, 1.

6. Matsuo, M., and Kurihara, K. (2021). Proliferating coacervate droplets as the missing link between chemistry and biology in the origins of life. Nat. Commun. *12*, 5487.

7. Slootbeek, A.D., Van Haren, M.H.I., Smokers, I.B.A., and Spruijt, E. (2022). Growth, replication and division enable evolution of coacervate protocells. Chem. Commun. *58*, 11183–11200.

8. Novichkov, A.I., Hanopolskyi, A.I., Miao, X., Shimon, L.J.W., Diskin-Posner, Y., and Semenov, S.N. (2021). Autocatalytic and oscillatory reaction networks that form guanidines and products of their cyclization. Nat. Commun. *12*, 2994.

9. Nakashima, K.K., Baaij, J.F., and Spruijt, E. (2018). Reversible generation of coacervate droplets in an enzymatic network. Soft Matter *14*, 361–367.

10. Ameta, S., Kumar, M., Chakraborty, N., Matsubara, Y.J., S, P., Gandavadi, D., and Thutupalli, S. (2023). Multispecies autocatalytic RNA reaction networks in coacervates. Commun. Chem. *6*, 91.