SELF-REPLICATING MICELLES — A CHEMICAL VERSION OF A MINIMAL AUTOPOIETIC SYSTEM

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Abstract. Reverse micelles hosting the internal production of the surfactant are proposed as experimentally feasible models of simple (or 'minimal') autopoietic systems. We describe the conditions under which these may be formed and their possible biological implications. The micellar systems considered here turn out also to exhibit a capacity for self-reproduction through fragmentation under plausible conditions, thus constituting also a minimal experimental model for prebiotic self-reproduction.

1. Introduction

The bacterial cell is the simplest living system because it possesses the capacity to produce, through a network of chemical processes, all the chemical components which lead to the constitution of a distinct, bounded unit. In other words, cells in their minimal expression are characterized by their *autopoietic organization.* An autopoietic system has been defined as a system which continuously produces the components that specify it, while at the same time realizing it (the system) as a concrete unity in space and time, which makes the network of production of components possible (Varela *et al.*, 1974; Maturana and Varela, 1980). Autopoiesis attempts to capture the mechanism that generates the *identity* of the living. This identity can be loosely described as self-produced coherence: the autopoietic mechanism will maintain itself as a distinct unity as long as its basic concatenation of processes is kept intact in the face of pertubations, and will disappear when confronted with a too drastic perturbation.

It is possible to argue that autopoiesis is a *necessary*, and arguably sufficient, condition to characterize life in its minimal form. For a detailed discussion we refer the reader to some recent literature (Varela, 1979; Maturana and Varela, 1980; Zeleny, 1981; Margulis and Sagan, 1986; Fleischaker, 1988).

This paper deals with the following issue: Whether and to what extent a simple molecular structure (in contrast to the complexity of a bacterial cell) can satisfy the criteria of autopoietic organization. Computer modeling (Varela *et al.*, 1974) and analytical calculations (Schwegler and Tarumi, 1986) suggest how an enzyme-mediated polymerization could in principle give rise to such minimal living unit. In fact, the encapsulation of macromolecules by lipid vesicles has been recently

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investigated (Deamer and Barchfield, 1982; Lazcano, 1986; Baeza *et al.*, 1987; see Deamer, 1986 for review). However, so far no chemical system has been synthesized which fully satisfies the autopoietic organization.

In this paper we will make the case that a reverse micellar system can come close to the mark. In particular we will discuss the case of a reverse micellar system hosting in its aqueous core a reaction which leads to the production of a surfactant, which is a boundary for the reverse micellar reaction. The interest of this case is that much is known about these chemical system making it possible to actually put into a operation such a minimal autopoietic system. The full experimental conditions are not yet fully worked out, and will be reported elsewhere (Mascolo *et al.*, 1988). In this paper we wish explore mostly the conceptual implications of this novel experimental developments for the minimal organization of cellular life.

We wish to make it clear from the outset that this paper does not address the question of the origin of life directly. However it does so indirectly by discussing a minimal implementation of an autopoietic unit. This issue can be dealt with in purely physico-chemical terms, i.e. which are boundary conditions which must be satisfied for a system to be autopoietic? This issues is both different and simpler than research attempting to reproduce pre-biotic chemistry of various kinds of other units, not necessarily autopoietic (Morowitz *et al.*, 1988; Eichberg *et al.*, 1978; Hargraves *et al.*, 1977; Yanagawa *et al.*, 1988). Hence we do attempt to take into account the overall problem of micellar systems in prebiotic life, but only those micellar system that seem relevant for an autopoietic implementation.

2. The Reverse Micellar System

The properties of reverse micelles are well reviewed in a number of places (Fendler, 1982; Eicko, 1980; Lindmann and Wennerström, 1980). We will briefly review only some essential aspects here. Surfactants can be viewed as molecules composed by a polar head group and a long aliphatic tail. Some surfactants, when dissolved in apolar solvents above a certain concentration, form spheroidal aggregates in which the tails of the surfactant molecules are directed towards the solvent and the polar heads are directed towards the interior of the aggregate, thus forming a polar core (Figure 1a). This is the opposite situation from that occurring in a usual water micelle, hence the name reverse micelle.

The polar core can solubilize water in the form of small droplets (water pools) whose dimensions for a given surfactant depend on the molar ratio defined as

$$w_0 = \frac{[H_2O]}{[SUR]}.$$

For the well-known surfactants bis-2-ethylhexyl sodium succinate (AOT) or cetyl trimethyl ammonium bromide (CTAB) in isoctane or isoctane-chloroform mixtures, and $5 \leq w_0 \leq 50$, the radius r of the micellar water pool is in the range of 10-

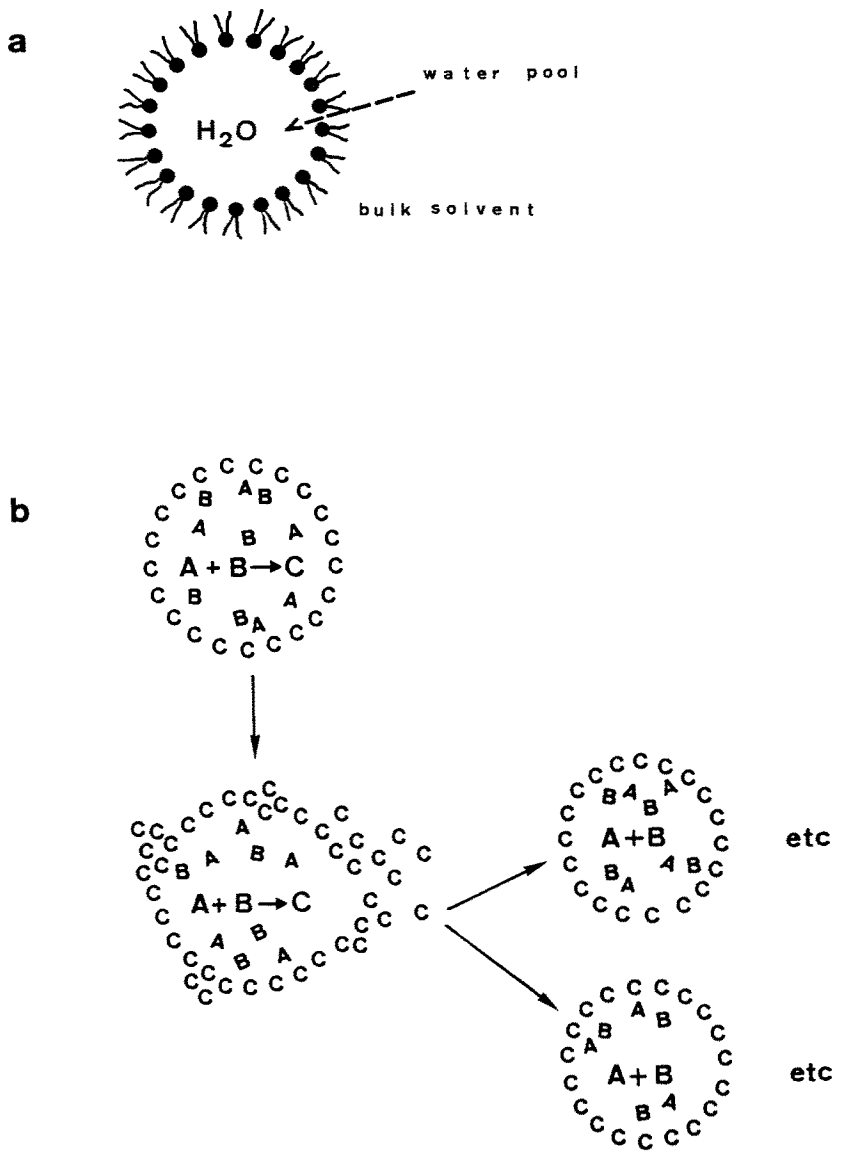


Fig. 1. (a) A diagram of a reverse micelle in cross section. (b) A reverse micelle hosting a reaction leading to the formation of C, the surfactant itself. This is the main idea behind micellar realization of autopoiesis. A and B are present in excess and due to fast micellar exchange they rapidly re-distribute overall the micelles present in solution.

70 Å (Fendler, 1982; Eicke, 1980). The aggregation number of AOT and CTAB also depends on w_0, and ranges between 50–400 for the aforementioned w_0 range. Thus a 200 mM CTAB solution will contain 0.5–4 mM of micelles.

These micelles are not rigid structures, or certainly more fluid than liposomes.

As a matter of fact they are endowed with a fair degree of mobility: not only the single molecules of surfactant are able to tumble and change within the same micelle, but the various micelles continuously coalesce by collision thus mixing and exchanging the content of their water pool (Fletcher and Robinson, 1981; Luisi *et al.*, 1988). It is also important to note that reverse micelles are generally monodisperse or nearly so; this is at variance with normal aqueous micelles. This relatively high degree of structuring (geometry, non-dispersion) is achieved spontaneously by the system. Thus the formation of reverse micelles is a typical example of the so-called spontaneous self-organization.

The reverse micellar system is characterized by two apparently contradictory but actually complementary properties. On the one hand the micelles are geometrically well-defined structures with an interior (water) which is chemically distinct from the external bulk solvent and with a stable boundary. On the other hand these micelles are extremely dynamic structures which rapidly interconvert one into the other. The solution as a whole is thermodynamically stable (generally for months or so) which warrants the topological integrity and constancy of the whole. The dynamic properties and fast-exchange kinetics permit each individual micelle to be in contact with many others in a brief period of time.

3. The Reverse Micelle as Autopoietic Unit

Whereas physical studies of reverse micelles have centered on thermodynamic and kinetic aspects, more chemically oriented studies have seen micelles as microreactors. The compulsory compartmentation of hydrophilic reagents in a water pool (i.e., the water inside the micellar volume), together with the observation that the water pool does not behave as normal bulk water, has elicited a great interest in chemical reactions occurring within this water pool (Fendler, 1982; Luisi *et al.*, 1988). Recently, enzymatic reactions have been described in reverse micelles, in which the enzyme is solubilized in it and maintains its full activity (Luisi *et al.*, 1988; Luisi, 1985; Martinek, 1986; Waks, 1986).

Consider a simple organic reaction taking place in the water pool of the type

$$A + B \longrightarrow C. \qquad [1]$$

Let C be the surfactant molecule, and A and B its constituents parts, for instance the polar head and the hydrocarbon chain. In other words, we can choose reactants and reaction conditions in the water pool of the reverse micelle such that surfactant molecules are being synthesized which in turn constitute the reverse micelle (Figure 1).

One illustrative example is the reaction leading to the formation of cetyltrimethylammonium bromide (CTAB), a well known surfactant for water-in oil microemulsions and reverse micelles:

$$N(CH_3)_3 + Br^-(CH_2)_{15}CH_3 \rightarrow (CH_3)_3\overset{+}{N}\,\overset{Br^-}{(CH_2)_{15}CH_3}\,, \qquad [2]$$

the starting reagents being trimethyl amine and cetylbromide respectively. This reaction is presently under further study in our laboratory.

Other reactions of this type are possible. For example the synthesis of AOT, the most commonly used surfactant for reverse micelles, starting from bisulfite and maleic anhydride:

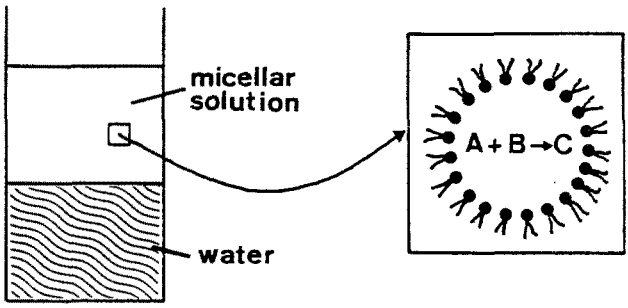
$$HSO_3^- + \begin{matrix} CH\text{-}COOR \\ \| \\ CH\text{-}COOR \end{matrix} \rightarrow \begin{matrix} CH_2COOR \\ | \\ {}^-O_2SO\text{-}CHCOOR \end{matrix} \qquad [3]$$

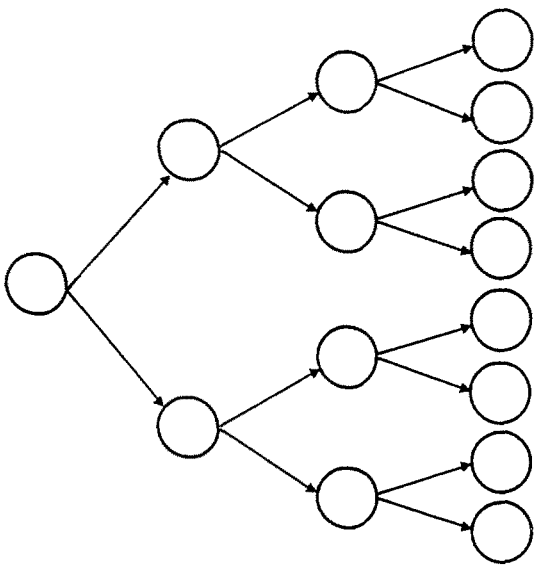
where R is the group 2-ethyl-hexyl.

Let us consider now how the system actually works. The micellar solution, as we have seen, is constituted by a bulk organic solvent (such as chloroform, isoctoane, or their mixtures), and by water droplets, stabilized in the organic solvent by a surfactant layer. For the reaction to occur, the micellar solution should contain a very large excess of A and B, so that it can proceed in a steady state over a relatively long period of time. From a practical standpoint, therefore, the solubility in the micellar system of either A and B must be large, and at least one of the reagents must be present in the water pool so as to ensure that the reaction takes place inside the micelle or at most at the micellar interphase. In the case of reaction [3], for instance, bisulfite is insoluble in the organic solvents used for the reverse micellar mixtures; since this reagent is indeed confined in the water pool.

Thus we start the reaction with a given concentration of $[A]$, $[B]$ and $[C]$. We assume this latter to be present in micellar form, and not free in the surrounding water. In this configuration we are dealing with a batch reaction, in which there is no need to feed externally the reagents after the initial addition of A and B. Following the formation of C, the number of micelles in the system will increase, but since the water concentration is fixed, an increase in the water pool can take place only at the expense of the original water pool. In other words the dimension of the micelles decreases from generation to generation.

The explicit dependency between the number of micelles and their dimensions is readily obtained since w_0 and $\mathbf{r}$, the micellar radius, are linearly related (Luisi *et al.*, 1988), and the volume of each water droplet assuming sphericity is given by $v = \frac{4}{3}\pi\mathbf{r}^3$. Thus, if the radius decreases by a factor of 2, the micellar volume will decrease by a factor of 8. Considering now a constant surface head group occupancy s_a, the aggregation number N will be given by the ratio $N = \frac{4\pi r^2}{s_a}$, and while $\mathbf{r}$ decreases by a factor of 2, N will decrease by a factor of 4, while the total number of micelles will increase by a factor of 8. All this is illustrated in Figure 2 showing in a qualitative fashion the change in [mic] and $\mathbf{r}$ with the number of generations. Details for the kinetics can be found in (Mascolo *et al.*, 1988).



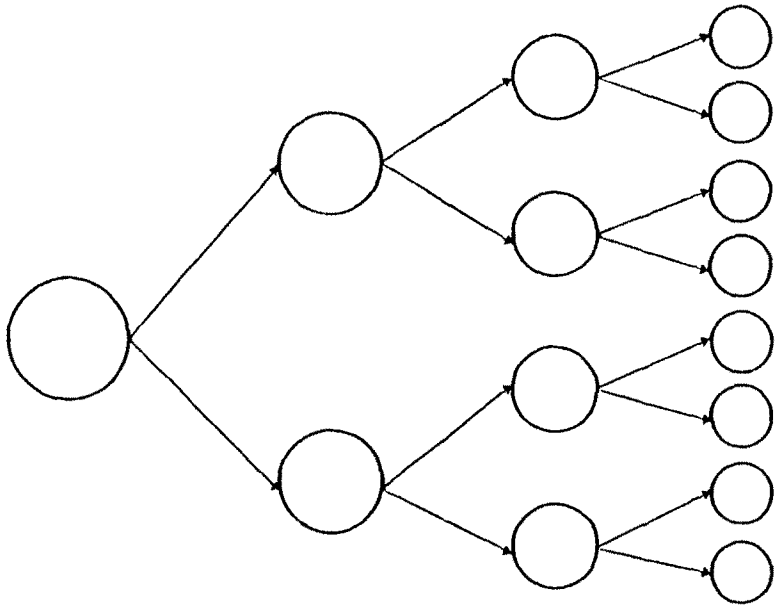


$$r_0 = r_1 = r_2 = r_3$$
$$N_0 = N_1 = N_2 = N_3$$
$$w_0 = w_1 = w_2 = w_3$$
$$[mic]_0 < [mic]_1 < [mic]_2 < [mic]_3$$

Relation initial / final state

$$r_0 = r_i$$
$$N_0 = N_i$$
$$w_0 = w_i$$
$$[mic]_3 = 8 \times [mic]_0$$

Fig. 2. The single phase autopoietic cycle, showing the relation between the radius r of micelles and the other relevant parameters. The water pool of the newly born micelle is created at the expense of the water initially present, leading a decrease in size. Due to fast equilibrium, all micelles will reach a final average size r_f.



								Relation initial / final state
micellar radius	r_0	>	r_1	>	r_2	>	r_3	$r_3 = \frac{1}{2} r_0$
concentration of micells	$[mic]_0$	<	$[mic]_1$	<	$[mic]_2$	<	$[mic]_3$	$[mic]_3 = 8 \times [mic]_0$
aggregationsnumber	N_0	>	N_1	>	N_2	>	N_3	$N_3 = \frac{1}{4} N_0$
	w_0	>	w_1	>	w_2	>	w_3	$w_3 = \frac{1}{2} w_0$

Fig. 3. The two-phase autopoietic cycle with a continuous and fast feed of water from the lower aqueous phase. In this case w_0 remains constant and so does the micellar radius.

It is then apparent that a reverse micellar solution hosting a reaction of type [1] or [2] can be considered to form a simple or minimal autopoietic system. But this system turns out *also* to be capable of replication, giving rise to increase number of units of identical organization. In practice this reaction has a yield on the order of 90–100%, comparable to the theoretical yields on which Figure 2 is based. Increasing the number of micelles by a factor of 8–10 is thus quite feasible. It must also be added that reaction [2] leading to CTAB is slow, typically taking hours of even days to reach completion.

There is an important problem with the chemical scheme described until now: the size of the micelles decreases over time. This drawback can be overcome by furnishing water externally during the reaction and is worthwhile to discuss its basic features.

Consider a biphasic system as illustrated in the top of Figure 3. Here we have a micellar-solvent system as described before, but overlaid by an aqueous solution. The two liquid phases are in equilibrium and exchange materials (in particular water and reagents) at the interphase until all components reach equilibrium. In particular, the w_0 value established in the micellar phase will correspond to the thermodynamically most stable distribution of water between the aqueous phase and the microemulsion. In typical experimental conditions, this w_0^{eq} is in the range 15–20.

If now the rection is allowed to proceed in the micellar phase, the production of surfactant in the micelles will continuously promote the transfer of more water into the organic phase, as the equilibrium condition will impose the w_0^{eq} value and maintain it. Since the transfer of water is a fast process relative to the chemical reaction, the system will be characterized at the end of the reaction by the same w_0^{eq}. The dependency of [mic] and **r** is represented in Figure 3, showing that the micellar numbers can grow wthout their size changing to any significant degree. The disadvantage from the experimental point of view is that w_0^{eq} cannot be freely set as in the previous set-up. On the other hand this case is more interesting biologically since water is continuously furnished and the replication process leads to units comparable in both having an autopoietic organization and similar sizes.

It is appropriate to address some of the problems that arise in the experimental feasibility of the micellar autopoietic proposed in this paper. An important problem is to find the conditions under which the reactions can proceed inside the micellar domain. For example in the case of CTAB reverse micelles (reactions [1]) which are usually built in chroloform/isooctane mixtures, we have realized that the partition coefficients are not completely favorable for the reaction to occur in the micelle itself, as both trimethyl amine (TMA) and cetyl bromide (CB) are much more soluble in the organic phase than in the water pool. Since TMA is also soluble in water, the reaction occurs to a smaller extent at the micellar interphase, thus limiting autopoietic growth.

In the case of reaction [2] with AOT, the problem is different: it is difficult to reach in the water pool the critical concentration of HSO_3^- which is necessary

to convert the unsaturated diester (which is mostly soluble in the organic phase) into novel AOT molecules.

We are currently investigating other reactions, for example those leading to the formation of ethers, starting from alcohols and sugars:

$$\text{Glu–OH} + \text{octyl alcohol} \leftrightarrow \text{Glu–O–C}_8\text{H}_{17} + \text{H}_2\text{O}$$

which also shows promise. Several other systems can be envisaged. Since all of these can readily studied with analytical techniques, it seems clear that a variety of autopoietic micellar systems are feasible, but that the right boundary condition must be found for each one. These more technical details of this research will be the subject of a next publication (Mascolo *et al.*, in preparation).

4. Discussion

The reverse micellar systems described above hosts a chemical reaction producing components which organize themselves into a boundary that provides the conditions for the reactions to take place. This is therefore a system that satisfies the autopoietic organization in a minimal form. To the best of our knowledge this is the first experimentally realizable chemical system proposed that exhibit autopoiesis in a manner that seems hard to simplify even farther. In this sense these micellar system correspond to the experimental implementation of what was until now a mere computer simulation possibility (Varela *et al.*, 1974).

An autopoietic system is not defined in relation to its reproduction (or lack of it), but as a mechanism of identy. However it is clear that once a system is established, the question of its duplication can be immediately posed. In other words, identity and reproduction are *not* linked *a priori* to one another, but once an identity is established various reproductive mechanisms can be envisioned. This is of course particularly important for any evolutionary history, including early life. In the present case, it is interesting that self-reproduction (two systems from an initial one from fragmentation) is a natural consequence of the establishment of a micelle (i.e. of its autopoietic identity).

To what extent are the systems described here relevant models for prebiotic reproduction? It is useful to envisage how micellar growth comes about. One possibility, particularly for the two-phase system were w_0 remains constant is that the growth takes place with the intermediate formation of larger micelles: CTAB being formed inside the micelles migrates towards the micellar interphase, and a somewhat larger micelle is formed before it decays into a thermodynamical more stable one characterized by w_0 at equilibrium. This is a typical case of reproduction by fragmentation of a distributed structure, that is, one in which the components are dispersed homogeneously, so that a fragment has roughly the same structural constitution than the original unit. In this sense it resembles cell reproduction where also a distributed structure is achieved by duplication of all components including those existing in single doses like nucleic acids. Thus, these minimal autopoietic

system exhibit an intrinsic capacity for reproduction and thus for an evolutionary history.

We do *not* claim that the reactions discussed in this paper are of prebiotic significance. In this sense the intention of this paper differs markedly from those dealing with lipid vesicles as analogues of prebiotic units. Our central point is to introduce the idea that a reversed micellar version of an autopoietic system as a good working model, both for simple autopoietic properties and for models of early self-reproduction.

In conclusion, it should be said that the micellar system described here can undergo some further sophistication. One most obvious step is the use of enzymatic or polyribonucleotide reactions inside the micelle as a promoter for micellar growth and stabilization. In view of the relative stability of enzymes in reverse micelles this is, in principle, possible. We are currently carrying out experiments using lipases to synthesize lechitines as surfactants. A further step, already initiated by the work of Morowitz *et al.* (1988), is to carry the same logic as discussed here to the use of liposomes as carriers for chemical rections which produce the lipids forming the liposomal boundary.

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