Functions and Possible Provenance of Primordial Proteins—Part II: Microorganism Aggregation in Clouds Triggered by Climate Change

Andrei P. Sommer* and N. Chandra Wickramasinghe†

Central Institute of Biomedical Engineering, University of Ulm, 89081 Ulm, Germany and Cardiff Centre for Astrobiology, Cardiff University, Cardiff, CF10 3DY, United Kingdom

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Current models predict that the elevation of the Earth’s surface temperature due to global warming is accompanied by a warming of the troposphere, and a thickening cloud cover associated with longer-lasting clouds, in particular over land. These effects can have an instant impact on the vitality level of microorganisms in clouds and the spreading of airborne diseases. Microorganisms could originate from locations on the Earth, or even arrive from space. Primordial proteins in nanobacteria, only recently identified in the atmosphere, could play a significant role in clouds—accelerating the formation of cloud droplets and interconnecting nanobacteria (and possibly nanobacteria and other microorganisms), thus enhancing their chances to eventually reach the Earth.

Keywords: global warming • primordial proteins • slime • light • living nanovesicles • nanobacteria • microorganisms • atmosphere

I. Sealing and Adhesion—Complementary Functions of Primordial Proteins

A prominent function of primordial proteins is adhesion, as described in our previous work.1 Adhesion is a prerequisite for establishing nonspecific contacts to the surface of various materials, hence providing favorable conditions for the realization of the evolutionary potential of primitive life-forming units, e.g., nanobacteria (NB).2 A second and less evident function of primordial proteins is surface sealing, recognized by us as vital for NB.3 Interestingly, there seems to exist a functional complementarity between adhesion and sealing. The primary role of sealing is to secure the survival of individual NB in a dry environment, as practically realized in the atmosphere external to clouds. The primary role of adhesion becomes clear from the biological advantage of colony formation. NB arriving from space, as might be indicated by their capture at an altitude of 41 km over sea level, have their first chance to meet in the wet environment of the clouds.1 Here, the slime enveloping NB is likely to soften and to become gradually more and more adhesive, depending on residence time, humidity, and the ambient cloud temperature. The slime consists of proteins (probably glycoproteins)3 and some minerals. An adhesive slime layer is certainly instrumental in promoting the formation of colonies on Earth, and ensures that whenever NB suspended in the clouds encounter each other, they will stick together. In this way, aggregates of NB can find a realistic chance to reach the Earth by the action of gravity. NB are presumably uniquely effective as cloud condensation nuclei (CCN). Due to their strongly hydrophilic shells (apatite), possibly carrying a glycoprotein film (probably extremely hydrophilic because of the sugars), NB are expected to form cloud droplets before the CCN coexisting with them. Under the action of gravity, accelerated droplet growth may result in the coalescence of some NB-nucleated droplets, preferentially at the base of the cloud—thus fostering the creation of larger rain drops, which may eventually reach the Earth.

Water is essential for microorganisms and is stored by them in various ways. Desiccation can inactivate bacteria and viruses, and most probably also living nanovesicles (NB) possessing internal compartments (nanocavities) suitable for the storage of aqueous fluids. Microorganisms passing through the terrestrial atmosphere can, in principle, be partially or completely desiccated via thermal effects involving the heating of the entire particle by solar radiation. Instantaneous water loss can result from absorption of light intensities of the order of 1000 Wm⁻², where the photons interact selectively with the water content of microorganisms. Both effects, in particular their synergistic interplay, can perform important physical and biological functions in the terrestrial atmosphere. On the basis of the prevalence of nanoscopic water layers on ice, found by Döppenschmidt and Butt,4 the aforementioned effects have been predicted by us to affect thundercloud electrification.5–7 Similar effects would be expected to control cloud albedos and indeed, most importantly, the vitality level of aerosolic biosystems. While the desiccation caused by absorption of solar radiation is clear, the direct effect of intensive sunlight on nanoscopic quantities of water contained in microorganisms has not yet been explored. In this paper, we establish a connection between results of experiments that could serve as a model for the latter effect, and their possible implication in mechanisms controlling the level of vitality of atmospheric NB. Previously, we have identified possibly still living nanovesicles at stratospheric
III. Desiccation of Living Nanovesicles: Laboratory Analogues

In previous laboratory experiments, we have provided evidence that the thickness of nanoscopic water layers, deposited at room temperature and normal humidity conditions from the air onto translucent polymer films, decreased in response to the exposure of such films to 670 nm laser light applied at an intensity of 1000 Wm\(^{-2}\), practically as low as the solar constant.\(^1\) The result clearly demonstrated (a) the existence of water layers in the air, and (b) that photons of 670 nm, which are only minimally absorbed by water, interact with the nanoscopic water layers attached to surfaces. A similar effect, which are only minimally absorbed by water, interact with the nanoscopic water layers attached to surfaces. A similar effect was predicted by us to play a role in the transfer of charge at the sun-exposed side of thunderclouds, where the intense sunlight could instantly deplete the nanoscopic water layers coating ice crystals.\(^7\) The interplay between the sunlight and atmospheric humidity would thus have a major impact in defining the state of vitality of airborne NB. From an experimental model, we concluded that NB, whose nanocrystalline mineral shells are optimized to serve as tiny light collectors,\(^13\) would respond to variations in solar irradiation and atmospheric humidity. For example, on leaving a cloud during periods of high solar intensity, they would react to an increase in irradiation intensity coupled with a drop in humidity by sealing the pores of their shells with slime—a programmed process, whose reversibility will presumably depend on the ambient conditions,\(^3\) and on the proper time-scale of the two competing processes: sealing and evaporation.

For a better understanding of the underlying process controlling the capacity of nanocavities to store water, it could be instructive to summarize phenomena related to the arrangement of water molecules at surfaces. Exceptional orientational effects were first reported for water in interfacial contact with hydrophobic liquids by Scatena et al.\(^14\) According to Fawcett et al., interfacial order can also be imposed to molecules by normal gravity.\(^15\) The concept of interfacial water layers, acting as an interlayer between a solid body immersed in water, and the bulk water surrounding it, has been introduced on the basis of an intuitive model,\(^16\) in which we predicted that the mobility of water molecules at solid surfaces is restricted, compared to bulk water. This concept was applied by us in the design of hydrophobic biosensors for uses in an aqueous milieu, and developed further to exploit the advantages of near-field optical analysis, promising to image, e.g., proteins in living cells.\(^17\)

The physical relevance of a layer of water molecules with unilaterally constrained mobility, existing between the bulk water and a hydrophobic surface, has been recently revealed in our lab. In a representative number of experiments performed with four to six 15 \(\mu\)L drops of pure water, successively placed on translucent hydrophobic substrates, where they evaporated under equal temperature and humidity conditions, one drop was continuously irradiated with a 670 nm laser, applied at an intensity of \(\sim 1000\) Wm\(^{-2}\). The light-exposed drop was placed always first on the substrate. Importantly, the light did not accelerate evaporation, as possibly might be anticipated, but clearly delayed it in comparison to the nonirradiated drops, illustrating implicitly the subaquatic persistence of ordered water layers at the liquid–solid interface. The differ-
ence has been correlated with a perturbation of the integrity of the ordered interlayer located at the interface between bulk water and substrate. In analogy to the modulation of the nanoscopic water layers masking substrates in air, achieved with virtually nondestructive light intensities, the subaquatic modulation was interpreted in terms of resonant fluctuations induced by the light into the layer of interfacial water molecules, changing their actual state transiently from “low mobility” to “bulk mobility”. From the fact that 670 nm laser light is minimally absorbed by water, a simple picture suggests itself: due to the presence of a number of less mobile interfacial water molecules, the probability that more than one photon can transfer identical momentum to a given water molecule is higher when this target molecule is in a quasi stationary state, than it would be in the case of more mobile target molecules. This concept receives justification from the observation that the evaporation of water drops irradiated with a laser (intensity \( \approx 1000 \text{ Wm}^{-2} \) operating at 650 nm, a wavelength which is even less absorbed than 670 nm, resulted in a similar delay. Considering the moderate light intensity employed, the delay-effect could not be traced back to the interaction of the light with “nanobubbles”—gas bubbles with a mean radius around 35 nm, and 20–30 nm in height, identified by Tyrrell and Attard via atomic force microscopy (AFM) on hydrophobic substrates immersed in water, using a closed fluid cell. Calculations predict inner pressures for such small bubbles far above atmospheric pressure, signifying stability. Therefore, even if present at the interface between evaporating drop and substrate, the applied laser light intensity is not likely to affect the state of the bubbles. Nanobubbles with a mean radius of 70 nm and a height of about 7 nm were imaged at the interface between polystyrene and water via AFM by Simonsen et al. In contrast to the stationary situation in imaging the nanobubbles, the interior of the water drops evaporating on hydrophobic substrates is constantly dynamic, with a powerful convective flow. The flow has been shown by us to transport 60–200 nm nanospheres suspended in water drops to the periphery of the drops, producing on both hydrophilic and hydrophobic substrates crystalline rings. We have demonstrated that in cases where the initial drop size coincided with the final ring size, the rings were formed prior to the complete evaporation of the drops, thus visualizing the action of the convective flow on nanoparticles deposited onto substrates. This flow may prevent the formation of nanobubbles in water drops. Nanosuspension drops, when irradiated with laser light intensities observed to delay the evaporation of drops of pure water, exhibited a significant retardation in evaporation, compared to nonirradiated drops. The similarity in the reaction of a representative number of drops of pure water and aqueous nanosuspensions to their irradiation with low level laser light indicates, however, that the light did not affect nanobubbles that might have been present, or such bubbles were completely absent.

We note that in experiments conducted with evaporating sessile drops (aqueous suspensions containing nano- or microparticles), the suspended particles tended to reach the initial substrate directly. Direct contact is energetically a costly solution because of dissipative shear-force effects. It becomes plausible that fluctuations induced in the partially immobilized interlayer by the laser light will affect both its rheological properties and its density profile. Obviously, a minimal change in density can have a major effect on the space required by an aqueous phase contained in a nanocavity—a relationship of an enormous potential importance in proteomics as well as in pharmaceutics. Immediate pharmaceutical applications include light-triggered pumping processes—providing production principles for porous nanovesicles for drug release, and methods to force microorganisms to uptake antiinfectives dissolved in their environment. Thus, the previously proposed desiccation model receives manifest justification from the experimental side, with evidence for a new photobiological effect which probably controls the capacity of nanocavities to store water.

IV. Application to Cloud Microorganisms

Applying the foregoing observations to nanoscale cavities in microorganisms containing water or aqueous liquids, it seems reasonable to expect, that to some extent, solar irradiation will affect the viscosity and density profiles of the fluid phase within such cavities. In combination with heating caused by the sun, such effects could desiccate microorganisms outside clouds. However, for desiccated microorganisms entering the clouds, the coupled, reciprocal change in irradiation intensity and humidity, could induce a rapid uptake of water, including dissolved nutrients, leading to their subsequent revitalization. The difference between the protective cloud cover (wet, nutrient-rich, and low irradiation) and the surrounding atmosphere could be responsible for important differences in the level of vitality of microorganisms reaching the Earth. In addition, it is expected that the manner in which they reach the Earth, e.g., by sedimentation from the dry atmosphere, or from a cloud dissolved by its evaporation, or incorporated in rain drops, will make a difference.

Discussion

The mechanism we have explored may be crucial in controlling the viability of NB returning from space to the Earth. Due to their size, predominantly ranging between 80 and 300 nm, a biologically vital aqueous content stored in internal nano-
cavities, is likely to respond instantaneously to alternations between the light-shielded wet environment of clouds and the luminous dry conditions that prevail outside. NB have been isolated from various terrestrial sources, including animals, humans, and apparently wastewater. Except the fossil structures identified by McKay et al. on Meteorite ALH84001, which could be ancient witnesses for a presence of NB on Earth and maybe on Mars, there has been, so far, no clear evidence for their presence in the atmosphere.

Recently, we presented environmental scanning electron microscope (ESEM) images showing a very large number of isolated from human serum by culturing in mammalian cell culture conditions for three weeks. Reprinted from ref 35 with permission. Image clearly shows how several NB are kept together by slime. Bar 100 nm.

![Image](image-url)
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