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Functions and Possible Provenance of Primordial Proteins

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Nanobacteria or living nanovesicles are of great interest to the scientific community because of their dual nature: on the one hand, they appear as primal biosystems originating life; on the other hand, they can cause severe diseases. Their survival as well as their pathogenic potential is apparently linked to a self-synthesized protein-based slime, rich in calcium and phosphate (when available). Here, we provide challenging evidence for the occurrence of nanobacteria in the stratosphere, reflecting a possibly primordial provenance of the slime. An analysis of the slime's biological functions may lead to novel strategies suitable to block adhesion modalities in modern bacterial populations.

Keywords: nanobacteria • living nanovesicles • primordial proteins • atmosphere

Evolutionary Functions of Primordial Proteins

Proteomics focuses on the structure and function of proteins. While it is impossible to analyze the structures without the adequate laboratory equipment, functional aspects, frequently dictating the structures, can be studied in models. Primordial biosystems represent here a particularly attractive field, as they can lead us to an understanding of some primal key-functions proteins must have had. This implies the identification of still existing and still vital primordial biosystems—survivalists.

Models predict that nanobacteria (NB) can survive extreme conditions in space by protecting themselves from desiccation with a self-synthesized slime that seals their mineral shells.[1] In the bacterial world, slime has principally one function: to establish stable bonds to surfaces encouraging the formation of complex multispecies communities (biofilms), thereby enhancing conditions for a physical contact between individuals, a precondition for horizontal gene transfer.[2] Biofilms are known to elevate the chances of survival of clinically relevant microorganisms.[3] It was proposed that the slime synthesized by NB consists of glycoproteins.[4] Indeed, glycoproteins can be linked to primordial proteins: the gene encoding antifreeze protein (AFGP) in an Antarctic fish provides evidence for such a link.[5−7] In NB, the slime is instrumental in performing three biological functions, each serving a different purpose, and their study promises to elucidate the evolutionary strategy initiating the protein synthesis in modern bacteria. Since NB are small, they can easily reach the atmosphere as aerosols where they need some protection from desiccation—the first function, as stated above. The second function results from their possible role as origin-of-life systems,[8] where slime becomes a paramount factor in facilitating colony formation, offering favorable conditions for growth and replication. The third function cannot be traced back to a primordial scenario. In it, the slime secretion seems to be a stress response, induced by a programmed survival mechanism, solely triggered by rapid physiological and/or biomechanical changes in their next environment.[4] The pathogenic nature of the NB can be derived to some extent from the last two functions, both securing their survival in a biological environment. Bacteria employ surface proteins in the initial phase of their interaction with a host. Possibly, they still employ the last two functions defined in NB. The question which clearly suggests itself is as follows: Are common bacteria using the first function? That is, do they possess a mechanism to use their protein content to seal their surface? In diseases related to NB, slime production has been possibly successfully reversed by repetitive exposure of parts of the body of a patient to intense laser light.[9] If indeed NB represent primordial biosystems and possibly even origin-of-life systems, it is plausible that modern bacteria will respond to the light irradiation parameters found in laboratory experiments to affect NB.[10,11] Laser cross-linking of proteins to nucleic acids is a known technique.[12] Laser inhibition of bacterial attachment to surfaces is less known, and may open completely new avenues to control adhesion of bacteria to host cells—a classical field for vaccines and anti-adhesion drugs. Progress in the recognition of the protein-induced adhesion mechanisms is now evident, and clearly manifests the multidisciplinarity of the subject, as can be seen in exemplary photographs showing the slime employed in the first phase of bacterial anchoring to a substrate.[13] NB isolated from the blood of mammals are known to possess central cavities, protected by mineral shells with diameters predominantly between 80 and 300 nm, consisting of nanocrystalline apatite.[14] The shells have geometries intermediate between spherical and coccoidal. Regulatory processes, contingent on the permeability of the apatite shells to fluids,

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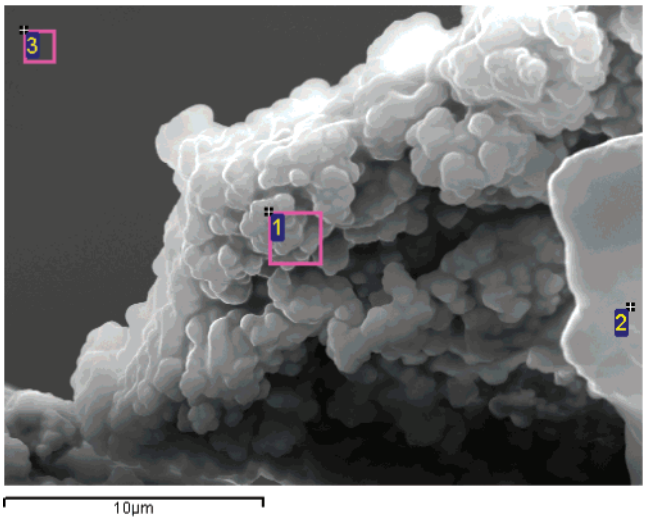

Figure 1. ESEM image of stratospheric microparticle on silicon wafer, identified as cometary micrometeorite.

have been observed in cultured NB, responding spontaneously to stress-provoking stimuli by the secretion of slime.[4] Three functions, all intrinsically related to the extreme survivability of NB, have been attributed to the adhesive nature of the slime. Adhesion permits the collection of the constituents of the mineral shell, provides a means of interconnection to form colonies, as well as attachment to surfaces to prevent potential elimination from a possible host. Clinical studies indicate that NB play an essential role in the nucleation of kidney stones,[16,17] and the formation of cardiovascular calcifications.[18,19] There is also growing observational evidence of NB acting as opportunistic infectious agents. Presumably, they aggravate the state of HIV-infected patients by inducing peripheral neuropathy,[9] nucleating kidney stones, and contributing to reduced bone mineral density levels.[20] A presence of NB in the terrestrial atmosphere could thus have profound epidemiological implications.[9] NB are equipped with unique capabilities for survival, in the terrestrial atmosphere,[1] and in a hypothetical extraterrestrial milieu.[8] Under favorable conditions, NB may even grow and replicate in clouds, in particular in warm clouds containing biomass particles. The presence of bacteria in the atmosphere of the Earth was confirmed 20 years ago,[21] and bacterial replication in clouds has been reported recently.[22]

NB can reach the atmosphere, in principle, via two routes: by winds and ascending hot air produced by natural fires, and/or injection from comets passing the Earth.[23] In the first scenario, horizontal winds could transport NB (as solitary aerosols or attached to biomass particles) across continents. Satellite measurements have shown that dust storms often cross the oceans, for example from the Sahara to the Americas.[24]

The resistance of NB to extreme temperatures, radiation, and extended periods of nutritional limitation is well attested,[8] suggesting their survival in both horizontal transport across the oceans and vertical transport from the stratosphere to the troposphere (or back). The comet-source hypothesis would explicitly imply the presence of NB at stratospheric altitudes, being part of the 100 tons of cometary debris introduced to the Earth on a daily basis.[25] Clearly, NB injected by comets in this way must require a heat shield for survival, and are therefore likely to resemble their cultured counterparts that are protected by a mineral shell. Tests suggest that very many humans and both wild and domestic animals are infected with NB.[26,27] Animal experiments have indicated that the main mode of their elimination from the body is via urine,[28]—a source for their passage into the atmosphere as aerosols.[9] NB without any mineral shells and consequently with smaller masses, welling up into the lower atmosphere from terrestrial sources could, however, survive without a mineral shield. This form could replicate in clouds via multiplication—a process that was observed in cultured NB.

In a recent project, a battery of 16 cryosampler tubes, each of 0.35 L capacity, designed to recover atmospheric microorganisms, was flown via balloon to an altitude of 41 km above sea level. Material was collected at preselected altitudes between 20 and 41 km. The geographical position of the launch site, Hyderabad in India, happens to be close to the Himalayas (1400 km south from Mt. Everest). In preliminary analyses, two cultures of microorganisms have been recovered,[29] and clumps of (nonculturable) microorganisms were detected using vital dyes and epifluorescence microscopy.[30,31]

Identification of Airborne Nanobacteria

Initial attempts to identify stratospheric aerosols collected over Hyderabad, using scanning electron microscopy (SEM), energy dispersive spectroscopy (EDAX)[29] and nanoscale mass spectroscopy (NanoSIMS) were hindered by the filamentous nature of the filters employed (glass fibers and cellulose acetate meshworks), into which the stratospheric particles were adsorbed after their extraction from one of the tubes. The complex textures did not allow for a safe discrimination between background and possible aerosols. We have now devised better methods for the transfer of loosely bound aerosols in their native forms, from the original filters on which they were collected, onto silicon wafers. One method involved establishing intimate contact between the filter carrying the collected material and a silicon wafer of 4×4 mm^2 mounted on a sample stub, and tapping the stub holder, while keeping the parts firmly together. The transfer was carried out under maintenance of maximum possible sterility in an aseptic environment. The silicon wafer was then sputter-coated with gold for examination by environmental scanning electron microscopy (ESEM).

The ESEM examination revealed a few giant particles with diameters reaching 30 μm, displaying morphologies characteristic of cometary micrometeorites. EDAX carried out on them revealed elemental compositions rich in calcium, carbon, copper, oxygen, phosphorus, and also uranium. The identification of uranium in one sample and the fact that the structure shown in the representative ESEM image (Figure 1) is unlike anything to be found on silicon wafers, gives confidence in asserting a prima facie case for a stratospheric origin. EDAX scans, performed at three different positions on the microparticle shown in Figure 1, are presented in Figure 2. Figures 3 and 4 display ESEM images of clusters of nanoparticles found on our sample stub. EDAX performed at two different spots collateral to the assembly shown in Figure 3 revealed in addition to gold and silicon, in both cases the presence of carbon, and in one case of fluorine.

Discussion

The identification of stratospheric NB appears to be compelling if the range of criteria is simultaneously considered. This includes the seven independent morphological parameters (size, shape, size distribution, interconnection, chain arrange-

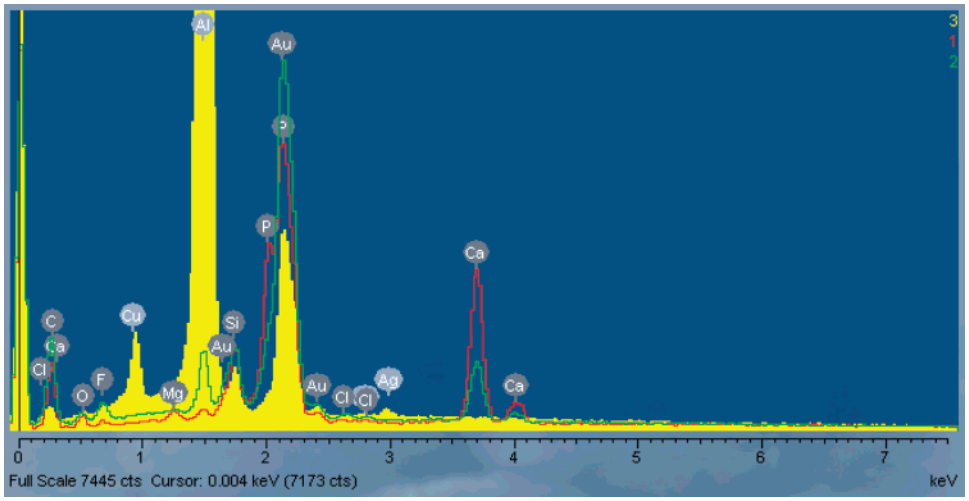

Figure 2. EDAX of sample shown in Figure 1. Red, green, and yellow scans correspond to spots marked with 1, 2, and 3 in Figure 1, respectively.

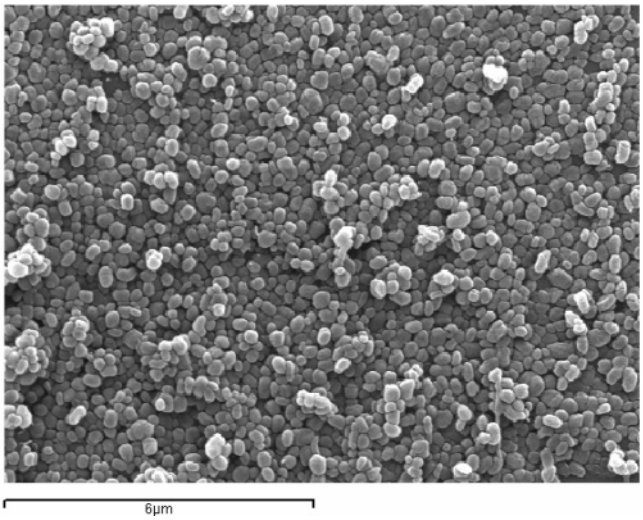

Figure 3. ESEM image showing numerous nanoparticles, probably NB, attached to silicon wafer. Formation of conglomerates is an indication of slime-mediated interconnections.

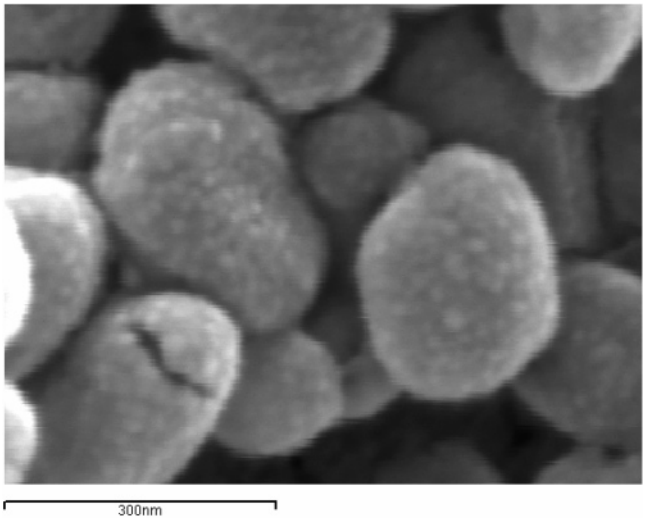

Figure 4. ESEM photography showing distinct nanoparticles of the typical size and shape of NB. Clearly visible is the crack in one of them, indicating the mineral nature of the shell. Such cracks can be regularly found in cultured NB.

ment, conglomeration, and cracking in apparently mineral shells) found in the Figures 3 and 4—identical to the corresponding structures that are widely regarded as characteristic of NB both in vitro,[8,14] and in vivo.[17,18] Figure 5, presenting an ESEM image of giant and normal-sized cultured NB, is included to allow a direct comparison with Figure 3.

NB have been described as ideal "origin-of-life systems",[8] and nanoscopic carbonate precipitates have been associated with possible fossil NB in meteorite ALH84001.[34,35] This latter role of NB is in conceptual agreement with our own findings and may also account for the overwhelming abundance of NB on Earth. Experimental studies of NB have focused so far on the analysis of their shells and identification of DNA and RNA.[17,18] Little attention has been paid to the precise composition of the slime and the mechanism of its production. Model simulations have provided evidence that NB cause disease primarily by adhesion—a process mediated by both the apatite and slime.[32,33]

The foregoing observations suggest that the slime produced by NB could be a property of the most primitive life form in the cosmos, possibly involving what might be regarded as primordial proteins.[36] The fundamental importance of this conjecture should justify a major effort to identify the composition of slime produced by NB. This may inspire de novo protein design experiments. It has been argued that early protein synthesis involved only the very simplest proteins necessary to accomplish a viable life system. The exceedingly complex protein synthesis displayed in present day biology could have evolved progressively from a primordial set by a process of gradual acquisition of new amino acids into an initial repertoire.[37] According to this point of view, NB might have served as a first principle platform in the evolution of early proteins.[38] Noting that light affects slime-production in NB, the nanovesicles commend themselves as model systems for studying the mechanisms of slime-inhibition. Slime-regulatory processes in NB, modulated by light intensities of the order of the solar constant (~1000 Wm^{-2}),[10,11] are facilitated by the optical properties of the apatite, the exclusive light-collecting geometry of the nanovesicles,[39] and the permeability of the nanoporous mineral shells for aqueous liquids. Powerful molecular flow processes in NB, with the capacity of pumping water and

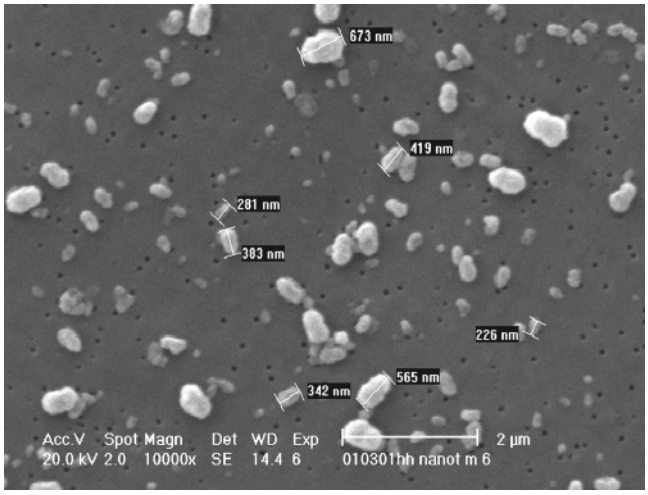

Figure 5. ESEM image of cultured NB on polycarbonate filter. The pores of the filter are visible as black spots. Reprinted from ref 10 with permission. As can be seen by comparison with Figure 3, the nanoparticles collected in the atmosphere are virtually indistinguishable from NB isolated from mammalian sources.

fluid components from the central cavity across the porous shell to the environment (and vice versa), have been formulated on this basis.[8] The stress-suppressive potential of low intensity light[40] on NB has been already exploited in the design of therapeutic strategies. Their response to light indicates that, depending on the environmental conditions, solar irradiation can modulate the vitality level of NB traversing the terrestrial atmosphere.

Conclusions

A more complete understanding of the slime formation process in NB could lead to fundamental insights into the nature of primordial living systems, even perhaps the origin of life itself. At a practical level, such an understanding could well be exploited for the development of therapies that block attachment of NB to tissues and hence alleviate the disease process in many serious illnesses that are caused by them. Of special interest is in this context a possible slime suppressive effect of light on bacteria. Inactivation of bacteria by low level laser light has already been reported for several species, in particular when exposure times were longer.[41−43]

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