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Heuristic consequences of a load of oxygen in microtubules☆

Pierre A. Denis*

SOS Médecins, 50 rue Ville-Pépin, 35400 Saint-Malo, France

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ABSTRACT

The current cell oxygen paradigm shows some major gaps that have not yet been resolved. Something seems to be lacking for the comprehensive statement of the oxygen distribution in the cell, especially the low cytoplasmic oxygen level. The entrapment of oxygen in microtubules (MTs) resolves the latter observation, as well as the occurrence of an extensive cytoplasmic foam formation. It leads to a novel oxygen paradigm for cells. During the steady-state treadmilling, the mobile cavity would absorb oxygenated cytoplasm forward, entrap gas nuclei and concentrate them. A fluorescence method is described to confirm the *in vitro* load of oxygen in MTs during their periodic growths and shrinkages. The latter operating mechanism is called the gas dynamic instability (GDI) of MTs.

Several known biosystems could rest on the GDI. (1) The GTP-cap is linked with the gas meniscus encountered in a tube filled with gas. The GTP hydrolysis is linked to the conformational change of the GTPase domain according to the bubble pressure, and to the shaking of protofilaments with gas particles (soliton-like waves). (2) The GDI provides a free energy water pump because water molecules have to escape from MT pores when foam concentrates within the MT. Beside ATP hydrolysis in motor proteins, the GDI provides an additional driving force in intracellular transport of cargo. The water streams flowing from the MT through slits organize themselves as water layers between the cargo and the MT surface, and break ionic bridges. It makes the cargo glide over a water rail. (3) The GDI provides a universal motor for chromosome segregation because the depolymerization of kinetochorial MTs is expected to generate a strong cytoplasmic foam. Chromosomes are sucked up according to the pressure difference (or density difference) applied to opposite sides of the kinetochore, which is in agreement with Archimedes' principle of buoyancy. Non-kinetochorial MTs reabsorb foam during GDI. Last, the mitotic spindle is imagined as a gas recycler. (4) The luminal particles within MTs (called MIPs) are imagined as a foam organizer, the luminal proteins being part of the borders and edges of identical bubbles. (5) Last, volatile anesthetics could destabilize MTs through anesthetic-induced bubble nucleation between protofilaments, and therefore causing shear stress and the opening of MT.

The load of oxygen in MTs might provide a major advance in this area of research.

> "For it is clear, first of all, that we do not merely want truth – we want more truth, and new truth. We are not content with "twice two equals four", even though it is true: we do not resort to reciting the multiplication table if we are faced with a difficult problem in topology or in physics. Mere truth is not enough; what we look for are *answers to our problems*."
> K. Popper (1902–94), Conjectures and Refutations (Popper, 1963)

1. Introduction and hypothesis

Microtubules (MTs) are hollow nanotubes present in all eukaryotic cells (Amos and Schlieper, 2005). Insinna et al. (1996) have admitted that "the mechanism underlying the dynamics of microtubules and related microtubular structures was still an unsolved mystery. . ." probably due to the "lack of heuristic hypotheses capable of opening the way to new experimental investigations." The present thesis aims to demonstrate that oxygen bubbles within the lumen of MTs in the cell could decipher many unresolved questions in biology. Indeed, according to Popper (1963) "Only if it is an answer to a problem – a difficult, a fertile problem, a problem of some depth – does a truth, or a conjecture about the truth, become relevant to science".

The first section of this article will reveal some major gaps in the current cell oxygen paradigm. Indeed, something seems to be lacking for the comprehensive statement of the oxygen distribution

* Tel.: +33 611810542.
E-mail address: pierredenisfr@yahoo.fr

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in the cell. The possible entrapment of oxygen in MTs throws light on the latter gaps and lead to a novel oxygen paradigm for cells. A fluorescence method will be described in order to confirm this hypothesis.

Several new working mechanisms linked to the heuristic presence of gas in MTs will be discussed; the dynamic instability will raise the issue of how intracellular transport could be improved, and how the mitotic spindle could work. Then, the GTP-cap and the luminal particles called MIPs within MTs will be discussed using concepts of foam physics. To conclude, I shall propose how volatile anesthetics could destabilize MTs.

2. Materials and methods

The scientific method is chosen according to the Inventor's Paradox from G. Pólya (1887–1985) and its heuristic approach, which offers a set of strategies for solving mathematical problems:

> "The more ambitious plan may have more chances of success... provided it is not based on a mere pretension but on some vision of the things beyond those immediately present." (Pólya, 1957, p. 121)

The heuristic approach has been complemented by Bayesian statistics (Bayes and Price, 1763) that are already used in many other areas of science.

3. Previous experimental procedures supporting the presence of a load of oxygen in MTs

There is an average of 150 MTs in a cultivated cell, each one measuring 50–100 μm (Hiller et al., 1978). MTs are built by polymerization of tubulin dimers, into long protein chains called protofilaments. Inner and outer diameters are respectively 240 and 150 Å. From 9 to 16 protofilaments (Chrétien et al., 1992; Andreu et al., 1994; Pierson et al., 1978; Amos and Schlieper, 2005) are non-covalently combined to form the wall of the MT.

First, I would like to cite the following experimental procedures found in the literature, which comply with a load of gas in MTs.

- Strikingly, MTs look like tiny cellulose fibers. Liger-Belair et al. (2002a) have demonstrated that those "tiny hollow elongated fibers" worked as sheltered crevices. When a glass was filled with champagne, authors demonstrated that the CO_2 could diffuse freely from the bulk into the cellulose microfibrils (Liger-Belair et al., 2004) then the cellulose fibers concentrated the gas into pockets.
- A solution containing MTs appeared as a bright layer to the light, and this property was related to the turbidity (Mandelkow and Mandelkow, 1992). This property could be explained (1) by the MT flotation, and (2) by the partial reflection at the gas–liquid interface, when light enters the foam inside MTs (see Fig. 10). Pictures of the brightness of the solution (see Mandelkow) are strikingly identical to the foam on the surface of a glass of champagne (see Liger-Belair and Le Champagne, 2009).
- Changes in birefringence retardation are observed in spindle microtubules (Salmon et al., 1975), and increased pressure produces rapid, reversible decreases in spindle birefringence, suggesting that the foam has been removed from the MTs under the pressure.
- When MTs are assembled in a magnetic field, they line up with their long axis parallel to the magnetic field (Bras et al., 1998; Sato et al., 1975; Vassilev et al., 1982). Currently, these experiments are not fully explained. Since oxygen is paramagnetic (Thery, 2006), MTs could have lined up because of their oxygen load, entrapped from the laboratory's atmosphere.

How high is the pressure in the predictive atmosphere of an MT? Laplace's law gives the pressure difference for sufficiently large bubbles.

$$\Delta P = 2 \cdot \frac{\gamma}{R} \tag{1}$$

with ΔP being the pressure difference across the fluid interface, ΔP is also the capillary pressure in a tube, γ the surface tension (or wall tension) and R the diameter.

I calculate the maximum pressure difference of a single bubble as large as the MT inner diameter: For $2R = 150$ Å, and into water $\gamma = 0.0728\ \mathrm{J \cdot m^{-2}}$ at 20 °C:

$$\Delta P \approx 1.94 \cdot 10^7\ \mathrm{Pa}$$

$$\Delta P \approx 200\ \mathrm{atm}.$$

One oxygen atmosphere could be contained into an MT, representing a 200 atm bubble in water at 20 °C. This in agreement with the response to pressure exerted on MTs (Salmon et al., 1975; Salmon, 1975). In one study, the degree of disorganization varied in the pressure range of 290–400 atm (Bourns et al., 1988). Further, this high pressure difference complies with the MT high rigidity (Kikumoto et al., 2006; Mizushima-Sugano et al., 1983).

4. How an oxygen load in MTs resolves the major gaps in the current cell oxygen paradigm

Model of gas anisotropy in cell. Hereafter, I shall briefly develop a novel approach toward gas anisotropy in solution, in order to prove that the current cell oxygen paradigm is unrealistic.

The rate of oxygen movement $\delta O_2/\delta t$ across muscle tissues has been shown to conform to the one-dimensional diffusion equation for gases as originally modified by Krogh (1919) (for more informations, see Sidell, 1998):

$$\frac{\delta O_2}{\delta t} = -K_{O_2} \cdot A \cdot \frac{\delta P_{O_2}}{x} \tag{2}$$

where K_{O_2} is the diffusion constant for oxygen, A is the surface area through which the exchange occurs, δP_{O_2} is the partial pressure gradient for oxygen across the diffusion path and x is the length of the diffusion path. For the purpose of the following demonstration, let an x-axis cross the cell membrane from the interstitial fluid to the cytoplasm, as depicted in Fig. 1. We shall assume that the latter equation is generally applicable to any cell.

Let δV_i and δV_a be two clusters of infinitesimal volumes, each one containing a few or no gas particles. Fig. 1 shows the 3D-grid pattern generated by cutting the studied compartment up to the δV volumes. We shall suppose that, during an infinitesimal time interval δt, the gas particles in the δV_a have a greater space position probability density than in the δV_i. This provides a straightforward mathematical description for the gas anisotropy. Eq. 2 indicates that an homogeneous bubble nucleation is expected to occur *over the whole length of the x-axis*, as long as the oxygen distribution meets the latter gas anisotropy conditions. In other words, the closest quantum bubbles (containing just one gas particle) combine into a bigger bubble (containing a few gas particles). According to the anisotropy of fluorescence quenching by oxygen in the cytoplasm (Koren et al., 2012), and with the previous enlightenment on gas anisotropy, a bubble nucleation is expected to occur everywhere in the cytoplasm, as well as inside the membrane!

Further, the presence of many crevices, membrane irregularities and lipid surfaces everywhere in the cell (Weathersby et al., 1982) increases the probability of an extensive bubble nucleation, in other words foam formation. All these unresolved problems are likely to lead to the cell dysfunction as depicted in Fig. 2. They should