

NANO - ARCHITECTURE AND MACROMOLECULAR STRUCTURE IN SOME VIRUS, BACTERIA AND PROTOZOA

¹K.O. Hovnanyan, ²N.S. Pryatkin, ¹N.L.Hovnanyan, ¹A.G.Asatryan, ³A.Trchounian

¹Institute of Molecular Biology of NAS RA, 0014 Yerevan, Armenia; ²Video TeasT, LTD. Image Analysis Systems, St.-Petersburg, 198035, Russia; ³Yerevan State University, 0025 Yerevan, Armenia

e-mail: hovkarl@mail.ru, info@videotest.ru

Abstract

The given work concerns some viruses, bacteria and protozoa nano-structures as well as their physical, chemical and bio-organic factors ultra structural analysis. There was used “Video-Test-5 structure nano-technology” program for electronic-microscopic picture size measure and compound analysis study.

The experience of received data ultrastructural analysis allowed classifying variety of some viruses, virus-symbiotes, bacteria and protozoa cellular nano-structures having an important role in cell morphogenesis. Electronic-microscopic identification of different physical, chemical and bioactive factors influence promotes the process of nano-particles discovery in biological objects.

Keywords: nano-structures, viruses, bacteria, protozoa, imaging, ultra-structures, microscopy, analysis.

Introduction

At present in nano-technology, nano-biology and nano-medicine many nano-particles and nano-materials are well known those radically differ from each other in physical, chemical and biological safety complex features comparing with the same materials of general macroscopic types [1]. Biological nano-structures classification from cellular elements micro-tubes, centrosomes, microfilaments, liposomes, poly-layers chitosan particles and other organic and non-organic formations.

In spite of radical changes in biology systems organization revealing and imaging, the questions concerning biological origin nano-structures and particularly nano-microbes remain studied not enough yet, even in fact when it's known the presence of numerous nano-microbes among new contagious agents for human being and animals [2-7]. The aim of the given work is the necessity to work out new approaches to identification of conditioned biological nano-structures by using of ultrastructural image analysis nano-technologic modes, to perform a comparative study of some viruses, microbes and protozoa nano-structures as well as physical, chemical and bio-organic different factors influence on them. We try to use the classification of

viruses, prokaryotes and eukaryotes structural elements nano-measuring in normal and contaminated circumstances factors.

Materials and Methods

Viruses, bacteria and protozoa; environmental physical factors and chemical reagents used.

As basic investigation objects there were used different strains of enteric viruses (rotaviruses), enteric bacteria *Escherichia coli*, *Salmonella typhimurium* pathogenic strains, pathogenic *Shigella flexnerii*, *Borrelia caucasica*, *Treponema pallida*, *Leptospira Pomona*, *Entamoeba histolytica* taken from patients and carriers, pathogenic for reptiles *Ent. invadens*, free-living *Ent. moshkovskii*, taken from sewage, as well as bioplates of the patients' mucous membrane who suffer from enteric amebiasis, *Lambliia intestinalis*, *Leishmania hertigi*, *Tetrahymena pyriformis* different strains cultures and separated appendages, cholesterol containing artificial liposome's in this work.

From physical, chemical and bioorganic factors there were used gamma-ionizing, ultraviolet irradiation, imidazole, mitomicine "C" and different antibiotics preparations influence.

Electronic microscopy assay

The methods of negative contrasting by 2 % phosphoric-tungsten acid solution at pH-6,8-7,0 and methods of ultrathin sections [8] both were used in the study. For preparing electronic-microscopic preparations of viruses, bacteria and protozoa suspensions, biological samples were fixed by 2.5 % solution of glutaraldehyde or the mixture of paraformaldehyde, glutaraldehyde and picric acid with further dehydration, saturating, polymerization in Araldite preparations. These were observed with electronic microscopes Tesla BS-500 (Tesla, Czechoslovakia) and JEM-100B (JEOL, Japan). Computer morphometric and stereo metric analysis of electronic microscopic pictures was performed according to the program "Video Test Structure", elaborated by "VideoTesT" Ltd (St. Petersburg, Russia).

Results and Discussions

The most important constructions of studied rotaviruses were capsomers of virions in diameter of 65-70 nm (Figs.1a,1b) and fibers structure of external cell surface of different bacteria (*Escherichia*, *Shigella*, *Salmonella*) (in diameter of ~20 nm).

Moving nano-constructions at spirochetes are observed to be fibrils in diameter of 17-20 nm, 20-40 nm diameter disk-like blepharoplasts and 20-40 nm in diameter axis fibrillas. Among

gram-negative bacteria *E. coli* only has adhesive features, on external cover of those there were observed nano-tubes-like fimbrias (pili) in diameter of 8 nm (Fig.2a), with the help of those plasmidous hoop-like DNA is passed to the donor microbial cell.

Structural observation of *Sh. flexnerii* culture growth different stages has revealed gram-negative nano-bacteria, size of which (in diameter of 20-50nm) (Fig.2b) correspond to nano-measuring involvement (in diameter of 20-200 nm). Such structural formations were also observed with *Salmonella* in case of different spectrum antibiotics suppressive doses influence. Electronic microscope analysis and compound measurement program calculation of entamoebae virus-symbiotes showed that the length of virus-symbiotes is of 160-180 nm, diameter is of 60-70 nm (Fig.3a), and the common surface area is of $\sim 11,036 \text{ nm}^2$. After the influence of gamma-ionizing irradiation (see Materials and methods) on entamoebae, in virus-symbiotes we discovered 2 types of osmium globe-shaped nano-particles “O₁” in diameter of $\sim 35 \text{ nm}$ and with $\sim 885 \text{ nm}^2$ surface and accordingly “O₂” with $\sim 5 \text{ nm}$ diameter and $\sim 45 \text{ nm}^2$ surface (Fig.3b) [6,7]. “O₂” type considerably big nano-particles seem to be placed under the membrane of virus-symbiotes.

The ultrastructural analysis showed that the ionizing irradiation contributes to viruses three-layers membrane outlines (in thickness of 9-10 nm) visibility defined pictures. By their structural special features virus-symbiotes are like *Rhabdoviridae* family viruses [9-12].

In cytoplasm of entamoebae of different species, the ribosomes in diffusive state and polyribosomes, diameter of which is 18-25 nm, were being imaged also as spiral-like windings congestion (Fig. 4a). Laser diffractometry of ribonucleoprotein crystal-like congestions showed that the winding distance from center to center is 40-42 nm.

In entamoebae vegetative types cytoplasm there were noticed nano-structures of $\sim 10 \text{ nm}$ inner diameter microtubes and 5-7 nm diameter microfilaments carrying the cell movable function (Fig.4b), but in cells of *Leishmania hertigi*, *Lambliia intestinalis* and *Tetrahymena pyriformis* there were noticed nanotubes of cell skeleton with 22-25 nm in diameter [11-13].

Electronic microscope studies with *Salmonella* lysogen strains of gram-negative viruses and the influence of mytomicin “C” antibiotics on ultraviolet rays as well as with entamoebae polygenic culture revealed their phagogenic, off-cysting and imidazole group preparations cell stimulating peculiarities.

In entamoebae nucleoplasma, we discovered ring-shaped plasmid DNA-like 30-50 nm by length filiform ring-shaped constructions (Fig.4c). It seems that nucleolus is formed individually (in diameter of 15-20 nm) and resulted as microfilament (in diameter of 2-5 nm). In nuclei of all studied entamoebae types, mitotic microtubes (in diameter of 20-25 nm) are in the

inner part of the nucleus, without withdraw from the nuclear membrane and forming the center of microtubes organization as shown by Shang et al. [13].

In count list bionanoparticles (diameter of 1 μm) enters also artificial membrane or liposomes stabilizing by digitonin having sizes less than erythrocyte (in diameter of 7 μm) for delivery different of chimiopreparatives and bioactive material in cells-targets. Us with using the methods negative contrasting, cryoultrathin sections and traditional ultrathin sections studied cholesterol containing liposomes and their interactions with entamoebas and bacteria (adhesion, dens-contacts membranes, penetration, phagocytosis [14-15].

Conclusions

Ultrastructural analysis of data obtained by electronic microscopy allowed classifying conditionally different types of viruses, microbes and protozoa of nano-sized structures which are involving in cell morphogenesis. The principle of performed nano-sized involvement of constructional agent classification is based on our retrospective analysis having a continuous property.

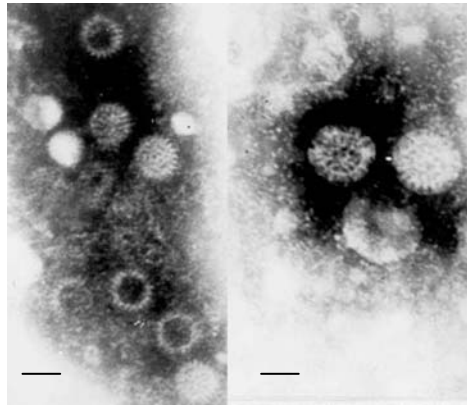
Acknowledgements

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a. b.

Fig.1. TEM of Rotavirus capsomers. Negative staining. Scale marker bars: 40 nm(a), 35 nm (b).

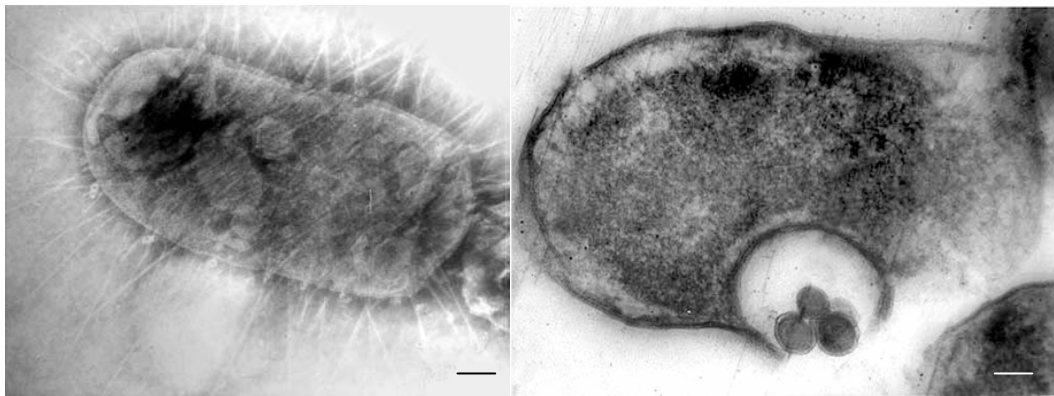


Fig. 2a. TEM. Fimbrii (pili) of cell *E. coli* (negative staining). Scale marker bar: 40nm.

Fig. 2b. TEM. *Sh. flexnerii* (strain). Ultrathin section. Breaking out (exit) nanobacterias. Scale marker bar: 40 nm.

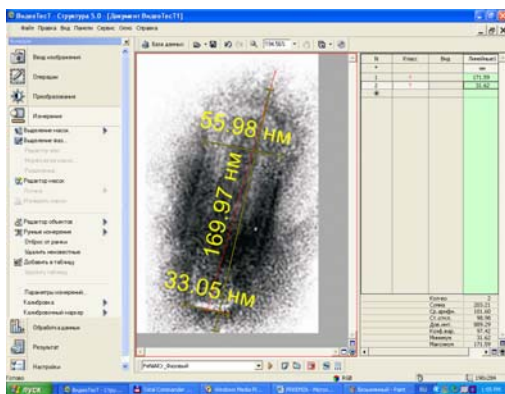


Fig. 3a. TEM. Virus-symbiont of vegetative form of *Ent. histolytica* (negative contrasting). For imaging analysis see Materials and methods).

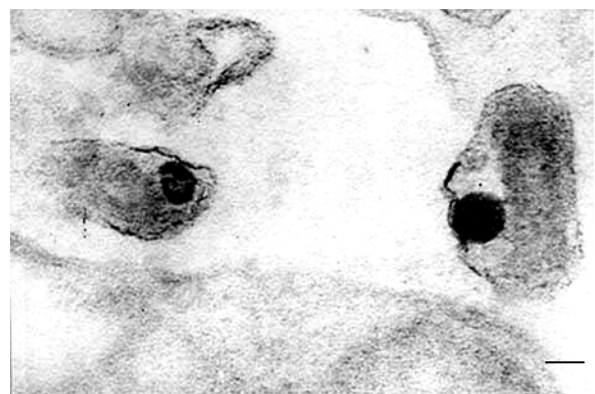


Fig.3b. TEM . Virus-symbionts *Ent. moshkovskii* after γ -ionizing radiation . O₁ –nanoparticles. Scale marker bar: 25 nm.

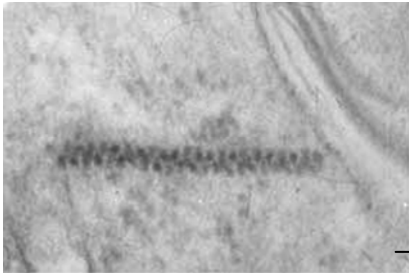


Fig. 4a. Ribonucleoprotein spiral nanostructure of *Ent. histolytica* (ultrathin section). Scale marker bar: 40 nm

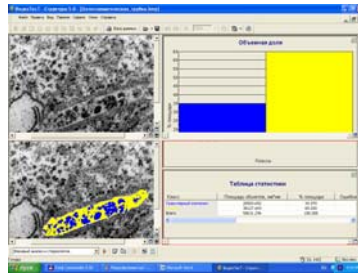


Fig. 4b. TEM. Hematophagy of *Ent. histolytica*. For imaging analysis see Materials and methods).

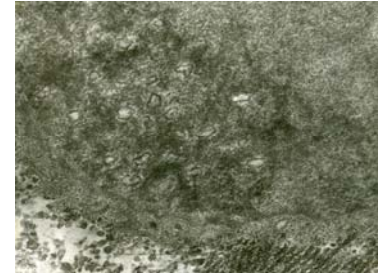


Fig. 4c. Internuclear ring-shaped DNA of *Ent. histolytica* (ultrathin section). Scale marker bar: 40 nm

**Contact adress: Karlen O. Hovnanyan , Dr.Sci
Head of Laboratory Electron Microscopy of Institute Molecular Biology of
National Academy Sciencs, Republic of Armenia,
President of Armenian Electron Microscopy Society,**

20/1 Vardanants str., Yertevan , 0070, Republic of Armenia,

Tel.: 550 209 apt.,

28 26 61 of.

E.mail: hovkarl@mail.ru

Tel.: 550 209

Mobil: 093647949