SCANNING FORCE MICROSCOPY STUDY OF THE PANCREATIC β-CELLS SURFACE MORPHOLOGY CHANGES AFTER EXPOSURE TO REACTIVE OXYGEN SPECIES

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In the research the insulin-producing pancreatic β-cells surface morphology before and after apoptosis promoter (H_2O_2) treatment was investigated by SFM. Hydrogen peroxide was used as well established model of a biologically active oxygen-derived intermediate at the concentration of 0.5 mM. Here we demonstrate that the observed by SFM cellular morphological changes of the pancreatic β-cells on glass after H_2O_2 exposure correspond, most likely, to apoptotic cell death.

1. Introduction

Due to the numerous advantages of scanning force microscopy (SFM), it is now applied to investigation of surfaces morphology changes of variety of biological cells in apoptosis researches in addition to traditional approaches of apoptotic cell death study [1]. SFM allows one to image the complicated surface morphology of cells in three dimensions at various resolution levels. For example, it is possible to image the shape of a whole cell and then zoom into a defined location on the same cell to image subcellular structures at high resolution of nanometer scale.

Apoptosis or programmed cell death (PCD) is a process of controlled cell removal in multi-cellular organisms. Recent studies have demonstrated that reactive oxygen species (ROS) play a pivotal role in apoptosis[2, 3]. H_2O_2 is one of a key oxygen metabolite, produced in several cellular compartments and is a intermediate in the reactions of reactive oxygen species. Besides its role in cellular toxicity, H_2O_2 has recently gained much attention as a possible signaling molecule involved in signal transduction pathways.Oxidative stress, through the production of oxygen metabolites, particularly H_2O_2 and other ROS, results in destruction of many cell types through putative necrotic\apoptotic processes. Although ROS have been implicated in cell death, the exact mechanism(s) are, as yet, unclear.

Insulin-producing pancreatic β-cells have been known to be particularly sensitive to oxidative stress (as pancreatic β-cells have been reported to be deficient in glutathione peroxidase, catalase, and superoxide dismutase relative to other tissues [5]), making these cells useful models to study of oxidative stress-induced apoptosis mechanism(s). In the research the pancreatic β-cells surface morphology before and after apoptosis promoter (H_2O_2) treatment was investigated by SFM. Hydrogen peroxide was used as well established model of a biologically active oxygen-derived intermediate at the concentration of 0.5 mM. Here we demonstrate that the observed by SFM

cellular morphological changes of the pancreatic β-cells on glass after H_2O_2 exposure correspond, most likely, to apoptotic cell death.

2. Experimental

Insulin-producing pancreatic β-cells were cultured on polylyzin-coated mica and glass slides. For AFM investigations, the β-cells before and after exposure to H_2O_2 (0.5 mM solution, during 20 min) were fixed with 2% glutaraldehyde for > 60 min and postfixed with 1% OsO_4 for 30min. The cells were then dehydrated in 30 – 100% ethanol and dried using a critical point dryer.

Surface morphology of pancreatic β-cells before and after hydrogen peroxide treatment was investigated by a Multi-Mode-SFM Nanoscope (R) IIIa (Digital Instruments, Santa Barbara, USA). SFM surface images were acquired in air using tapping imaging mode. Commercially available, 123–μm-long silicon cantilevers with spring constants of 29–57 N/m were used for most experiments (Nanosensors GmbH).

3. Results and Discussion

Apoptosis is characterized by a lot of biological and morphological changes of cells such as nuclear condensation, cell shrinkage, membrane blebbing, cell fragmentation and release of vesicles, apoptotic bodies, which are further ingested by phagocytes without inflammation reaction.The use of SFM reveals new potentials for surface morphological features studies which accompany the process of apoptosis induced ROS.

Fig.1 (a) shows the SFM image of fixed with glutaraldehyde control β-cell on mica, which was not exposed to H_2O_2. The diameter of the cell is approximately 10 μm and its height is about 2.5 μm. After using the zoom-in mode of the SFM an analysis of the plasma membrane of this β-cell revealed its globular structure with globular particles of irregular size of from ~ 30 nm to 100 nm (Fig. 1,b).

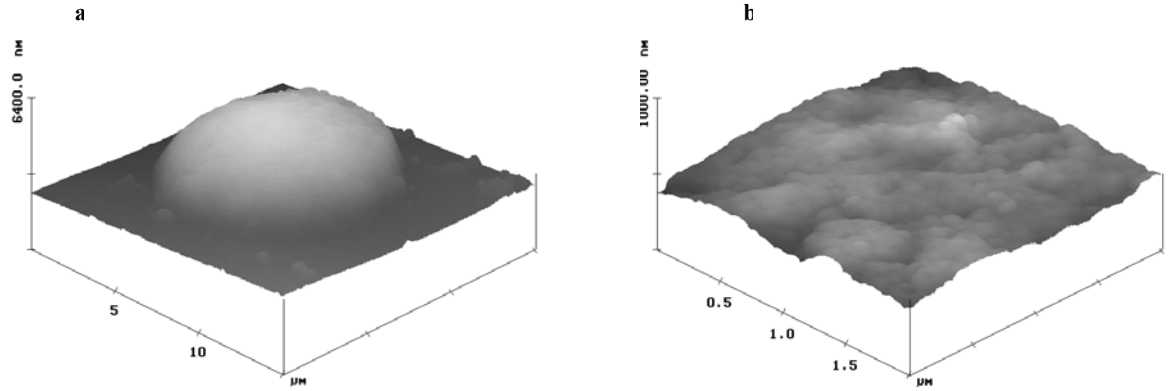

Fig.1. SFM images of fixed with glutaraldehyde control pancreatic β-cell (a) and its plasma membrane (b) on mica

Fig.2 (a) presents the SFM image of β-cell on mica after H_2O_2 exposure. It is worth noting that SFM investigations demonstrate marked changers in surface morphology of β-cells on mica before and after H_2O_2 exposure. As illustrated by Fig. 2,a

β-cell treated with H_2O_2 on mica swells and its size increases up to ~13 μm. The height of the cell is extremely varied, increasing from 2.5 μm to 5.5 μm. Smoothing of the plasma membrane and its early lyses are visualized (Fig. 2,b). Observed cellular morphological changes of the pancreatic β-cells on mica after H_2O_2 treatment correspond, most likely, to necrotic cell death. Probably, due to mica layer structure some H_2O_2 can remain in mica and thus to prolong exposure time and result in necrotic cell death.

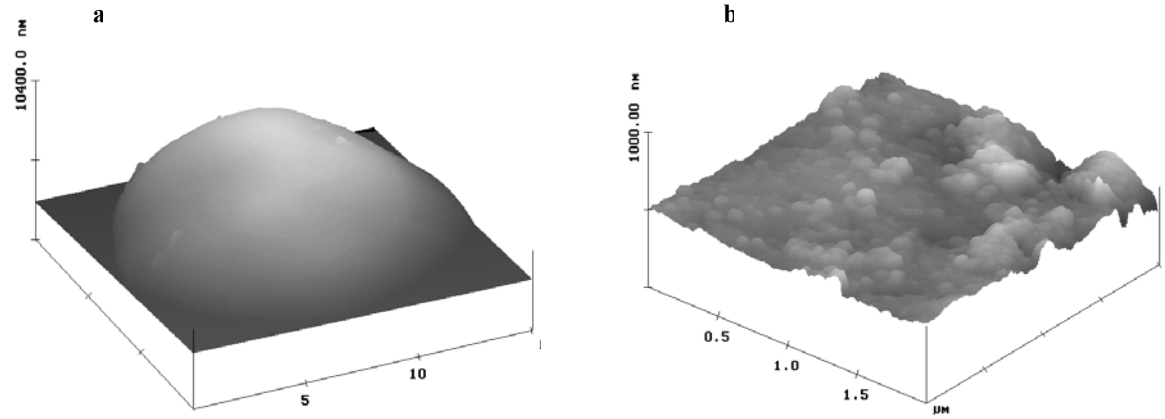

Fig.2. SFM images of fixed with glutaraldehyde pancreatic β-cell (a) and its plasma membrane (b) on mica after exposure to H_2O_2

A control pancreatic β-cell on glass is shown in Fig. 3,a. The dimensions of the cell are ~ 10 μm in width and ~2.5 μm in height. Zooming in on the same cell the globular structure of the plasma membrane was examined. The *xy* size of the particles was generally between ~30 and 100 nm. Damages of the β-cells plasma membrane before H_2O_2 exposure were not observed (Fig. 3,b).

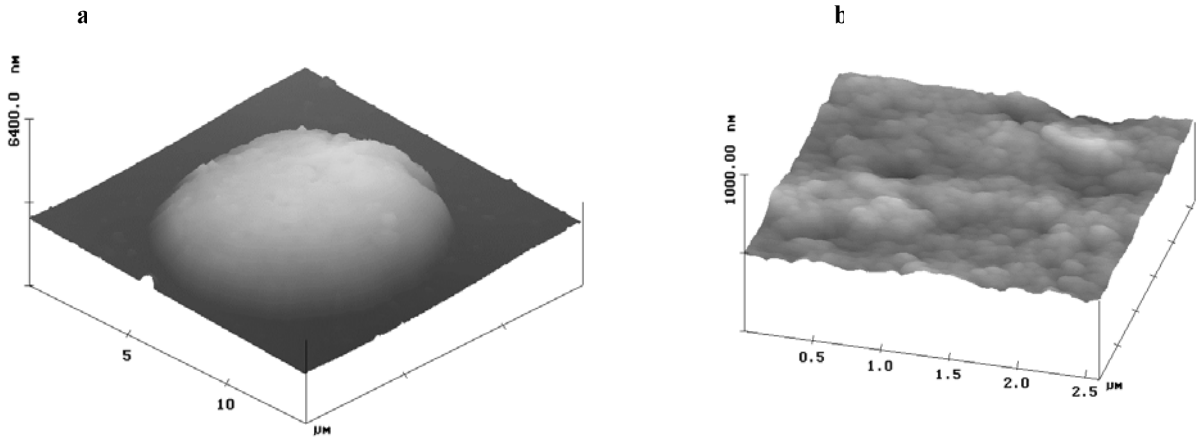

Fig. 3. SFM image of fixed with glutaraldehyde control pancreatic β-cell (a) and its plasma membrane (b) on glass

The β-cell SFM image on glass after H_2O_2 treatment is given in Fig. 4,a. In contrast,

after exposure to H_2O_2 β-cell shrinkage and cell fragmentation are visualized. Treated with H_2O_2 β-cell on glass flattens. Drastic reduction in cell body height (from 2.5 μm to 0.5 μm) following exposure to H_2O_2 is vividly seen. On the cell surface the local membrane convexities are observed. Possibly, the fragments of cell organelles are located under these convexities. Most likely, the formation of apoptotic bodies is visualised. The decreasing of scan area allows to observe plasma membrane blebbing originated from H_2O_2 treatment (Fig. 4,b). The presence of typical features of apoptotic morphological changes of the pancreatic β-cells treated with H_2O_2 on glass as visualized by SFM let us conclude that apoptotic cell death was observed.

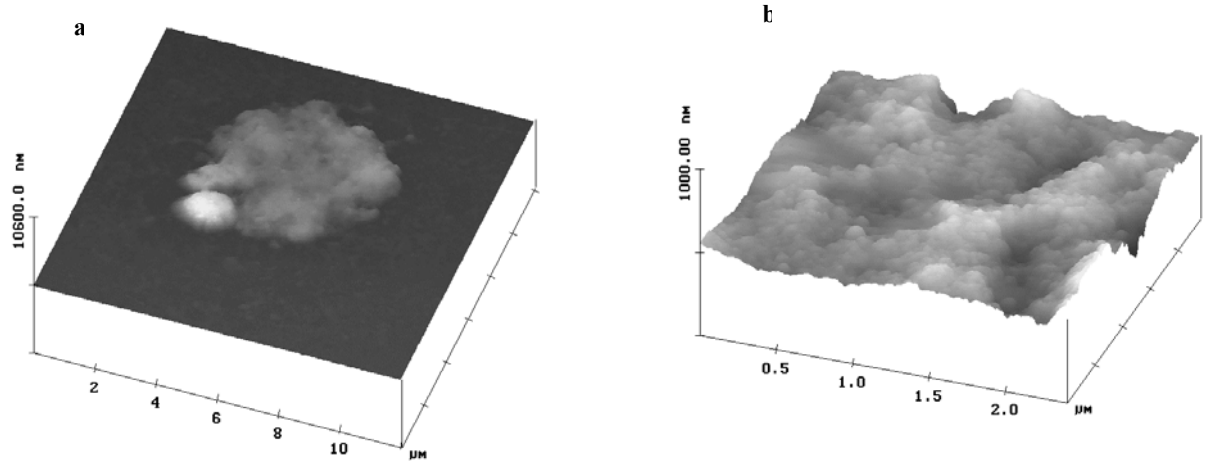

Fig.4. SFM image of fixed with glutaraldehyde pancreatic β-cell (a) and its plasma membrane (b) on glass after exposure to H_2O_2

Previous researches have demonstrated that H_2O_2, most probably through the generation of ROS, causes the sustained elevation of intracellular Ca^{2+} levels [6,7] and activation of Ca^{2+} -dependent catabolic enzymes that cause apoptotic cell death [8]. There is no clear consensus in the literature indicating the likely mechanism by which H_2O_2 causes intracellular Ca^{2+} levels rise. Without doubt mitochondria, which are responsible for the initial calcium response under oxidative stress play major role in apoptosis [9,10]. Further SFM investigations should be direct for study surface morphology changes of intracellular organelles, in particular mitochondria under oxidative stress.

In conclusion, using SFM, we could visualize the β-cells surface morphology changes originated from H_2O_2 treatment. The present study revealed that the observed cellular morphological changes of the pancreatic β-cells on glass after H_2O_2 exposure correspond, most likely, to apoptotic cell death. These results indicate that SFM can be utilized for the analyses of cellular surface morphological changes which accompany the process of apoptosis induced ROS.

Acknowledgments

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References

1. Kukharenko L.V., Fuchs H., Ilyich G.K., Gronskaya R.I., Cherenkevich S.N., Martinovich G.G. // Phys. Low-Dim.Struct. 2002. № 5/6. P. 115.
2. Song J.Y., Lim J.W., Kim H., Morio T., Kim K.H. // J. Biol. Chem. 2003. № 278. P. 36676.
3. Kelvin J.A. Davies // Life 2000. № 50. P. 241.
4. Reth M. // Nature immunology. 2002. № 3. P. 1129.
5. Tiedge M., Lortz S., Drinkgern J., Lensen S. // Diabetes. 1997. № 46. P. 1733.
6. Krippeit-Drews P., Kramer C., Welker S., Lang F., Ammon H.P., Drews G. // J. Physiol. Lond., 1999. № 514. P. 471.
7. Maechler P., Jornot L., Mollheim C.B. // J. Biol. Chem. 1999. № 274. P. 27905.
8. Dröge W. // Physiol.Rev. 2002. № 82. P. 47.
9. Douglas R. Green and John C. Reed // Science. 1998. № 281. P. 1309.
10. Desagher S., Martinou J.-C. // Cell Biology. 2000. № 10. P. 369.