UPTAKE AND INTRACELLULAR LOCALIZATION OF $TPPS_4$ IN LIVING CELLS

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Meso-tetra(4-sulfonatophenyl) porphyrin ($TPPS_4$) is a synthetic water-soluble organic macrocycle compound, composed of a porphyrin ring (which consists of four pyrrole rings interconnected via methine bridges at their α carbon) and four sulfonatophenyl substitutes. $TPPS_4$ has been studied as a potential photosensitizer in photodynamic therapy (PDT) of cancer [1]. Due to its high triplet yield, this porphyrin is an efficient singlet oxygen generator. However, in an acidic environment, $TPPS_4$ tends to form J or H aggregates, which affect the optical and energetic properties by decreasing the quantum yields and lifetimes of excited singlet and triplet states [1]. J-aggregates have a low yield of generating reactive oxygen species. The aggregation properties of the dye also play a role in the accumulation and retention of the dye inside cells. Therefore, it is important to know the conditions at which such aggregates form. Recently, it has been predicted that $TPPS_4$ sensitizer has a potential application in non-linear microscopy (second and third harmonic generation).

This study aims to determine the localization and uptake of $TPPS_4$ in cells as well as to determine optical properties inside living cells. Some hydrophilic sensitizers are taken up by the endocytic pathway and are mainly localized in the lysosomes [2]. The initial experiment was carried out with embryonic mice fibroblast NIH/3T3 cells. After the addition of $TPPS_4$, the cells were cultivated for 24 h at 37°C, 5% CO_2. The results of confocal microscopy imaging showed that this sensitizer accumulates in vesicles around the nucleus of the cell and probably entered the cell *via* the endocytic pathway. $TPPS_4$ is distributed in a relatively uniform pattern inside the cells.

We recorded fluorescence spectra in living cells, when excited with 404 nm and 488 nm lasers, and showed that $TPPS_4$ molecules exist in a monomeric form inside NIH/3T3 cells. We compared $TPPS_4$ spectra measured inside the living cells with the spectra of the monomeric form of $TPPS_4$ in a solution (pH~7). We noticed that the peaks of the fluorescence spectra inside the cells have a shift of approximately 10 nm towards the longer wavelength of the spectrum. This is due to interactions between $TPPS_4$ and the biomolecules inside the cells.

We plan to carry out further experiments with tumor cells and perform live-cell imaging by using second and third harmonic generation microscopy, as well as PDT treatment after accumulation of $TPPS_4$ inside the cells. The study will validate a possibility of using $TPPS_4$ as a theranostic agent for targeted cancer therapy.

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