A setup consisting on a laser scanning microscope (LSM 410, Zeiss, Germany) equipped with appropriate detection units was developed for time-resolved intracellular fluorescence spectroscopy and fluorescence lifetime imaging (FLIM) for on-line detection of structural changes of various biomolecules. Short-pulsed excitation was performed with a diode laser which emits pulses at 398 nm with 70 ps duration (LDH-C 400, PicoQuant, Germany). The laser was coupled to the laser scanning microscope. For time resolved spectroscopy a setup consisting on a Czerny Turner spectrometer and a MCP-gated and -intensified CCD camera was used. Time-gated spectra within the cells were acquired by placing the laser beam in “spot scan” mode. In addition, a time-correlated single photon counting module (SPC-730, Becker & Hickl, Germany) was used to determine the fluorescence lifetime of different photosensitizers from single spots and to record lifetime images (τ-mapping) [1].

Within this work, we investigated the time-resolved fluorescence characteristics of 5-ALA (5-aminolevulinicacid) induced protoporphyrine IX (PPIX). 5-ALA is an important precursor of the photosensitizer PPIX, the latter being synthesized in the mitochondria of cells and used in photodynamic therapy (PDT) of superficial tumours. Alternatively to 5-ALA lipophilic derivatives, which are taken up in a higher amount into the cells are promising candidates, as 5-ALAhe (5-aminolevulinicacid hexylester). It is also reported that 5-ALA derivatives may induce a higher phototoxicity. It is however unclear, whether this enhanced effect is solely due to the increased uptake or to a different subcellular accumulation and metabolism. Therefore, we performed FLIM of cells incubated with 1mM 5-ALA or 50 µM 5-ALAhe, respectively, before and during PDT. For cells which were incubated with 5-ALA, a component with a fluorescence lifetime of about 7 ns was correlated with a structured fluorescence, possibly attributed to mitochondria, whereas a shorter lifetime was found in the cytoplasm. In the case of 5-ALAhe the lifetime of PPIX was longer, which could be due to different subcellular localization or synthesis of other porphyrins. During PDT the component with the longer lifetime completely vanished, whereas the shorter lifetime was retained. Because the photodynamic effects can be correlated with the lifetime of the excited states it seems that FLIM, using ps diode lasers and TCSPC is a valuable method to selectively identify and localize the photodynamically active photosensitizer.


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