MEROCYANINES AS FLUORESCENT PROBES FOR THE DETECTION IN MICROSCOPY

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Nowadays, the study of the drug distribution in cells is becoming an increasingly actual method of biomedical research. Far-red fluorescent probes are highly applicable due to certain advantages such as minimum photo-damaging of biological samples, high tissue penetration and allow imaging with minimal to no autofluorescence. The aim of the research was to study the novel series of far-red merocyanine dyes containing modified polymethine bridges by fluorescence microscopy.

Studied dyes are low to moderate fluorescent when free. The maxima of excitation spectra are located at 624–714 nm with the fluorescence emission maxima lie in the far-red area of the spectrum between 644–730 nm. The fluorescence intensity of all dyes increases in number of times in the presence of bovine, human and equine albumins. The equilibrium constant of dye binding with HSA (binding constant, K_b) was determined for the most promising merocyanine based on experimental measurements of the fluorescence intensity of the dye with increasing amounts of HSA. Based on the titration curve, the binding constant of dye to HSA was estimated to be $K_b = (1.2\pm0.1)\cdot10^5\ M^{-1}$. The protein detection range of HSA for this dye monotonically increases from 0.007 to 4 mg/ml, which indicates the high sensitivity of the dye to this protein at low concentrations. The quantum yield value was 31 % in the presence HSA comparing to 2 % for the free dye.

To study the ability of merocyanine dyes to penetrate cell membrane, human ovarian cancer cell line A2780 were used. Fluorescence microscopy revealed that the studied dyes able to pass through biological membranes and stain the cell components in cytoplasm. Both dyes are not accumulated in nuclei as shown by co-staining with Hoechst 33342: no co-localization with nuclear DNA dye is observed. Co-staining of the studied dyes with Rhodamine 123 (a dye specific for mitochondria), was carried out to understand the organelle-specificity of the dyes. The subsequent colocalization analysis indicate that the studied dyes colocalize very poorly with the Rhodamine 123.

The lack of specificity of dyes to some specific components of the cell (for example, mitochondria) may be an advantage for this class of compounds in their use as labels for studying the drugs distribution.

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