Photochemical behaviour and Na^+,K^+-ATPase sensitivity of voltage-sensitive styrylpyridinium fluorescent membrane probes

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Styrylpyridinium dyes are widely used probes of electric field strength changes in biological membrane systems. They can be used as optical probes in the imaging of electrical activity in nerve and muscle cells, and for the investigation of the mechanisms of electrogenic ion pumps. A limit to their application is, however, their photochemical stability. Probes with improved stability and voltage sensitivity are required in order to extend their areas of application.

Exposure of the voltage-sensitive membrane probe RH421 to continuous illumination with 577 nm light from an Hg lamp leads to an increase in its steady-state fluorescence level when bound to lipid membranes. The increase occurs on the second time-scale at typical light intensities and was found to be due to a single-photon excited-state isomerisation. In order to suppress this undesirable reaction, modifications to the dye structure are, therefore, necessary. The related probe ANNINE 5, which has a rigid polycyclic structure, shows no observable photochemical reaction when bound to DMPC vesicles on irradiation with 436 nm light. The voltage sensitivity of ANNINE 5 was tested using Na^+,K^+-ATPase membrane fragments. As long as ANNINE 5 is excited on the far red edge of its visible absorption band, it shows a similar sensitivity to RH421 in detecting charge-translocating reactions triggered by ATP phosphorylation. Unfortunately, the wavelengths necessary for ANNINE 5 excitation are in a region where the Hg lamps routinely used in stopped-flow apparatus have no significant lines available for excitation.