Live-cell imaging of new polyene sterols for improved analysis of intracellular cholesterol transport.

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Rok wydania

2018

Czasopismo

Journal of Microscopy

Numer woluminu

271

Strony

36-48

DOI

10.1111/jmi.12691

Kolekcja

Naukowa

Język

Angielski

Typ publikacji

Artykuł

Streszczenie

Analysis of intracellular cholesterol transport by fluorescence microscopy requires suitable fluorescent analogues of cholesterol. Most existing cholesterol analogues contain lipophilic dyes which can compromise the sterol properties in membranes. An alternative strategy is to introduce additional double bonds into the sterol ring system resulting in intrinsic fluorescence, while at the same time keeping the cholesterol-like properties of the analogues. Existing polyene sterols, such as dehydroergosterol (DHE) or cholestatrienol (CTL), however, contain only three double bonds and suffer from low brightness, significant photobleaching and excitation/emission in the ultraviolet region. Thus, special equipment is required to image such sterols. Here, we describe synthesis, characterization and intracellular imaging of new polyene sterols containing four conjugated double bonds in the sterol ring system. We show that such analogues have red-shifted excitation and emission by ∼20 nm compared to DHE or CTL. The red shift was even more pronounced when preventing keto-enol tautomer equilibration by protecting the 3'-hydroxy group with acetate. We show that the latter analogue can be imaged on a conventional wide field microscope with a DAPI/filipin filter cube. The new polyene sterols show reduced photobleaching compared to DHE or CTL allowing for improved deconvolution microscopy of sterol containing cellular membranes.

Słowa kluczowe

cholesterol, electronic structure calculation, fibroblasts, fluorescence, imaging, intracellular transport, Niemann–Pick disease type C2, photobleaching, synthesis

Adres publiczny

http://dx.doi.org/10.1111/jmi.12691