Title
Visualizing HDL particles removing cholesterol from artificial membranes by Laurdan GP and two-photon microscopy.

Journal
BIOPHYSICAL JOURNAL, 88(1)

ISSN
0006-3495

Authors
Sanchez, SA
Tricerri, MA
Gratton, E

Publication Date
2005

Susana A Sánchez, M Alejandra Tricerri, and Enrico Gratton.

Visualizing HDL particles removing cholesterol from artificial membranes by Laurdan GP and two-photon microscopy.

49th Annual Meeting of the Biophysical Society, Long Beach, California, 2005.
Biophys J. 2005; Suppl, 2862-Pos/B143.

Abstract

The traditional protocols to study cholesterol removal are done by incubation of the acceptor with a donor containing radioactive cholesterol, and measurement of radioactivity distribution after the reaction has occurred. This type of measurements disrupt the original equilibrium and give the average cholesterol that is being removed, i.e., the cholesterol coming from all the membrane independently from the original spatial location of the sterol. Here we report the use of a technique to quantify cholesterol removal at equilibrium and with high spatial resolution using two-photon microscopy. Laurdan is a fluorescent dye that gives information about the membrane water content which is related to fluidity. Laurdan generalized polarization (GP) is the parameter used to quantify water penetration. GP measurements in a two-photon microscope give the spatial resolution of water penetration and then indirectly of the membrane fluidity. We show changes in GP of GUVs made of POPC and 33% cholesterol with time, after incubation with reconstituted HDL particles (rHDL). Apolipoprotein A-I labeled with Alexa 488 was used in reconstituted HDL particles. The changes in the diffusion coefficient of the particles during the cholesterol removal were following by Fluorescence Correlation Spectroscopy (FCS). The autocorrelation analysis showed a decrease in diffusion coefficient during cholesterol removal. The Photon Counting Histogram analysis shows that the decrease in lateral mobility is due to the increase in size of the particle and not to particle aggregation. In GUVs made of DOPC/DPPC/cholesterol presenting domain coexistence we have observed and quantified the changes in GP produced by the addition of ... [truncated at 250 words]