Detection and characterization of calcium induced PI(4,5)P2 clustering in membranes. Application of advanced fluorescence and microscopy methodologies

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The lipid phosphatidylinositol 4,5-biphosphate is a critical minor component of the plasma membrane, since it is associated to e.g., membrane trafficking, endocytosis and exocytosis, as well as to the remodeling of the actin cytoskeleton. Although most of these processes are related to its localized lateral enrichment in the membrane, the mechanisms involved in this lipid clustering are however not fully elucidated. We carried out a fluorescence study of a derivatized PI(4,5)P2 in several types of model membrane systems, and determined that its lateral distribution is very sensitive to the calcium concentrations in the low micromolar concentration, i.e., in the physiological range. This was determined from steady-state and time-resolved fluorescence techniques, using fluorescence self-quenching and energy migration (energy homotransfer) interactions. This effect was observed in both liquid-disordered (cholesterol poor), or liquid-ordered (cholesterol rich/raft model membranes). A model was developed to rationalize the fluorescence depolarization, and its fitting to the data allowed to conclude that the average cluster size is about 15 molecules of PI(4,5)P2. Evidence for clustering was also directly obtained from fluorescence correlation spectroscopy (FCS), which showed a slower diffusion in the membrane due to the formation of the aggregates. In this way, PI(4,5)P2 could operate as a reliable sensor for calcium concentrations at the plasma membrane.