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Coverage-dependent changes of cytochrome *c* transverse location in phospholipid membranes revealed by FRET☆

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Abstract

The method of fluorescence resonance energy transfer (FRET) has been employed to monitor cytochrome *c* interaction with bilayer phospholipid membranes. Liposomes composed of phosphatidylcholine and varying amounts of anionic lipid cardiolipin (CL) were used as model membranes. Trace amount of fluorescent lipid derivative, anthrylvinyl-phosphatidylcholine was incorporated into the membranes to serve energy donor for heme moiety of cytochrome *c*. Energy transfer efficiency was measured at different lipid and protein concentrations to obtain extensive set of data, which were further analyzed globally in terms of adequate models of protein adsorption and energy transfer on the membrane surface. It has been found that the cytochrome *c* association with membranes containing 10 mol% CL can be described in terms of equilibrium binding model (yielding dissociation constant K_d=0.2–0.4 μM and stoichiometry n=11–13 lipid molecules per protein binding site) combined with FRET model assuming uniform acceptor distribution with the distance of 3.5–3.6 nm between the bilayer midplane and heme moiety of cytochrome *c*. However, increasing the CL content to 20 or 40 mol% (at low ionic strength) resulted in a different behavior of FRET profiles, inconsistent with the concepts of equilibrium adsorption of cytochrome *c* at the membrane surface and/or uniform acceptor distribution. To explain this fact, several possibilities are analyzed, including cytochrome *c*-induced formation of non-bilayer structures and clusters of charged lipids, or changes in the depth of cytochrome *c* penetration into the bilayer depending on the protein surface density. Additional control experiments have shown that only the latter process can explain the peculiar concentration dependences of FRET at high CL content.
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