Cytochrome *c* Interaction with Cardiolipin/Phosphatidylcholine Model Membranes: Effect of Cardiolipin Protonation

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ABSTRACT Resonance energy transfer between anthrylvinyl-labeled phosphatidylcholine as a donor and heme moiety of cytochrome *c* (cyt *c*) as an acceptor has been employed to explore the protein binding to model membranes, composed of phosphatidylcholine and cardiolipin (CL). The existence of two types of protein-lipid complexes has been hypothesized where either deprotonated or partially protonated CL molecules are responsible for cyt *c* attachment to bilayer surface. To quantitatively describe cyt *c* membrane binding, the adsorption model based on scaled particle and double layer theories has been employed, with potential-dependent association constants being treated as a function of acidic phospholipid mole fraction, degree of CL protonation, ionic strength, and surface coverage. Multiple arrays of resonance energy transfer data obtained under conditions of varying pH, ionic strength, CL content, and protein/lipid molar ratio have been analyzed in terms of the model of energy transfer in two-dimensional systems combined with the adsorption model allowing for area exclusion and electrostatic effects. The set of recovered model parameters included effective protein charge, intrinsic association constants, and heme distance from the bilayer midplane for both types of protein-lipid complexes. Upon increasing CL mole fraction from 10 to 20 mol % (the value close to that characteristic of the inner mitochondrial membrane), the binding equilibrium dramatically shifted toward cyt *c* association with partially protonated CL species. The estimates of heme distance from bilayer center suggest shallow bilayer location of cyt *c* at physiological pH, whereas at pH below 6.0, the protein tends to insert into membrane core.