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INTERACTIONS BETWEEN CYTOCHROME c AND PHOSPHOLIPID VESICLES STUDIED BY RESONANCE RAMAN SPECTROSCOPY

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INTRODUCTION

Binding of cytochrome c to negatively charged surfaces such as metal electrodes, polyanions, and phospholipid vesicles can produce significant conformational changes in the heme pocket[1-3]. Detailed resonance Raman (RR) studies have revealed that in such complexes cytochrome c exist in a conformational equilibrium between two states (I, II). While in state I the structure of the heme pocket is unchanged compared to the unbound protein, in state II the heme crevice opens, weakening the coordinative bond of the heme iron with the methionine-80 ligand. Thus, a thermal coordination equilibrium between a six-coordinated low-spin (6cLS) and a five-coordinated high-spin configuration (5cHS) is established in state II. These structural changes were also detected in complexes with the physiological redox partner cytochrome c oxidase[4], suggesting that they are of functional significance for the biological electron transfer.

In the present work we have continued these studies by systematically analyzing the interactions of ferri-cytochrome c with negatively charged phospholipid vesicles. RR spectroscopy was employed to determine the conformational equilibria in cytochrome c formed in complexes with different lipid systems. The relative contributions of the various species of cytochrome c were determined from the relative intensities of those RR bands which have been found to be characteristic markers for the individual conformational states[3]. The effect on protein binding on the lipid bilayer structure was analyzed by ^{31}P-NMR spectroscopy. The main goal of this study was to find correlations between the structural changes in the protein and in the lipid upon complex formation. A detailed description of the experimental methods, data analysis, and sample preparation is given elsewhere[3,5].

RESULTS AND DISCUSSION

Fig. 1 shows the so obtained concentration ratio of state II (including both the 5cHS- and the 6cLS-configuration) and state I of cytochrome c bound to dioleoyl phosphatidylglycerol (DOPG) - dioleoyl glycerol (DOG). The admixture of uncharged lipids (DOG) significantly lowers the fraction of state II. At 10% DOG, the equilibrium constant has decreased by a factor of ~ 2, but a further increase of DOG up to 30% lowers the equilibrium constant only from ~ 1.6 to ~ 1.2. On the other hand, the binding energy is approximately the same (~ 39 kJ/M) in pure DOPG and DOPG-DOG (70:30). Since the main contribution to the binding energy originates from the electrostatic interactions between the anionic phopholipid headgroups and the positively charged lysine residues around the heme crevice of cytochrome c, these findings imply that complex formation with cytochrome c induces an asymmetric distribution of charged and uncharged lipids at the protein binding site. This would in turn affect the bilayer structure of the phospholipid vesicles which is, in fact, reflected by the ^{31}P-NMR spectra. While in the absence of cytochrome c the ^{31}P-NMR spectra reveal the

characteristic signature of a regular bilayer structure, admixture of DOG favors the the formation of an inverted hexagonal phase which is prevailing at 50% DOG. In the presence of cytochrome c, however, all the spectra are dominated by an isotropic peak which may result from local curvatures on the bilayer structure. Such curvatures may facilitate the electrostatic interactions with the lysine residues on the surface of the spherically-shaped cytochrome c.

The state II/state I ratio of cytochrome c in the pure DOPG system is constant in the temperature range between 15 and 30°C (Fig. 2). On the other hand, in the dimyristoyl phosphatidylglycerol (DMPG) system there is an abrupt increase of the concentration ratio at ~ 25°C. This temperature corresponds to the phase transition gel→fluid of DMPG, which in the case of DOPG is about 20° lower. Below and above this temperature, the concentration ratio is largely constant and in both phases of DMPG clearly larger than in the DOPG system.

It is not possible to correlate the equilibrium constants of the conformational equilibrium with the surface charge density of the various lipid systems since in the absence of specific ion binding effects, the lipid surface charge density is expected to be greater in the gel phase than in the fluid phase of DMPG and yet smaller in the fluid phase of DOPG. Hence, there must be some other effect contributing to the electrostatic control of the conformational equilibrium. Based on previous studies of cytochrome c bound to charged interfaces it was argued that depending on the individual lysine residues which are involved in the complex formation either the conformational state II or I are stabilized. Thus, not only the number but also the specific spatial arrangement of the phospholipid headgroups at the protein binding site may govern the conformational equilibrium. The latter parameter should strongly depend on the surface flexibility of the lipid bilayer which is substantially different in the fluid and in the gel phase as well as in saturated and unsaturated lipid systems. Thus, surface charge density and the structural flexibility may exert opposite effects on the conformational distribution of the bound cytochrome c.

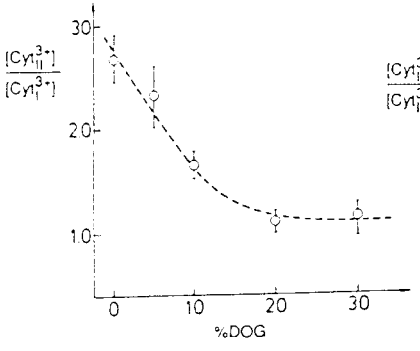

Fig. 1:
Concentration ratio of state II and state I in cytochrome c/DOPG-DOG, as a function of DOG content.

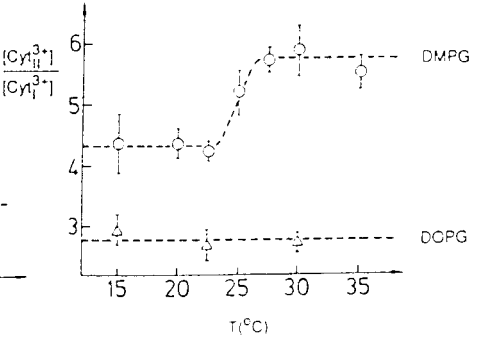

Fig. 2:
Temperature-dependence of the conformational equilibrium state II/state I for cytochrome c bound to DMPG and DOPG.

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