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Membrane Binding Induces Destabilization of Cytochrome *c* Structure†

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ABSTRACT: The effect of membrane binding on the structure and stability of ferricytochrome *c* was studied by Fourier-transform infrared spectroscopy and differential scanning calorimetry. Association of cytochrome *c* with phospholipid membranes containing phosphatidylglycerol as a model acidic phospholipid results in only slight, if any, perturbation of the protein secondary structure. However, upon membrane binding, there is a considerable increase in the accessibility of protein backbone amide groups to hydrogen–deuterium exchange, which suggests a lipid-mediated loosening and/or destabilization of the protein tertiary structure. A lipid-induced conformational perturbation of ferricytochrome *c* is also indicated by a marked decrease in the thermodynamic stability of the membrane-bound protein. Upon binding to membranes containing dimyristoylphosphatidylglycerol (DMPG) or dioleoylphosphatidylglycerol (DOPG) as a single lipid component, the denaturation temperature of ferricytochrome *c* decreases by approximately 30 °C. This is accompanied by a decrease in the calorimetric enthalpy of denaturation, particularly for the DMPG-associated protein. With ferricytochrome *c* bound to membranes containing a mixture of DMPG (or DOPG) and zwitterionic phosphatidylcholine, the extent of structural perturbation depends on the surface density of the negatively charged lipid head groups, becoming smaller with decreasing proportions of acidic phospholipid in the membrane. The observed destabilization of protein structure mediated by acidic phospholipids (and possibly formation of folding intermediates at the membrane surface) may represent a general property of a larger class of water-soluble proteins for which membrane binding is governed by electrostatic forces.

Cytochrome *c*, a 104 amino acid protein, is an essential component of the respiratory chain in mitochondria. Due to its location on the surface of the inner mitochondrial membrane, as well as its ability to associate with negatively charged membrane phospholipids, cytochrome *c* is considered a typical extrinsic membrane protein. In this context, cytochrome *c*/lipid systems have been studied extensively with the aim of understanding the role of phospholipids in the activation of peripherally bound proteins. Interest in the interaction between cytochrome *c* (as well as its heme-free precursor, apo-

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cytochrome *c*) and lipids has been further stimulated by recent indications that acidic phospholipids may be functionally involved in the import of proteins into mitochondria (Rietveld & de Kruijff, 1986; Rietveld et al., 1983; Demel et al., 1989).

Although the interaction of cytochrome *c* with different lipids in model membranes has been studied by numerous physicochemical techniques (Kimelberg et al., 1970; Brown & Wüthrich, 1977; Rietveld et al., 1983; MacNaughtan et al., 1985; Devaux et al., 1986; Waltham et al., 1986; Szebeni & Tollin, 1988; Lee & Kim, 1989), some fundamental aspects of this interaction remain unclear and/or controversial. No general consensus has been reached regarding the degree of protein penetration into the lipid bilayer. Furthermore, surprisingly little attention has been paid to the conformational properties and thermodynamic stability of the protein molecule itself in the cytochrome *c*/lipid complexes. Recent resonance Raman (Vincent & Levin, 1986; Hildebrandt & Stockburger, 1989b; Hildebrandt et al., 1990) and magnetic resonance experiments (Vincent et al., 1987; Soussi et al., 1990) indicate that binding of cytochrome *c* to acidic phospholipids induces changes in the conformation and coordination within the heme group. However, no data exist to establish whether this perturbation of the prosthetic group represents a local effect only, or whether it reflects more extensive changes in the conformation and/or thermodynamic state of the protein backbone.

In this study, we have used Fourier-transform infrared spectroscopy and differential scanning calorimetry to probe the effect of membrane binding on the conformational properties and thermodynamic stability of the oxidized form of cytochrome *c*. Our data clearly indicate that association of ferricytochrome *c* with liposomes containing acidic phospholipids results in a loosening and destabilization of the overall protein structure.

Materials and Methods

Horse heart cytochrome *c* (type VI) and sodium dodecyl sulfate were obtained from Sigma (St. Louis, MO). Dimyristoylphosphatidylglycerol (DMPG),[1] DOPG, DMPC, and DOPC were from Avanti Polar Lipids (Birmingham, AL). Prior to use, cytochrome *c* was converted to the fully oxidized form by addition of excess $K_3Fe(CN)_6$ and then was dialyzed for approximately 10 h at 4 °C against buffer (50 mM HEPES/100 mM NaCl, pH 7.4).

To prepare samples for infrared spectroscopy, protein solution in buffer (15 mg/mL) was added to dry lipid films of the required composition, so that a lipid to protein weight ratio of approximately 1.5:1 was achieved. The mixture was gently vortexed for 5 min and incubated at approximately 25 °C for an additional 30 min. Lipid/protein complexes were then collected by centrifugation (16000*g*, 5 min), washed 2–3 times with a buffer prepared in ^2H_2O (containing 50 mM HEPES/100 mM NaCl, p^{2}H 7.4), and finally resuspended in the same buffer at a concentration of 20–30 mg of protein/mL. Samples for differential scanning calorimetry were prepared by essentially the same procedure except that the buffer was made in H_2O. The concentration of ferricytochrome *c* in samples used for calorimetric measurements was 1.5–3 mg/mL for protein complexes with DMPG or DMPG/DMPC, and 0.5–1.5 mg/mL for protein complexes with DOPG or DOPG/DOPC. In order to prevent peroxidation, samples containing unsaturated phospholipid were kept under nitrogen during preparation and measurement. Lipid content in the samples was determined by phosphorus assay (Chen et al., 1956), and protein concentration was measured according to Lowry et al. (1951). Cytochrome c/SDS complexes were perpared by mixing SDS with the protein solution at a surfactant to cytochrome *c* molar ratio of 100:1. The mixture remained visually transparent.

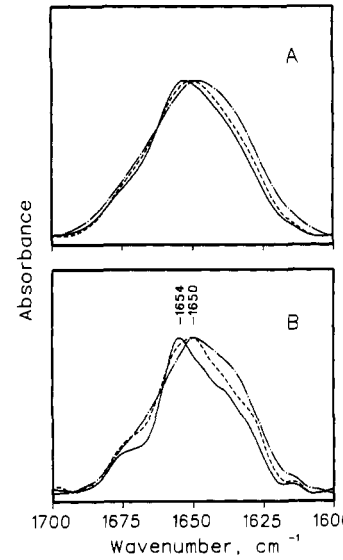

FIGURE 1: Infrared spectra in the amide I region of ferricytochrome *c* in buffer (—), ferricytochrome *c* complexed with DMPG at a lipid to protein molar ratio of 25:1 (- - -), and ferricytochrome *c* in the presence of sodium dodecyl sulfate at a detergent to protein molar ratio of 100:1 (-·-). (A) Original spectra; (B) same spectra after band-narrowing by Fourier self-deconvolution by use of a 15 cm^{-1} Lorentzian line and a band-narrowing factor of 2.

Infrared spectra were recorded on a Digilab FTS-60 instrument, with samples assembled in a 50-μm path-length calcium fluoride cell. Typically, 100 interferograms were co-added and Fourier-transformed to give a resolution of 2 cm^{-1}. Temperature was controlled by the computer and was stable to within 0.2 °C. The average heating rate was 10 °C/h. To eliminate spectral contributions of atmospheric water vapor, the instrtrument was continuously purged with dry nitrogen. Overlapping infrared bands were resolved by using Fourier self-deconvolution procedures (Kauppinen et al., 1981). Circular dichroism spectra were acquired at 28 °C on a Jasco J-600 spectropolarimeter. The differential scanning calorimetry experiments were performed with a high-sensitivity Microcal MC-2D instrument at a scanning rate of approximately 50 °C/h. Calorimetric data were converted to heat capacity versus temperature functions and analyzed with software provided by Microcal. Base lines were subtracted by using a cubic splines interpolation procedure.

Results and Interpretation

Infrared Spectroscopy. The conformation-sensitive amide I band of the aqueous solution of ferricytochrome *c* (in ^2H_2O buffer at p^{2}H 7.4) exhibits a maximum at 1653 cm^{-1}, together with low- and high-wavenumber shoulders (Figure 1A). As with many other proteins, the amide I contour of cytochrome *c* is a composite of several overlapping component bands. Some of these components can be resolved by the computational procedure of band-narrowing by Fourier self-deconvolution (Kauppinen et al., 1981). The deconvolved spectrum of ferricytochrome *c* in solution (solid trace, Figure 1B) shows a dominant band at 1654 cm^{-1} which is characteristic of the

[1] Abbreviations: DMPG, dimyristoylphosphatidylglycerol; DOPG, *dioleoyl*phosphatidylglycerol; DMPC, dimyristoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; SDS, sodium dodecyl sulfate; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; CD, circular dichroism; DSC, differential scanning calorimetry; FT-IR, Fourier-transform infrared.

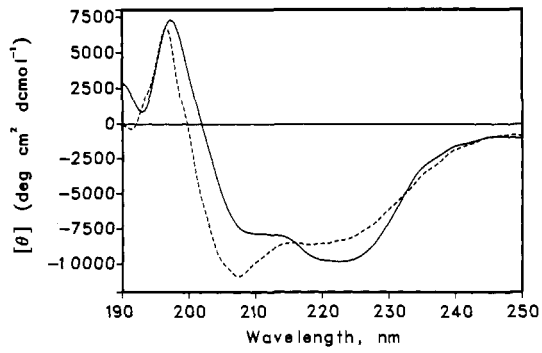

FIGURE 2: Circular dichroism spectra of ferricytochrome c in buffer (—) and ferricytochrome c in the presence of sodium dodecyl sulfate at a detergent to protein molar ratio of 100:1 (---). The concentration of protein was 0.5 mg/mL.

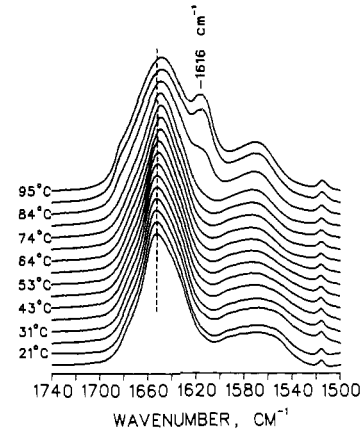

FIGURE 3: Temperature dependence of the amide I band contour of ferricytochrome c in buffer. Infrared spectra were recorded during the heating cycle at a heating rate of approximately 10 °C/h.

protein backbone amide groups in α-helices (Surewicz & Mantsch, 1988). The assignment of the weaker component bands below 1640 and above 1670 cm^{-1} is less straightforward. While such bands are generally associated with β-sheets, cytochrome c is known to contain very little β-structure in the generally defined sense (Dickerson et al., 1971). It has been previously proposed (Byler & Susi, 1986) that bands around 1630–1638 and 1675 cm^{-1} in the infrared spectrum of cytochrome c be assigned to short, extended chains connecting the helical cylinders.

Upon binding to DMPC membranes, the overall amide I band contour of ferricytochrome c shifts by ca. 3 cm^{-1} toward lower wavenumbers (Figure 1A); the deconvolved spectrum of the DMPG-associated protein reveals the major component band at 1650 cm^{-1} (Figure 1B). A qualitatively similar although slightly less pronounced shift of the amide I band maximum (to 1651 cm^{-1}) occurs upon binding of cytochrome c to membranes composed of an equimolar mixture of DMPG and DMPC (spectrum not shown for brevity).

The observed changes in the amide I band contour of cytochrome c and, in particular, the shift of the major component band from 1654 to 1650/1651 cm^{-1} clearly indicate a perturbation of the native structure of the protein resulting from binding to lipid membranes. Useful clues as to the nature of this structural perturbation may be obtained from a comparison of the infrared spectrum of the membrane-bound protein with that of the protein associated with micelles of an acidic surfactant, sodium dodecyl sulfate (SDS). The changes in the amide I band of cytochrome c induced by SDS are qualitatively similar to those found for the membrane-bound protein, and even more pronounced as indicated by the shift of the band maximum to 1648 cm^{-1} (Figure 1A). Yet, the micellar system has the advantage of being optically transparent which allows for an independent assessment of alterations in protein structure by circular dichroism spectroscopy.

The far-UV region of the CD spectrum of cytochrome c in aqueous solution shows a double minimum at 222 and 209 nm, which is typical of a helical conformation. Upon addition of excess SDS, the two minima are preserved although their relative magnitudes are somewhat altered (Figure 2). This is in agreement with the previously published results of Hiramatsu and Yang (1983). While this relatively small change of the circular dichroism spectrum may indicate some alterations within the helical segments of the protein, the magnitude of the CD bands argues against any significant loss of helicity in the presence of SDS. In view of this, one can discard the otherwise possible interpretation of the low-wavenumber shift of the major amide I component in the infrared spectrum of the ferricytochrome c/SDS system (and most likely also of the cytochrome c/DMPG complex) as being indicative of a helix to random structure transition. This shift is rather suggestive of some rearrangement within the helices and/or the overall loosening of the protein tertiary structure, which results in increased accessibility of the helical segments to hydrogen–deuterium exchange. The latter possibility is strongly supported by a more complete displacement of the amide II band from its original position around 1550 cm^{-1} in the spectra of the cytochrome c/DMPG mixtures compared to the displacement observed in the spectrum of the free protein. After 18-h incubation in the ^2H_2O buffer, the amide II to amide I intensity ratio for ferricytochrome c in solution is 0.13, compared with the respective value of 0.08 for the DMPG-associated protein.

Further insight into the effect of membrane binding on the structure and stability of cytochrome c may be obtained from examination of the temperature dependence of the amide I band. Inspection of infrared spectra of an aqueous solution of ferricytochrome c at increasing temperatures (Figure 3) reveals major changes in the amide I mode between approximately 80 and 90 °C. These changes include broadening and a low-wavenumber shift of the overall band contour which is accompanied by the emergence of a well-defined component band at 1616 cm^{-1}. This latter band is highly characteristic of thermally denatured proteins; it is believed to represent hydrogen-bonded extended structures which are formed upon aggregation of thermally unfolded proteins (Surewicz et al., 1990). The intensity ratio of the amide I band at 1616 cm^{-1} and around 1650 cm^{-1} may be used as a convenient parameter to follow the thermal unfolding of cytochrome c (Figure 4). The midpoint denaturation temperature for ferricytochrome c in buffer, as identified by this parameter, is 83 °C. Binding to DMPG results in a marked decrease in the thermal stability of the protein. Changes in the amide I band, similar to those described above for free ferricytochrome c, occur between approximately 50 and 65 °C, with the apparent midpoint temperature of the denaturation event at 56 °C (Figure 4). The decrease in thermal stability is also observed upon binding of cytochrome c to membranes consisting of equimolar amounts of DMPG and DMPC, although the effect is less pronounced than that observed with a pure DMPG membrane. The midpoint of the temperature-induced changes in the infrared spectra of the DMPG/DMPC-associated protein is at approximately 69 °C.

Data of Figure 4 also suggest a substantial difference in the behavior of denatured cytochrome c in buffer and in the

Table I: Thermodynamic Parameters of Ferricytochrome *c* Denaturation

system	lipid:protein molar ratio	T_m (°C)	ΔH^{cal} (kcal/M)	ΔH^{vH} (kcal/M)	$\Delta H^{vH}/\Delta H^{cal}$ (kcal/M)
buffer (pH 7.4)		63.8,[a] 80.7	38,[a] 94	44,[a] 88	1.1,[a] 0.9
DMPG/DMPC (1:3)	290	76.3	79	130	1.6
DMPG/DMPC (1:1)	62	68.2	70	128	1.8
DMPG	25	52.4	24	156	6.5
DOPG/DOPC (1:3)	890	74.4	119	104	0.8
DOPG/DOPC (1:1)	135	63.3	104	80	0.8
DOPG	80	54.6	82	52	0.6

[a] Low-temperature endotherm in the DSC scan of free ferricytochrome *c* in buffer.

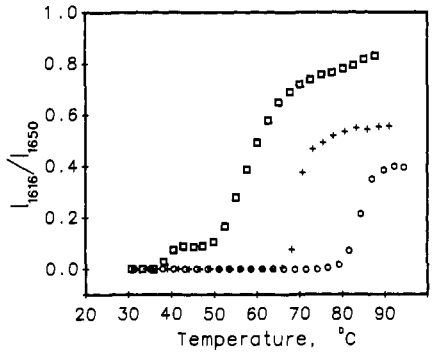

FIGURE 4: Temperature dependence of the ratio of the amide I band intensity at 1616 cm^{-1} to that at 1650 cm^{-1} for ferricytochrome *c* in solution (○), ferricytochrome *c* associated with DMPG/DMPC (1:1) membranes (+), and ferricytochrome *c* associated with pure DMPG membranes (□). The molar ratio of DMPG to protein in both samples is (25 ± 3):1.

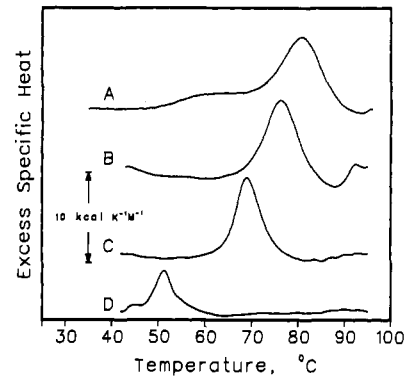

FIGURE 5: Tracings of typical differential scanning calorimetry scans for ferricytochrome *c* in buffer (A) and ferricytochrome *c* bound to membranes composed of a 1:3 mixture of DMPG and DMPC (B), a 1:1 mixture of DMPG and DMPC (C), and DMPG alone (D).

presence of DMPG (or DMPG/DMPC) liposomes. The higher I_{1616}:I_{1650} ratio at postdenaturation temperatures for DMPG-associated protein is indicative of increased aggregation of the denatured species when the protein is bound to DMPG.

Differential Scanning Calorimetry. The excess specific heat versus temperature curve for ferricytochrome *c* in an aqueous buffer (pH 7.4) shows a major endothermic transition at 81 °C, which is preceded by a weaker endotherm centered at aproximately 64 °C (Figure 5A). This denaturation profile is generally similar to the DSC scan of ferricytochrome *c* at neutral pH reported recently by Santucci et al. (1989). These authors also found that the denaturation of the protein is essentially independent of the scan rate. However, the previous calorimetric data were beset with difficulties in defining the posttransitional base line, most likely due to an extensive aggregation of the unfolded protein. Under our experimental conditions, the posttransitional aggregation of cytochrome *c* (or at least its effect on the base line of DSC scans) appears to be much less significant. This allows a more precise evaluation of the thermodynamic parameters of cytochrome *c* denaturation. Deconvolution of the calorimetric curve of Figure 5A, based on a model of two independent transitions, yields thermodynamic parameters shown in Table I. For each of the two resolved endothermic peaks, the ratio of the integrated calorimetric enthalpy to the van't Hoff enthalpy is close to 1, indicating that both thermotropic events may be approximated by a simple two-state model (Privalov 1979; Sturtevant, 1987).

The presence of two distinct steps in the thermal denaturation of ferricytochrome *c* at neutral pH is consistent with earlier observations based on circular dichroism (CD) measurements. Analysis of the temperature dependence of CD spectra (Meyer, 1968; Myer et al., 1980, 1987; Santucci et al., 1989) revealed a major denaturation event at about 83 °C, preceded by a low-temperature transition between approximately 50 and 60 °C. While the transition at 83 °C was identified as a major unfolding of the polypeptide backbone, the low-temperature event was believed to represent mainly the loosening of protein structure in the vicinity of the heme crevice (Meyer, 1968; Myer et al., 1980, 1987). The above assignment of spectroscopically detected transitions provides a basis for the interpretation of the two endotherms in the DSC traces of ferricytochrome *c*. The correspondence between the spectroscopic and calorimetric results is particularly close for the main (high-temperature) transition, for which the midpoint temperatures determined by CD, infrared, and calorimetric measurements are at 83, 83, and 81 °C, respectively. However, for the low-temperature event, the midpoint temperatures derived from circular dichroism and calorimetric experiments differ by as much as 10 °C. Elucidation of this temperature gap will require further studies. The low-temperature transition can also be detected, though with inferior resolution, by infrared spectroscopy. It appears as a small (2–3 cm^{-1}) downward shift of the amide I band maximum, with an apparent midpoint temperature of 60–62 °C (data not shown). This shift likely reflects an increased accessibility of the polypeptide backbone to hydrogen–deuterium exchange.

Upon binding of ferricytochrome *c* to liposomes containing DMPG as the sole lipid component, the DSC trace of the protein shows a single endotherm[2] with a midtransition tem-

[2] Figure 5 displays only the high-temperature portions of the DSC traces for cytochrome *c* complexes with DMPG or DMPG/DMPC. At lower temperatures, the DSC scans are dominated by very strong features which represent thermotropic transitions of the lipid. These lipid transitions are affected by cytochrome *c*, and their detailed analysis will be presented elsewhere. Nevertheless, inspection of these low-temperature regions (not shown) does not provide any indication of the second protein endotherm. The absence of a low-temperature transition (corresponding to the first endotherm in the DSC scan of ferricytochrome *c* in solution) is more clearly documented for the protein bound to DOPG or DOPG/DOPC (see Figure 6), a system for which there is no lipid transition above 0 °C.

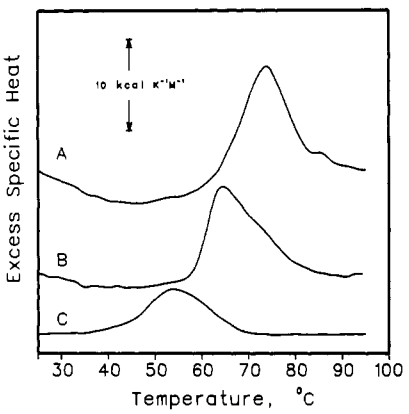

FIGURE 6: Tracings of typical differential scanning calorimetry scans for ferricytochrome c associated with membranes composed of a 1:3 mixture of DOPG and DOPC (A), a 1:1 mixture of DOPG and DOPC (B), and DOPG alone (C).

perature of only 52 °C and a calorimetric enthalpy of 24 kcal/M. This single calorimetric peak represents the unfolding of DMPG-bound ferricytochrome c, i.e., the event corresponding to the second endotherm in the DSC scan of the protein in solution. Such an assignment is strongly supported by a close correspondence between the midtransition temperature of this endotherm and the temperature of the major unfolding step of DMPG-associated cytochrome c as determined by FT-IR spectroscopy (cf. Figure 4). Further characterization of the denaturation of the membrane-bound cytochrome c using techniques such as circular dichroism or absorption spectroscopy in the ultraviolet or visible region was precluded by high turbidity of the lipid/protein complexes. Nevertheless, the large decrease in the temperature and calorimetric enthalpy of the major unfolding event provides strong evidence of a pronounced destabilization of the DMPG-bound ferricytochrome c. A destabilizing effect is also seen upon binding of the protein to membranes containing mixtures of DMPG and DMPC in different proportions (Figure 5 and Table I). In accord with the FT-IR data, the extent of this structural destabilization depends strongly on the surface density of negatively charged lipid head groups: it decreases as the proportion of acidic DMPG in the membrane decreases.

A qualitatively similar destabilization of the protein structure, as indicated by a large decrease in the denaturation temperature, occurs also upon binding of ferricytochrome c to liposomes composed of DOPG or DOPG/DOPC (Figure 6). However, detailed inspection of the calorimetric data (Table I) reveals subtle differences in the interaction of ferricytochrome c with membranes containing DMPG and those containing DOPG. First, the drop in the calorimetric enthalpy of denaturation for DOPG-associated ferricytochrome c is less pronounced than that for the DMPG-bound protein. For ferricytochrome c associated with mixed DOPG/DOPC membranes, the calorimetric enthalpy of denaturation even exceeds the ΔH^{cal} value determined for the second endotherm in the DSC scan of the protein in solution (although it remains smaller than the combined enthalpy of the two steps involved in the denaturation of the free protein). Second, for the protein bound to DMPG-containing liposomes, ΔH^{vH} significantly exceeds ΔH^{cal}. This effect is particularly strong if DMPG is used as a sole lipid component of the membrane. The unusually high cooperativity of the thermal denaturation of the DMPG-associated ferricytochrome c suggests strong intermolecular interactions (and likely self-association) of protein on the membrane surface (Sturtevant, 1987). These strong protein/protein interactions may be rationalized by a particularly low lipid to protein ratio (and thus close proximity of protein molecules) found for cytochrome c bound to liposomes containing DMPG as a sole lipid component (Table I). The apparent tendency to self-associate of DMPG-bound protein (and to a much smaller extent also of DMPG/DMPC-bound protein) may be contrasted with the behavior of ferricytochrome c bound to DOPG or DOPG/DOPC, for which ΔH^{vH} is somewhat smaller than ΔH^{cal}. In the latter case, the denaturation process cannot be approximated by a simple two-state model. However, due to relatively high noise levels in the DSC scans of DOPG or DOPG/DOPC-associated protein and the resulting difficulty in defining the base lines, no attempt was made to deconvolve the calorimetric curves into the underlying two-state components.

Discussion

The results of this study demonstrate that binding of ferricytochrome c to model membranes containing an acidic phospholipid, phosphatidylglycerol, results in an overall destabilization of the protein structure. This destabilization is indicated by (i) an increased accessibility of the backbone amide groups to hydrogen–deuterium exchange (resulting in a low-wavenumber shift of the protein amide I vibrational mode) and (ii) the decreased thermal stability of the membrane-bound protein, as evidenced by the temperature dependence of infrared spectra and direct thermodynamic measurements with differential scanning calorimetry.

Cytochrome c is a highly polar protein. In the oxidized (ferri) form, it carries, at neutral pH, a net positive charge of approximately +9. Characteristically, most of the basic residues are segregated into two well-defined patches on the surface of the protein molecule (Dickerson et al., 1971). This rather unique charge distribution is likely to play a crucial role in electrostatic interactions that govern associated of cytochrome c with liposomes or its binding to physiological redox partners on the surface of the inner mitochondrial membrane. The most notable feature of the interaction between ferricytochrome c and lipid vesicles is the relationship between the stability of the protein and the surface density of negatively charged lipid head groups. It should be pointed out here that special care has been exercised in this study to ensure that essentially all (more than 95%) protein present in samples prepared for spectroscopic or calorimetric measurements is bound to the membrane (see Materials and Methods). Therefore, the differences in protein stability found in the presence of vesicles of different lipid compositions are unrelated to factors such as variations of binding constants or differences in the proportion of the free and associated protein. The observed surface potential dependent destabilization and loosening of the tertiary structure appears to represent an intrinsic property of membrane-bound ferricytochrome c. Unravelling the detailed mechanism of this electric field dependent protein destabilization will require further studies. Nevertheless, the present data point to the critical importance of electrostatic interactions in maintaining the stable three-dimensional structure of cytochrome c. It may be postulated that upon binding to the membrane there is a competition between electrostatic interactions within the protein (that involve oppositely charged amino acid residues) and the interactions between basic protein residues and negatively charged lipid head groups. The latter interactions gain in strength as the negative surface potential of the membrane increases; they may weaken the internal stabilizing forces within cytochrome c (e.g., by rupturing intramolecular salt bridges), leading to an overall loosening and destabilization of the protein structure.

Of direct relevance to the subject of this study are recent resonance Raman experiments. In a series of reports (Hildebrandt & Stockburger, 1989a,b; Hildebrandt et al., 1990), it has been demonstrated that binding of cytochrome *c* to a variety of negatively charged interfaces, including phospholipid vesicles, affects the conformation and immediate environment of the heme group. The structural rearrangements deduced from resonance Raman spectra include alterations in the coordination pattern of the heme iron and a partial opening of the heme crevice. However, our data indicate that structural perturbations in ferricytochrome *c* which accompany its binding to negatively charged lipids are considerably more extensive. In particular, these perturbations are not restricted to the immediate environment of the heme group; they appear to affect the thermodynamic properties (and possibly the folding) of the entire protein molecule. Specific local changes within the heme moiety may be one of the consequences of an overall destabilization of cytochrome *c* structure.

The in vivo role of cytochrome *c* is to transfer electrons between the mitochondrial enzyme complexes cytochrome reductase and cytochrome oxidase. This dynamic function requires that the protein remains in close contact and interacts with various components of the inner mitochondrial membrane. The formation of less stable folding intermediates in an interfacial environment of phospholipid vesicles is thus likely to be of direct physiological relevance. In this context, we attach particular significance to the observation that conformational changes in lipid-bound cytochrome *c* are modulated by the membrane surface potential. Electric field induced structural perturbations could provide an effective mechanism for controlling the functional state of cytochrome *c* at different docking sites on the mitochondrial membrane. Although details of such a mechanism remain to be explored, one specific model was proposed recently (Hildebrandt & Stockburger, 1989b) that postulates coupling of electric field induced conformational changes within the heme group of cytochrome *c* and the electron-transfer processes in mitochrondria.

Observations made in the present study may also have more general ramifications. They raise an intriguing possibility that conformational destabilization on the membrane surface, similar to that found for ferricytochrome *c*, may represent a general property of a larger class of polar proteins for which membrane binding is governed by electrostatic interactions. If so, this could have far-reaching biological implications. A noteworthy example is the possible involvment of lipid-induced destabilization in the mechanism of protein translocation across biological membranes, as recently proposed by Endo et al. (1989). On the basis of observations with an artificial mitochondrial precursor protein, these authors have postulated that lipid-mediated conformational changes may add to factors responsible for inducing and maintaining a loose, translocation-competent conformation of posttranslationally translocated proteins.

Registry No. DMPG, 61361-72-6; DOPG, 62700-69-0; DMPC, 18194-24-6; DOPC, 4235-95-4; cytochrome *c*, 9007-43-6.

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