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Thermodynamic Characterization of Interactions between Ornithine Transcarbamylase Leader Peptide and Phospholipid Bilayer Membranes†

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ABSTRACT: The interactions of the targeting sequence of the mitochondrial enzyme ornithine transcarbamylase with phospholipid bilayers of different molecular compositions have been studied by high-sensitivity heating and cooling differential scanning calorimetry, high-sensitivity isothermal titration calorimetry, fluorescence spectroscopy, and electron microscopy. These studies indicate that the leader peptide interacts strongly with dipalmitoylphosphatidylcholine (DPPC) bilayer membranes containing small mole percents of the anionic phospholipids dipalmitoylphosphatidylglycerol (DPPG) or brain phosphatidylserine (brain PS) but not with pure phosphatidylcholines. For the first time, the energetics of the leader peptide–membrane interaction have been measured directly by using calorimetric techniques. At 20 °C, the association of the peptide with the membrane is exothermic and characterized by an association constant of 2.3×10^6 M^{-1} in the case of phosphatidylglycerol-containing and 0.35×10^6 M^{-1} in the case of phosphatidylserine-containing phospholipid bilayers. In both cases, the enthalpy of association is −60 kcal/mol of peptide. Additional experiments using fluorescence techniques suggest that the peptide does not penetrate deeply into the hydrophobic core of the membrane. The addition of the leader peptide to DPPC/DPPG (5:1) or DPPC/brain PS (5:1) small sonicated vesicles results in vesicle fusion. The fusion process is dependent on peptide concentration and is maximal at the phase transition temperature of the vesicles and minimal at temperatures below the phase transition.

Mitochondria contain approximately 200 proteins of which about 90% are synthesized in the cytosol and then transported across one or both mitochondrial membranes to their functional sites [for a review, see Hay et al. (1984), Wickner and Lodish (1985), and Rapoport (1986)]. However, the manner in which the newly synthesized proteins interact with the mitochondrial membranes to facilitate this translocation is not completely understood. Recent studies (Horwich et al., 1985a,b; Hurt et al., 1985; Ono & Tuboi, 1986) have demonstrated that most proteins of the inner membrane, matrix, and intermembrane space are synthesized in precursor form with a 15–30 amino acid N-terminal extension referred to as a target or leader sequence. This presequence not only targets the newly synthesized protein to the mitochondria but also is essential for its subsequent transfer into the organelle. After translocation, the leader sequence is cleaved by a divalent cation-dependent protease, and the mature protein assumes its role in mitochondrial function (Hay et al., 1984; Rapoport, 1986). Several workers (Ito et al., 1985; Gillespie et al., 1985) have shown

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using in vitro transfer assays that synthetic leader sequences are able to competitively inhibit the import of precursor proteins. Conversely, Horwich et al. (1985a,b) and Hurt et al. (1985) are among those who have shown translocation of nonmitochondrial proteins into mitochondria when hybridized to a presequence from a mitochondrial protein such as ornithine transcarbamylase.

The mitochondrial leader peptides are a diverse group in terms of length and amino acid sequence. One common feature appears to be a preponderance of basic and hydroxyl-carrying residues; generally, there are several positively charged amino acids at the N-terminus followed by more neutral amino acids interspersed with other basic residues (Ito et al., 1985; Rapoport, 1986). Often, the C-terminal region is more polar, probably signifyng the protease cleavage site. In recent papers, Roise et al. (1986) and von Heijne (1986) have suggested that there are structural elements in these leader sequences that are similar to other peptides known to interact with membranes, specifically those with lytic properties such as melittin and α-hemolysin. These peptides are thought to exist as amphiphilic helices at water–membrane interfaces with one highly charged and one hydrophobic face. The manner in which these peptides interact with lipid components of the membrane is just beginning to be elucidated. Epand and co-workers (Epand et al., 1986) have recently reported that the peptide corresponding to the first 27 residues of ornithine transcarbamlyase becomes more α-helical in conformation in the presence of anionic lipids [cardiolipin and dimyristoylphosphatidylglycerol (DMPG)] but not zwitterionic lipids [dimyristoylphosphatidylcholine (DMPC)].

In this paper, we report the results of a systematic investigation of the interactions between a synthetic peptide representing the first 15 residues of the ornithine transcarbamylase leader sequence and liposomes of varying compositions. Utilizing high-sensitivity differential scanning calorimetry, high-sensitivity isothermal titration calorimetry, fluorescence spectroscopy, and electron microscopy, we have studied the physicochemical basis of the interaction between the peptide and phospholipid bilayer membranes.

Materials and Methods

Materials. The peptide used in these experiments is representative of the first 15 residues of the transit sequence of the mitochondrial matrix enzyme ornithine transcarbamylase. The sequence is Met-Leu-Trp-Asn-Leu-Arg-Ile-Leu-Leu-Asn-Asn-Ala-Ala-Phe-Arg.

The third-position Trp replaces a Phe; this was done to facilitate fluorescence measurements. This is a rather conservative change and does not introduce a significant perturbation in the hydrophobicity of the peptide. The peptide was prepared at the Analytical Facilities Laboratory (University of Tennessee, Knoxville) by standard Merrifield resin chemistry using a Biosearch SAM Two peptide synthesizer. Purification was accomplished by using a Milliport Waters high-performance liquid chromatography (HPLC) system with a C-18 reverse-phase column and an acetonitrile gradient. This peptide will be referred to as pOTC15.

Dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylglycerol (DPPG), brain phosphatidylserine (PS), egg phosphatidylcholine (PC) and egg phosphatidylethanolamine (PE) were purchased from Avanti Biochemicals (Birmingham, AL) and used without further purification. The purity of all of the lipids was checked by thin-layer chromatography on silica gel plates (E. Merck). Lipid concentrations were estimated by a modified Bartlett phosphate assay as described by Marinetti (1962). 1,6-Diphenyl-1,3,5-hexatriene (DPH) for use in anisotropy studies was purchased from Molecular Probes (Junction City, OR).

Liposome Preparation. Most of the membranes used were small unilamellar vesicles (SUV's) and were produced by first drying the lipid(s) from a chloroform stock solution and resuspending them in 50 mM KCl with 1 mM ethylenediaminetetraacetic acid (EDTA) to the desired concentration. The lipid suspensions were then sonicated by using a bath sonicator (Laboratory Supplies, Model G112, SO1G, Hicksville, NY) and centrifuged at 15000*g* for 60 min above the phase transition temperature of the lipid to remove any residual multilamellar vesicles. The upper one-third of the supernatant was used immediately for experimental work; the peptide was added to the vesicles from a concentrated aqueous stock. Dilutions were made with 50 mM KCl with 1 mM EDTA. Other vesicles used were large unilamellar vesicles prepared as described earlier (Myers & Freire, 1985).

High-Sensitivity Differential Scanning Calorimetry. All temperature-scanning calorimetric experiments were performed with a Microcal MC2 differential scanning calorimeter. The calorimeter is interfaced to an IBM-PC microcomputer system using a Data Translation DT2801 A/D converter board for automatic data acquisition, analysis, and instrument control. The calorimeter used for these studies has been specially modified to operate in both heating and cooling modes. Cooling operation is achieved with a high-precision refrigerated circulating water bath (Haake F3C) operating under computer control at the desired scanning rate. For these experiments, lipid concentrations ranging from 2 to 5 μmol/mL with a calorimeter cell volume of 1.2 mL were used. A scanning rate of 20 °C/h was used for heating scans and −30 °C/h for cooling scans.

High-Sensitivity Isothermal Titration Calorimetry. A newly developed differential titration module has been used for these studies. This module is similar to the one previously developed by us (Ramsay et al., 1986) except that it operates in a differential mode; i.e., titration is made simultaneously in both the sample and reference cells, and only differential heat effects are measured. With this configuration, the effective sensitivity is not limited by the reproducibility of mechanical heat effects such as stirring and injection. Conventional titration systems require separate experiments to estimate the magnitude of the mechanical heat effects (Ramsay et al., 1986). Also, the differential configuration of the new titration module allows constant stirring of the sample which is especially important for membrane suspensions and other materials that tend to settle with time. With this configuration, heat effects as small as 25 ncal/s (0.1 μW) can readily be measured during an actual titration experiment.

Steady-State Fluorescence Anisotropy. The effect of pOTC15 on the thermotropic behavior of membrane preparations was studied by steady-state fluorescence depolarization. The change in the anisotropy of DPH incorporated into membranes in the presence and absence of pOTC15 was monitored as a function of temperature. The experiments were performed with a Perkin-Elmer LS-5 spectrofluorometer equipped with a thermostated cuvette holder and 3M 105–M1 glass polarizers in the excitation and emission beams. The sample temperature was controlled by a Precision RDL 20 refrigerated bath circulator and the temperature monitored within ±0.1 °C by a Keithley digital thermometer. The DPH was dissolved in acetonitrile and added to liposome preparations at a ratio of 1 probe molecule per 500 lipid molecules. The total lipid concentration was 0.25 mM; samples were incubated with the probe for 1 h at 45 °C to assure equili-

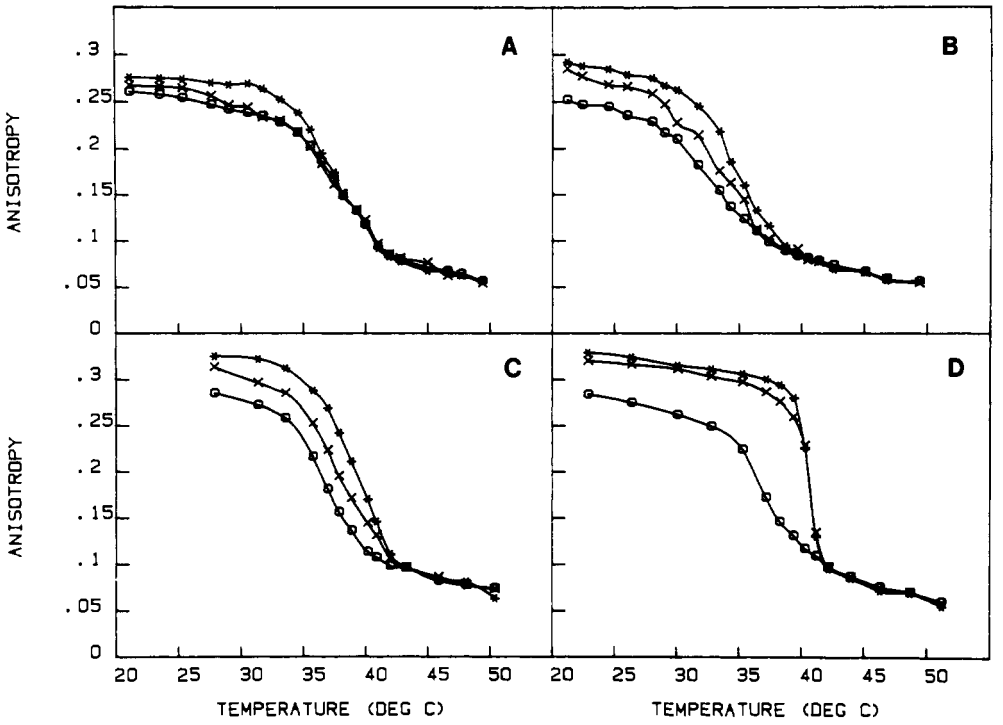

FIGURE 1: Steady-state DPH fluorescence anisotropy as a function of temperature for pure DPPC vesicles (panel A), DPPC/brain PS (5:1) vesicles (panel B), and DPPC/DPPG (5:1) vesicles (panels C and D) at peptide:lipid molar ratios of 0 (○), 1:200 (×), and 1:100 (asterisks). The data in panels A–C were obtained immediately after the addition of the peptide and the data in panel D after 8-h incubation above T_m.

bration. The samples were excited at 360 nm, and the emission intensity at 430 nm parallel and perpendicular to the plane of excitation was measured. Anisotropy was calculated as described by Barenholz et al. (1976).

Electron Microscopy. Lipid preparations (1 μmol/mL) with and without pOTC15 were examined by negative-stain electron microscopy after incubation at the desired temperature. Samples were placed on formvar, carbon-coated grids and stained with 2% phosphotungstic acid adjusted to pH 7.4 with NaOH. An H–600 Hitachi electron microscope was used. In samples containing pOTC15, the peptide:lipid (P/L) mole ratio was 1:200.

RESULTS

Steady-State Fluorescence Experiments. The effect of pOTC15 on the physical state of phospholipid bilayer membranes was first studied by observing the temperature dependence of the steady-state fluorescence anisotropy of DPH. These studies revealed that the peptide had only a very small effect on the anisotropy of DPH incorporated into phosphatidylcholine vesicles but a rather dramatic effect on vesicles containing small mole fractions of negatively charged phospholipids such as phosphatidylserine and phosphatidylglycerol. These effects are illustrated in Figure 1 for a series of cooling scans obtained with pure DPPC vesicles, DPPC/PS (5:1) vesicles, and DPPC/DPPG (5:1) vesicles at 0, 1:200, and 1:100 peptide:lipid molar ratio. For phosphatidylcholine vesicles (panel A), the temperature dependence of the DPH anisotropy remains largely unaffected except for a slight anisotropy increase in the gel phase of the vesicles containing the peptide. The situation is completely different for vesicles containing small molar fractions of anionic lipids such as phosphatidylserine or phosphatidylglycerol. In this case, the phospholipid melting profiles become sharper and shifted to higher temperatures immediately after addition of the peptide above T_m. These peptide-induced changes in the lipid melting profiles are not instantaneous and continue for a period of several hours until final equilibrium is achieved (panel D). Inspection of the samples after equilibration revealed an increase in turbidity similar to the one observed for vesicle fusion.

Upon increasing the peptide concentration, the steady-state DPH anisotropy remained approximately constant in the fluid phase of the membrane but showed a large increase in the gel phase. This type of behavior is also characteristic of the one observed when small unilamellar vesicles fuse into large vesicles (Suurkuusk et al., 1976). Measurements of the fluorescence emission spectrum of the peptide in aqueous solution yielded an emission maximum at 358 nm. Addition of DPPC/DPPG (5:1) vesicles resulted in a blue shift in the emission maximum of 4 nm. The magnitude of the shift was the same below (20 °C) or above (48 °C) the phase transition temperature of the lipid molecules. The existence of a blue shift is an indication that the peptide is in a more hydrophobic environment; however, the maximum occurs at higher wavelengths than those found for tryptophan residues in highly hydrophobic environments (Lakowicz, 1983). These results suggest that the peptide region where the tryptophan is located is not deeply buried in the hydrophobic core of the bilayer.

High-Sensitivity Differential Scanning Calorimetry. Figure 2 shows the excess heat capacity function associated with the gel–liquid-crystalline transition of small, sonicated unilamellar vesicles (SUV's) of DPPC containing 16 mol % of DPPG. The phase transition of the vesicles is characterized by a transition temperature (T_m) of 37.8 °C and a transition enthalpy (ΔH) of 5.9 kcal/mol of lipid. As shown in the figure, the transition is relatively broad ($\Delta T_{1/2}$ = 4.5 °C) and characterized by a cooperative unit of ~30 lipid molecules. Contrary to the case of pure DPPC vesicles, the DPPC/DPPG (5:1) vesicles are stable and show no hysteresis as demonstrated by repeated calorimetric cooling and heating scans. Presumably, the negative surface charge of the DPPC/DPPG vesicles precludes vesicle aggregation, therefore inhibiting vesicle fusion. The addition of pOTC15 at a peptide:lipid molar ratio of 1:200 causes the immediate appearance of a second, sharp peak at 40.9 °C in the calorimetric scans. In these experiments, the peptide was added above the phospholipid transition tem-

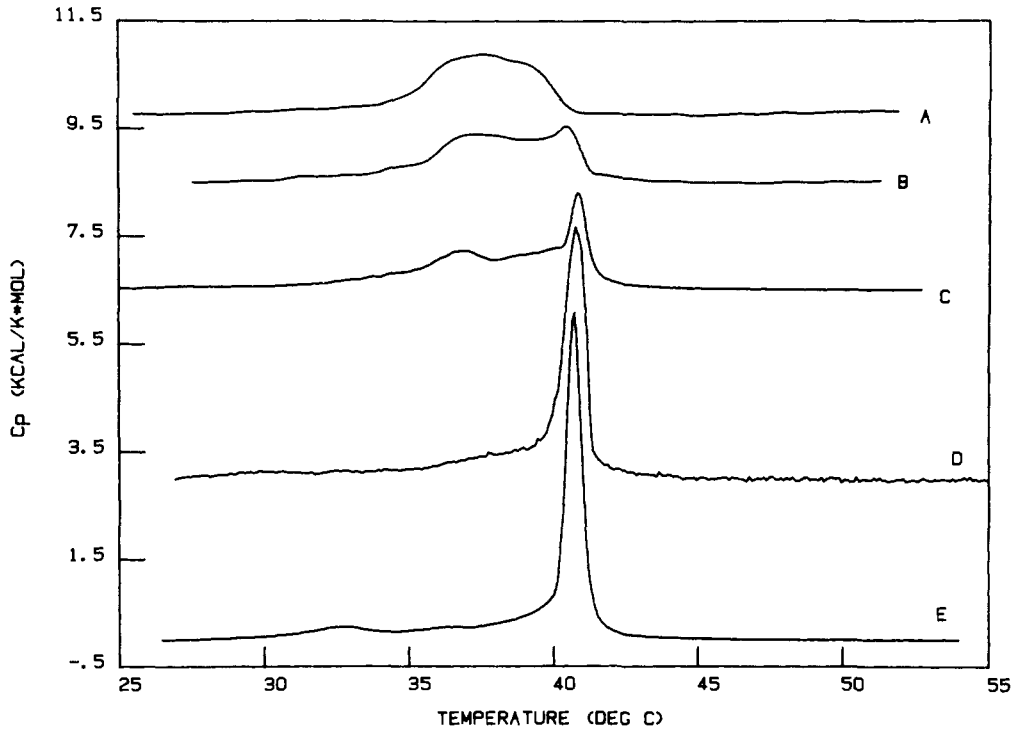

FIGURE 2: Heat capacity function vs. temperature for DPPC/DPPG (5:1) vesicles in the absence (curve A) and after addition of pOTC15 at a peptide:lipid molar ratio of 1:200 (curves B–E). Curves A, B, and D were obtained in cooling scans, and curves C and E were obtained in heating scans.

perature (50 °C) and the sample immediately scanned downward in the cooling calorimeter. This second peak becomes more prominent with time, and eventually it constitutes the single component in the transition profile. At this point, the transition is very cooperative ($\Delta T_{1/2}$ = 0.6 °C, cooperative unit = 150 lipids) and characterized by an enthalpy change of 7.4 kcal/mol. The measured increase in the enthalpy change associated with the gel–liquid-crystalline transition of the phospholipid molecules is similar to the enthalpy change increase observed for pure DPPC-sonicated vesicles after vesicle fusion. This time-dependent behavior, similar to the one observed in the fluorescence experiments, indicates that pOTC15 is not inducing a simple phase separation type of phenomenon but a more complex process most likely associated with peptide-induced vesicle fusion.

The effect of pOTC15 on the thermotropic behavior of DPPC vesicles containing phosphatidylserine was similar to the one observed with the DPPC/DPPG vesicles. On the other hand, pOTC15 had no effect on the thermotropic behavior of phospholipid vesicles prepared with pure DPPC. Figure 3 shows the cooling calorimetric scans of pure DPPC vesicles and vesicles that have been incubated with pOTC15 at a peptide:lipid molar ratio of 1:200 for 2 h at 50 °C. In both cases, the vesicles were always maintained above T_m in order to prevent the spontaneous fusion characteristic of DPPC vesicles that have been allowed to be below their transition temperature. The results shown in Figures 2 and 3 indicate that at the peptide concentrations used in these experiments (10^{-5}M) the peptide–membrane interaction requires the presence of negatively charged lipids and that the resulting effect is independent of whether the lipid is phosphatidylserine or phosphatidylglycerol.

pOTC15-Induced Vesicle Fusion. The fusogenic effect of the peptide was studied by negative-stain electron microscopy. The results of these studies are shown in Figure 4 for DPPC/PS (5:1) vesicles incubated for 2 h at three different temperatures in the absence and in the presence of pOTC15 at a protein:lipid molar ratio of 1:200. As shown in the vesicle size distribution histograms in Figure 4, in the absence of the peptide the vesicles are characterized by an average radius of 270 Å. In the presence of the peptide, however, vesicle fusion occurs in a manner dependent on the incubation temperature. Below the phospholipid transition temperature (20 °C), very little fusion is observed. At temperatures above the phase

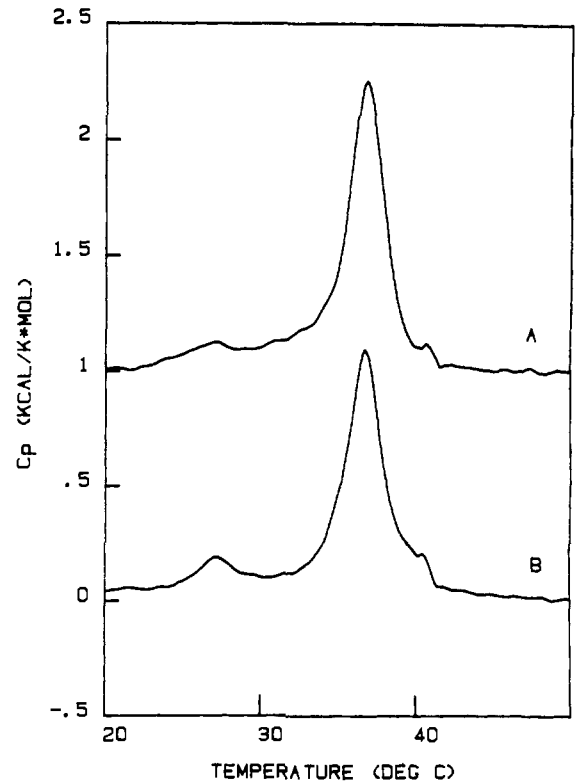

FIGURE 3: Heat capacity function vs. temperature for DPPC vesicles in the absence and in the presence of pOTC15 at a peptide:lipid molar ratio of 1:200. Both curves were obtained in calorimetric cooling scans.

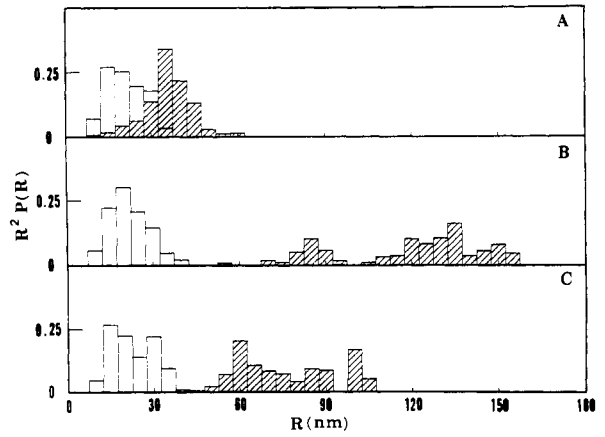

FIGURE 4: Weight-average vesicle size distribution, for DPPC/brain PS (5:1) vesicles, in the absence (open bars) and in the presence of pOTC15 at a peptide:lipid ratio of 1:200 (hatched bars). Data were obtained after 2-h incubation at 20 °C (panel A), 37 °C (panel B), and 52 °C (panel B).

transition temperature, essentially all the vesicles have undergone fusion, resulting in a broad size distribution with a mean radius of 750 Å after 2 h of incubation. Incubation at the phase transition temperature (37 °C) resulted in a maximal rate of fusion with a resulting vesicle population of an average radius of 1300 Å after 2 h of incubation.

High-Sensitivity Isothermal Titration Calorimetry. The energetics of the interaction between pOTC15 and the phospholipid bilayer membrane were measured directly by using a newly designed titration calorimeter module. These experiments were performed at 20 °C using a lipid concentration of 2 mM in a total reaction volume of 1 mL. The titration experiments were performed under computer control and consisted of a sequence of 30-μL injections at predetermined time intervals, each one containing the desired amount of peptide, typically 3 nmol. The calorimeter reference cell also contained phospholipid vesicles, but only buffer was loaded into the injection device. The calorimetric titration was performed under continuous stirring of sample and reference cells, and the differential heat effects between both cells were measured and stored for subsequent analysis.

The results of a typical titration experiment are shown in Figure 5. Shown in this figure is the differential power output in microwatts (1 μW = 1 μJ/s; 1 μJ = 0.239 μcal) as a function of time for each injection in the titration process. From left to right, these thermograms represent the heat effects measured after successive injections of pOTC15 to DPPC/DPPG (5:1) vesicles at equal intervals of 1 h. Two major features are immediately apparent in the data. First, the association of the peptide with the phospholipid vesicles is exothermic at all concentrations and exhibits saturation as expected for a binding process obeying the law of mass action. Second, there is a second, slower, exothermic process that becomes clearly visible after the fourth injection in Figure 5. This slower process appears as a broad envelope extending over a period of 3–4 h and is associated with the fusion of the phospholipid vesicles. The correspondence of the slow process with vesicle fusion was established by measuring the thermotropic behavior of the vesicles by differential scanning calorimetry and observing the appearance of the sharp peak at 41 °C (see Figure 2) and also by examining the turbidity of the vesicle suspensions.

The energetics of the two processes shown in Figure 6 were resolved by repeating the vesicle titration experiments using different amounts of peptide per injection, and increasing the time interval between injections up to 4 h in order to achieve complete return to base line after each injection. In every case, the curves were deconvoluted, and the areas corresponding to the fast and slow processes were calculated. Figure 6 shows the cumulative areas for the peptide–vesicle association (fast process) as a function of the total concentration of peptide for two separate titration experiments performed with different amounts of peptide per injection. This curve represents the total amount of heat released up to the indicated concentration of peptide and is similar in shape to a ligand binding isotherm. In fact, at each concentration of peptide, the amount of heat released, Q, is equal to the amount of peptide bound multiplied by the enthalpy of binding:

$$Q = \Delta H_b V[P]_b \tag{1}$$

where ΔH_b is the molar enthalpy of binding, V the reaction volume, and $[P]_b$ the concentration of bound peptide. For a system of identical, independent binding sites, eq 1 can be written as

$$Q = \Delta H_b V[L]\frac{nK[P]}{1 + K[P]} \tag{2a}$$

$$Q = \Delta H_b V[L]\frac{nK([P]_T - [P]_b)}{1 + K([P]_T - [P]_b)} \tag{2b}$$

where K is the association constant, $[P]$ and $[P]_T$ are the free and total concentrations of peptide, respectively, and $[L]$ is the lipid concentration. The product $n[L]$ is equal to the total concentration of binding sites. Equation 2b was used to fit the experimental data using a nonlinear least-squares procedure based upon the simplex algorithm (Nelder & Mead, 1986; Masserini & Freire, 1986). The solid line in Figure 6 is the best-fitted curve to the experimental data and corre-

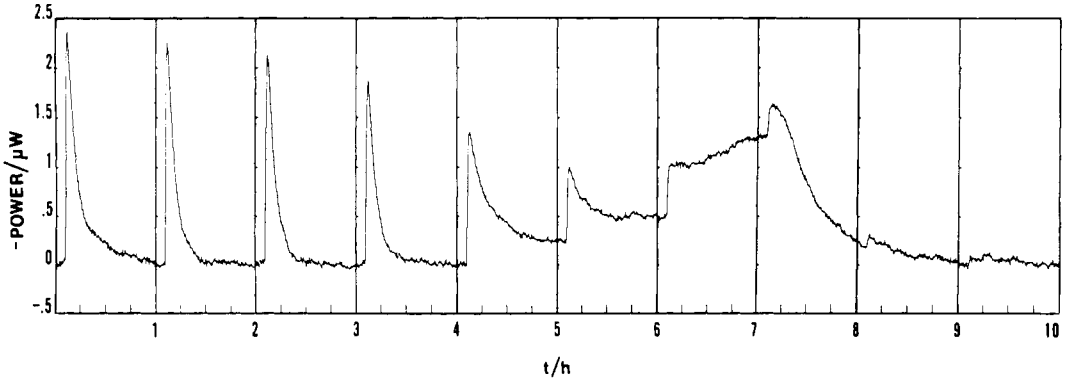

FIGURE 5: High-sensitivity isothermal calorimetric titration of DPPC/DPPG (5:1) vesicles with pOTC15. Shown in the figure is the power output in microwatts as a function of time for successive injections of pOTC15 (3.6 nmol per injection) at equal intervals of 1 h. The amount of lipid in the calorimeter cell is 2 μmol.

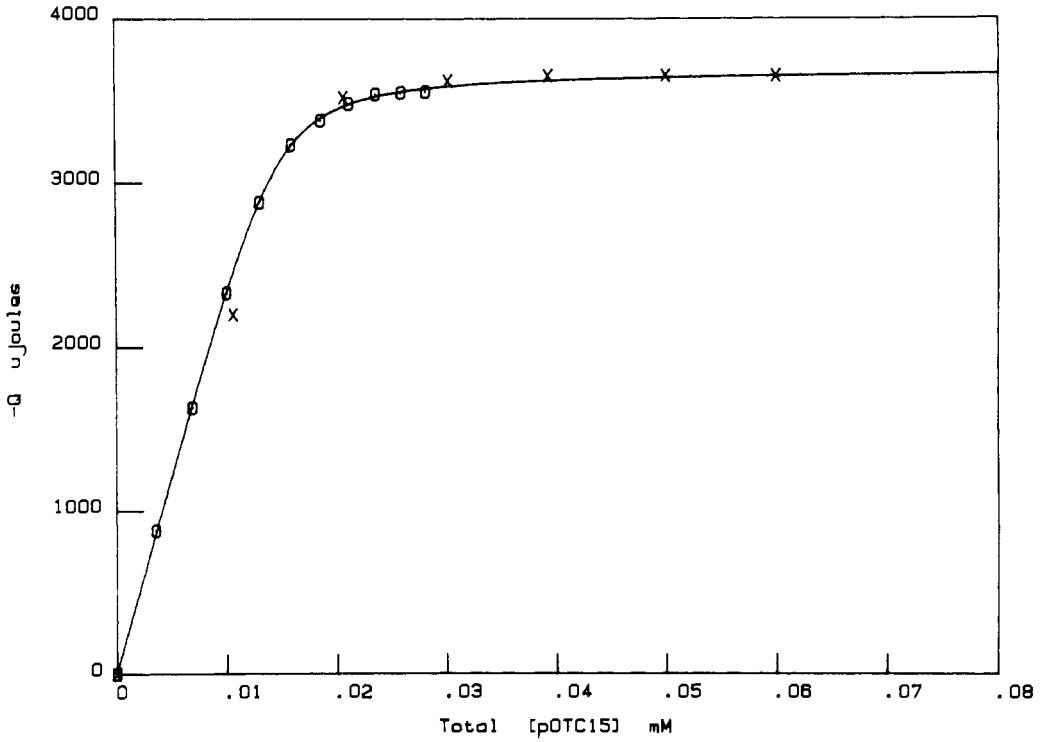

FIGURE 6: Heat of association of pOTC15 to DPPC/DPPG (5:1) vesicles at 20 °C as a function of the total peptide concentration. The values plotted are the cumulative areas associated with the binding phase of the titration peaks for two independent experiments. The solid line is the best-fitted curve using eq 2b.

sponds to an association constant K of 2.3×10^6 M^{-1}, a molar enthalpy of binding of −59.9 kcal/mol of peptide, and an n value of 0.0074, corresponding to 1 peptide molecule for every 135 lipid molecules (1 peptide for every 22 DPPG molecules). Additional titration experiments performed with DPPC/brain PS (5:1) vesicles yielded similar results except that the association constant was almost 1 order of magnitude smaller ($K = 0.35 \times 10^6$ M^{-1}). The enthalpy of binding was essentially the same (−63 kcal/mol) and the saturation stoichiometry equivalent to 1 peptide molecule every 105 phospholipid molecules (1 peptide for every 18 PS molecules). These experiments demonstrate that the binding of the leader peptide is qualitatively similar in both cases even though it exhibits a much stronger affinity for phosphatidylglycerol than for phosphatidylserine. Preliminary calorimetric experiments performed with the leader peptide of cytochrome c oxidase subunit IV using phospholipid vesicles in which the phosphatidylcholine bilayer matrix is either DPPC (gel phase) or egg PC (fluid phase) suggest that the peptide has 2–3 times larger affinity for phospholipid vesicles in the gel phase.

The total enthalpy associated with the slow process, assigned to vesicle fusion, amounts to −1.3 kcal/mol of lipid. This value is similar in magnitude to the value of −0.9 kcal/mol obtained by incubating sonicated pure DPPC vesicles in the calorimeter cell at 20 °C and measuring the heat effects associated with the spontaneous fusion of these vesicles over a period of 6 days. Also, the value of −1.3 kcal/mol of lipid is similar to the difference in transition enthalpies between the small and fused vesicles obtained in this work for DPPC/DPPG (5:1) vesicles and, previously, for pure DPPC vesicles (Suurkuusk et al., 1976). These results indicate that the gel state of large vesicles is enthalpically lower than that of small vesicles. This conclusion is in agreement with the larger anisotropy values measured in the gel phase of the fused vesicles [see Figure 1 and also Suurkuusk et al. (1976)] and with previous observations that the gel phase of small vesicles is more disordered than the gel phase of large vesicles.

DISCUSSION

Ninety percent of the mitochondrial proteins are synthesized by free ribosomes in the cytosol and then transferred to their final destination within the mitochondria. Two fundamental questions posed by this process are the following: (1) how the newly synthesized proteins find the mitochondria and (2) once in the mitochondria, how the various proteins find their proper compartment. It is well-known that most mitochondrial proteins are synthesized in precursor form containing a 15–30 amino acid N-terminal extension. There is considerable evidence [see Rapoport (1986) for a review] indicating that this presequence contains the necessary targeting information to direct the new protein to the mitochondria and then guide it to its final location. This processing requires molecular interactions between the leader peptides and specific proteins or lipid components in the mitochondrial membrane. The results presented in this paper indicate that a synthetic peptide corresponding to the first 15 amino acids of the leader sequence of ornithine transcarbamylase interacts very strongly with phosphatidylcholine vesicles containing small amounts of phosphatidylglycerol and to a lesser extent with phosphatidylcholine vesicles containing phosphatidylserine. In previous studies, Epand et al. (1986) have shown that both phosphatidylglycerol and diphosphatidylglycerol (cardiolipin), but not phosphatidylcholine, induce a significant conformational change in the ornithine transcarbamylase leader peptide.

The association constant of pOTC15 to DPPC/DPPG (5:1) phospholipid vesicles is 2.3×10^6 M^{-1} ($K_d = 4.3 \times 10^{-7}$ M). This is indeed a very strong association constant similar in magnitude to those required for in vivo mitochondrial protein import. In fact, Gillespie et al. (1985) have recently observed that 5–10 μM synthetic leader peptide is able to prevent the mitochondrial import of pre-ornithine transcarbamylase in a reversible fashion. This is a competitive inhibition and can be overcome by increasing the concentration of precursor

protein. Gillespie et al. (1985) also showed that the leader peptide of pOTC is capable of inhibiting the import of other mitochondrial proteins destined to the matrix or inner membrane, suggesting a common import pathway for some of these proteins.

The association of the leader sequence to phospholipid bilayers containing negatively charged phospholipids is strongly exothermic and characterized by an enthalpy change of −60 kcal/mol of peptide. In addition to the intrinsic peptide–lipid binding enthalpy, the measured value contains any enthalpic contributions associated with a conformational change of the peptide upon binding. In this respect, Epand et al. (1986) have recently observed that the association of the leader sequence of ornithine transcarbamylase with phosphatidylglycerol vesicles is accompanied by a large increase in the helical content of the peptide. Similar conclusions have been obtained for the 25-residue leader sequence of subunit IV of yeast cytochrome *c* oxidase (Tamm, 1986) and the signal sequence of the *Escherichia coli* λ phage receptor protein (Briggs et al., 1986). For a 15-residue peptide, α-helix formation should contribute on the order of −30 to −40 kcal/mol to the overall enthalpy of binding. The large affinity of these peptides for negatively charged phospholipids strongly suggests that the initial events in the peptide–phospholipid bilayer association is of an electrostatic nature and that this event is followed by α-helix formation and peptide insertion into the hydrophobic core of the bilayer. From surface area measurements in phospholipid monolayers, Tamm (1986) has concluded that the leader peptide of subunit IV of yeast cytochrome *c* oxidase adopts a helical conformation and orients with its long axis parallel to the plane of the monolayer. Previously, Gierasch et al. (1982) have suggested that small hydrophobic peptides reside in the interfacial region of the hydrocarbon chains near the phospholipid head groups. In our experiments, the absence of a large wavelength shift in the tryptophan fluorescence emission maximum suggests that at least that part of the peptide is not deeply buried in the hydrophobic core of the bilayer.

In agreement with studies on other mitochondrial leader peptides (von Heijne, 1986; Gillespie et al., 1985), we have found that pOTC15 is surface active and able to cause vesicle fusion. This apparently general property (von Heijne, 1986) indicates that the leader peptide has a destabilizing effect on the bilayer. With the isolated peptide, i.e., without the bulk of the protein present, this bilayer destabilization leads to vesicle fusion at high enough peptide concentrations. With the intact precursor molecule, however, this destabilization may play an important role in facilitating phospholipid packing rearrangements required for protein insertion and translocation.

To the best of our knowledge, the studies reported in this paper constitute the first thermodynamic characterization of the interaction between a leader peptide and a membrane system. Considering that the leader peptide binds with high affinity to phosphatidylglycerol ($K_d \sim 10^{-7}$ M) and that both the outer and inner mitochondrial membranes are rich in anionic phospholipids, especially diphosphatidylglycerol, whereas other subcellular organelles lack or contain very little amounts of these lipids, then it is very feasible that these negatively charged phospholipid molecules constitute the primary site of membrane attachment even if further processing of the precursor proteins involves protein receptors.

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