Specific Binding of Cinnamycin (Ro 09-0198) to Phosphatidylethanolamine. Comparison between Micellar and Membrane Environments†

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ABSTRACT: Cinnamycin (Ro 09-0198) is a tetracyclic peptide antibiotic that binds specifically to phosphatidylethanolamine (PE). Formation of a complex with phosphatidylethanolamine follows a 1:1 stoichiometry. Using high-sensitivity isothermal titration calorimetry (ITC), we have measured the thermodynamic parameters of complex formation for two different PE environments, namely, PE dissolved either in octyl glucoside (OG) micelles or in a 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) bilayer membrane. We have compared diacyl-PE with lyso-PE and have varied the carbon chain length from 6 to 18. Binding requires both a PE headgroup and at least one fatty acyl chain. The optimum chain length for complex formation (*n*) is eight. Longer chains do not enhance the binding affinity; for shorter chains, the interaction is weakened. The cinnamycin−PE complex has a binding constant K_0 of $\sim 10^7-10^8$ M^{-1} in the POPC membrane and only $\sim 10^6$ M^{-1} in the octyl glucoside micelle. The difference can be attributed to the nonspecific hydrophobic interaction of cinnamycin with the lipid membrane. Complex formation is enthalpy-driven in OG micelles, whereas enthalpy and entropy make equal contributions in bilayer membranes. However, for the optimum chain length (*n*) of eight, the binding reaction is also completely enthalpy-driven for the bilayer membrane.

Cinnamycin (Ro 09-0198) is a polypeptide bacteriocin which is active against Gram-positive bacteria but also lyses other cells (*1*, *2*). Related structures are the duramycins and ancovenin (*3*). The chemical structure of cinnamycin has been determined by NMR (*4*, *5*) and by chemical methods (*6*) (cf. Figure 1). Posttranslational modifications result in unusual amino acids that are typical for the whole group of lantibiotics (*2*). Cinnamycin and duramycin B and C interact specifically with phosphatidylethanolamine but not with phosphatidylcholine or other lipids (*7*−*10*). The binding constant of the 1:1 complex has recently been determined with isothermal titration calorimetry to be 10^7-10^8 M^{-1} for the 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine matrix (*11*). In the study presented here, we compare the binding of PE[1] in the lipid bilayer with that in octyl glucoside micelles to elucidate the role of lipid polymorphism in the binding process. We also vary systematically the acyl chain length of the phosphatidylethanolamines and compare lyso- and diacylphosphatidylethanolamine. In contrast to biological assays, isothermal titration calorimetry reveals a distinct influence of these structural modifications on the binding process.

MATERIALS AND METHODS

Materials. 1,2-Dihexanoyl-*sn*-glycero-3-phosphoethanolamine (C_6-PE), 1,2-dioctanoyl-*sn*-glycero-3-phosphoethanolamine (C_8-PE), 1,2-didecanoyl-*sn*-glycero-3-phosphoethanolamine (C_{10}-PE), 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine (C_{12}-PE), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (C_{14}-PE), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) were purchased from Avanti Lipids (Alabaster, AL). Cinnamycin (Ro 09-0198) (MW = 2078.78, hydrochloride salt) was a generous gift of R. Imhof (Hoffmann-La Roche Ltd., Basel, Switzerland). Octyl glucoside (octyl β-D-glucopyranoside, OG) was from Fluka (Buchs, Switzerland).

Preparation of Lipid Vesicles. Vesicles were prepared as POPC/POPE mixtures (10:1, w/w) at a final lipid concentration of 10 mg/mL. For this purpose, stock solutions of POPC an dPOPE in methanol and chloroform were prepared. The appropriate amount of POPC was added to a 5 mL round-bottomed flask and was dried down under nitrogen. Residual solvent was removed by applying high vacuum. The amount of POPC was controlled by weighing. Next, POPE dissolved in chloroform and methanol was added, and the procedure was repeated. A total of 2 mL of buffer [10 mM Tris-HCl and 0.1 M NaCl (pH 7.4)] was added to the dry film, and the suspension was extensively vortexed. Next, small unilamellar vesicles (SUV) were prepared by sonication of the lipid suspension for 20−40 min until an almost clear solution was obtained (at 4 °C under a nitrogen atmosphere). Metal

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[1] Abbreviations: ITC, isothermal titration calorimetry; PE, phosphatidylethanolamine; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; LUV, large unilamellar vesicles ~100 nm in diameter; SUV, small unilamellar vesicles ~30 nm in diameter; OG, octyl glucoside, octyl β-D-glucopyranoside; C_6-PE, 1,2-dihexanoyl-*sn*-glycero-3-phosphoethanolamine; C_8-PE, 1,2-dioctanoyl-*sn*-glycero-3-phosphoethanolamine; C_{10}-PE, 1,2-didecanoyl-*sn*-glycero-3-phosphoethanolamine; C_{12}-PE, 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine; C_{14}-PE, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine.

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debris of the titanium tip was removed by centrifugation in an Eppendorf centrifuge (10 min at 3000 rpm). Sonication produces vesicles with an average diameter of ∼30 nm.

For the preparation of 100 nm large unilamellar vesicles (LUV), the lipid suspension was first subjected to at least four freeze−thaw cycles and was then extruded 12 times through two stacked polycarbonate filters with a pore size of 0.1 μm (Nucleopore, Whatman).

High-Sensitivity Titration Calorimetry. Isothermal titration calorimetry (ITC) was performed with an OMEGA titration calorimeter (Microcal, Northampton, MA). Solutions were degassed under vacuum before use. The calorimeter was calibrated electrically. Injection volumes varied between 3 and 20 μL. Peptide concentrations varied between 30 and 200 μM. The calorimeter cell had a reaction volume of 1.3353 mL. The titration pattern was analyzed in terms of a chemical complex formation according to the equation P + L → PL, where P, L, and PL denote the peptide, the PE lipid, and the 1:1 peptide−lipid complex, respectively. The chemical equilibrium can be written as

$$\frac{C_{PL}}{C_P C_L} = \frac{C_{PL}}{(C_P^0 - C_{PL})(C_L^0 - C_{PL})} = K_0 \qquad (1)$$

$$C_{PL} = \frac{1}{2}\left(C_L^0 + C_P^0 + \frac{1}{K_0}\right) - \frac{1}{2}\sqrt{\left(C_L^0 + C_P^0 + \frac{1}{K_0}\right)^2 - 4C_L^0 C_P^0} \qquad (2)$$

where C_P^0 and C_L^0 are the total peptide and lipid concentrations, respectively, in the sample cell. In a lipid (syringe)-to-peptide (calorimeter cell) titration experiment, C_L^0 increases in steps of δC_L^0 while the peptide concentration C_P^0 remains constant (except for small dilution effects). The total heat absorbed or released after i injections is

$$\sum_i h_i = \Delta H^\circ C_{PL}^i V_{cell} \qquad (3)$$

where ΔH° is the reaction enthalpy and V_{cell} is the volume of the reaction vessel. Via combination of eqs 2 and 3 (including dilution effects), it is possible to fit the experimental titration curve h_i versus N_{inj} with the proper set of thermodynamic parameters ΔH° and K_0 (*12*).

As a control reaction, POPC/POPE vesicles were injected into buffer without cinnamycin. The heat of dilution was very small (ca. −1 μcal) and was subtracted from the heat of reaction of the actual titration experiment.

A different result is observed when OG micelles are injected into pure buffer. Micelles are highly dynamic structures, and the more than 100-fold dilution upon injection into buffer results in a disintegration of the micelles which is associated with a considerable release of heat (*13*). The large heat of demicellization completely obscures the small heat of cinnamycin−PE binding. Hence, both cinnamycin and PE were dissolved in the same OG solution (36 mM) before titration.

RESULTS

Cinnamycin and phosphatidylethanolamine were each dissolved in 36 mM octyl glucoside (OG). The critical micellar concentration (CMC) of OG is ≈23 mM at 30 °C (*13*, *14*). At this temperature, micelle formation is endothermic with a $\Delta H^{\circ,w\rightarrow m}$ of 1.85 kcal/mol. The molar heat capacity change $\Delta C_p^{w\rightarrow m}$ is ca. −100 cal mol^{-1} K^{-1}. The superscript w→m indicates the transition of OG from water (w) into the micelle (m). Figure 1 shows two titration curves obtained with cinnamycin concentrations of 25 μM (Figure 1A) and 50 μM (Figure 1B). The calorimeter cell (V_{cell} = 1.3353 mL) contained the cinnamycin solution, and the phosphatidylethanolamine solution (0.5 mg of PE/mL) was added to the injection syringe. The specific phosphatidylethanolamine that was employed was 1,2-decanoyl-*sn*-glycero-3-phosphoethanolamine (1,2-dicapryl-*sn*-glycero-3-phosphoethanolamine, C_{10}-PE). Each titration peak reflects the heat flow generated by the injection of 6 μL of PE solution. The reaction heat is exothermic, and the height of the titration peaks decreases as the concentration of free cinnamycin is reduced during the course of the titration. Figure 2 displays the heats of reaction, h_i, obtained by integration of the heat flow peaks of Figure 1. The binding equilibrium between cinnamycin (Cyn) and PE in the OG

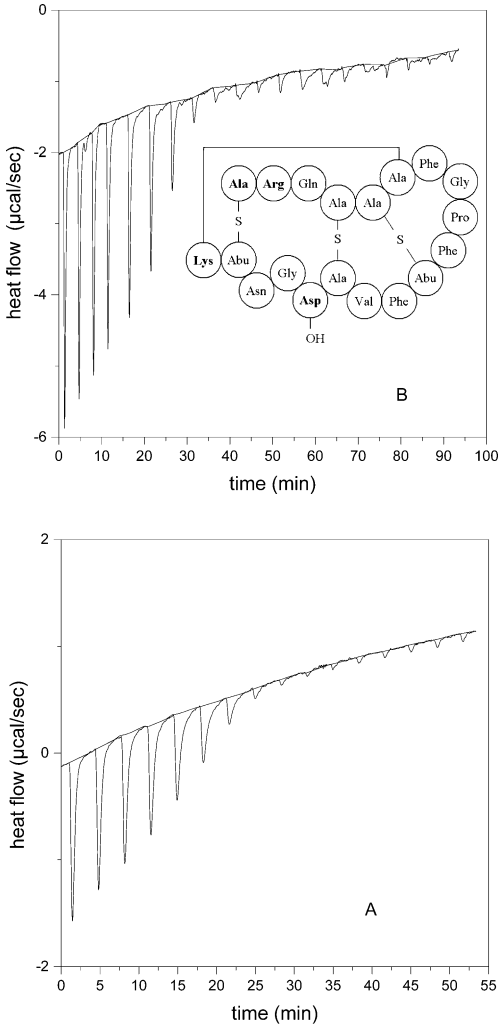

FIGURE 1: Isothermal titration calorimetry. The lantibiotic cinnamycin (Ro 09-0198) is titrated with C_{10}-PE. Both compounds are dissolved in 36 mM octyl glucoside. The peptide concentration in the reaction cell is 25 (A) or 50 μM (B). The concentration of the injected lipid is 0.955 (A) or 1.72 mM (B). Each peak corresponds to the injection of 6 μL of the lipid solution into the reaction cell (V_{cell} = 1.3353 mL). The temperature is 37 °C.

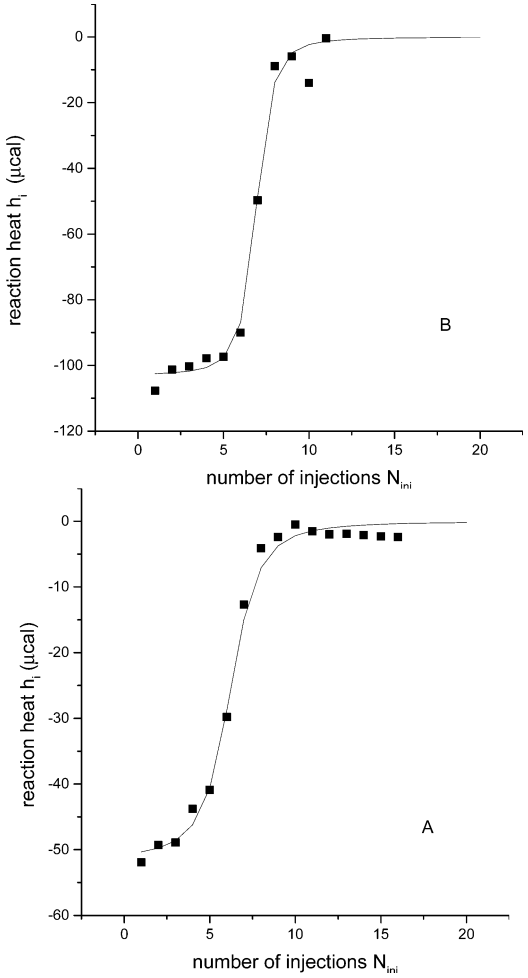

FIGURE 2: Heats of reaction, h_i, as evaluated from the areas underneath the calorimeter tracings shown in Figure 1. The symbols represent the experimental data. The solid lines are the theoretical calculations based on formation of a 1:1 complex. (A) $C_{peptid} = 25$ μM, and $C_{PE} = 0.955$ mM. In the theoretical analysis, $\Delta H° = -9.0$ kcal/mol and $K_0 = 2 \times 10^6$ M^{-1}. (B) $C_{peptid} = 50$ μM, and $C_{PE} = 1.72$ mM. In the theoretical analysis, $\Delta H° = -10.0$ kcal/mol and $K_0 = 4 \times 10^6$ M^{-1}.

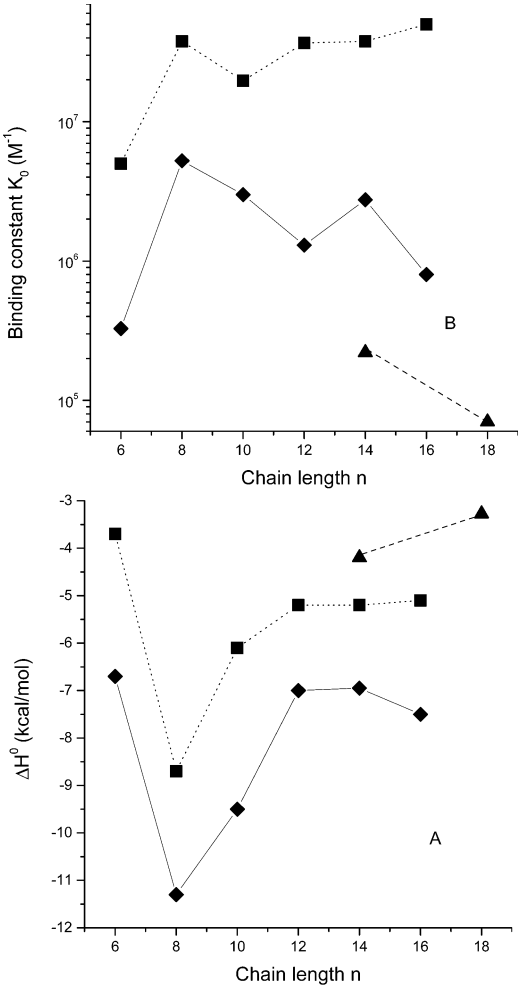

FIGURE 3: Thermodynamic parameters as a function of fatty acyl chain length at 37 °C: (■) C_n-PE and cinnamycin dissolved in 36 mM octyl glucoside, (◆) 1-acyl-2-lyso-PE and peptide dissolved in 36 mM OG, and (▲) lipid vesicles (100 nm in diameter) composed of POPC and diacyl-PE at a 9:1 molar ratio. Vesicles and peptide were in buffer [100 mM NaCl and 10 mM Tris (pH 7.4)]. (A) Variation of the reaction enthalpy $\Delta H°$ with chain length n. (B) Variation of the binding constant K_0 with chain length.

solution can be described by simple chemical equilibrium with a 1:1 stoichiometry according to

$$PE + Cyn \overset{K}{\rightleftharpoons} PE{\cdot}Cyn$$

with the corresponding equations given above. The solid lines in Figure 2 were calculated with this model taking into account the dilution of the total concentrations during the course of the titrations. Doubling of the cinnamycin concentration from 25 to 50 μM reveals an initial plateau area (Figure 2B). The average reaction enthalpy $\Delta H°$ equals -9.5 ± 0.7 kcal/mol, and the binding constant K_0 equals $(3 \pm 1) \times 10^6$ M^{-1} at 37 °C.

Figure 3A summarizes the results obtained for the chain length dependence of the reaction enthalpy ($\Delta H°$). Data obtained in micellar OG solution are compared with those where the same PE lipid was embedded in a membrane composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) at a PE:POPC molar ratio of 1:10 or 1:100 (100 nm unilamellar vesicles). The two curves run parallel to each other over the whole range investigated with the $\Delta H_{OG}°$ being more exothermic by ca. -2 kcal/mol than the $\Delta H_{POPC}°$. The reaction enthalpy is constant with a $\Delta H_{OG}°$ of ca. -7.0 kcal/mol for carbon chain lengths of 6, 12, 14, and 16 but goes through a distinct minimum at chain lengths of 8 and 10 with a $\Delta H_{OG}°$ of -11.3 kcal/mol for 8 (all data at 37 °C). The PEs employed in this study have two identical, saturated fatty acyl chain. The only exception is 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) which has a saturated 16-carbon chain at the *sn*-1 position and an unsaturated 18-carbon chain (oleic acid) at the *sn*-2 position. Since the *sn*-1 chain determines the transmembrane extension, POPE is counted as a 16-carbon lipid in Figure 3. The data depicted in Figure 3 together with other details are presented in numerical form in Table 1.

Figure 3B summarizes the variation of the binding constant K_0 as a function of the lipid chain length. Again, the data for PE dissolved in an OG solution are compared with those for POPE in the POPC membrane (100 nm vesicles). The binding constant is clearly larger for PE in a lipid matrix than for PE in an OG solution. In addition, there is almost no influence of the chain length for membrane-bound PEs. In contrast, K_0 exhibits a clear maximum for C_8-PE in an OG solution and decreases either sharply toward C_6-PE or

Table 1: Thermodynamic Data of Cinnamycin Binding to Phosphatidylethanolamine Lipids

Diacyl-PE in Octyl Glucoside							
chain length *n*	lipid	temp (°C)	$\Delta H°$ (kcal/mol)	SD (kcal/mol)	K_0 (M^{-1})	$\Delta G°$ (kcal/mol)	$T\Delta S°$ (kcal/mol)
6	PE(6:0)	37	−6.7	0.7	3.27×10^5	−7.79	1.09
8	PE(8:0)	37	−11.3	1.7	5.25×10^6	−9.50	−1.80
10	PE(10:0)	37	−9.5	0.7	3.00×10^6	−9.15	−0.35
12	PE(12:0)	37	−7	0.0	1.30×10^6	−8.64	1.64
14	PE(14:0)	37	−7	0.4	2.75×10^6	−9.10	2.10
16	POPE[a]	10	−13.1		3.00×10^6	−8.36	−4.74
16	POPE	20	−10.3		3.00×10^6	−8.65	−1.65
16	POPE	30	−5.51		2.00×10^6	−8.70	3.19
16	POPE	37	−7.2	0.3	8.00×10^5	−8.34	1.14
16	POPE	40	−4.7		8.00×10^5	−8.42	3.72
16	POPE	50	−4.5		6.00×10^5	−8.51	4.01

Diacyl-PE in a POPC Bilayer[b]						
chain length *n*	POPC:PE ratio	temp (°C)	$\Delta H°$ (kcal/mol)	K_0 (M^{-1})	$\Delta G°$ (kcal/mol)	$T\Delta S°$ (kcal/mol)
6	13:1	28	−3.7	5.00×10^6	−9.19	5.49
8	10:1	28	−6.4	7.00×10^7	−10.77	4.37
8	80:1	28	−9	3.20×10^7	−10.30	1.30
8	80:1	28	−10.8	1.10×10^7	−9.66	−1.14
10	10:1	28	−5.2	3.00×10^7	−10.26	5.06
10	84:1	28	−6.4	1.70×10^7	−9.92	3.52
10	84:1	28	−6.7	1.20×10^7	−9.71	3.01
12	10:1	28	−5.56	7.00×10^7	−10.77	5.21
12	113:1		−4.8	2.00×10^7	−9.09	4.29
12	87.5:1	28	−5.2	2.00×10^7	−10.02	4.82
14	10:1	28	−3.4	7.00×10^7	−10.77	7.37
14	88:1	28	−6.4	1.30×10^7	−9.76	3.36
14	88:1	28	−5.8	3.00×10^7	−10.26	4.46
16	84:1	28	−5.1	5.00×10^7	−10.57	5.47

1-Acyl-2-lyso-PE in Octyl Glucoside							
chain length *n*	lipid	temp (°C)	$\Delta H°$ (kcal/mol)	SD (kcal/mol)	K_0 (M^{-1})	$\Delta G°$ (kcal/mol)	$T\Delta S°$ (kcal/mol)
14	lyso-PE(14:0)	37	−4.2	1.2	2.20×10^5	−7.55	3.35
18	lyso-PE(18:0)	37	−3.3	0.9	7.00×10^4	−6.85	3.55

1-Acyl-2-lyso-PE in a POPC Bilayer[b]					
lipid	temp (°C)	$\Delta H°$ (kcal/mol)	K_0 (M^{-1})	$\Delta G°$ (kcal/mol)	$T\Delta S°$ (kcal/mol)
lyso-PE 14:0	37	−12	5.00×10^6	−9.47	−2.53

[a] POPE has a C-16 *sn*-1 chain and a C-18 *cis*-unsaturated *sn*-2 chain. [b] Half-sided binding!

more gradually toward C_{16}-PE. The influence of the fatty acyl chains was further investigated with 1-acyl-2-lyso-*sn*-glycero-3-phosphoethanolamine (*lys*-PE) with either a 14- or 18-carbon fatty acyl chain at the *sn*-1 position. As seen in panels A and B of Figure 3, the reaction enthalpy is less exothermic and the binding constant smaller than those of diacyl lipids. Also, the 18-carbon chain is binding less tightly than the 14-carbon chain, which parallels the observation for diacyl PEs in OG.

The temperature dependencies of $\Delta H°$ and K_0 have been studied for the cinnamycin−POPE system, again dissolved in 36 mM OG. The results for $\Delta H°$ are displayed in Figure 4 for the temperature interval from 10 to 45 °C. The reaction enthalpy *increases* with increasing temperature (Figure 4A) and can be described by linear regression analysis according to the equation $\Delta H°(t)$ (kcal/mol) = $0.217t$ (°C) − 14.45. The complex formation in OG micelles is hence characterized by a *positive* heat capacity $\Delta C_p°$ of 217 cal/mol. The temperature dependence of the binding of cinnamycin to POPE embedded in a POPC bilayer was measured previously (*11*) and is also included in Figure 4A. The reaction enthalpy *decreases* with temperature and has a *negative* molar heat capacity $\Delta C_p°$ of −240 cal/mol which is in distinct contrast to those for OG micelles.

The binding constant of an exothermic reaction is expected to decrease with increasing temperature, which is indeed borne out by the experimental data shown in Figure 4B for OG micelles. The solid line in Figure 4B is the variation of the binding constant K_0, as calculated with the temperature-dependent $\Delta H°$ taken from Figure 4A. Within the accuracy of the measurements, a good agreement is obtained between theory and experiment.

DISCUSSION

Cinnamycin has no unique spectroscopic properties, and in most cell biological assays, the molecule is modified by covalent attachment of a suitable reporter group such as biotin (*15*, *16*) or by conjugating biotinylated Ro 09-0198 to fluorescence-labeled streptavidin (*17*). The role of the reporter group in the binding process is usually ignored which may not always be justified. For example, the binding of a small charged peptide, heptalysin (lys_7), to lipid bilayers was assessed for unmodified lys_7 and for the peptide with a fluorescent label attached to it. The binding constant of the latter was 3 orders of magnitude larger than that of lys_7 (*18*). In this respect, isothermal titration calorimetry has an advantage in that the level of unmodified cinnamycin can be measured.

The molecular conformation of phosphatidylethanolamine has been determined for C_{12}-PE crystals with X-ray diffraction (*19*), for C_{18}-PE lipid bilayers in the gel state with

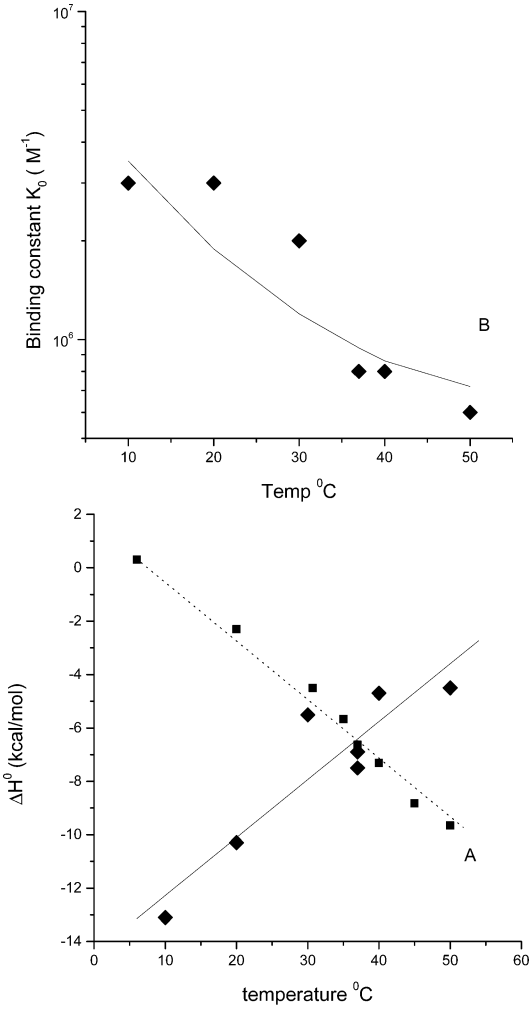

FIGURE 4: Temperature dependence of the thermodynamic parameters of formation of the PE−cinnamycin complex in OG micelles and POPC membranes: (A) reaction enthalpy $\Delta H°$ and (B) binding constant K_0. All measurements were taken with POPE. (◆) POPE and cinnamycin were dissolved in 36 mM octyl glucoside. (■) POPE was embedded in the POPC lipid bilayer (10:1 POPC:POPE molar ratio). The 100 nm lipid vesicles were prepared by extrusion. The POPC/POPE data were taken from ref *11*. The lipid vesicles and the cinnamycin solution were prepared with buffer [100 mM NaCl and 10 mM Tris (pH 7.4)]. The solid line in panel B is the predicted temperature dependence of K_0 calculated with the temperature-dependent $\Delta H°$ of panel A.

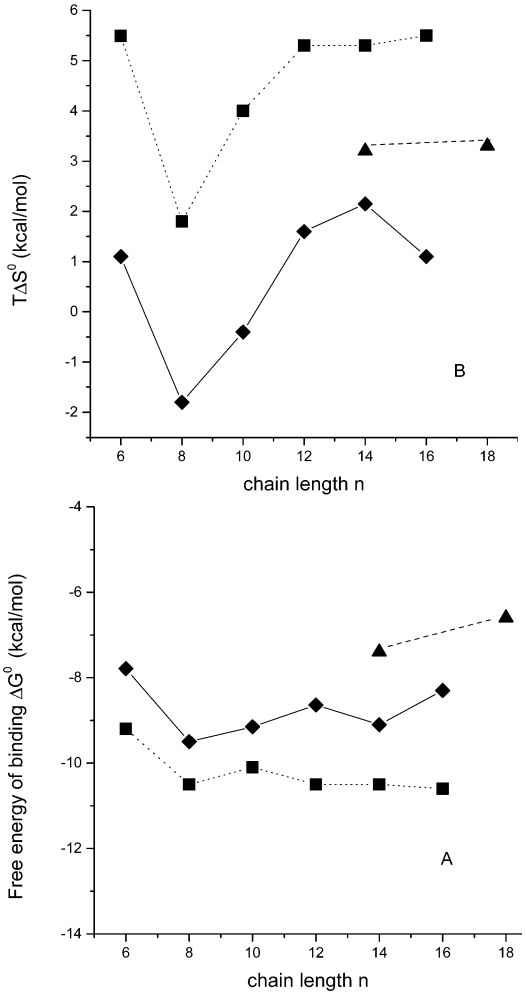

FIGURE 5: Variation of the reaction entropy, $T\Delta S°$, of formation of the cinnamycin−PE complex as a function of chain length in (■) 36 mM octyl glucoside micelles and (◆) POPC bilayer membranes [100 nm vesicles; 10:1 POPC:POPE molar ratio; 100 mM NaCl and 10 mM Tris (pH 7.4)]. (▲) 1-Acyl-2-lyso-*sn*-glycero-3-phosphoethanolamine dissolved in 36 mM octyl glucoside.

neutron diffraction (*20*), and for liquid-crystalline C_{18}-PE membranes with deuterium and phosphorus NMR (*21*). The molecular arrangement of the fatty acyl chains resembles that of an asymmetric tuning fork. The glycerol backbone and the *sn*-1 chain are in line and extend perpendicular to the membrane surface. The *sn*-2 chain starts out parallel to the membrane surface and is bent by 90° at the C-2 segment. From C-3 onward, the *sn*-2 chain runs parallel to the *sn*-1 chain. Finally, the $^-P-N^+$ dipole of the PE headgroup is parallel to the membrane surface. The essential features of the crystal structure are retained in the gel state and in the fluid-like lipid membrane. The conformation of PE in the micelle has not yet been determined, but the membrane conformation can be expected to carry over into the micellar environment.

In the study presented here, we have investigated the question of the extent to which cinnamycin binding to PE is influenced by the long-range order of the "solvent", i.e., if there are differences between a densely packed lipid bilayer and an isotropic micellar environment. Intuitively, one would argue that a PE molecule inserted into a planar lipid bilayer is less easily accessible to a water-soluble molecule such as cinnamycin than a PE molecule dissolved in a loosely packed micellar environment.

Considering first the reaction enthalpy (Figure 3A), one observes indeed a tighter enthalpic cinnamycin−PE interaction in OG micelles than in the POPC bilayer membrane. At all the chain lengths that have been investigated, $\Delta H°$ is by approximately −2 kcal/mol more exothermic in the OG micelle. However, this situation is reversed when the free energy of binding, $\Delta G°$, is considered (Figure 5A, $\Delta G° = -RT \ln K_0$). PE in the bilayer membrane has a higher affinity for cinnamycin than PE in the micelle. $\Delta G°$ is by approximately −1 to −2 kcal/mol more favorable in the membrane than in the PE−OG system. As a consequence, there is an even larger difference in the entropic contribution to the binding process as summarized in Figure 5B. For PE in the POPC bilayer, binding to cinnamycin is accompanied by a large positive $T\Delta S°$ term of approximately 5 kcal/mol. Entropy and enthalpy make approximately equal contributions to the total binding affinity $\Delta G°$. The same process in

the OG micelle entails a $T\Delta S°$ term of only −1 to 1.5 kcal/mol. Formation of the PE−cinnamycin complex in OG micelles is almost completely driven by enthalpy.

A molecular explanation of these differences is provided by the molar heat capacities of the two reactions. Inspection of Figure 4A demonstrates that $\Delta C_p°$ is negative with a $\Delta C_p°$ of ca. −240 cal mol^{-1} K^{-1} for the reaction in a membrane environment but positive with $\Delta C_p°$ of ca. 220 cal mol^{-1} K^{-1} for a micellar environment. A large negative $\Delta C_p°$ is the characteristic signature of the hydrophobic effect (*22*−*24*). The transfer of a hydrophobic compound from an aqueous environment into a nonpolar solvent leads to a loss of the hydration shell and to a corresponding reduction of the molar heat capacity. In contrast, charge neutralization reactions have substantial positive $\Delta C_p°$ values (*25*). If the titration is carried out in OG micelles as a solvent, both the PE lipid and the peptide are at first embedded in a rather hydrophobic environment. The hydrophobic contribution to be binding reaction can be expected to be reduced, whereas the electrostatic attraction is presumably increased because of the lower dielectric constant. These data therefore suggest that the interaction of cinnamycin with PE in octyl glucoside micelles is predominantly electrostatic in nature.

NMR studies have described the interaction of cinnamycin with lysophosphatidylethanolamine in dimethyl sulfoxide (*26*), the conformation of cinnamycin in the absence of PE in different environments (*27*), and the conformation of the cinnamycin−lyso-PE complex, again in DMSO (*28*). In these studies, only the β-hydroxyaspartic acid 15 was found to form an ion pair with the free amino group of lyso-PE. A second ionic interaction, that between the ammonium group of lysinoalanine and the PE phosphate group, appeared to be unlikely on the basis of the NMR data for lyso-PE in DMSO (*28*). However, diacyl-PEs have a distinctly higher binding constant for cinnamycin, and the large positive heat capacity suggests that a second ionic interaction should be possible.

The interaction between cinnamycin and C_n-PE in the lipid bilayer appears to be dominated by hydrophobic interactions. It should be recalled that cinnamycin is partly hydrophobic and binds also to POPC bilayers not containing PE lipid, albeit with a much lower binding constant K_0 of ∼100−300 M^{-1} and a binding enthalpy $\Delta H°$ of ca. −1 kcal/mol (*11*). The corresponding free energy change ($\Delta G°$) ∼ −2.7 to −3.4 kcal/mol at 25 °C. This type of interaction is missing if the complex is formed in OG micelles. Inspection of Table 1 reveals that the free energy of the cinnamycin−PE complex is more favorable by approximately −2 kcal/mol in POPC membranes than in an OG micelle.

On the basis of NMR studies in a dimethyl sulfoxide solution, it was concluded that the fatty acyl chain of C_{12}-lyso-PE does not interact with the peptide (*26*). Likewise, measurements of the hemolytic activity of cinnamycin (*7*) and of the permeability of liposomal membranes (*7*) showed a similar efficiency of dicacyl phosphatidylethanolamine and 1-acyl-lysophosphatidylethanolamine. These conclusions must be modified in light of the more precise measurements presented here. The length of the hydrocarbon chains clearly influences the binding process. An optimum interaction is observed for the C-8 chain, both in octyl glucoside and in POPC membranes (Figure 5A). A further increase in chain length leaves the affinity, $\Delta G°$, almost constant, whereas the binding affinity is clearly reduced for the C-6 chain. These results indicate that the interaction between cinnamycin and the hydrocarbon chain is limited to a stretch of 8−10 carbon atoms. Longer chains extend beyond the complex, and the additional methylene segments do not contribute to binding. The methyl terminal of fatty acyl chains with more than 10 carbon atoms should hence be free to move. On the other hand, the binding affinity of C_6-PE is reduced since the hydrocarbon chain is too short for maximum interaction. It should be recalled that glycerophosphoethanolamine which has no hydrocarbon chain does not interact (*11*). Likewise, the number of fatty acyl chains also matters. As shown for lipids with the myristoyl chain, the diacyl lipid binds better than the lyso lipid ($\delta\Delta G°$ ∼ −1 to −2 kcal/mol) both in OG micelles and in a POPC matrix (at PC:PE ratios between 110:1 and 10:1) (Table 1).

It has been pointed out that cinnamycin has an unusual amphiphilic structure. Most of the lipophilic amino acids are positioned at one side of the peptide, whereas the hydrophilic ones are located on the opposite side (*29*). Inspection of Figure 1 (inset) reveals that the charged amino acids on the left side of the structure constitute the binding site for the phosphoethanolamine dipole. The fatty acyl chains can then be buried in the hydrophobic cleft constituted by hydrophobic amino acids on the right side of the structure shown. The size of this cleft corresponds to 8−10 methylene segments and is consistent with the chain length dependence of the binding enthalpy.

In conclusion, the thermodynamic measurements not only yield the binding affinity, $\Delta G°$, and related parameters but also provide details about the molecular structure of the complex. Both the PE headgroup and, in contrast to previous contentions, the hydrocarbon chains are important. The first 8−10 segments of each hydrocarbon chain are in direct contact with a hydrophobic peptidic surface or cleft, and two hydrocarbon chains are better than one. The lipid environment is also important. For PE dissolved in a membrane environment, the peptide binds specifically to PE and the binding affinity is further enhanced by a nonspecific hydrophobic interaction of the peptide with the lipid membrane.

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