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Review

# Thermodynamics of lipid–peptide interactions

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**Abstract**

This review is focused on peptide molecules which exhibit a limited solubility in the aqueous phase and bind to the lipid membrane from the aqueous medium. Surface adsorption, membrane insertion, and specific binding are usually accompanied by changes in the heat content of the system and can be measured conveniently with isothermal titration calorimetry, avoiding the necessity of peptide labeling. The driving forces for peptide adsorption and binding are hydrophobicity, electrostatics, and hydrogen bonding. An exclusively hydrophobic interaction is exemplified by the immunosuppressant drug cyclosporine A. Its insertion into the membrane can be described by a simple partition equilibrium $X_b=K_0C_{eq}$. If peptide and membrane are both charged, electrostatic interactions are dominant leading to nonlinear binding curves. The concentration of the peptide near the membrane interface can then be much larger than its bulk concentration. Electrostatic effects must be accounted for by means of the Gouy–Chapman theory before conventional binding models can be applied. A small number of peptides and proteins bind with very high affinity to a specific lipid species only. This is illustrated for the lantibiotic cinnamycin (Ro 09-0198) which forms a 1:1 complex with phosphatidyethanolamine with a binding constant of $10^8$ $M^{-1}$. Membrane adsorption and insertion can be accompanied by conformational transitions facilitated, in part, by hydrogen bonding mechanisms. The two membrane-induced conformational changes to be discussed are the random coil-to-α-helix transition of amphipathic peptides and the random coil-to-β-structure transition of Alzheimer peptides.


*Keywords:* Isothermal titration calorimetry; Cyclosporine A; Nisin Z; Cinnamycin; Magainin; Alzheimer peptide

**Contents**



## 1. Introduction

Thermodynamics of peptide–membrane interactions is a wide field. It depends on the chemical nature of the lipids, peptides, and carbohydrates involved and also on the mechanistic nature of the processes investigated. Different

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rules apply for trans-membrane insertions than for half-sided embedding. Electrostatic forces (both coulombic attraction/repulsion and dipolar interactions), hydrogen bond formation, and hydrophobic interactions play equally important roles. The enormous interest in "lipid rafts" has sensitized the scientific community to realize that the lipid part of the membrane is not simply a homogenous grease but comprises an enormous variety of lipid molecules of hitherto unknown functions. Depending on the membrane composition, groups of specific lipids may aggregate into patches with physical properties distinctly different from those of other membrane domains. A concept related to "lipid rafts" has been proposed almost 30 years ago under the headings of "boundary lipids" and "annular lipids" but faded away because of insufficient evidence for functional consequences.

Only a limited number of topics can be discussed in this review. The interaction of a peptide with the lipid membrane will be divided into three thermodynamic steps as illustrated in Fig. 1. In a first step, binding is initiated by the electrostatic attraction of the cationic peptide to the anionic membrane. Depending on the peptide charge, $z_p$, and the size of the membrane surface potential, $\psi_0$, electrostatic attraction (repulsion) will increase (decrease) significantly the peptide concentration near the membrane surface, $C_M$, compared to the bulk concentration, $C_{eq}$.

Of course, electrostatic attraction is not a prerequisite for binding. Binding can also occur between a non-charged peptide and a neutral membrane. Under these conditions the peptide concentration near the membrane surface is identical to that in bulk solution.

The next step is the transition of the peptide into the plane of binding. The exact location of this layer is difficult to define and depends on the hydrophobic/hydrophilic balance of the molecular groups and forces involved. "Binding" means that $n_p$ peptides are in some direct contact with a membrane composed of a total of $n_L^0$ lipids, all accessible for binding. The *degree of binding*, $X_b$, can thus be defined as

$$X_b = n_p/n_L^0 \quad (1)$$

The main task in the thermodynamic analysis is to measure $X_b$ as a function of the peptide equilibrium concentration, $C_{eq}$, and to derive judicial models which describe $X_b$=$X_b(C_{eq})$ as a function of membrane composition, temperature, and other parameters of interest. It is then possible to derive the binding constant and the associated thermodynamic parameters.

"Binding" in a conventional chemical reaction denotes the specific complex formation between two or more interacting species with a well-defined stoichiometry. In membrane equilibria, the term binding is used more loosely and refers to physical phenomena such as partitioning and adsorption. Complex formation between a peptide and a specific lipid is not encountered too often. An interesting example will be discussed below.

The third step in the binding process is a change of the conformation of the bound peptide. In many instances peptides are in a random coil conformation in solution but adopt an α-helical conformation when associated with the lipid membrane. A particularly well-documented example is the bee venom melittin whose CD spectrum changes from almost completely random coil in buffer to about 80% α-helix upon addition of neutral phospholipids vesicles [1]. Conformational changes entail changes in the thermodynamic parameters. For the molecular interpretation of the binding process, it is hence important to partition the measured thermodynamic quantities into the binding process proper and the ensuing conformational transition.

α-Helix formation is not the only conformational change possible. In the case of Alzheimer peptides, binding to negatively charged lipid bilayers leads first to β-sheet structures (at low lipid-to-protein ratios) and then to α-helix formation (at high lipid-to-protein ratios) [2]. Hence, an additional complication is encountered since β-structure formation usually requires the aggregation of molecules. The thermodynamics of the binding process must then be divided into binding, β-structure formation, and aggregation.

The scheme outlined above will be illustrated in the following with examples of increasing complexity. We shall start with the *hydrophobic partitioning* of a neutral peptide (cyclosporine A) into a neutral membrane. In a second example, involving the charged lantibiotic nisin Z, we combine *hydrophobic partitioning* with *electrostatic attraction*. Next we shall discuss one of the very few examples of a *specific complex formation* between a small peptide (cinnamycin) and a phospholipid (phosphatidylethanolamine). The last sections of this review then describe *membrane-induced conformational changes*, namely (i) the *random coil-to-α-helix* transition, illustrated with antibacterial peptides of the magainin class, and (ii) the *random coil-to-β-structure* transition of Alzheimer peptides.

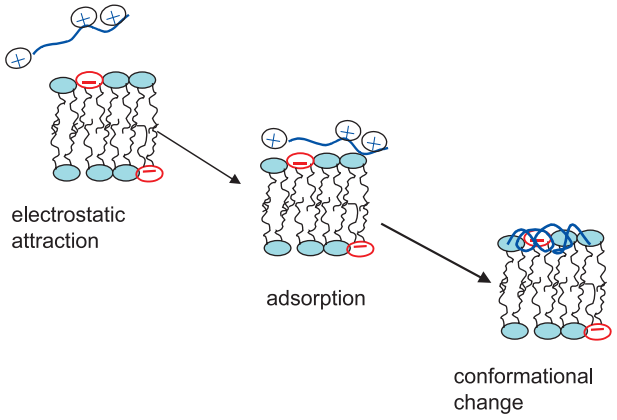


Fig. 1. Molecular recognition at the membrane surface. The diagram shows different stages of peptide binding. The charged peptide is attracted electrostatically to the membrane surface followed by a conformational change to an α-helix. The surface potential of a lipid bilayer composed of neutral and negatively charged lipids (typically ~20–25% monovalent anionic lipid) at physiological salt concentration is about $\psi \approx -50$ mV. The concentration of a peptide of charge $z$=+3 at the membrane surface, $C_M$, is about 350-fold larger than that in bulk solution ($C_M \sim 350 C_{eq}$).

Mainly spectroscopic methods are employed to measure peptide–membrane equilibria. Most common are UV-absorbance spectroscopy, fluorescence spectroscopy, and surface plasmon resonance. In addition, equilibrium dialysis, gel chromatography, and isotope labeling methods are also applied. The primary parameter deduced from all these methods is a binding constant, $K_0$ (also called partition constant, association constant), which is then converted into the free energy of binding, as $\Delta G^0 = -RT\ln K_0$. (The definition of $K_0$ may vary from author to author. A comparison of different definitions may be found in Ref. [3]). In order to derive the reaction enthalpy, $\Delta H^0$, spectroscopic measurements must be repeated at different temperatures and $\Delta H^0$ then follows from the temperature dependence of $K_0$ via the van't Hoff analysis. However, the reaction enthalpy, $\Delta H^0$, is usually temperature-dependent itself and the temperature dependence of $K_0$ must thus be measured with extreme precision. It should also be noted that a reporter group attached to yield a fluorescence signal may itself contribute to the binding reaction, not giving an unbiased report of the equilibrium. For example, unlabeled heptalysine ($Lys_7$) binds to charged lipid membranes with an intrinsic binding constant of $K \sim 600\ M^{-1}$, the same molecule with a fluorescence label attached to it binds 100-fold stronger with $K \sim 6\times10^4\ M^{-1}$ [4].

In contrast to spectroscopic methods, high sensitivity isothermal titration calorimetry (ITC) allows the simultaneous measurement of the binding constant, $K_0$, and the reaction enthalpy, $\Delta H^0$, without the need for labeling [5]. Almost all chemical reactions and physical adsorption processes exhibit some exo- or endothermic $\Delta H^0$, and ITC measures directly the heat of reaction. Under appropriate experimental conditions, ITC measures not only $\Delta H^0$ but also the full binding isotherm from which $K_0$ can then be deduced. Moreover, the temperature dependence of $\Delta H^0$ yields the molar heat capacity, $\Delta C_p^0$, of the binding reaction with high precision (for a review see Ref. [6]). The examples reported in this review were all measured with ITC.

## 2. Hydrophobic partitioning: a simple membrane equilibrium

Cyclosporin A (CyA) is an efficient and clinically used immunosuppressive agent. It is the drug of choice in transplantation medicine and the treatment of autoimmune diseases. The cyclic peptide is composed of 11 amino acids, with seven of them N-methylated. The structure of the molecule is shown in Fig. 2. Two conformations have been reported for CyA: one for pure CyA, the other bound to immunophilin or calcineurin [7]. In addition, CyA in solution also appears to adopt two different conformations [8]. As suggested by the chemical structure, CyA is only sparingly soluble in saline solutions ($c_{sol} \sim 15$ μM at 25 °C in 0.164 M NaCl) but exhibits a high lipophilicity [9]. Interestingly, the solubility of CyA decreases with increasing temperature, indicating an *exothermic* heat of solubilization for this hydrophobic compound. $\Delta H_{sol}$ was found to be −16.4 and −12.2 kcal/mol for CyA in water and 0.164 M NaCl, respectively [9].

In pharmaceutical industry lipid membranes are used as drug carrier systems in the form of liposomes. From a pharmaceutical point of view, liposomes can be regarded as nontoxic and biodegradable carriers. Because of the high lipophilicity of CyA, one may take advantage of liposomes

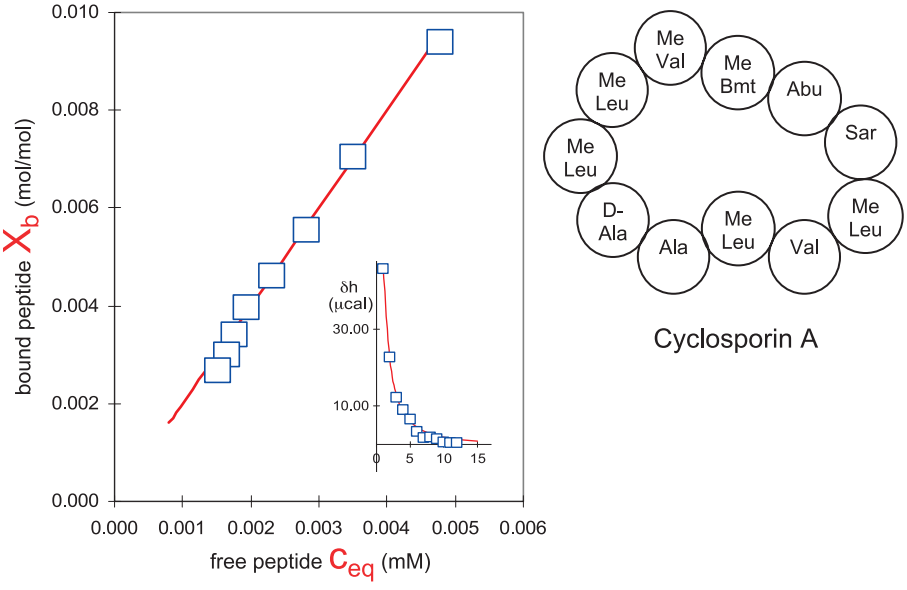


Fig. 2. Hydrophobic partition equilibrium of the immunosuppressant drug cyclosporine A (CyA) with phosphatidylcholine SUVs. Lipid vesicles ($c_{Lipid}$=37.7 mM) were titrated into a CyA solution (CyA=7.3 μM) and the heats of reaction, $\delta h_i$, were recorded calorimetrically as a function of the injection number (small insert). The $\delta h_i$s are endothermic and can be used to deduce the binding isotherm. The figure shows the degree of binding $X_b = n^{bound}_{CyA}/n^0_{Lipid}$ as a function of the CyA equilibrium concentration, $C_{eq}$. A straight line through the origin is obtained which can be described according to $X_b = K_0 C_{eq}$. The thermodynamic parameters for the example chosen are $\Delta H^0$=12.9 kcal/mol and $K = 2\times10^3\ M^{-1}$ (at 20 °C) [9].

as carrier systems for CyA and it is thus of general interest to measure the binding of CyA to bilayer membranes.

CyA carries no chromophor suited for quantitative UV absorbance or fluorescence detection. However, partitioning of CyA into liposomes is associated with the consumption of a considerable amount of heat, making ITC a most convenient tool for binding studies [9]. The insert in Fig. 2 shows the actual heats of titration as 10-μl aliquots of single-walled phospholipid vesicles (composed of POPC=1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; vesicle diameter 100 nm; $c_{lipid}$=37.7 mM) are injected into a dilute (7.3 μM) CyA solution. Each lipid injection removes a considerable fraction of CyA from the solution and the heats of reaction decrease rapidly. After about 10 injections, almost all CyA appears to be membrane-bound. The sum of the individual heats yields the molar reaction enthalpy of $\Delta H^0$=12.9 kcal/mol (at 20 °C). The titration isotherm can be translated into a binding isotherm as detailed elsewhere [6,9]. Fig. 2 then shows the membrane-bound peptide, $X_b$, as a function of the free CyA concentration, $C_{eq}$, yielding a straight line. CyA 'binding'
to POPC liposomes can thus be described by a simple partition law according to

$$X_b = K_0 C_{eq} \quad (2)$$

The binding constant, $K_0$, is given by the slope of the straight line and is $K_0$=2000 $M^{-1}$ (at 20 °C) for the data shown in Fig. 2. The corresponding free energy is $\Delta G_0 = -RT\ln 55.5K_0 = -6.7$ kcal/mol. (The factor 55.5 is the molar concentration of water. $K^*=55.5K_0$ is the binding constant if the concentration of CyA is referred to 1 mol $H_2O$.) Since $\Delta H^0$ is endothermic, the driving force for CyA partitioning into the lipid phase is a large entropy gain suggesting the following molecular picture. As CyA enters the hydrophobic membrane, it sheds off a large hydration shell. The gain in the translational and rotational freedom of the water molecules then generates sufficient entropy to more than compensate the unfavorable enthalpy term. This is also confirmed by the large negative heat capacity change of the reaction, which is about $\Delta C_p^0 \approx -200$ cal/K mol (at 20 °C), indicating the 'melting' of a large hydration shell (J. Seelig and P. Ganz, unpublished results). Because of the negative $\Delta C_p^0$, the reaction enthalpy becomes smaller and CyA binding better with increasing temperature.

## 3. Electrostatic attraction and surface partitioning

The interaction of a peptide with the lipid membrane can be purely hydrophobic as for cyclosporine A or purely electrostatic as, for example, with polylysines [10,11], but for most charged peptides an intermediate situation will be encountered. Charged peptides will be attracted electro-

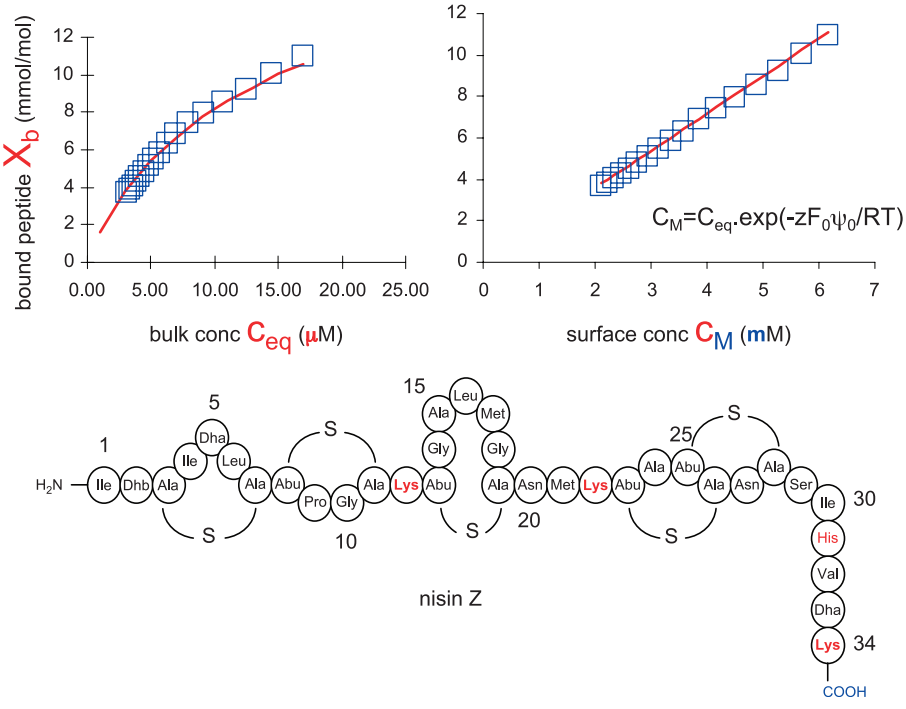


Fig. 3. Partition equilibrium modified by electrostatic attraction. Isothermal titration calorimetry was used to measure the binding of the lantibiotic nisin Z to membranes composed of neutral and negatively charged lipid (membrane composition POPC/POPG 3:1 molar ratio). Nisin Z carries an electric charge of $z$=+3.5 and is attracted electrostatically to the membrane surface. The surface concentration $C_M$ (calculated with the Gouy–Chapman theory) is much larger than the bulk concentration $C_{eq}$. The figure shows plots of the degree of binding $X_b=n^{bound}_{nisin}/n^0_{Lipid}$ as a function of either the bulk concentration, $C_{eq}$, or the surface concentration, $C_M$. For the latter case a straight line is obtained and the binding process can be described by a surface partition equilibrium $X_b=K_0C_M$. The thermodynamic parameters for the experiment shown are $K_0$=1.8 $M^{-1}$ and $\Delta H^0$=−8.5 kcal/mol (at 28 °C) [12].

statically to the membrane surface and will then penetrate to some extent into the hydrophobic part of the membrane.

A typical example is the antimicrobial cationic peptide nisin Z (34 amino acids), which has found application as a food preservative. The peptide has four intramolecular thioether bridges and its chemical structure is depicted in Fig. 3. The peptide renders the bacteria cytoplasmic membrane permeable to ions, amino acids, and ATP and interacts with most membrane lipids in a nonspecific manner. Recently, a highly specific interaction between nisin Z and lipid II has also been discovered [12].

The interaction of nisin Z with small unilamellar vesicles (SUVs), prepared by sonication (vesicle diameter, $d$~30 nm) and composed of neutral POPC lipid, yields only a weak but nevertheless measurable heat signal. Addition of negatively charged POPG (=1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol) drastically improves the binding [13]. Fig. 3A shows the binding isotherm as deduced from calorimetric titrations. The extent of bound nisin Z expressed by the degree of binding, $X_b$, is plotted versus the equilibrium concentration of free nisin, $C_{eq}$. The binding constant as defined by Eq. (2) is no longer constant but decreases with increasing peptide concentration. This is a consequence of a screening of the electrostatic attraction at high peptide concentrations. In the absence of peptide, the electric surface potential of a mixed POPC/POPG (3:1) membrane is about $\psi_0 \approx -50$ mV (0.11 M NaCl, pH 7.4, $T$=20 °C). At low peptide concentration, the electrostatic attraction is strong which is translated into a large apparent binding constant. With increasing peptide concentration more peptide is bound, the membrane surface potential is reduced, and $K_{app}$ decreases smoothly.

The influence of electrostatic attraction can be corrected by calculating the surface concentration of the peptide, $C_M$, according to the Boltzmann equation

$$C_M = C_{eq} e^{-z_p F_0 \psi_0 / RT} \quad (3)$$

where $z_p$ is the signed charge of the peptide, $F_0$ is the Faraday constant, and $RT$ is the thermal energy. Unfortunately, the membrane surface potential is not known. However, $\psi_0$ can be calculated from the surface charge density, $\sigma$, of the membrane using the Gouy–Chapman theory [14]. The surface charge density $\sigma$ is given by the sum of contributions of anionic lipid, bound cationic peptide, and all other bound cations such as $Na^+$ [15]. The result of this analysis is shown in Fig. 3B where the degree of binding, $X_b$, is now plotted against the surface concentration, $C_M$, of the peptide. The latter is about two orders of magnitude larger than the bulk equilibrium concentration (note the different concentration scales). The figure shows that by replacing $C_{eq}$ by $C_M$, the binding isotherm becomes a straight line and follows the modified partition equilibrium:

$$X_b = K_0 C_M \quad (4)$$

The effective peptide charge was $z_p \approx 3.5$ and the intrinsic binding constant derived from Fig. 3B is $K_0$=1.8 $M^{-1}$. Fig. 3B demonstrates that $K_0$ is independent of the peptide concentration. The reaction enthalpy is $\Delta H^0$=−9.1 kcal/mol.

If one ignores the specific role of electrostatic attraction, an apparent binding constant may be defined in Fig. 3A according to

$$K_{app} = X_b / C_{eq} \quad (5)$$

comprising both electrostatic attraction and hydrophobic adsorption. Obviously, $K_{app}$ varies with the peptide concentration. Nevertheless, $K_{app}$ is useful to calculate the contribution of the electrostatic free energy to peptide binding according to:

$$\Delta G_{el} = -RT \ln\left(K_{app}/K_0\right) = -RT \ln\left(C_M/C_{eq}\right) \quad (6)$$

Since $K_{app}$ depends on the peptide concentration (cf. Fig. 3A), $\Delta G_{el}$ is also not constant.

## 4. Specific lipid–peptide interactions via complex formation

Most amphiphilic peptides bind to lipid membranes by a physical adsorption mechanism. In contrast, *specific* phospholipid–peptide interactions are rare. One recent example is the interaction of nisin Z with lipid II [12]. Another exception is provided by the 19-amino-acid tetracyclic peptide Ro 09-0198 (cinnamycin) which forms a tight equimolar complex with phosphatidylethanolamine [16,17]. The chemical structure is shown in Fig. 4. Posttranslational modifications result in unusual amino acids that are typical for the whole group of lantibiotics. Cinnamycin interacts specifically with phosphatidylethanolamine but not with phosphatidylcholine or other lipids. The binding constant of the 1:1 complex can be determined with isothermal titration calorimetry and a typical experiment is shown in Fig. 4 where the calorimeter cell contains a 40 μM solution of cinnamycin in buffer [18]. Each titration peak corresponds to the injection of 10-μl aliquots of sonicated phospholipids vesicles ($d$=30 nm) composed of phosphatidylcholine/phosphatidylethanolamine 9:1. The titration curve exhibits a very steep transition, and a binding constant of $K_0 = 7 \times 10^7$ $M^{-1}$ can be derived for this 1:1 complex. The reaction enthalpy is $\Delta H^0$=−8.5 kcal/mol and is the major contribution to the free energy of $\Delta G^0$=−10.7 kcal/mol at room temperature.

Since cinnamycin is an amphiphilic molecule, it will also partition nonspecifically into bilayer membranes, a fact which has been overlooked in the past. Indeed, partitioning is much weaker than complex formation and cannot be detected with spectroscopic methods. However, cinnamycin partitioning into POPC membranes can be measured easily with isothermal titration calorimetry. The

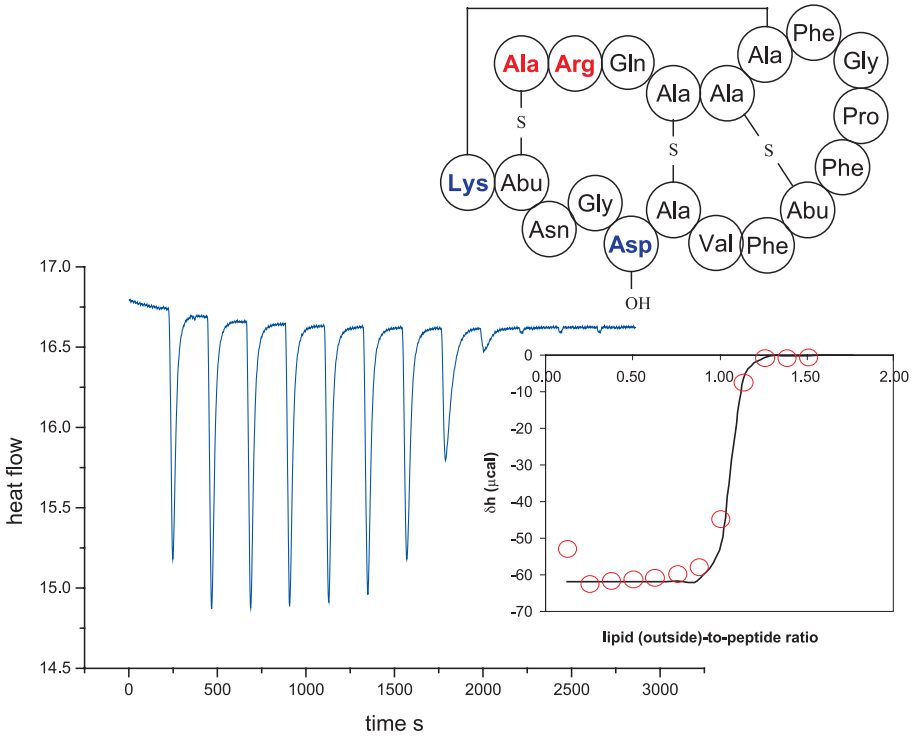


Fig. 4. Lipid–peptide complex formation. The figure shows the calorimetric titration pattern obtained for the titration of cinnamycin (40 μM) with phospholipids vesicles composed of POPC/PE (9:1 molar ratio). The cinnamycin reacts specifically with the phosphatidylethanolamine on the membrane outside. The insert displays the heat of reactions, $\delta h_i$, as a function of the lipid (outside)-to-peptide ratio and reveals a 1:1 stoichiometry. The binding constant is $K_0=7\times10^7$ $M^{-1}$ and the reaction enthalpy $\Delta H^0=-8.5$ kcal/mol [17,18].

partition constant is only $K_0 \approx 100$–$300$ $M^{-1}$. The partition enthalpy is also small with $\Delta H^0 \approx -0.5$ to $-1$ kcal/mol (at room temperature).

The question may be raised as to which extent cinnamycin binding to phosphatidylethanolamine (PE) is influenced by the long-range order of the "solvent", i.e., if there are differences between a densely packed bilayer and an isotropic micellar environment. Intuitively, one would argue that a PE molecule in a bilayer is less accessible to a water-soluble molecule such as cinnamycin than PE in a micelle. However, the contrary is observed experimentally. PE dissolved in micelles composed of octylglucosides reacts specifically with cinnamycin with binding constants which are about a factor of 100 *smaller* compared to those obtained for the lipid bilayer. The 1:1 stoichiometry remains unchanged [19], leading to the following explanation. When cinnamycin binds to PE embedded in a lipid matrix, it experiences two types of interactions, namely the specific interaction with PE and the nonspecific hydrophobic interaction with the hydrophobic bilayer. As indicated above, the latter is characterized by a binding constant of 100–300 $M^{-1}$. This interaction with the bilayer is, however, missing when PE and cinnamycin are dissolved in the micellar environment, explaining the reduced free energy of binding. On the other hand, as a general consequence it follows from these results that it is possible to study the interaction of water-insoluble membrane proteins with lipids with ITC by dissolving both compounds in a micellar environment.

## 5. Membrane-induced conformational changes

### 5.1. Random coil-to-α-helix transition

Amphipathic α-helices are the membrane-binding motif in many proteins. The corresponding peptides are random coil in solution but are folded into a α-helix upon interaction with the membrane. The reaction path following electrostatic attraction can then be divided into two distinct steps: (i) the binding of the random coil peptide to the membrane and (ii) the transition of the bound peptide to adopt a well-defined α-helix.

The thermodynamics and cooperativity of the random coil-to-α-helix transition in solution have been investigated extensively and are well understood. In contrast, the corresponding membrane-induced processes have been approached only recently. A systematic study was undertaken with the antibacterial frog peptide magain 2 amide (M2a=GIGKF LHSAK KFGKA FVGEI MNS($NH_2$)). M2a is random coil in buffer but adopts an essentially α-helical conformation when bound to a POPC/POPG (3:1) membrane [20]. The spectral deconvolution of the CD spectrum of the bound peptide yields an α-helix content of about 74% at room temperature. It is then possible to systematically vary the helix content by substituting two adjacent amino acids by their D-enantiomers. For d4,5 M2a the helix content is 53%, for d11,12 M2a only 29%. Next, using titration calorimetry it is possible to measure the

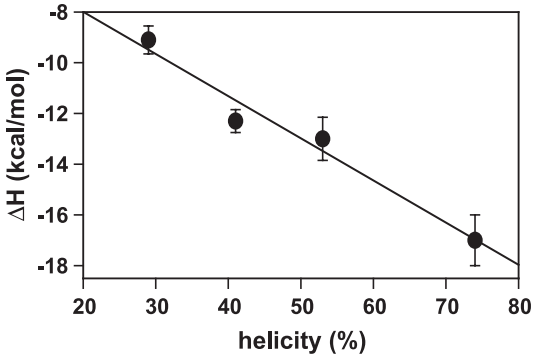


Fig. 5. Helix formation at the membrane surface. The peptide magainin 2 amide (M2a) binds to the membrane surface and adopts an essentially α-helical structure (73% α-helix). A series of diastereomers were synthesized in which two adjacent amino acid residues were replaced by their D,D enantiomers. D,D substitution interrupts the α-helix, and the various analogs display different helix contents upon interaction with the membrane. The figure shows the variation of the standard reaction enthalpy, $\Delta H^0$, with the helix content. Extrapolation to 0% helicity provides the binding enthalpy of the (hypothetical) random coil M2a as $\Delta H^0_{rc}=-4.7$ kcal/mol. Membrane-induced helix formation is an exothermic process and each helical residue contributes an additional enthalpy of $\Delta H^0_{helix}=-0.72$ kcal/mol to the total binding process as deduced from the slope of the straight line.

corresponding binding enthalpies and binding isotherms [21]. Fig. 5 then shows a plot of the experimental standard reaction enthalpies, $\Delta H^0$, as a function of the helix content. To a good approximation a straight line is obtained. From the intercept with the ordinate (0% helicity) the binding enthalpy of a hypothetical random coil magainin 2a peptide can be estimated to be $\Delta H^0_{rc}=-4.7$ kcal/mol. This reaction enthalpy is then further decreased by α-helix formation since the conformational transition is an exothermic process. Linear regression analysis of the data in Fig. 5 yields $\Delta H^0=-0.72n_{helix}-4.7$ (kcal/mol) (at 30 °C) where $n_{helix}$ is the average number of helical segments in the polypeptide chain. Each helical residue thus contributes $\Delta H^{suv}_{helix}=-0.72\pm0.09$ kcal/mol per residue to the reaction enthalpy [21]. The results described above were obtained with SUVs with an average diameter of $d\approx30$ nm (prepared by sonication). SUVs are characterized by a non-ideal lipid packing due to their high membrane curvature. The analogous study performed with large unilamellar vesicles (LUV) with $d\approx100$ nm (prepared by extrusion through polycarbonate filters) yields $\Delta H^0_{LUV}=-0.80n_{helix}+10.5$ (kcal/mol) (at 30 °C) [22]. The enthalpy of helix formation per peptide residue is $\Delta H^{LUV}_{helix}=-0.80\pm0.02$ kcal/mol and thus very similar to $\Delta H^{SUV}_{helix}$.

The membrane-induced helix formation was also studied with the 23-amino-acid presequence of rat mitochondrial rhodanese (RHD) with the sequence MVHQV LYRAL VSTKW LAESI RSG. Again a systematic variation of the helix content was induced by double D-substitution and a linear dependence of the binding enthalpy on the helicity of the membrane-bound peptide was observed [23]. The incremental changes were $\Delta H^{SUV}_{helix}=-0.53$ kcal/mol and $\Delta H^{LUV}_{helix}=-0.63$ kcal/mol [23].

A linear relation between the experimental $\Delta H^0$ and the helicity can be expected if the enthalpy of helix formation is the same for each helical segment within a peptide chain. This is strictly valid only for homopolymers in an isotropic solvent. The slightly different values of $\Delta H_{helix}$ obtained for M2a and RHD can presumably be explained by the fact that the peptides are no homopolymers.

Membrane-induced α-helix formation is an exothermic process. An increase in temperature will thus produce a melting of the α-helix. In the case of M2a the helicity of the bound peptide decreases from ~83% at 5 °C to ~33% at 95 °C [21]. The Zimm–Bragg theory for the helix–coil transition of homopolymers can be used to analyze the thermal unfolding [24]. The theory describes the reversible random coil $\rightleftarrows$ α-helix transition in terms of the nucleation parameter $\sigma$, the growth parameters $s$, and the total chain length $n$. For thermal unfolding the growth parameter $s$ changes with temperature according to

$$s = s_0 e^{-\Delta H_{helix}/RT} = e^{-\left(\Delta H_{helix}/R\right)\left(\frac{1}{T}-\frac{1}{T_c}\right)}$$

where $\Delta H_{helix}$ is the enthalpy of helix formation per residue and $T_c$ is the midpoint of the transition for an infinitely long polymer $\left(n>>\sqrt{1/\sigma}\right)$. Using CD spectroscopy the variation of helicity with temperature can be measured. For M2a bound to POPC/POPG vesicles (3:1 molar ratio) the Zimm–Bragg analysis yields $\Delta H_{helix}=-0.81$ kcal/mol, in excellent agreement with the calorimetric measurements [21]. The nucleation parameter is $\sigma=2.3\times10^{-3}$ and the midpoint of the transition is $T_c\approx150$ °C. This latter value is much higher than those obtained for peptides in solution and indicates an increased stability of the membrane-bound α-helix. It is, however, beyond the scope of this review to compare in detail α-helix formation in a membrane environment with that in aqueous solution.

The thermodynamic analysis can be extended to include the free energy of binding. To this purpose, the binding isotherms of the various peptides with D,D-substitution are measured, the corresponding binding constants $K_0$ are derived (after correction for electrostatic effects) and the standard free energies, $\Delta G_0=-RT\ln55.5K_0$ are deduced. A plot of $\Delta G^0$ vs. the helix content (analogous to that shown in Fig. 5 for $\Delta H^0$) then yields the incremental contribution, $\Delta G_{helix}$, per helical residue to the total free energy. For M2a the regression analysis yields $\Delta G_{helix}=-0.14$ kcal/mol (SUVs) [21] and −0.12 kcal/mol (LUV) [22], for RHD the corresponding numbers are −0.2 kcal/mol (SUV) and −0.23 kcal/mol (LUV) [23]. Two other estimates of $\Delta G_{helix}$ may be found in the literature. A comparison of native melittin with a diastereomeric analog with four D-amino acids suggests $\Delta G_{helix}\sim-0.4$ kcal/mol [25]. Likewise, the thermodynamic analysis of the binding of analogues of the influenza hemeagglutinin fusion peptide leads to $\Delta G_{helix}=-0.25\pm0.05$ kcal/mol per residue [26]. Notwithstanding numerical differences, the main conclusion from

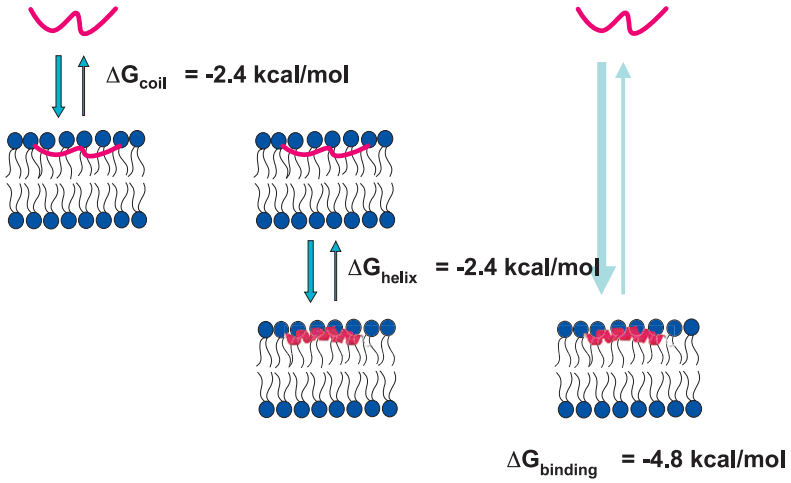


Fig. 6. Consequences of α-helix formation for the membrane-binding equilibrium as exemplified with M2a. The binding process is dissected into two steps. First, the unstructured M2a binds to the membrane with a free energy of $\Delta G_{\text{random coil}}$=−2.4 kcal/mol. Next, the peptide adopts an α-helical conformation which releases an additional free energy of $\Delta G_{\text{helix}}$=−2.4 kcal/mol. The total free energy change for binding is thus $\Delta G_{\text{bind}}$=−4.8 kcal/mol. The peptide binds from the region of increased interfacial concentration $C_M$, that is, $\Delta G_{\text{bind}}$ does not include the free energy of electrostatic attraction.

these different studies is obvious: α-helix formation at the membrane surface reduces the free energy considerably and is thus an important driving force for membrane binding.

The consequences of α-helix formation for the M2a (solution) ⇄ M2a (membrane) equilibrium are illustrated in Fig. 6. For the sake of the argument, binding and α-helix formation are again divided into two consecutive steps even though they probably occur simultaneously. In the first step, the peptide is locked into the random coil conformation. Its binding to the membrane is characterized by a free energy change of $\Delta G_{\alpha}^{0}$=−2.4 kcal/mol. Next, the transition to the α-helical conformation occurs which contributes an additional free energy change of $\Delta G_{\text{helix}}^{0}$=−2.4 kcal/mol, leading to a total free energy of binding of $\Delta G^{0}$=−4.8 kcal/mol. Expressed in terms of binding constants, M2a binding without helix formation has a binding constant of $K_0$~60 $M^{-1}$, whereas M2a binding with helix formation is characterized by $K_0$~3600 $M^{-1}$. The biological advantage of α-helix formation thus becomes obvious. In order to disrupt the bacterial membrane, a certain threshold of M2a ($X_b \cong 13$ mmol peptide/mole lipid) in the membrane is needed. To reach this threshold with M2a fixed in a random coil conformation a 60-fold higher concentration of M2a in the aqueous phase would be needed than is observed experimentally for M2a with α-helix formation (~1.1 μM).

### 5.2. Random coil-to-β-structure transition.

A main component of Alzheimer plaques is the βAP(1–40) peptide with the sequence $^{+}NH_3$DAFR HDSGY EVHHQ KLVFF AEDVG SNKGA IIGLM VGGVV-$COO^{-}$. In spite of its seven negative and four positive charges and its three histidines, the peptide exhibits only a limited solubility in water or buffer. It is monomeric up to a concentration of about 15 μM [27]. The CD spectrum of the monomer reflects a predominantly random coil structure. Increasing the peptide concentration leads to an aggregation and a simultaneous transition to a β-structured conformation. The isosbestic point of the CD spectra provides evidence for a simple two-state transition, that is, only two types of limiting conformations are involved in the aggregation process and all CD spectra can be described by a linear combination of the two basic spectra [28]. A quantitative analysis of the aggregation process is possible with a cooperative aggregation model [28].

Increasing the temperature from 10 to 80 °C produces only a small change of the CD spectra, with a tendency to increase the β-structured conformation. This suggests that aggregation and formation of β-structure are associated with a $\Delta H_{\beta}^{0}$ close to zero or slightly negative. These observations are consistent with a recent study on some unfolded peptides which comes to the conclusion that increasing the temperature leads to an extended β-conformation propensity of these peptides [29]. However, different results may be obtained if the peptide solution is prepared at low pH, at low temperature, or at different buffer conditions [30].

In Alzheimer disease the βAP peptides are found as fibrillar plaques of ~0.2-μ extension on the outer cell surface. The fibrils show a diffuse β-structure eluding a detailed X-ray analysis. The interaction of βAP with the cell membrane can be modeled with pure lipid membranes. However, particular attention must be given to the method of preparation. If βAP is co-dissolved with lipid in an organic solvent and buffer is added to the dry mixture, a transmembrane orientation of βAP appears to be induced [31]. On the other hand, if the βAP peptide (in buffer) is added to a pre-formed lipid bilayer (also in buffer) a spontaneous insertion into the lipid bilayer does not occur. Under these conditions, the Alzheimer peptide remains superficially attached to the membrane surface. Indeed, there is almost no interaction with a neutral POPC membrane as judged on the basis of ITC, monolayer, and CD experiments [2].

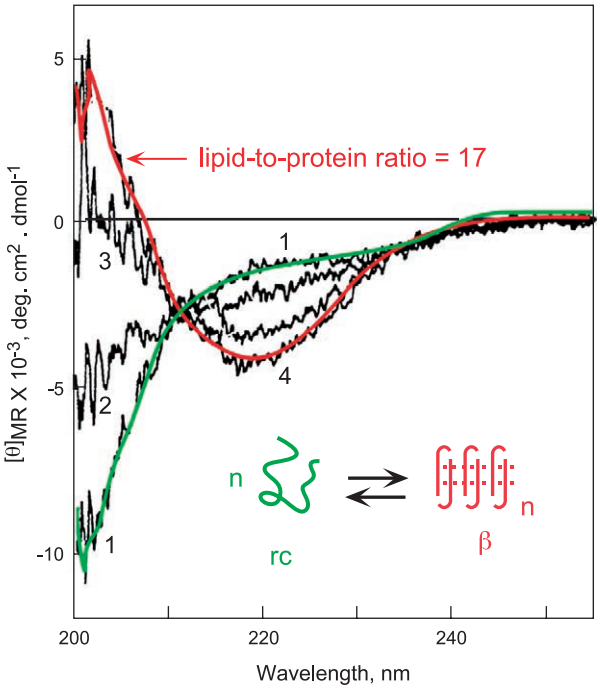


Fig. 7. Lipid-induced random coil ⇄ β-structure transition of an Alzheimer peptide. Small unilamellar lipid vesicles composed of POPC/POPG (3:1) ($C_L^0$=55 mM) were titrated into a 50 μM solution of βAP(1–40). The CD spectra correspond to different lipid-to-protein ratios. In the absence of lipid, the CD spectrum is typical for a predominantly random coil conformation. With increasing lipid concentration, the CD spectra indicate the transformation to a β-structured conformation [25].

A different result is obtained if the membrane contains an anionic lipid such as phosphatidylglycerol (PG) as is illustrated in Fig. 7 [28]. The figure displays CD spectra of βAP(1–40) at a constant concentration of 50 μM to which increasing amounts of lipid vesicles are added. In the absence of lipid (spectrum 1) the CD spectrum reveals a predominantly random coil conformation. Addition of unilamellar vesicles (~30 nm) composed of POPC/POPG (3:1) induces a dramatic spectroscopic change, leading to a CD spectrum with ~70% β-structure at a lipid-to-peptide ratio of ~17. The molecular origin of this spectral change can be traced back to the combined effects of electrostatic attraction, concentration increase at the membrane surface, and aggregation. The strong negative surface potential of the lipid vesicles attracts the βAP(1–40) with its positively charged residues to the membrane surface thereby increasing the peptide concentration by more than 10-fold. The peptide, now accumulated at higher concentration at the lipid–water interface, starts to aggregate much as it would do so free in solution and forms β-structures.

As the titration with lipid is continued, a second transition is observed [2]. The conformation now changes from β-structure to α-helix, again with an isosbestic point. This transition is completed at a lipid-to-peptide ratio of about 50. This result suggests that the βAP(1–40) aggregates are dissociated again at higher lipid-to-protein ratios. The monomeric peptides then fold into an amphipathic α-helix as discussed above for M2a.

What do we know about the thermodynamics of the membrane-induced β-structure formation of Alzheimer peptides? Unfortunately, very little. It is possible to perform the titration of βAP(1–40) with sonicated phospholipids vesicles in the titration calorimeter and to determine a binding enthalpy $\Delta H^0$ and the binding isotherm [28]. Using POPC/POPG SUVs (3:1 molar ratio) the heats of titration are typically −15 to −17 kcal/mol, depending on temperature and buffer conditions. The binding is dominated by electrostatic attraction and the intrinsic (hydrophobic) binding constant is only ~10 $M^{-1}$. However, the lipid-into-peptide titration experiment spans the whole conformational space of βAP(1–40). Initially, the L-to-P ratio is small and the peptide will aggregate into a β-structured conformation. As more lipid is titrated in, the aggregates are dissolved and an α-helix is formed. Hence, the titration isotherm comprises (i) two conformational transitions, (ii) a binding reaction, and (iii) an aggregationdissociation equilibrium.

Using a simple model peptide, an attempt has been made to estimate at least the free energy of β-structure formation [32]. Again it appears difficult to separate binding from aggregation and structural transitions. This unsatisfactory situation of the membrane-induced β-structure formation is perhaps not surprising since the same difficulty is encountered for the thermodynamics of β-folded proteins in solution [33].

## 6. Some general conclusions

The examples discussed above describe the thermodynamics of rather simple lipid–peptide interactions where the bilayer membrane remains essentially intact. Membrane micellization [34,35] and peptide pore formation [23,36], which can also be detected with calorimetric methods, have been excluded from the discussion. From an experimental point of view isothermal titration calorimetry appears to be an ideal method to study the thermodynamics of lipid–protein interactions. ITC has matured into a highly sensitive technique requiring only small volumes (~1 ml) and low concentrations (μM), and reasonably short measuring times. A complete titration isotherm can be measured within 60–90 min.

Lipid binding studies can be performed with either sonicated lipid vesicles ($d$~30 nm; SUV) or extruded vesicles ($d$~100 nm or larger; LUV). The binding constants and free energies for the binding of a given peptide are very similar for both systems; however, there are large differences in enthalpy and entropy. SUVs exhibit a much more exothermic $\Delta H^0$ than LUVs, and this makes SUVs a more sensitive system for titration calorimetry. In addition, SUVs allow the simultaneous recording of CD spectra while LUVs produce a large scattering background. Since the free energies of

binding, $\Delta G^0$, for SUVs and LUVs are almost identical, the large differences in $\Delta H^0$ must be compensated by corresponding changes in the entropy term, $T\Delta S^0$. The origin of this enthalpy–entropy compensation effect is not clear yet but has been related to the different packing densities of SUVs and LUVs [22].

From the available studies it can be concluded that amphipathic peptides bind to SUVs with a distinctly exothermic reaction and that $\Delta H^0$ is the major driving force (non-classical hydrophobic effect) [37]. Typical $\Delta H^0$ values are of the order of −5 to −20 kcal/mol but depend very much on the type of peptide involved. This is in contrast to the common belief that amphipathic peptides bind to lipids because of the entropy-driven hydrophobic effect. For highly charged peptides, electrostatic attraction is predominant and electrostatic binding is characterized by small $\Delta H^0$ values. Typical examples are the binding of pentalysine to POPG SUVs with $\Delta H^0 = +1.0$ kcal/mol [11] and of heparan sulfate to POPG containing SUVs with $\Delta H \sim -2$ kcal/mol [38]. No other general rules have emerged so far.

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