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Interaction of enkephalin peptides with anionic model membranes

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Abstract

According to the model for passive transport across the membranes, the total flow of permeant molecules is related to the product of the water–membrane partition coefficient and the diffusion coefficient, and to the water–membrane interfacial barrier. The effect of membrane surface charge on the permeability and interaction of analgesic peptide ligands with model membranes was investigated. A mixture of zwitterionic phospholipids with cholesterol was used as a model membrane. The lipid membrane charge density was controlled by the addition of anionic 1-palmitoyl-2-oleoylphosphatidylserine. Two classes of highly potent analgesic peptides were studied, c[D-Pen^2,D-Pen^5]enkephalin (DPDPE) and biphalin, a dimeric analog of enkephalin. The effect of increased surface charge on the permeability of the zwitterionic DPDPE is a relatively modest decrease, that appears to be due to a diminished partition coefficient. On the other hand the binding of the dicationic biphalin ligands to membranes increases proportionally with increased negative surface charge. This effect translates into a significant reduction of biphalin permeability by reducing the diffusion of the peptide across the bilayer. These experiments show the importance of electrostatic effects on the peptide–membrane interactions and suggest that the negative charge naturally present in cell membranes may hamper the membrane transport of some peptide drugs, especially cationic ones, unless there are cationic transporters present.

Keywords: Peptide–membrane; Permeability coefficient; Partition coefficient; Electrostatic effect

1. Introduction

A crucial step in the development of peptide based opioid ligands for therapeutic purposes is the design of ligands with desirable biological properties coupled with the proper biophysical properties to permit access from the blood to receptor sites in the brain. The primary objective of this research is to determine the nature of the interaction of receptor selective opioid ligands with lipid bilayer membranes. In doing so we seek to elucidate which molecular characteristics of peptide and peptidomimetic ligands may be modified to increase the membrane permeability of these ligands.

It is accepted that interaction of peptides with membranes arises from both hydrophobic and electrostatic effects [1–5]. The Gouy–Chapman theory (see [6,7] for reviews) has been successfully applied to describe the electrostatic interactions of peptides with membranes [1,2,5,8,9], and it has been shown that applicability of the Gouy–Chapman theory depends on the distribution of charge on the membrane surface and its distance from the membrane [10]. According to the Gouy–Chapman theory, the non-specific electrostatic interaction may enhance peptide solubility in the diffuse double layer. An experimen-

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tal manifestation of this effect is an increase in the peptide partition coefficient [5,8,11].

According to the diffusion model for the membrane transport, permeability coefficients are related to the product of the water–membrane partition coefficient of the permeant and the normal component of the diffusion coefficient [12]. Diffusional properties of the membrane are strongly heterogeneous and the partitioning process between water and membrane is more complex than that between water and a non-polar solvent [3]. At least two distinct regions can be identified, the highly polar interface and hydrocarbon interior [12]. To identify the rate-limiting domain it is necessary to analyze partitioning and diffusion within these regions. Diffusivity and partitioning in the bilayer strongly depend on the permeant size and shape [13,14] and models have recently been developed [15,16].

This report is part of a study of the relations between opioid peptide structure, the energetics of interaction with the membrane, and the membrane permeability. The general formalism for permeation is outlined elsewhere [12,13,17]. The permeability coefficient, P, is proportional to the diffusion coefficient, D, and partition coefficient, K:

$$P = \frac{DK}{X} \tag{1}$$

where X is the membrane thickness.

The water–membrane partition coefficient is defined as $K = [C_{mem}]/[C_{\infty}]$, where $[C_{mem}]$ and $[C_{\infty}]$ are the concentration of the solute in the membrane and in the bulk solvent, respectively. This model treats the membrane as a homogeneous non-polar medium, whereas the actual flux across a membrane is also dependent on interactions of the solute with the phospholipid headgroups, or the membrane surface, constituting an interface between aqueous and non-polar phases. Experimentally the observed permeability coefficient reflects both intramembrane and interfacial processes [12]:

$$P^{-1} = \int_x \frac{1}{D(x)K(x)} \mathrm{d}x + R \tag{2}$$

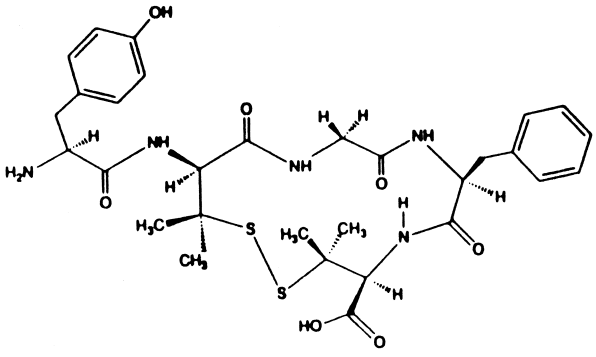
where R is the interfacial resistance. Its dimension is that of a reciprocal permeation coefficient, and both diffusion and partition coefficients are functions of position in the membrane, x. This relationship indicates that transmembrane drug delivery can be enhanced by: (i) increasing water–membrane partitioning, (ii) increasing the diffusion constant within a membrane interior, and/or (iii) controlling the interfacial resistance of the membrane. Previously, we have focused on (i), increased peptide partitioning, as a tool to increase permeability [18,19]. The partition coefficient is dependent on the free energy change upon the water-to-membrane transfer, and therefore can be related to the thermodynamics of peptide–solvent and intramolecular interaction, characteristics that appear to be controllable by an appropriate molecular design.

In this study we analyze how the surface charge, contributing to the interfacial resistance (iii) affects the transmembrane flow of two classes of enkephalin analogues. These peptides represent different approaches in the design of biologically active peptides: the biphalins, which are flexible dimeric peptides, that are among the most potent analgesic enkephalin analogues [20–22]; and the conformationally constrained cyclic enkephalin analogues, the c[D-Pen^2,D-Pen^5]enkephalins (DPDPEs) [23,24]. Both peptides and their analogues were previously studied in this laboratory [18,19,25,26]. By comparative analysis of the parent peptides, along with their modifications, we are able to infer specific mechanisms for peptide permeation across phospholipid bilayers.

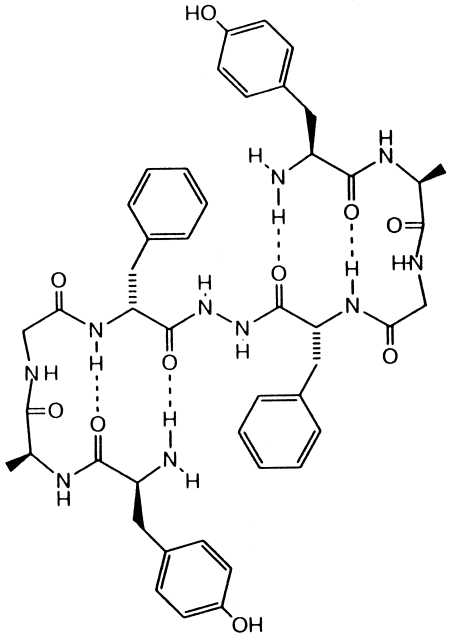
2. Materials and methods

The following lipids: 1,2-dipalmitoylphosphatidylcholine, 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE), 1-palmitoyl-2-oleoylphosphatidylserine (POPS), and cholesterol were obtained from Avanti Polar Lipids and used to prepare lipid bilayers. HEPES and HEPPS were purchased from Calbiochem, sodium azide and sodium chloride from Aldrich, cholesterol and Triton TX-100 from Sigma. Degassed HEPES buffer (10 mM HEPES, 150 mM sodium chloride, 1 mM sodium azide, 0.1 mM EDTA, pH 7.4) or HEPPS buffer (10 mM HEPPS, 150 mM sodium chloride, 1 mM sodium azide, 0.1 mM EDTA, pH 8.9) was used throughout the experiments. Dialysis membranes were purchased from Spectrum, Houston, TX, USA. Liposomes were

prepared from 20 mg of a dried mixture of lipids and cholesterol hydrated in 1 ml of buffer and vigorously stirred to form homogeneous dispersion. The dispersion was frozen and thawed 10 times to form extended bilayers, which were then extruded at 45°C through Nuclepore filters with a pore size of 200 nm [27]. Predominantly unilamellar liposomes of uniform size and narrow polydispersity were formed as shown by quasi-elastic light scattering (QELS) and supported by negative stain electron microscopy [25].



[D-Pen², D-Pen⁵]-enkephalin (DPDPE)



Biphalin in a folded structure

2.1. Permeability assay

Liposomes for this assay were prepared in a HEPPS buffer (pH 8.9), that contained the peptides. These circumstances impart a negative surface charge to POPC, POPE and cholesterol liposomes that minimizes liposome aggregation at high lipid concentrations when the chromatography is initiated. Liposomes, with trapped peptides, were separated from untrapped peptides by size exclusion chromatography on a Sephacryl S-300 column. A 2 ml fraction of liposome suspension was placed in a dialysis membrane bag, and the pH was adjusted to 7.4. The pH gradient across the lipid bilayer relaxes several orders of magnitude faster than the peptide permeation [19,25]. The dialysis was performed at 25°C as described by Romanowski et al. [19]. The amount of peptide molecules released from the vesicles into the dialysate was determined by a fluorescamine assay [28]. The permeability data were analyzed by the procedure of Johnson and Bangham [29] which yields the following equation:

$$\ln\left[\frac{NV_{out}}{V_{in}+V_{out}}-N(t)\right]=\ln\frac{NV_{out}}{V_{in}+V_{out}}-k\frac{V_{out}+V_{in}}{V_{out}}t \quad (3)$$

where $N(t)$ is the number of peptide molecules in dialysate at a given time, N is the number of peptide molecules in liposomes at time = 0, V_{out} is the volume of the dialysate, V_{in} is the volume of the liposome suspension contained in the dialysis bag and, finally, $k=(A/V)P$, where A is the surface area of the inside liposomes, V is the internal volume of the liposomes and P is the permeability coefficient. The number of peptide molecules initially trapped in the liposomes was estimated by the following procedure. The liposomes were sized by dynamic light scattering [30]. The apparent liposome diameter with entrapped peptides varied between 146 and 155 nm. The lipid content was determined by the ammonium ferrothiocyanate assay [31]. The total liposome volume was calculated from the average liposome diameter together with the total lipid content. Finally, the peptide concentration inside the liposomes was assumed to be equal to the initial concentration of peptides in the lipid suspension before extrusion, thereby giving the trapped amount of peptide, N. Eq. 3 allows for calculation of the permeability coefficient, P, by the method of least squares. The above method is not corrected for any peptide that resides in the lipid bilayer phase, the magnitude of this error is similar

or less than the uncertainties cited in Table 1, as discussed previously [19].

2.2. Equilibrium dialysis

We have developed an equilibrium dialysis assay to determine partitioning of the opioid peptides between the aqueous phase and model membranes [19]. This experimental method also allows for determination of thermodynamic characteristics of the partitioning process, such as van't Hoff enthalpy and entropy of peptide transfer. Each dialysis cell is separated by a cellulose dialysis membrane, average MW cut off 12 000–14 000. One milliliter of ca. 0.1 mM peptide solution in buffer is placed on the *cis* side of the membrane, whereas 1 ml of a liposome suspension in the same buffer is placed on the *trans* side. Liposomes for this assay were prepared in HEPES buffer (pH 7.4), without peptides. Dialysis is performed overnight in a water bath, using five cells mounted in a rotor that ensures homogeneity of peptide and lipid distribution in the dialysis cells. In the experiments performed, the lipid concentration was varied from 0 to 40 mM. Concentrations of peptides on the *cis* side (therefore unbound) are determined by measurements of the intrinsic peptide fluorescence (all peptides under study contain tyrosine). Data are analyzed using a non-saturable partitioning model, which yields the partition coefficient:

$$\frac{I_0}{I} = \frac{K[\mathrm{L}]}{2[\mathrm{W}]} + 1 \quad (4)$$

where I is the intensity of fluorescence, I_0 is the intensity of fluorescence in the cell with no lipids on the *trans* side, [L] is the concentration of lipids, [W] is the concentration of water, and K is the partition coefficient. The described method actually measures the water–membrane partition coefficient, where the membrane phase includes both the non-polar interior and any possible associations of the solute with the lipid headgroups.

3. Results

The lipids used in this study to prepare liposomes were either a mixture of POPC, POPE and cholesterol in the molar ratio 65:25:10, or the same lipids plus POPS as indicated in Tables 1 and 2. The compositions approximate those of neutral or anionic lipid membranes, respectively. The published phase diagrams of PC–cholesterol mixtures indicate that the lipid bilayer is in the liquid disordered phase when the cholesterol content is between 10 and 20 mol% and the temperature is at least 15°C above the main phase transition temperature (T_m) of the phospholipid [32,33]. Since the T_m values for POPC and POPE are below or near room temperature, respectively [34], and there is no evidence of a phase transition in the POPC:POPE:cholesterol mixture up to 60°C, it appears reasonable that the model membranes used in these studies exist in the liquid disordered phase at the experimental temperature. POPS lipid was chosen to introduce the negative charge to the model membrane. It has been shown that binding of short (up to five amino acid residues) basic peptides does not exhibit specificity between PS and PG lipids [8].

The permeability of the bilayer membranes to various solutes may be measured by dialysis of the liposome-entrapped solute provided the rate of permeation through the dialysis bag, which in this case was

Table 1
Permeability of selected peptides across neutral and anionic membranes

Peptide	Permeability coefficient (10^{-12} cm/s)	
	0% POPS	10% POPS
DPDPE(SH)$_2$	3.24 ± 0.39	2.88 ± 0.56
[D-Phe^3]DPDPE	2.91 ± 0.38	2.40 ± 0.44
DPDPE	0.38 ± 0.11	0.30 ± 0.08
[*p*-F-Phe$^{4,4'}$]Biphalin	2.58 ± 0.67	1.20 ± 0.23
[(2*S*,3*R*)β-Me-Phe$^{4,4'}$]Biphalin	1.63 ± 0.15	0.50 ± 0.06
Biphalin	0.68 ± 0.15	0.21 ± 0.19

Permeability coefficients determined using POPC:POPE:cholesterol liposomes, with or without addition of anionic POPS.

Table 2
Water–membrane partition coefficient of DPDPE analogues

Peptide	Partition coefficient, K		Ratio $K_{10\%}/K_{0\%}$
	0% POPS	10% POPS	
[L-Trp^6]DPDPE(SH)$_2$	1240 ± 100	820 ± 70	0.66
[L-Trp^6]DPDPE	800 ± 80	580 ± 60	0.73
DPDPE(SH)$_2$	780 ± 70	380 ± 50	0.48
DPDPE	155 ± 10	< 100	< 0.67
[*p*-F-Phe$^{4,4'}$]Biphalin	2190 ± 180	3610 ± 200	1.65
Biphalin	860 ± 80	1350 ± 150	1.56

Partition coefficients determined using POPC:POPE:cholesterol liposomes, with or without addition of anionic POPS.

ca. 10^{-4} cm s^{-1}, is much larger than the rate through the bilayer. The peptide concentration in the buffer was assayed by the rapid reaction of the peptide amino group with fluorescamine to yield highly fluorescent derivatives (excitation 380 nm, emission 475 nm). The partition coefficient was determined by equilibrium dialysis, using the intrinsic fluorescence of peptides, due to tyrosine (excitation 275 nm, emission 305 nm) or tryptophan (excitation 285 nm, emission 340 nm). This method allows for determination of partition coefficients of ca. 100 or greater, which is similar to the limit of other methods based on equilibrium dialysis [4]. The apparent partition coefficient, as determined in these experiments, does not differentiate between peptides that penetrate into the membrane interior and those that merely adsorb at the membrane surface.

3.1. Peptide structures

The permeability coefficients of selected peptides are listed in Table 1. Along with the parent compounds, DPDPE and biphalin, the other peptides represent different modifications known to increase permeability across neutral membranes. Those modifications include more hydrophobic side chains (Gly^3–Phe^3 replacement in DPDPE, [Phe^3]DPDPE); methylation of Phe$^{4,4'}$ in biphalin ([(2*S*,3*R*)β-Me-Phe$^{4,4'}$]-biphalin), halogenation of Phe$^{4,4'}$ in biphalin, and removing conformational restrictions in DPDPE by reducing the D-Pen^2,D-Pen^5 disulfide bridge (DPDPE(SH)$_2$). These modifications also increase the peptide permeability across anionic membranes; however, the permeability coefficients of biphalins and DPDPEs decrease when the anionic lipid is added (Table 1). This change is more significant for the dicationic biphalins, where the addition of 10% of POPS reduces the permeability coefficients to less than 50% of its initial value, whereas in the zwitterionic DPDPEs the analogous change is much less. In the latter case the decrease may not be statistically different than the permeability of a neutral membrane.

The magnitude of the change is similar for each peptide within a class, i.e. DPDPEs or biphalins. Thus the permeability of the DPDPEs is decreased to a similar extent whether the peptide is cyclic or acyclic. The addition of the tryptophan residue in position 6 increases the partition coefficient of DPDPE and its reduced acyclic form, as expected

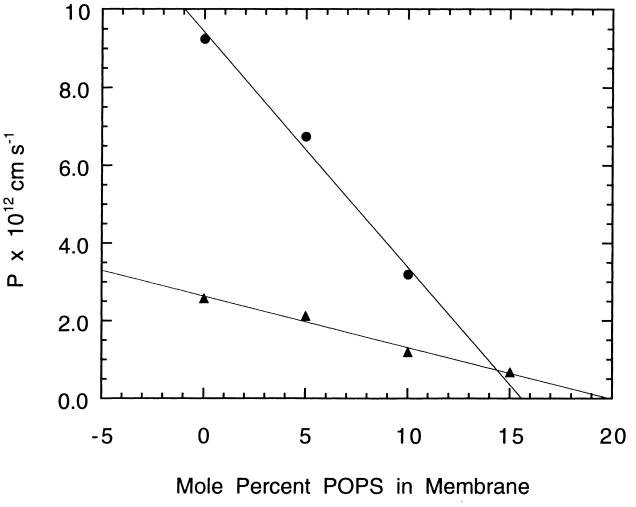


Fig. 1. Effect of increasing anionic content on the membrane permeability of [(2*S*,3*R*)2′β-diMe-Tyr$^{4,4'}$]biphalin (●) and [*p*-F-Phe$^{4,4'}$]biphalin (▲). Permeability coefficients, *P*, were determined using POPC:POPE:cholesterol liposomes and the anionic content was controlled by addition of POPS, 0–15 mol%. 10 mol% POPS corresponds to approximately 0.025 C/m^2.

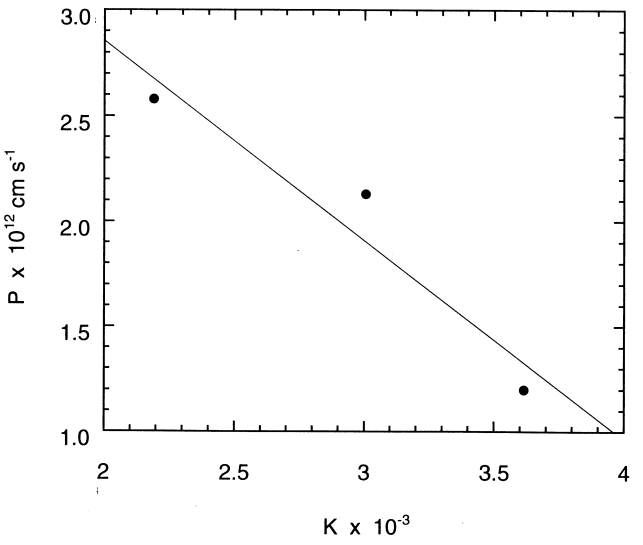


Fig. 2. Comparison of the partition coefficient, K, and the permeability coefficient, P, as the anionic lipid (POPS) content is increased from 0, 5, to 10 mol%. Both partition and permeability coefficients were determined for p-F-Phe$^{4,4'}$-biphalin with POPC:POPE:cholesterol liposomes, with the addition of POPS to control the anionic content of the membrane.

from the hydrophobic character of tryptophan (Table 2). The water–membrane partition coefficient of DPDPE and analogues is lower for the anionic model membrane than for the neutral one (Table 2), indicating that fewer peptide molecules enter the negatively charged membranes. This observation suggests an explanation for the diminished permeability of DPDPEs across the membrane. In contrast to the zwitterionic DPDPEs, the partition coefficient of the dicationic biphalins is greater for anionic rather than neutral membranes. This is consistent with the postulated electrostatic nature of peptide–interface interaction. Here the electrostatic attraction forces adsorb biphalin at the membrane surface. However, the electrostatic attraction does not increase the transmembrane flux of the dicationic peptides.

To further analyze the contribution of interfacial interactions to the overall permeation process we analyzed the permeability coefficients of [p-F-Phe$^{4,4'}$]biphalin and [(2S,3R)2′β-diMe]Tyr$^{4,4'}$-biphalin as a function of the fraction anionic lipid, POPS, in the bilayer membranes. The permeability coefficient of these peptides decreases with added anionic lipids (Fig. 1), linear correlation coefficients being −0.9908 and −0.9951, respectively. A similar type of analysis shows that the partition coefficient [p-F-Phe$^{4,4'}$]biphalin increased as the fraction of anionic lipid increased (Fig. 2), linear correlation coefficient = 0.9961. These results suggest that the negative surface charge of the model membrane reduces the transmembrane flux of the dicationic peptides by increasing the population of electrostatically adsorbed peptides.

4. Discussion

Our efforts to date have concentrated on the modifications intended to affect the population of analogues within a membrane, treated as a homogeneous medium, with the implied assumption that this is the rate-limiting process of the overall transmembrane transport. Introducing a membrane surface charge, that can be controlled by the fraction of anionic phospholipids such as POPS, can provide insights into the nature of membrane–water interfacial barrier and help to determine the limiting step of the transmembrane movement of selected peptides. Understanding the rate-limiting step of transmembrane transport is very important for the future design of therapeutic peptides. Peptide flux limited by the membrane interior can be enhanced by, for example, increased hydrophobicity of this analog, whereas flux limited by the interfacial barrier may require modification of a cation/anion equilibrium and distribution within the peptide. We previously demonstrated that the conformational flexibility of a ligand promotes its transfer across the membrane [19,25]. Our data show that permeability of different peptide classes require different bases of comparison related to the hydrophobicity of the amino acids [18]. The analysis of peptide permeability across anionic model membranes, as shown here, demonstrates the importance of peptide–membrane electrostatic interactions for design of permeable therapeutic agents.

A qualitative analysis of electrostatic interactions between peptide and membrane can be described by means of the electrostatic potential. Following the Gouy–Chapman approximation [6,7,11] the surface potential of uniformly charged membrane surface is given by:

$$\psi_0 = \frac{\sigma\lambda}{\varepsilon\varepsilon_0} \quad (5)$$

where σ is the surface charge density and λ is the Debye screening length. The spatial profile of the electrostatic potential can be then computed within the framework of the Gouy–Chapman approximation as follows:

$$\psi(x) = \psi_0 \exp(-x/\lambda) \quad (6)$$

where x is the distance from the membrane surface. The local concentration $[C(x)]$ of any Z-valent ion present in the aqueous phase can be calculated using the Boltzmann equation, provided the electric potential $\psi(x)$ is known.

The anionic lipid mixture used in the experiments that contain 10 mol% of negatively charged lipids corresponds to a surface charge density $\sigma = -0.025$ C/m^2 = 641^{-1} e/Å^2. The Debye screening length λ at the room temperature was estimated following Cevc [11]: $\lambda = 0.304/Z[C]^{1/2} = 0.785$ nm, where $[C] = 150$ mM is the concentration of monovalent ($Z = 1$) NaCl in buffer. According to Eq. 6, the surface potential of the uniformly charged liposome equals −26.4 mV.

The experimental data clearly shows a very different effect of the membrane surface charge on the partitioning of DPDPEs and biphalins. The partition coefficient of biphalin increases, whereas that of DPDPE decreases with addition of negatively charged lipids to the membrane, since at pH 7.4 these peptides show the opposite ionic character. Qualitatively, biphalin carries a positive charge whereas DPDPE is negative. Biphalin contains two amino termini due to tyrosines, with typical pK_a for deprotonation of -NH_3^+ of 9. At pH 7.4 the overall character of biphalin is essentially dicationic ($Z = +2$) due to the two amino termini. DPDPE contains two ionizable groups, the amino terminus of tyrosine in position 1, and the carboxyl terminus of penicillamine in position 5. The approximate pK_a for deprotonation of -COOH is 1.8. It appears that the anionic character of DPDPE can be explained by the fact that the average p$K_a = 5.5$ is below the pH of the experiments.

In our experiments the concentration of the peptides was low (ca. 0.1 mM) compared to the concentration of NaCl in buffer (150 mM). Therefore the spatial profile of the electrostatic potential within the Gouy–Chapman approximation (Eq. 6) can be reliably computed using the NaCl concentration alone. The concentration of peptides as a function of the peptide valency, $Z = -1$, 0, +2, was calculated according to the Boltzmann distribution. Under the conditions used in the experiments (buffer containing 150 mM NaCl and liposomes containing 10% negatively charged lipids) this analysis predicts that at 1 nm from the membrane surface the concentration of dicationic peptide ($Z = 2$) such as biphalin, is 1.89 times greater than that of a neutral compound. On the other hand, the concentration of an anionic peptide ($Z = -1$) is 0.73 that of a neutral compound.

The data presented in Table 2 indicates that the partition coefficient of biphalin increases by a factor of ca. 1.6 when going from neutral to negatively charged (10 mol% PS) membranes. The partition coefficient of DPDPEs (except the acyclic analog) is reduced by a factor of 0.7. Both of these observations are in good agreement with the predicted values. The water–membrane partition coefficient is independent of the peptide concentration in the bulk phase. However, a local change of the peptide concentration at the membrane surface induced by the electrostatic interaction will affect the apparent partition coefficient measured in the bulk phases. Let $[P_{mem}] = K_0[P_{face}]$ define the intrinsic partition coefficient K_0, where $[P_{mem}]$ and $[P_{face}]$ are molar concentrations of peptides in the membrane and in the aqueous phase at the arbitrarily defined interfacial distance δ. The measured (bulk phase) partition coefficient is equal to:

$$K = [P_{mem}]/[P_\infty] = K_0[P_{face}]/[P_\infty] = K_0 \exp(-ZF\psi(\delta)/RT) \quad (7)$$

where the last equation combines the Boltzmann equation and Gouy–Chapman approximation, $[P_{face}] = [P_\infty]\exp(ZF\psi(\delta)/RT)$ and $K_0 = K$ in the absence of the electric field. Therefore the experimentally observed changes of the partition coefficient are directly proportional to the changes of the peptide concentration at the interfacial distance from the membrane. This observation suggests a simple empirical relation between ionic character of peptide,

expressed by its valency Z, and the peptide partition coefficient. However, an experimental data analysis by the method of best fit to the above expression led to the conclusion that the effective valency Z is lower than that deduced from the structure of the solute [2]. This appears to be a common observation for ionic peptides [35].

The proton concentration within the vicinity of a negatively charged membrane surface is higher than bulk solution. Qualitatively, this effect shifts the anion/cation equilibrium at the membrane surface toward more positively charged species, as compared to those present in the bulk phase. This effect however is partially screened by the salt present in water and by the distance from the membrane surface. At the surface of a liposome that contains 10 mol% negatively charged lipids and immersed in a 150 mM solution of monovalent salt, the pH value would be 0.45 less than bulk pH. The same effect at 1 nm from that membrane surface lowers the pH by 0.12, compared to the bulk value, which does not seem to be a significant change.

We have discussed how the anionic character of membranes may influence the local concentration of peptides and how in turn this concentration controls the partition coefficient. Finally, we shall examine the model of diffusion controlled membrane permeability, Eq. 2. Previously we analyzed the relation between the partition coefficient and permeability coefficient of a series of enkephalin analogues interacting with a neutral model membrane [18,19]. We found good correlation between these quantities, consistent with the model given by Eq. 2. However, introducing the electrostatic charge results in changes of the permeability coefficient that seem to be related to the diffusion across the interfacial barrier rather than partition coefficient itself.

As pointed out by Diamond and Katz [12], permeation in biological membranes depends on equilibrium solute partition, solute mobilities in the membrane interior and interfacial rate processes. The permeation of DPDPE across neutral and anionic membranes seems to be predominantly controlled by the partition coefficient (Tables 1 and 2). In the case of biphalin, the permeability decreases while the electrostatic attraction increases the number of peptides associated with membrane, as evident from the comparison of partition and permeability data (Tables 1 and 2). We assume that the electrostatically controlled layer is characterized by a relatively slow diffusion rate creating a kinetic barrier for the transmembrane movement, therefore preventing these molecules from penetrating and crossing the membrane. Another possibility is that the addition of the anionic lipids changes the organization of lipid molecules within the model membrane, rendering the membrane less permeable for any type of solute, anionic, cationic, or neutral. It has been shown however that lateral packing density and molecular area of charged and uncharged lipids in the fluid state are essentially the same [36], suggesting that organization of lipid molecules remains similar in anionic and neutral model membranes.

In conclusion, the strong correlation between the ionic character of peptides, the anionic lipid content of the model membrane, and the observed partition coefficients suggest that electrostatic interactions control the water–membrane partition of the enkephalin peptides by electrostatic repulsion or attraction forces. However, the addition of anionic lipids to the membrane composition decreases the permeability of the peptides, both zwitterionic and dicationic. This observation suggests that the electrostatic attraction of the dicationic peptide at the water–membrane interface reduces the transmembrane flux of such peptides by creating a kinetic barrier. The observed effects indicate that the anionic lipids naturally present in cell membranes may reduce the transport of some peptide drugs across the membrane. Our results suggest that by controlling the isoelectric point of the peptide one can partially overcome this difficulty. The effect of the negatively charged membrane surface is much smaller for zwitterionic peptides (such as DPDPE) than for dicationic ones (biphalins). This observation explains why the span between biphalin and DPDPE permeability coefficients, obtained with neutral liposomes, diminishes in experiments in vitro, using bovine brain microvessel endothelial cell systems [37].

Acknowledgements

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