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Association of the wasp venom peptide mastoparan with electrically neutral lipid vesicles

Salt effects on partitioning and conformational state

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We have measured circular dichroism signals of aqueous mastoparan and mastoparan-X when titrated with electrically neutral phospholipid unilamellar vesicles. The data could be converted into association isotherms (binding curves) under various conditions of salt content. In spite of the absence of a net charge in the lipid moiety, substantial salt effects have been observed regarding the partition coefficient of the peptide and its conformation in the associated state. These results are discussed on the basis of a general thermodynamic approach for peptide association with lipid bilayers.

Mastoparan; Peptide–lipid interaction; Binding structure; Salt effect

1. INTRODUCTION

Mastoparan is an amphipathic peptide of 14 amino acid residues having the sequence, Ile-Asn-Leu-Lys-Ala[5]-Leu-Ala-Ala-Leu-Ala[10]-Lys-Lys-Ile-Leu-NH_2 (M_r 1,480). It makes up the major constituent of wasp venom (*Vespula lewisii*) and has been found to cause degranulation of mast cells [1]. The N-terminal part up to position 10 is largely hydrophobic, whereas the C-terminal end exhibits a highly polar nature due to its two Lys residues. In this respect the molecule displays distinct similarities with melittin, a well-known membrane-modifying peptide from bee venom composed of 26 residues [2]. Mastoparan also binds to phospholipid bilayers, generating a substantial extent of helical conformation [3]. Furthermore, channel formation in planar lipid membranes has been reported [4], a phenomenon also observed with melittin [5,6]. However, in contrast to the latter peptide, an α-helical mastoparan molecule is not long enough to span a phospholipid bilayer. This point naturally poses questions regarding the pertinent pore architecture when the currently favored channel model, involving a bundle of individual peptide helices, is considered [7].

A basic issue in the quantitative analysis of any effect caused by membrane-active agents concerns the partitioning of such a substance between the lipid and external aqueous media. Certainly the possible efficiency of action must be related to the amount of agent actually associating with the membrane. In our laboratory we have for some time investigated relevant theoretical and experimental problems. Most recently we could link melittin-induced pore formation kinetics to the concentration of membrane-bound peptide, thus getting some useful insights into the underlying molecular mechanism [8].

In the present article we report results related to the partitioning equilibrium of mastoparan in a buffer solution/lipid vesicle system. The phospholipid used, namely pure dioleoylphosphatidylcholine (DOPC), is electrically neutral so that no direct electrostatic interaction with the positively charged peptide occurs. Nevertheless, we have observed rather pronounced salt effects.

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2. MATERIALS AND METHODS

2.1. *Peptides*

Mastoparan (MP) was supplied by Bachem Feinchemikalien (Bubendorf BL, Switzerland). In addition we obtained an analogous peptide, Mastoparan-X (MPX), from Peninsula (St. Helens, Merseyside, UK). MPX is a constituent of the venom of another wasp (*Vespa xanthoptera*) involving 5 amino acid exchanges compared with MP, namely Leu[3]→Trp, Ala[5]→Gly, Leu[6]→Ile, Leu[9]→Met, Ile[13]→Leu [9].

2.2. *Lipid vesicles*

The pure phospholipid, DOPC, was a product of Avanti Polar Lipids (Birmingham, AL, USA) and was used without further purification. Small unilamellar vesicles (SUV) were made by the sonification technique [10]. A lipid dispersion in Tris buffer (10 mM Tris-HCl, pH 7.4, 0.5 mM EDTA and a given NaCl concentration in the 0.0–0.4 M range) was irradiated for 40 min under a flow of nitrogen gas at a temperature of 10°C. Afterwards metallic debris from the titanium tip

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was removed by centrifugation. The so prepared stock solutions were immediately used for measurements or stored at 8–10°C. We determined the lipid concentration through phosphate analysis [11].

2.3. *Titration experiments*

A peptide of given concentration (in a range of about 3–10 μM) was titrated with vesicles at 20°C. Association with the lipid bilayer could be monitored by the change in circular dichroism (CD) that results from the induced increase of the secondary structure. The CD measurements were done using a Cary 61 instrument (Varian, UK). We have registered appropriate values of $[\Theta]_{222}$, i.e. the molar ellipticity per residue at a wavelength of 222 nm, which is characteristic for the helix content. The signal at 260 nm was taken as a reference in order to correct for possible shifts of the baseline.

3. RESULTS

With mastoparan (MP) the starting value of $[\Theta]_{222}$ (no vesicles added) was found to be $[\Theta]^{\circ}_{222} = -7{,}410$ ($\pm$ 25) deg · cm^2/dmol, practically independent of the peptide concentration, c_P (up to 20 μM), and salt content. This is in contrast to the case of larger c_P and different buffer/salt conditions where appreciable CD changes have been observed, indicating increase of helix content even in an aqueous medium (possibly due to aggregation) [12].

In the course of our titration experiments we determined, as a basic measuring signal, the change of the negative molar ellipticity per residue, $F = -([\Theta]_{222} - [\Theta]^{\circ}_{222})$, taken at the respective ratio of the total lipid to peptide concentrations, c_L/c_P (after having corrected for the slight dilution effect when vesicle stock solution was added). From these data the appropriate association isotherms have been evaluated, namely the ratio of the actually associated peptide to total lipid, $r = c_{as}/c_L$, plotted vs. the concentration of the free aqueous peptide, c_f.

The evaluation procedure was applied and substantiated in previous work [13,14]. Here a brief review will be presented. First we note that generally the relationship

$$F = F_\infty \cdot r \cdot (c_L/c_P) \quad (1)$$

must hold true where F_∞ stands for the signal of the associated peptide projected to infinite binding affinity. Under simple circumstances (only one mode of association, for instance) this F_∞ would be a constant parameter of the system. At any rate, however, it can be shown that F_∞ as well as r, must be definite functions of c_f alone (provided equilibrium is established). The quantity, $Q = F \cdot (c_P/c_L)$, which can be directly calculated from the titration data, will accordingly also be determined by c_f, and vice versa, the set of variables, c_f, r and F_∞, must be the same for all different sets of c_P, c_L with an identical value of Q. The routine to be followed is illustrated in Fig. 1. Measured Q taken from three different titration experiments (involving diverse c_P) are plotted vs. c_L. The dashed horizontal line indicates a level of constant Q where the respective individual F_∞, r, c_f must be identical. The corresponding three sets of c_P, c_L at the

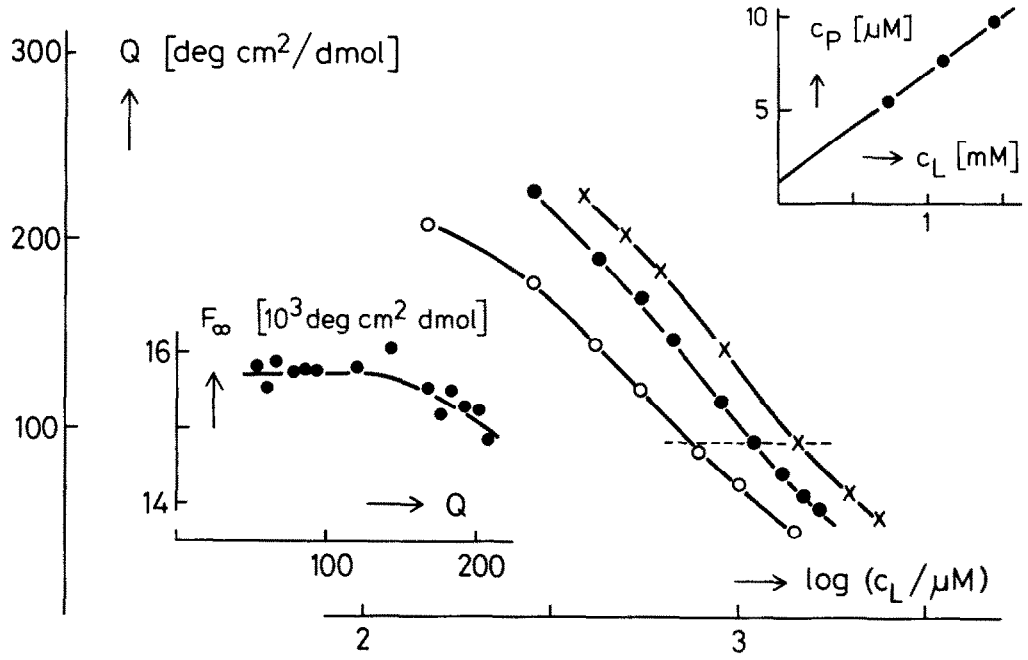

Fig. 1. Evaluation of association isotherm parameters from titration data F measured as relative change of negative molar ellipticity at 222 nm (see text). The experimental quantity, $Q = F \cdot (c_P/c_L)$ has been plotted vs. lipid concentration, c_L, for three different (initial) peptide concentrations, $c_P/\mu M$ = 6.25 (○), 9.26 (●), 10.93 (×) in the case of MP/DOPC with 0.1 M NaCl. The dashed horizontal line indicates points of constant Q where r (bound peptide per lipid) and c_f (concentration of free peptide) are constant. Then the respective c_P, c_L fall on a straight line (because of mass conservation, Eqn. 2) as demonstrated in the inset on the upper right. Its slope (= r) leads to F_∞ (value of F projected to full binding) by means of Eqn. 3. The resulting F_∞, as a function of Q, are plotted in the inset on the lower left.

intersection points necessarily have to satisfy the condition of mass conservation

$$c_P = r \cdot c_L + c_f \quad (2)$$

implying that c_P must fall on a straight line when plotted vs. c_L. This is demonstrated by the diagram in the inset on the upper right of Fig. 1. The proper values of r and c_f can apparently be taken from the slope and intercept, respectively. Once r is so obtained, F_∞ can be readily determined by means of the relationship

$$Q = F_\infty \cdot r \quad (3)$$

as derived from Eqn. 1. In the inset on the lower left of Fig. 1 we present values of F_∞ for a broader range of Q. They appear to remain largely constant for a lower extent of association ($r < 10^{-2}$) and tend to decrease when the associated peptide molecules approach each other more closely. The F_∞ at 'low Q' are listed in Table Ia for various NaCl concentrations, demonstrating a rather pronounced drop at higher salt content. Note that the relevant ellipticity of the associated peptide would be equal to $[\Theta]^{as}_{222} = [\Theta]^{o}_{222} - F_\infty$.

The apparent salt effect exerted on the association isotherms is shown in Fig. 2. The individual data points were calculated from the original Q, c_P and c_L, utilizing Eqns. 2 and 3 with the appropriate F_∞. The solid curves have been theoretically computed applying a model described in more detail elsewhere [14]. It considers the association process in terms of a thermodynamic partitioning equilibrium implying the basic relationship

$$r = (\Gamma/\alpha) \cdot c_f \quad (4a)$$

This involves a pertinent partition coefficient, Γ (a measure of the free energy of the peptide–lipid interaction) and an activity coefficient, α (reflecting peptide–peptide interaction in the associated state). This α can be mainly attributed to the electrostatic repulsion of the positive charges on neighboring peptide molecules. Assuming univalent ions in the aqueous electrolyte moiety, a Gouy–Chapman model approach then leads to the expression

$$\ln \alpha = 2\nu \cdot \sinh^{-1}(\nu b r) \quad (4b)$$

with ν standing for the effective number of elementary charges on the bound peptide monomer and b a parameter that is essentially determined by the ionic strength, I, of the bulk electrolyte [14]. In our present case we have $b = 3.54 \cdot \sqrt{M/I}$ (a contribution of the buffer to the ionic strength of the aqueous solution was estimated as 0.005 M). Eqns. 4a,b can be well fitted to the experimental association isotherms of Fig. 2, yielding sets of the parameters, Γ, ν as they are also specified in Table Ia. Some relevant results applying to MPX instead of MP are added in Table Ib. In case two or more associated modes exist, as we have reason to believe here (see section 4), the various quantities in Table I are actually averaged values.

Table I

Ellipticity parameters, partition coefficient and effective charge number of the associated peptide at various salt concentrations with ranges of uncertainty as indicated (buffer, 10 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 20°C)

	NaCl content (M)	F_∞; $[\Theta]^{as}_{222}$ (deg·cm²/dmol)[a]	Γ ($10^3 \cdot M^{-1}$)[b]	ν[c]
(a) MP/DOPC	0.0	22,600; −30,000	1.65	0.95
	0.02	19,300; −26,700	3.1	1.5
	0.1	15,700; −23,100	7.0	1.4
	0.4	12,800; −20,200	17	1.75
(b) MPX/DOPC	0.1	15,500; −19,100	13	1.2
	0.4	14,700; −19,000	27	1.2

In this case $[\Theta]^{o}_{222} = -3{,}625$ deg · cm²/dmol at 0.1 M NaCl, changing slightly to −4,300 deg · cm²/dmol at 0.4 M NaCl.

[a] ±5%, [b] ±10–20%, [c] ±15%.

4. DISCUSSION

Let us first consider the mastoparan (MP) case. In view of the modest value of $[\Theta]^{o}_{222}$, remaining unchanged when the salt content and concentration are varied, we gather that the peptide molecules in the aqueous medium are largely unordered and monomeric. On the other hand, the observed values of $[\Theta]^{as}_{222}$ reflect the existence of substantial helical order in the lipid associated state. Its extent evidently depends on the amount of NaCl added to the system. This may be estimated taking into account a value of −30,000 deg · cm²/dmol for an α-helix, and negligible contributions by β-sheet and random coil [15]. Then we obtain practically 100% helix at no salt content, decreasing to approximately 89, 77 and 67% when the NaCl concentration is raised to 0.02, 0.1 and 0.4 M, respectively. These results apply to a moderate degree of binding ('low Q'), above which some loss in the percentage of helical structure appears to occur. By all means we can conclude that there are association states differing with regard to secondary structure the stability of which depends on ionic strength.

Under such circumstances a salt effect of the measured partition coefficient can naturally be expected. In order to analyze it quantitatively we introduce pertinent expressions of the chemical potentials of the free and of one associated monomeric peptide [14]

$$\mu_f = \mu^\infty_f + RT \cdot \ln c_f \quad (5a)$$

$$\mu_{as} = \mu^\infty_{as} + RT \cdot \ln (\alpha r) \quad (5b)$$

This involves standard potentials, μ^∞_f, μ^∞_{as}, standing for the respective molar free energies of the peptide at infinite dilution (or, in other words, in the absence of peptide–peptide interaction). The condition of equilibrium, $\mu_f = \mu_{as}$, readily leads to the relationship

$$\Gamma = \exp\{-\Delta G^\Theta_{as}/RT\}, \text{ where } \Delta G^\Theta_{as} = \mu^\infty_{as} - \mu^\infty_f \quad (6)$$

Accordingly any effect on Γ must be attributed to

changes of the standard chemical potentials. Considering μ_f^∞, essential factors to be examined arise from the basic interactions between solute and solvent (i.e. water), the inherent electrostatic energies of the charges on the peptide molecule, and the work done in the process of setting up an ionic atmosphere owing to an added electrolyte. The two former contributions would be affected only upon conformational changes, which in the present case appear to be negligible. The third determinant, however, naturally, is sensitive to the given salt content. By all means, it will contribute a negative term to the free energy. A rigorous quantitative treatment is not attempted here since it would have to go far beyond the scope of the Debye–Hückel limiting law. Nevertheless we can at least argue that the effect of added salt causes a decrease of μ_f^∞, implying a drop in Γ. The observed increase of the partition coefficient therefore definitely suggests an even larger decrease of μ_{as}^∞. A discussion of the latter issue has to take into account the apparent existence of associated states with different structures. The experimentally registered value of Γ simply becomes $\Gamma = \Gamma_1+\Gamma_2+\ldots$, i.e. it sums up the individual partition coefficients related to the various possible states, $s(=1,2,\ldots)$. The actual distribution of these states as described by their molar fractions $x_s = \Gamma_s/\Gamma$, is determined by the magnitude of the particular terms, $\exp\{-\mu_{as}^\infty/RT\}$. In other words, the most favorable state has the lowest standard potential. With no extra salt, only the interactions with solvent (water and lipid), as well as an energetically advantageous arrangement of the discrete charges, will be of influence. Once more and more electrolyte is added to the system the μ_{as}^∞ will decrease because of the interactions with the ionic atmospheres. The resulting salt effect could doubtlessly exceed the analogous one in the aqueous surroundings, owing to a change of significant physical parameters, e.g. structural properties and a decrease of the dielectric constant close to the water–lipid interface. Then Γ will in fact be raised. Furthermore we emphasize the possibility that originally less likely states of association experience a comparatively larger decrease of free energy, and therefore become more favorable. Such a situation is certainly indicated by our present experimental data. The preferred state of associated MP in the absence of salt apparently has a maximum of helicity. Yet there must be at least another possible state of about 65% or less helical content which is promoted at higher ionic strength so that a conformational change of bound peptide takes place. Whether this transition involves more than two structurally defined states can, however, not be decided at the present stage.

The above considerations are of course generally applicable to similar peptide/lipid systems, particularly those specified in Table 1b. When instead of MP the analogous peptide MPX interacted with DOPC within the range of 0.1–0.4 M NaCl, the absolute magnitudes of Γ became slightly larger whereas the salt effect was practically the same as far as the relative change is concerned. Interestingly, however, the ellipticity of bound peptide remains practically unaltered suggesting no change of secondary structure.

So far we have focussed on the properties of associ-

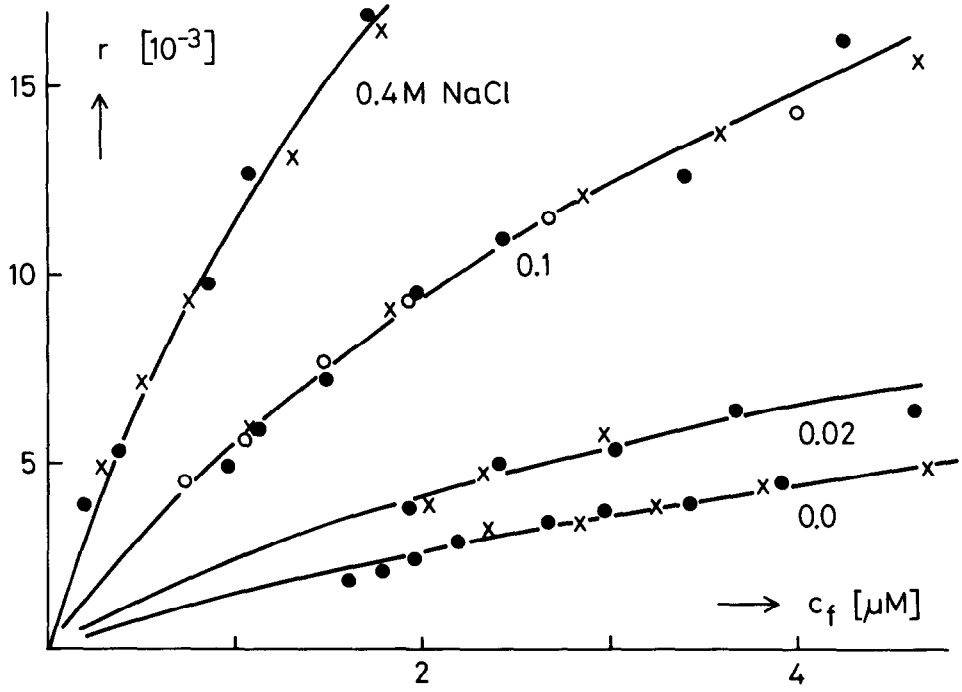

Fig. 2. Salt effect on association isotherms for the MP/DOPC system. Points calculated from titration experiments with different (initial) peptide concentrations are distinguished by different symbols (×, ●, ○).

ated peptide at lower concentrations. When the degree of binding is gradually increased, mutual electrostatic repulsion will eventually become effective, causing a continuous flattening out of the association isotherm which is rather well described by the activity coefficient formulated in Eqn. 4b. The apparent (effective) charge numbers, ν, listed in Table I, evidently fall well below the true physical charge number equal to a value somewhere between 3 and 4 at the given pH. This is indeed a well-known phenomenon reported previously by various authors in the case of melittin [14,16–19]. It appears to be caused by the fact that the involved charges are not evenly smeared out in the buffer/membrane interface as assumed by the Gouy–Chapman model. These charges are instead discretely distributed and presumably are some distance from that interface depending on the respective structural arrangements of the associated peptide molecule. Such conditions would provoke a reduction of the effective charge [16,18]. In addition, the finite size of the adsorbed molecules can substantially contribute to the effect [20]. Looking at the data of Table Ia we see a distinct increase of the effective charge when the salt content is raised, going parallel to a decrease of the ellipticity. This suggests that the underlying conformational changes imply a displacement of charges closer to the interface. On the other hand, we note that for MPX/DOPC a step from 0.1 to 0.4 M NaCl changes neither the ellipticity nor the effective charge, suggesting largely alike conformations in both cases. This does of course still allow changes of Γ, owing to the ionic atmosphere interactions, as pointed out above.

Let us finally summarize the principal conclusions of the present work. Mastoparan, a positively charged amphipathic peptide of only 14 amino acid residues, strongly associates with zwitterionic lipid vesicles, taking different structural states depending on the salt content in the buffer solvent. The partition coefficient (measuring the affinity of binding in the limit of high dilution) increases with the ionic strength. This indicates that the peptide charges interact more favorably with their ionic atmospheres when located close to the bilayer than they can do in purely aqueous surroundings.

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