# MELITTIN AND THE 8–26 FRAGMENT

## Differences in Ionophoric Properties as Measured by Monolayer Method

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ABSTRACT Melittin is a major (~50%) prtoein component of bee venom. This peptide is an amphiphilic protein, because, while the amino acid residues 1–20 are predominantly hydrophobic (with the exception of Lys-7), residues 21–26 are hydrophilic. The binding properties to vesicles and lipid bilayers of melittin have provided much useful information regarding biological (hemolytic) activity (Habermann, E., 1972, *Science* [*Wash. DC* ], 177:314–322). Recent studies have convincingly established that the melittin monolayer (at air-water interface) model membrane sytem allows one to analyze the various forces present in such structures. We present comparative monolayer studies of melittin and the peptide fragment 8–26 regarding the channel formation for the selective anion ($Cl^-$) penetration in monolayers, analogous to melittin (tetramer) channel function in lipid bilayer. The differences in surface pressure and surface potential of monolayers between native melittin and the 8–26 fragment suggest that these may be ascribed to Lys-7.

### INTRODUCTION

The interaction of melittin, a peptide with 26 amino acids, with bilayers and vesicles has been the subject of many current studies (1–10). The primary sequence of melittin shows some unusual characteristics (11) as discussed herein

(+)Gly-Ile-Gly-Ala-Val-Leu-Lys(+)-Val-Leu-Thr-Thr-Gly-
(10)

Leu-Pro-Ala-Leu-Ile-Ser-Trp-Ile-Lys(+)-Arg(+)-Lys(+)-
(15)

Arg(+)-Gln-Gln-$NH_2$.
(25)

Recently the molecular mechanism of melittin's action was reported from the changes in the electrical properties of planar lipid bilayers induced by melittin (7). From these studies it was concluded that the channels were formed by melittin tetramers. These channels were more permeable to anions ($Cl^-$) than to cations (7). Melittin is reported to form stable monolayers at the surface of water (12, 13, 14,). Because a monolayer is a very useful membrane model system, we report here the comparative studies of melittin and its 8–26 fragments. This is of interest since several studies report that while melittin causes rupture of lipid membranes, fragment 8–26 does not possess lytic properties (13, 15, 16). Here we report on the different physical properties of monolayers of melittin and 8–26 fragment in comparison with their biological activities.

### RESULTS AND DISCUSSION

Melittin and 8–26 fragment were found to form stable monolayers at the air-water interface. This is in accord with the literature data for melittin (12–14). It is also known from other biopolymers (proteins) monolayers that these molecules when carefully applied to the air-water interface give stable monolayers (i.e., very negligible loss to the bulk phase) (3, 17). Since the surface pressure ($\Pi$) vs. area per molecule ($A$) data were the same when using solid melittin or as dispersion in $CHCl_3$, we found added evidence that almost all of melittin added was present at the interface. If any appreciable desorption did take place, then these methods would have given varying results.

$\Pi$ vs. $A$ isotherms of both melittin and the 8–26 derivative are given in Fig. 1 *a*. The surface potential, $\Delta\psi$, data are also given in Fig. 1 *b* for the same monolayers as a function of added electrolyte concentration, KCl, in the aqueous subphase. The $\Pi$-$A$ and the $\Delta\psi$-$A$ data are quite different for the two peptides, both on subphase water and on 1 M KCl (at 25 °C).

The $\Pi$ of any monolayer is given as (3, 17)

$$\Pi = \Pi_{kin} + \Pi_{el} + \Pi_{coh}, \quad (1)$$

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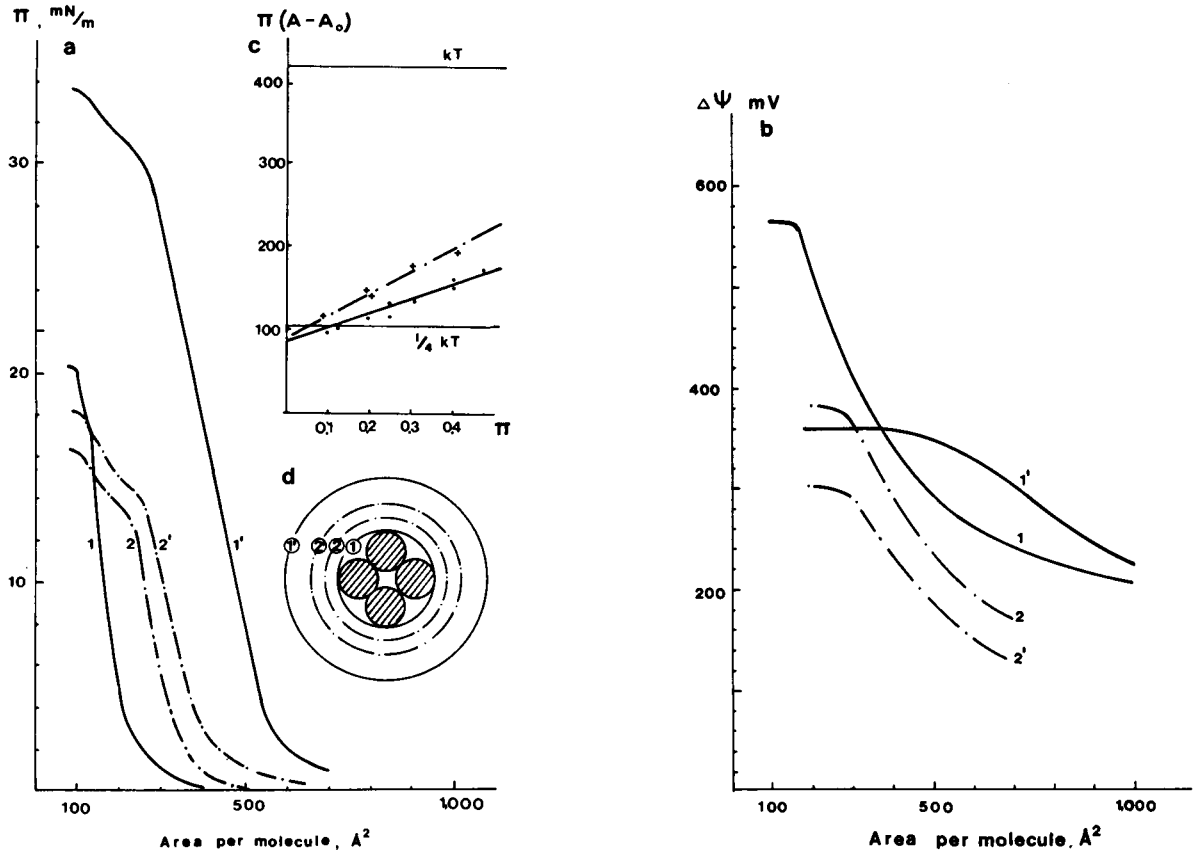


FIGURE 1 (*a*) Surface pressure (Π) vs. area per molecule (*A*) isotherms of melittin (1, 1') and 8–26 fragment (2, 2') on water (1, 2) and 1 M KCl (1', 2') subphase at 25°C. The monolayers were formed by applying the peptide to the surface in solid form (or as 170 μg/ml $CHCl_3$ dispersion [6]. (*b*) Surface potential ($\Delta\psi$) vs. area (*A*) isotherms of melittin (1, 1') and 8–26 fragment (2, 2') at 25°C as measured simultaneously with Π-*A* data under *a*. The experimental details have been given elsewhere (6). (*c*) $\Pi(A-A_0)$ vs. Π plots of melittin (—) and fragment 8–26 (---) on subphase water (at 25°C). The plots corresponding to values ($kT$) and ($kT/4$) are indicated. (*d*) Relative area per tetramer at a surface pressure equals 11 mN/m, for melittin on water (1) and 1 M KCl (1'); for 8–26 fragment (2, 2').

where $\Pi_{kin}$ arises from the kinetic forces, $\Pi_{el}$ is related to the electrostatic charge repulsion, and $\Pi_{coh}$ arises from the van der Waals' attraction forces between the alkyl parts of the monolayer forming molecules. For noncharged monolayers, at low surface concentration (where $\Pi_{coh}$ is negligible), the following equation is valid (3)

$$\Pi(A - A_0) = kT, \qquad (2)$$

where $A_0$ is the co-surface area correction and $k$ is the Boltzmann constant.

The melittin and 8–26 fragment monolayers gave plots of $\Pi(A - A_0)$ vs. Π (Fig. 1 *c*), where $(\Pi[A - A_0])_{\Pi\to 0} = kT/4$, which suggests that the peptides are present as neutral tetramer. This observation is in agreement with the lipid bilayer studies (8), where the formation of voltage-dependent ion-selective channels by melittin tetramers were suggested. Evidence for the tetramer state is also provided from the analyses of the area at the collapse state.

It is well established from monlayer studies of proteins and synthetic poly-amino acids (3, 17) that the value of area per amino acid for unfolded protein molecules is ~15 Å²/residue (equivalent to the data from x-ray diffraction results). If these peptides were completely unfolded and oriented flat on the surface, then the value of area per molecule at the collapse state would be ~400 Å² (i.e., 26 amino acids × 15 Å²/residue). Because much lower values of areas at the collapse state are actually observed (~90 Å²), this suggests that the peptides are indeed in the vertically oriented tetramer state (Fig. 1). The orientation where the polar end (20–26 residues) is situated inside water while the hydrophobic part is oriented away from the aqueous phase gives the lowest energy for the system. Very few current studies report where the surface potential, $\Delta\psi$, of melittin monolayers is measured (6). Our studies on various protein and peptide monolayers have clearly shown that Π and $\Delta\psi$ measurements provide useful information regarding molecular weight, number of charges, and molecular forces between molecules (van der Waals, electrostatic) (2–6, 17).

The limiting values of the $\Delta\psi$ for the native melittin and 8–26 fragment are 560 and 380 mV, respectively (Fig. 1 *b*). Because in monolayers these peptides differ in their orientation (i.e., dipoles along the vertically packed α-

helix) from other globular proteins (3), the large magnitudes of $\Delta\psi$ can be ascribed to this difference. Further, the peptides will be oriented parallel with the polar end (i.e., residues 21–26) inside the aqueous phase, and the predominantly apolar part will point away from the aqueous phase, such that the system arrives at a minimum surface energy. This packing arrangement is obviously different than in crystal structure (18), where antiparallel orientation is reported. Hence, the ratio of limiting $\Delta\psi$ on pure water is proportional to the number of amino acids in the peptide, thus 560/26 = 21 for melittin, while 380/19 = 20 for the 8–26 fragment.

The dynamical picture on the reaction of the native melittin tetramers and the 8–26 derivative on the addition of KCl to the subphase is given in Figs. 2 and 3 as a function of initial surface pressure in monolayers. The plots in Fig. 2 show that in the case of melittion the anion penetration phenomenon is independent of the initial value of $\Pi$, i.e., addition of KCl leads to an appreciable increase in the value of $\Pi$ with time (Fig. 2).

On the other hand, the $\Delta\psi$ data show a more complicated reaction mechanism. Initially an abrupt decrease in $\Delta\psi$ is observed, which is independent of the initial $\Pi$ value. If the value of $\Pi$ is high, then $\Delta\psi$ remains constant after reaching a steady state. However, if the value of $\Pi$ is low, ~5 mN/m (millinewton per meter), then a minimum in $\Delta\psi$ is observed, followed by an increase (Fig. 2). These differences in the shape of $\Delta\psi$-$\tau$ depend on the $\Pi$, which indicates a change in the conformation of melittin as $Cl^-$-ion penetration takes place in accordance with the $\Pi$-area isotherms in Fig. 1. The addition of KCl to the 8–26 fragment has much weaker effect on both $\Pi$ and $\Delta\psi$ (Fig. 3) in comparison with melittin monolayers (Fig. 2). Further, a recent study reported (8) that melittin in tetramer state in lipid bilayers exhibits stronger affinity to $Cl^-$ ion than to the larger anion $CH_3COO^-$. In Fig. 4 we give the plots of $\Pi$–$\tau$ for penetration of ions in melittin and 8–26 fragment monolayers. Here the rate of $CH_3COO^-$ penetration is lower than for $Cl^-$ anion for the melittin monolayer.

With the $\Delta\psi$ results we conclude that potential gradient exists both along the channels, in the case of native melittin tetramers, and as well in the case of tetramers as formed by the 8–26 derivative. The observation that the shortened

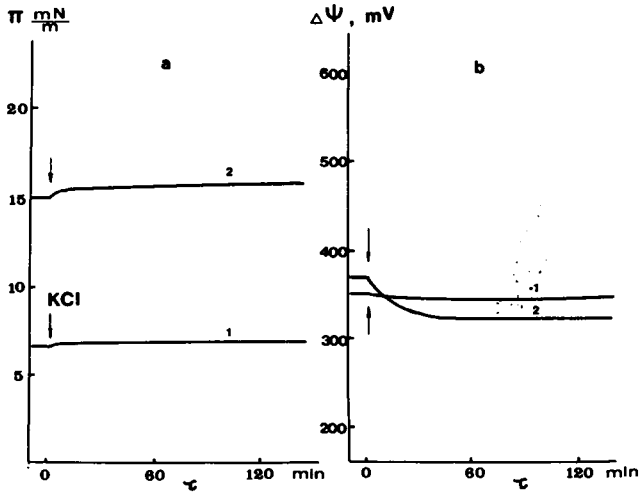


FIGURE 3 (*a*) Variation of $\Pi$ with time and (*b*) of $\Delta\psi$ with time of 8–26 fragment after the addition (at arrows) of KCl (0.125 M) to water as subphase (see details under Fig. 2) at 25°C.

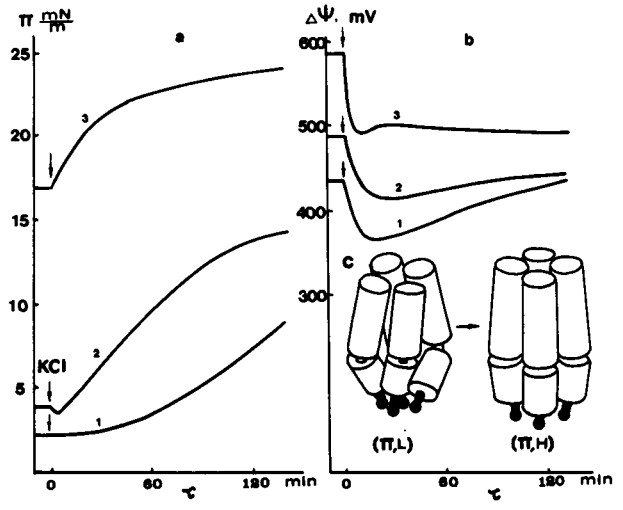


FIGURE 2 (*a*) Variation of $\Pi$ with time (minutes), and (*b*) of $\Delta\psi$ vs. time (minutes) for melittin monolayers at varying initial $\Pi$ values after the subphase concentration of KCl was changed (at arrows) from 0 to 0.125 M (by addition of the appropriate amount of KCl under stirring) at 25°C; (*c*) schematic drawings show the change in conformation of the tetramer when at low $\Pi$ ($\Pi$,*L*) or high $\Pi$($\Pi$,*H*).

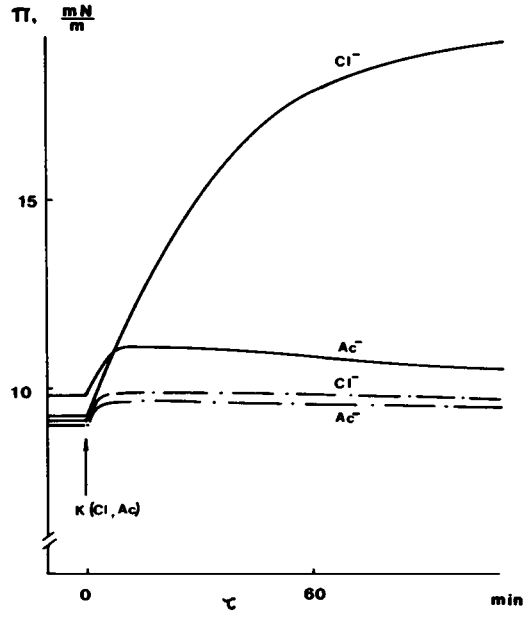


FIGURE 4 Variation of $\Pi$ (~10 mN/m) of monolayers of melittin (—) and 8–26 fragments (—·—) on the addition (at arrows) of KCl ($Cl^-$) and $CH_3COOK$ ($Ac^-$). Electrolyte concentration equals 0.125 M.

derivative exhibits a lower surface potential by a factor 1.4 provides evidence for this.

The $\Delta\psi$ data in Fig. 1 show additionally that when $Cl^-$ ion is present in the subphase, the limiting value of $\Delta\psi$ decreases both in the case of native melittin and 8–26 fragment. However, the degree of decrease in $\Delta\psi$ is much lower in the case of 8–26 fragment. These data are not easily explained at this stage regarding the differences in the shapes of the various isotherms (Fig. 1). However, if we note that two of the hydrophilic residues (e.g., Lys-7 and Gly-1) are absent in the 8–26 peptide from the $NH_2$ terminus, one may expect that the channels inside the tetramers will be more hydrophobic. As a result the ions from the subphase cannot easily penetrate these channels, which have now been deprived of the hydrophilic-ion fixation residue, Lys-7. This would be expected to give rise to a weaker repulsion force between the neighboring tetramers at the interface on the addition of the electrolyte $Cl^-$. These data suggest that for $Cl^-$-ion penetration to take place, the presence of Lys-7 is necessary in melittin, however, more studies are needed before this can be fully understood.

It may be safe to assume that when tetramers exist at low $\Pi$, both of the two $\alpha$-helical domains of melittin molecules are displaced relative to the vertical axis of tetramer at a given angle. This is based on the reported nonlinear structure of melittin (9, ,15, 18, 19). Recent studies suggest (20) that the $\alpha$-helix backbone possess large net dipole moment. The more linear conformation of the tetramer would most likely lead to an increase in $\Delta\psi$, if the dipole charges along the $\alpha$-helix dominate the screening effect due to the anion penetration inside the channel (Fig. 2 *c*). In the case of films with high $\Pi$ values, it may be safe to assume that the tetramers are already in a more linear state, such that the penetration of $Cl^-$ ion inside the channel leads to the decrease in the field inside these channels yielding a much simpler change in $\Pi$–$\tau$ and $\Delta\psi$–$\tau$ curves as also observed. The $\Pi$–$\tau$ and $\Delta\psi$–$\tau$ data for the fragment 8–26 suggest that the electrostatic charge repulsion is smaller than in melittin, independent of the initial value of $\Pi$ (Fig. 3) as expected from the isotherms in Fig. 1.

We conclude that independent of the initial surface pressure, small changes in $\Pi$ with the addition of salt to 8–26 fragment can be ascribed in this case with the negligible penetration of anions into tetramers. The differences in both the $\Pi$–$A$ and the $\Delta\psi$–$A$ isotherms suggest that tetramers of fragment 8–26 are in a different conformation as compared with the tetramers of native melittin. The pressure of KCl in the subphase does not cause much divergence in the shape of $\Pi$–$A$ isotherms for 8–26 fragment in comparison with native melittin. This suggests that the conformation in peptide 8–26 remains unchanged. On the other hand, the presence of KCl in the subphase causes an increase in area per molecule in the case of native melittin. This can be ascribed to $Cl^-$ penetration in the melittin tetramers, which become charged, and leads to a change in the peptide conformation.

In conclusion, these data of monolayers show that while melittin exhibits strong affinity for anions ($Cl^-$), 8–26 fragments show rather poor anion interaction. The data for different anion penetration (Fig. 4) are in accord with the melittin behavior in lipid bilayers (8) and thus provide additional support to the conclusions as described herein. It allows us to postulate that the absence of Lys-7 in 8–26 fragment most likely is the reason for the decrease in $Cl^-$-ion binding, and this is in agreement with the poor lytic activity of 8–26 fragment, as reported by other investigators (15).

The authors wish to thank Mrs. J. Klausen for her excellent technical help. V. S. Gerod gratefully acknowledges a "Danish State Scholarship," awarded during the period of September 1982 to June 1983.

*Received for publication 5 April 1983 and in final form 8 November 1983.*

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