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PHYSICOCHEMICAL STUDIES OF THE PROTEIN-LIPID INTERACTIONS IN MELITTIN-CONTAINING MICELLES

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Summary

Complexes of melittin with detergents and phospholipids have been characterized by fluorescence, circular dichroism, ultracentrifugation, quasi-elastic light scattering and ^{1}H nuclear magnetic resonance (NMR) experiments. By ultracentrifugation and quasi-elastic light-scattering measurements it is shown that melittin forms stoichiometrically well-defined complexes with dodecylphosphocholine micelles consisting of one melittin molecule and approximately forty detergent molecules. Evidence from fluorescence, circular dichroism and ^{1}H nuclear magnetic resonance experiments indicates that the conformation of melittin bound to micelles of various detergents or of diheptanoyl phosphatidylcholine is largely independent of the type of lipid and furthermore appears to be quite closely related to the conformation of melittin bound to phosphatidylcholine bilayers. ^{1}H NMR is used to investigate the conformation of micelle-bound melittin in more detail and to compare certain aspects of the melittin conformation in the micelles with the spatial structures of monomeric and self-aggregated tetrameric melittin in aqueous solution. The experience gained with this system demonstrates that high resolution NMR of complexes of membrane proteins with micelles provides a viable method for conformational studies of membrane proteins.

Introduction

Although the possible importance of lipid-protein interactions to the functioning of biological membranes has long been recognized and much discussed, detailed information regarding the nature of lipid influence on protein structure is largely lacking. Lipid influences on the activity of a variety of mem-

brane-bound enzymes have been demonstrated, but research into the structural basis of such influences has been confined mostly to the disturbances in lipid organization occasioned by membrane-bound proteins. A major reason for this situation is that methods for detailed conformational studies of membrane-bound proteins are largely lacking and consequently much work is yet to be done in this area.

The two techniques most widely applied to detailed conformational studies of water-soluble proteins, namely X-ray diffraction and high resolution NMR, have not as yet yielded comparable information for membrane-bound proteins. For both techniques, this arises because of the difficulty of obtaining membrane proteins in a form suitable for application of the method. Since obtaining three-dimensional crystals of membrane proteins in an environment similar to that of a membrane appears rather problematic, high resolution NMR may be a more promising approach to study the conformation and orientation of membrane proteins. In previous experiments [1—5] with native membranes and reconstituted systems consisting of proteins bound to phospholipid vesicles, protein ^{1}H NMR signals were not observed. One purpose of the present paper is therefore to demonstrate, using melittin as an example, that complexes with micellar lipid systems provide a suitable system for high resolution NMR studies of membrane proteins.

A second principal aspect of this paper is to investigate the structural basis for the biological functions of melittin. Melittin is a polypeptide of 26 amino acids which constitutes about 50% of the dry weight of bee venom. Its physiological function appears to be two-fold. Firstly, at sufficiently high concentrations melittin causes lysis of native and artificial lipid membranes [6—10]. Secondly, melittin was reported to be an activator for the phospholipase A found in bee venom and the available evidence suggests that this activation is a result of the influence of melittin on the organization of lipid bilayers [11—13]. The primary sequence of melittin is highly amphiphilic, which has led to the proposal that the physiological functions of melittin might be based on detergent-like properties [7,14]. This does not seem a sufficient description for several reasons. Firstly, there is no simple correlation between the surface activity and the lytic properties of melittin and various melittin derivatives [15, 16]. Secondly, measurements of the influence of melittin on the organization of lipid bilayers [17] and measurements of the activation of various phospholipases A by melittin [11,12] suggest each melittin molecule may influence up to several hundred lipid molecules. Finally, the ability of melittin and several melittin derivatives to activate phospholipase A or to effect hemolysis are not correlated [13]. It therefore seems pertinent to ask whether melittin has a defined conformation when bound to lipids and if so, how the influence of lipid on melittin conformation and conversely the influence of melittin on lipid organization may be related to the physiological effects of the peptide.

Materials and Methods

Chemicals used. Melittin was either purchased from ICN or purified from lyophilized bee venom (H. Mack, Illertissen, F.R.G.) according to the procedure of King et al. [18]. Residual phospholipase A activity was removed by the

method of Mollay et al. [11]. A sample of purified melittin was also obtained as a gift from Prof. M. Lazdunski. Decyl-, dodecyl- and tetradecyldimethylamine oxides were synthesized by the method of Herrmann [19]. Dodecyldimethylamine oxide was also purchased from Fluka. Dodecylphosphocholine was synthesized by the method of Hirt and Berchtold [20] as modified by Eibl et al. [21]. Dodecyl-(oxyethylene)$_{7,8}$-glycol was a gift from Dr. M. Chabre and L-α-diheptanoyl phosphatidylcholine a gift from Dr. A.J. Slotboom. L-α-Dilauroyl phosphatidylcholine was purchased from Fluka. All detergents and phospholipids were pure using thin-layer chromatography with several different solvent systems.

Ultracentrifugation and light scattering. Unless otherwise stated these measurements were all performed in $5 \cdot 10^{-2}$ M phosphate buffer, pH 7.0, with $2 \cdot 10^{-2}$ M dodecylphosphocholine and with or without $2 \cdot 10^{-4}$ M melittin. For dodecylphosphocholine, the critical micelle concentration of $1.1 \cdot 10^{-3}$ M at 22°C was determined by the method of Bonsen et al. [22] using Rhodamine 6G (Merck), and the partial specific volume, $\bar{\nu} = 0.937$ cm^3/g, was measured by the method of Tanford et al. [23]. The partial specific volume of melittin, $\bar{\nu} = 0.782$ cm^3/g, was calculated from the amino acid composition [24].

Ultracentrifugation experiments were performed on an MSE Mark 2 analytical ultracentrifuge. Sedimentation equilibrium experiments with detergent only were performed with double-sector cells using Schlieren optics. In mixed detergent/melittin solutions, the melittin concentration was followed by ultraviolet scanning at 280 nm. Underfill, synthetic-boundary cells and Schlieren optics were used for diffusion constant measurements and the data evaluated by the height-area method [25]. The zero-time shift was under 100 s. The boundary was between a solution with $2 \cdot 10^{-2}$ M dodecylphosphocholine and a second solution with $1 \cdot 10^{-3}$ M dodecylphosphocholine to avoid detergent concentrations below the critical micelle concentration. The measurements were performed at 21°C and the diffusion constant corrected to $D_{20^\circ C}$. Sedimentation equilibrium experiments for dodecylphosphocholine micelles were made at 20°C and evaluated by a plot of ln $[(1/r) \cdot (dC/dr)]$ versus r^2. Sedimentation equilibrium experiments for dodecylphosphocholine-melittin complexes were made at 20°C by the method of Reynolds and Tanford [26]. An apparent absorption of the reference detergent solution caused by the optical system hindered determination of the base-line, therefore the absorption of the melittin-dodecylphosphocholine complex was measured against both buffer and detergent without melittin in order to provide an estimate of the baseline error.

Quasi-elastic light-scattering measurements utilized an argon laser (Spectra Physics) at 514.5 nm and a 96 channel correlator (Malvon). The autocorrelation function was fit with a single exponential function [27–29]. For the light-scattering experiments and for ultracentrifugation experiments using Schlieren optics, the detergent to melittin ratio was chosen to provide sufficient detergent to bind all melittin, but to avoid large excesses of detergent micelles without bound polypeptide.

Fluorescence spectroscopy. Fluorescence measurements were performed at 25°C on an Aminco-Bowman SPF fluorometer which automatically corrected for non-linearities in the optical system. The excitation wavelength was 280

nm. 3 ml of a $2 \cdot 10^{-6}$ M melittin solution in 0.05 M phosphate buffer at pH 7.0 was titrated with the detergents by the addition of aliquots of 1—30 μl of concentrated detergent solution.

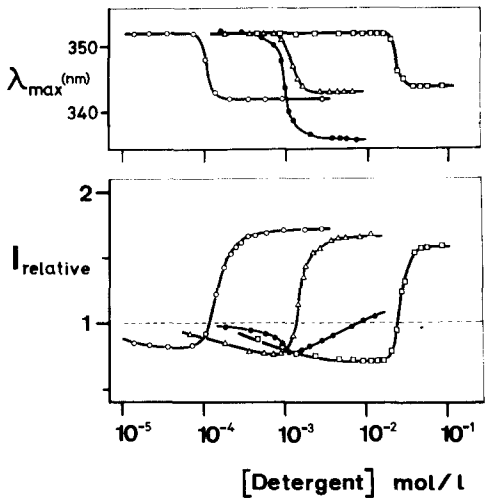
Circular dichroism spectroscopy. Circular dichroism measurements were performed at 25° C on a Jasco J-40AS instrument. Quartz cells with pathlengths between 0.01 and 1.0 cm were used to keep total absorbance below 2 units over the spectral region investigated. Melittin-micelle complexes were prepared by direct dissolution of melittin and lipid at the desired concentration and pH. No time-dependent variation of the circular dichroism spectra was observed. Melittin-liposome complexes were prepared by evaporation of a solution of melittin and lipid in chloroform to give a thin film which was subsequently lyophilized and taken up in water by mechanical shaking at 50° C. A homogeneous solution of melittin and lipid in chloroform was obtained by adding a minimal volume of melittin dissolved in dimethylsulfoxide to the lipid dissolved in chloroform. The approximate amount of α-helical content in the various melittin preparations was calculated from the molar ellipticity at 222 nm [30].

NMR spectroscopy. 360 MHz Fourier transform ^{1}H NMR spectra were recorded on a Bruker HX-360 instrument using internal ^2H_2O for the lock system. Chemical shifts are in parts per million (ppm) relative to the internal reference sodium 3-trimethylsilyl[2,2,3,3-^{2}H]propionate at p^{2}H 7.0 [31]. Spectral resolution was improved by multiplication of the free induction decay by a sine bell [32]. The NMR samples contained $1—4 \cdot 10^{-3}$ M melittin in ^2H_2O and were adjusted to the desired p^{2}H directly in the NMR tubes. Values of p^{2}H are pH meter readings uncorrected for isotope effects.

Results

Tryptophan-19 fluorescence of melittin in the presence of detergents

In order to test the interaction of melittin with various detergents, the relative intensity and emission maximum for fluorescence of the single tryptophan residue at position 19 of the melittin amino acid sequence have been measured as a function of detergent concentration (Fig. 1). For decyl-, dodecyl- and tetradecyldimethylamine oxides, minor changes in the relative intensity of the fluorescence emission of tryptophan-19 were observed below the critical micelle concentrations of these detergents. As discussed further below in connection with the NMR results, this probably reflects weak interaction of these detergents with aggregated melittin. Above the critical micelle concentrations, strong increases in the fluorescence intensity and an approximately 10 nm blue shift for the wavelength of the emission maximum were observed. Similar changes in the fluorescence properties of tryptophan-19 occurred in the presence of dodecylphosphocholine. These results indicate that below the critical micelle concentration of the detergent there are at most weak interactions between the detergent and melittin whereas in the concentration range where detergent micelles are formed, melittin binds to these micelles. Implicit in this interpretation is that melittin does not have strong binding sites for these detergents, but rather accommodates itself to a lipid-water interface. Similar changes in the fluorescence properties of tryptophan-19 have previously



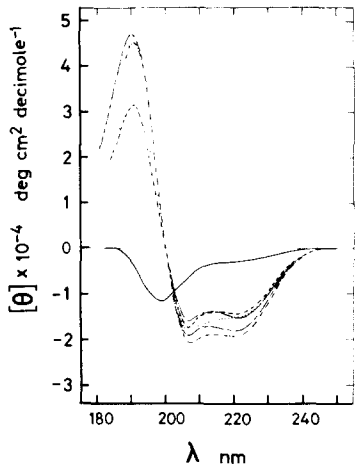


Fig. 1. Relative intensity and wavelength of maximum emission for the fluorescence of tryptophan-19 of melittin as a function of the concentration four different detergents. Measured with $2 \cdot 10^{-6}$ M melittin in 0.05 M phosphate buffer at pH 7.0 and 25°C. The excitation wavelength was 280 nm. □, decyldimethylamine oxide; △, dodecyldimethylamine oxide; ○, tetradecyldimethylamine oxide; ●, dodecylphosphocholine. The respective critical micelle concentrations are approx. $2 \cdot 10^{-2}$ M, $2 \cdot 10^{-3}$ M and $2 \cdot 10^{-4}$ M for the dimethylamine oxides [19] and approx. $1 \cdot 10^{-3}$ M for dodecylphosphocholine (see Materials and Methods).

Fig. 2. Circular dichroism spectra showing the mean residue molar ellipticities as a function of wavelength for melittin and melittin-lipid complexes at pH 7.0 and 25°C. ———, $5.8 \cdot 10^{-6}$ M melittin in the monomeric form in salt-free aqueous solution; · · · · · ·, $6.7 \cdot 10^{-4}$ M melittin in the tetrameric form in 0.05 M phosphate buffer; - - - - - -, $3.8 \cdot 10^{-5}$ M melittin plus $2.1 \cdot 10^{-3}$ M tetradecyldimethylamine oxide; · — · — ·, $4.2 \cdot 10^{-4}$ M melittin plus 0.022 M dodecylphosphocholine; - — - — -, $3.7 \cdot 10^{-4}$ M melittin plus 0.026 M L-α-diheptanoyl phosphatidylcholine; · · — · · —, $6.4 \cdot 10^{-6}$ M melittin plus $3.1 \cdot 10^{-4}$ M L-α-dilauroyl phosphatidylcholine and 0.1 M NaCl. At the low concentrations of peptide and L-α-dilauroyl phosphatidylcholine used to avoid excessive light scattering of the liposomes, addition of salt was necessary to ensure full binding of melittin to the lipid. Even under these conditions, light scattering distorted the CD spectrum for wavelengths shorter than 200 nm and this region of the spectrum is therefore not shown.

been observed for the binding of melittin to native and artificial phospholipid membranes [11,33,34].

Since for decyl-, dodecyl- and tetradecyldimethylamine oxides the length of the alkyl chain appears to have little or no influence on the fluorescence properties of tryptophan-19 when melittin is bound to these micelles (Fig. 1) and since these detergents form comparatively small micelles with molecular weights of approx. 7000, 17 000 and 33 000, respectively [19], it appears that rather small micelles may accommodate melittin. For dodecylphosphocholine, additional physicochemical studies (see below) have confirmed that the micelle-melittin complex has a relatively small size. For binding of melittin to phosphatidylcholine bilayers, it has been previously observed that the fluorescence properties of tryptophan-19 for melittin bound to dilauroyl, dimyristoyl or dipalmitoyl phosphatidylcholine are identical as long as the lipid bilayer is in the fluid state [34].

Circular dichroism of melittin in aqueous solution and in lipid complexes

In order to characterize the conformation of melittin bound to a lipid-water

interface and to see whether this conformation is dependent on the nature of the lipid-water interface, CD spectra have been measured for melittin in solution, bound to various detergent micelles and bound to phosphatidylcholine micelles or bilayers. The results are shown in Fig. 2.

Dependent on conditions, melittin in aqueous solution exists either as a monomer or as a self-aggregated tetramer (Lauterwein, J., Brown, L.R. and Wüthrich, K., unpublished results). The CD spectrum shown in Fig. 2 for monomeric melittin is characteristic of an extended flexible polypeptide chain, which is consistent with NMR results obtained for monomeric melittin (Lauterwein, J., Brown, L.R. and Wüthrich, K., unpublished results). The CD spectrum of tetrameric melittin indicates a major conformation change upon self-aggregation and the melittin tetramers show the usual CD characteristics of an α-helical conformation [35]. This is compatible with NMR observations which indicated that the melittin molecules in the tetrameric aggregate adopted a non-random conformation, which also contained a number of slowly exchanging amide protons (Lauterwein, J., Brown, L.R. and Wüthrich, K., unpublished results).

In the presence of various detergent micelles and either micelles or bilayers of phosphatidylcholine, i.e. diheptanoyl phosphatidylcholine micelles [36] or dilauroyl phosphatidylcholine bilayers, the CD spectra are of the type usually correlated with an α-helical conformation (Fig. 2). Only minor variations in the CD spectra were observed for melittin bound to the various lipids used in the present study and similar spectra have been previously observed for melittin bound to sodium dodecyl sulfate micelles [37]. It thus appears that an α-helix type CD spectrum [30] is a consequence of binding melittin to lipids and does not strongly depend on the exact type of lipid. It should be noted in particular that on the basis of the CD spectra, the conformation of melittin appears to be quite similar when bound to either phosphatidylcholine bilayers or detergent micelles. There is also a striking similarity between the CD spectra of tetrameric melittin and melittin bound to lipids, suggesting it may be appropriate to regard tetrameric melittin as a micelle in which melittin-lipid contacts are replaced by melittin-melittin contacts.

Ultracentrifugation and quasi-elastic light scattering

In view of the close similarity between the CD spectra of tetrameric melittin and of melittin bound to lipid aggregates of various types, the question of the state of aggregation of melittin in complexes with lipids arises. Furthermore, for detailed spectroscopic studies of the conformation of lipid-bound melittin, it is important that the size and stoichiometry of the melittin-lipid complex is well-defined. We have used equilibrium ultracentrifugation and diffusion constant measurements by both ultracentrifugation and quasi-elastic light scattering to characterize the size and stoichiometry of dodecylphosphocholine micelles and of the complex formed between melittin and these micelles.

Table I shows the data obtained for dodecylphosphocholine micelles. Comparison of the diffusion constant obtained by quasi-elastic light scattering and by ultracentrifugation shows good agreement between these two methods. Since heterogeneous size distribution is manifested differently in the two methods [25,29], these experiments also indicate that dodecylphosphocholine

TABLE I

PHYSICOCHEMICAL CHARACTERIZATION OF DODECYLPHOSPHOCHOLINE MICELLES AND THE COMPLEXES FORMED BETWEEN MELITTIN AND DODECYLPHOSPHOCHOLINE MICELLES

For the diffusion constant, when comparing D_{UC}^{20} and D_{LS}^{20}, the systematic deviations arising from the different averages over heterogeneous samples obtained by the two methods should also be considered ([25, 29], see text).

Parameter measured	Experimental method	Dodecylphosphocholine micelles	Melittin-dodecylphosphocholine complex
Diffusion constant	Analytical ultracentrifuge	$D_{UC}^{20} = 9.2 \cdot 10^{-7}$ cm^2/s	$D_{UC}^{20} = 8.8 \cdot 10^{-7}$ cm^2/s
Diffusion constant	Quasi-elastic light scattering	$D_{LS}^{20} = 7.8 \cdot 10^{-7}$ cm^2/s	$D_{LS}^{20} = 7.6 \cdot 10^{-7}$ cm^2/s
Particle weight	Analytical ultracentrifuge	19 500 ± 1700	peptide: 3250 ± 600 * detergent: 11 200 ± 3500 **
Partial specific volume	Density determination	0.937 cm^3/g	0.905 cm^3/g ***

* By equilibrium ultracentrifugation in $H_2O/^2H_2O$) mixtures according to Reynolds and Tanford [26].
** From the slope of the curve in Fig. 3.
*** Calculated from Fig. 3 for $M_\rho (1-\phi'\rho) = 0$.

micelles have a narrow size distribution, which remains virtually constant over the range of concentrations and temperatures likely to be of interest for NMR experiments, i.e. approx. 0.005—0.1 M, and 10—50°C, respectively. Diameters of equivalent spheres have been calculated on the one hand from the diffusion constants using the Stoke's-Einstein equation, and on the other hand from the micelle molecular weight using the partial specific volume (Table II). Con-

TABLE II

STOICHIOMETRY, SIZE AND SHAPE OF DODECYLPHOSPHOCHOLINE MICELLES AND THEIR COMPLEXES WITH MELITTIN DETERMINED FROM THE EXPERIMENTS IN TABLE I

Structural parameter	Experimental basis (see Table I)	Dodecylphosphocholine micelles	Melittin-dodecylphosphocholine complex
Stoichiometry	Particle weight	56 ± 5 detergent molecules/micelle	1.1 ± 0.2 peptide molecules and 32 ± 10 detergent molecules per micelle
Diameter of equivalent sphere *	Diffusion constant D_{UC}^{20}	47 Å	49 Å
	Diffusion constant D_{LS}^{20}	55 Å	56 Å
	Particle weight and partial specific volume	39 ± 3 Å	35 ± 3 Å
Maximum thickness of hydration shell *	D_{UC}^{20} D_{LS}^{20} Particle weight	6 ± 2 Å *	—
Maximum asymmetry a/b of prolate ellipsoid *	D_{UC}^{20} D_{LS}^{20} Particle weight	$a/b \sim 6$ *	—

* See Ref. 25 for the procedures used in these calculations. Maximum hydration was calculated assuming spherical shape of the particle. Maximum asymmetry was computed with the assumptions that there is no hydration and that the shape of the particle is a prolate ellipsoid with axes a and b.

sidering that the diameters determined from the diffusion constants include the hydration shell and would be systematically too large if the species were not spherical [25], the diameters of 47 Å and 55 Å determined from the two diffusion studies and the diameter of 39 ± 3 Å determined from the micelle molecular weight are in reasonable agreement. The value of 6 Å for the maximal thickness of the hydration shell of the dodecylphosphocholine micelles (Table II) also compared well with the value of 6 Å determined for phosphatidylcholine [38]. The size of the dodecylphosphocholine micelles determined by these measurements appears to be quite typical for zwitterionic detergents of this chain length [19,39,40].

The state of aggregation of melittin bound to dodecylphosphocholine micelles has been determined by means of equilibrium ultracentrifugation in $H_2O/^2H_2O$ mixtures [26]. This method is based on the principle that at a solvent density which matches that of the detergent, the apparent molecular weight of the complex is determined by the molecular weight of the protein component. Fig. 3 shows a plot of the apparent value of $M_P(1 - \phi'\rho)$ as a function of the solvent density. The molecular weight obtained from these data indicates that monomeric melittin is incorporated into the micelle (Tables I and II).

The following approaches were used to determine the number of dodecylphosphocholine molecules/micelle. A relatively unreliable estimate of the number of detergent molecules associated with each melittin molecule can be obtained from the slope of the plot in Fig. 3 [26]. This yields a value of 32 ± 10, as compared to 56 ± 5 detergent molecules found for the dodecylphosphocholine micelles without melittin (Table II). NMR titrations of melittin with dodecylphosphocholine (see below) indicated that the number of detergent molecules associated with each melittin molecule is about 40. Furthermore, the diffusion constants measured for the melittin-micelle complex by

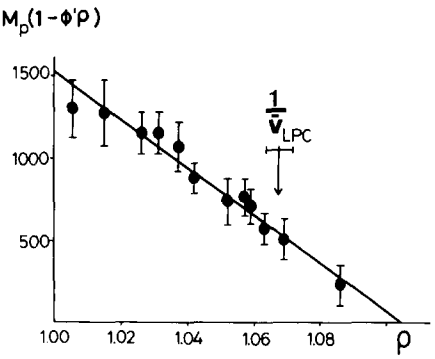


Fig. 3. Sedimentation equilibrium measurements by the method of Reynolds and Tanford [26] for a solution containing $2 \cdot 10^{-4}$ M melittin and 0.02 M dodecylphosphocholine in 0.05 M phosphate buffer at pH 7.0 and 20°C. The value $M_P(1 - \phi'\rho)$ is plotted versus the solvent density ρ, which was altered by mixing various proportions of H_2O and ^2H_2O. M_P is the molecular weight of the protein component of the complex and ϕ' is the volume increment/g protein. The arrow indicates the solvent density for which $\rho = 1/\bar{v}$ of the dodecylphosphocholine. Error limits for the value of $M_P(1 - \phi'\rho)$ at a given solvent density were calculated from the two different reference solutions used in the determination of the melittin concentration by ultraviolet scanning (see Materials and Methods).

ultracentrifugation and quasi-elastic light scattering indicated that the micelles with and without bound melittin are of similar size (Table II). This suggests that melittin might simply displace a certain number of detergent molecules when the complex with the micelle is formed. This hypothesis would be compatible with a melittin-micelle complex containing approximately 40 detergent molecules, as indicated by the NMR studies.

Nuclear magnetic resonance experiments

In Fig. 4 the 360 MHz ^1H NMR spectra of monomeric and self-aggregated tetrameric melittin in aqueous solution and of the complex of melittin with dodecyldimethylamine oxide micelles are compared. The NMR spectra of monomeric and tetrameric melittin have been extensively analyzed and a considerable number of resonances assigned (Lauterwein, J., Brown, L.R. and Wüthrich, K., unpublished results). Fig. 4 shows that the conformational differences between monomeric and tetrameric melittin evidenced in the circular dichroism experiments (Fig. 2) are also manifested in the NMR spectra. The advantage of the NMR method is that resonances arising from amino acids distributed throughout the sequence of melittin can be resolved and assigned, and thus allow a many-parameter comparison of the melittin conformations in the different species (Lauterwein, J., Brown, L.R. and Wüthrich, K., unpub-

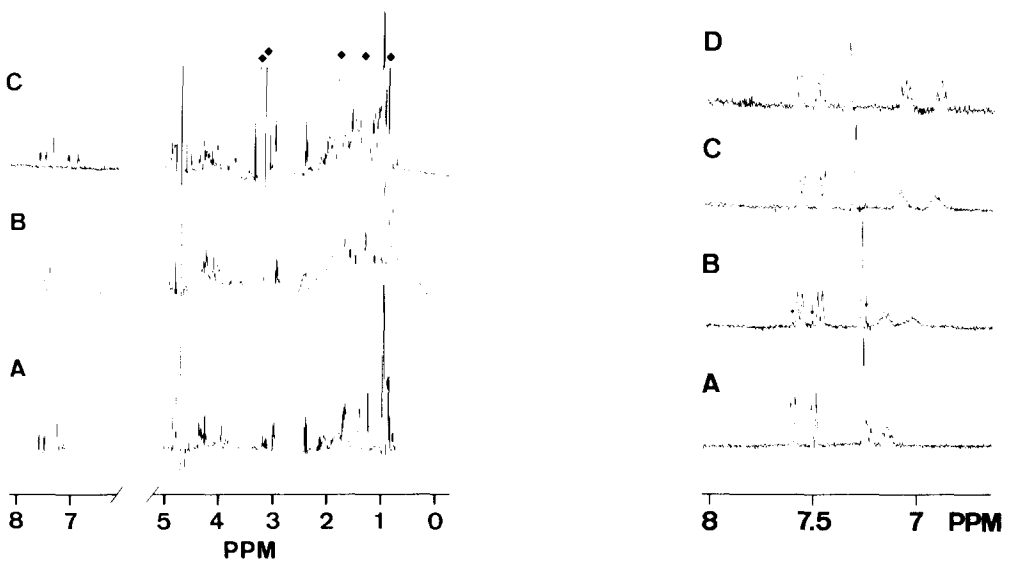


Fig. 4. 360 MHz ^1H NMR spectra of $4 \cdot 10^{-3}$ M melittin in ^2H_2O at p^{2}H 7.0 and 30°C. (A) Monomeric melittin in salt-free solution. (B) Tetrameric melittin in 0.05 M phosphate buffer. (C) Micelle-bound melittin in the presence of 0.135 M dodecyldimethylamine oxide. The resonances of the indole ring of tryptophan-19 at 6—8 ppm are shown at twice the intensity of the remainder of the spectrum. The intense resonances from residual solvent protons (◇) and from the detergent (◆) have been truncated.

Fig. 5. 360 MHz ^1H NMR spectra of the resonances of the indole ring of tryptophan-19 of melittin as a function of the concentration of dodecyldimethylamine oxide at p^{2}H 7.0 and 30°C. (A) $4 \cdot 10^{-3}$ M monomeric melittin in salt-free aqueous solution. (B—D) $4 \cdot 10^{-3}$ M melittin and dodecyldimethylamine oxide at concentrations of (B) 0.005 M, (C) 0.019 M and (D) 0.135 M. The arrows in spectrum B indicate the resonances which are not shifted by the presence of dodecyldimethylamine oxide (see text).

lished results). In the spectrum of melittin bound to dodecyldimethylamine oxide micelles strong resonances arise from the detergent molecules, but resolved resonances from melittin are also observable. The protons of the indole ring of tryptophan-19 are particularly well resolved between 6 and 8 ppm (Fig. 4C), and a number of melittin resonances can also be observed in other regions of the spectrum. Even though the intense resonances of the detergent molecules obscure much of the melittin spectrum, Fig. 4C clearly illustrates that high resolution ^{1}H NMR spectra can be obtained for micelle-bound polypeptide chains and hence NMR can be used for conformational studies in these systems in a similar way as for investigations of peptides and proteins in solution [41].

Two general conclusions result from inspection of those melittin resonances in Fig. 4C which are not overlapped by the detergent lines. Firstly, the NMR spectrum of melittin bound to dodecyldimethylamine oxide micelles differs from the NMR spectra of both monomeric and tetrameric melittin in aqueous solution, which emphasized the need for a membrane-like environment to obtain information on the conformation of membrane-bound melittin. Secondly, that melittin bound to dodecyldimethylamine oxide micelles gives rise to a single spectrum suggests that one well-defined conformational state predominates for the micelle-bound polypeptide chain. In the following NMR is used to further analyze the melittin-lipid interactions in the micelles.

Fig. 5 shows the resonances of the indole ring protons of Trp-19 of melittin in the presence of increasing concentrations of dodecyldimethylamine oxide. It can be seen that increasing detergent concentration causes continuous changes of the chemical shifts. This shows that exchange of melittin between the micelle-bound and free forms is in the fast exchange limit on the NMR time scale *. Sharp resonance lines prevail for the monomeric melittin as well as for melittin in the presence of saturating detergent concentrations. Line broadening is observed, however, at intermediate detergent concentrations (Fig. 5). A variety of motions could contribute to the observed linewidths. These include overall motion of the micelle, motion of the polypeptide within the micelle, motion of the lipid chains within the micelle and exchange between micelle-bound and free forms of detergent and polypeptide. Since all of the resonances in Fig. 5 arise from a single aromatic ring and thus have comparable rotational mobility, the different broadening of the individual lines must be related with exchange phenomena, and thus suggests that the rate of exchange between free and micelle-bound melittin is not very fast. This interpretation is consistent with the observation that the resonances which have the

* Evidence was found that approximately 10% of the monomeric melittin does not participate in the rapid exchange between micelle-bound and free peptide. In the monomeric form, the tryptophan resonances of the two melittin species are identical. In the presence of low detergent concentrations, the bulk of the intensity of the tryptophan resonances is shifted and slightly broadened, whereas the remaining intensity is observed as sharp resonances at the positions found for monomeric melittin (Fig. 5B, the arrows indicate the weak resonance lines which are not shifted by the detergent). The weak resonances decrease in intensity and disappear as the detergent concentration is increased (Fig. 5). More detailed evidence for the coexistence of two different, slowly exchanging monomeric forms of melittin, which were tentatively correlated with cis-trans isomers of proline-14, is presented elsewhere (Lauterwein, J., Brown, L.R. and Wüthrich, K., unpublished results).

largest changes in chemical shift between free and micelle-bound melittin show the largest line-broadening effects.

The continuous variation of the chemical shifts with increasing detergent concentration makes it possible to identify corresponding resonances in the spectra of monomeric and micelle-bound melittin. Since numerous resonances were individually assigned for the monomeric peptide (Lauterwein, J., Brown, L.R. and Wüthrich, K., unpublished results), resonance assignments for the micelle-bound melittin are also obtained. In the titration experiment of Fig. 6, some resonances could be followed through the entire transition from free to micelle-bound melittin while other melittin resonances became obscured by detergent lines at the higher detergent concentrations. Fig. 6 shows that virtually identical spectra for micelle-bound melittin were obtained in titrations

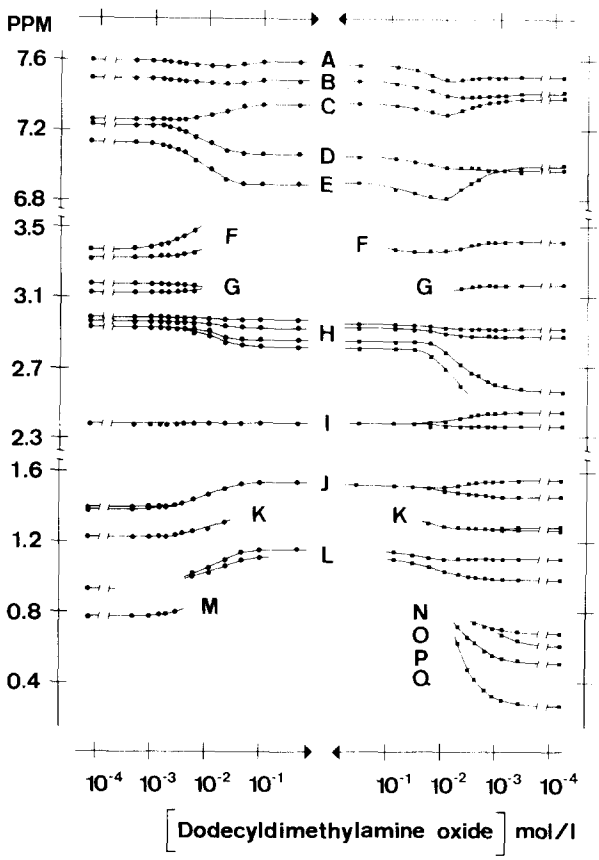


Fig. 6. ^{1}H NMR chemical shifts of the resonances of $4 \cdot 10^{-3}$ M melittin versus the concentration of dodecyldimethylamine oxide at p^{2}H 7.0 and 30°C. The titration with detergent was begun either with monomeric melittin in salt-free aqueous solution (left side, ●———●) or with tetrameric melittin in 0.05 M phosphate buffer (right side, ■———■). The points shown at the extreme left and right of the plots are the chemical shifts observed in the absence of detergent for the monomeric and tetrameric forms of melittin, respectively. The assignments of the resonances are: A—E, Trp 19 indole ring, H4, H7, H2, H6, H5; F, Trp 19, βCH_2; G, Arg 22 and 24, δCH_2; H, Lys 7, 21 and 23, ϵCH_2 (see text); I, Gln 25 and 26, γCH_2; J. Ala 4 and 15, βCH_3; K, Thr 10 and 11, γCH_3; L, Val 5 and 8, γCH_3; M, Ile 17 or 20, γCH_3; N and O, unidentified; P and Q, Ile or Leu, CH_3.

started either with monomeric or tetrameric melittin. The very slight differences may arise because 0.05 M phosphate buffer was added to induce aggregation (Lauterwein, J., Brown, L.R. and Wüthrich, K., unpublished results) in the experiment with tetrameric melittin. Since the NMR spectrum of melittin bound to dodecyldimethylamine oxide micelles is very similar to that of melittin bound to dodecylphosphocholine micelles, where melittin has been shown to be monomeric (Tables I and II), these data indicate that complexation with dodecyldimethylamine oxide micelles leads to dissociation of the melittin tetramer. Another interesting observation in Fig. 6 is that at detergent concentrations below the critical micelle concentration there are virtually no spectral changes for monomeric melittin, but small chemical shift variations occurred for a number of resonances of tetrameric melittin. This difference between monomeric and tetrameric melittin was also observed with other detergents and will be further analyzed in Discussion.

Titrations of the type shown in Fig. 6 have been carried out with a variety of detergents (see Table III) in order to test how the NMR spectrum of micelle-bound melittin depends on the type of detergent. For all the detergents used except dodecylphosphocholine (see below), rapid exchange of melittin between free and micelle-bound states was observed over the entire range of detergent concentrations used, allowing titrations of the type in Fig. 6 to be performed. As with dodecyldimethylamine oxide (Fig. 6), the presence of strong detergent resonances allowed only a few melittin resonances to be followed through the entire transition from free melittin to melittin bound to a particular type of detergent micelle. Furthermore, the melittin resonances which can be followed throught the complete transition vary from one detergent to another dependent on the resonance positions of the detergent protons. To increase the number of spectral parameters available for comparing melittin bound to different detergents, the extrapolation procedure outlined in the following was employed. The resulting chemical shifts of numerous resonances of melittin bound to four different detergent micelles and to L-α-diheptanoyl phosphatidylcholine micelles are presented in Table III.

The extrapolation procedure used to obtain chemical shifts for those resonances of micelle-bound melittin which could be observed only at low detergent concentrations was based on the assumption that the same type of dependence on the detergent concentration prevailed for all the resonances of the melittin spectrum. In the general case of rapid exchange on the NMR time scale between the unbound, monomeric form of melittin and j different bound forms for melittin interacting with detergent, the observed chemical shift for the i^{th} melittin resonance at a given detergent concentration, δ_i, can be written as:

$$\delta_i - \delta_{i0} = \sum_j X_j^i(\delta_{ij} - \delta_{i0}) \qquad (1)$$

where X_j^i is the mol fraction of resonance i in the detergent-bound form with chemical shift δ_{ij}, and δ_{i0} is the chemical shift of resonance i in free melittin. If only one detergent-bound state were populated, Eqn. 1 would become:

$$\delta_i - \delta_{i0} = X_m^i(\delta_{im} - \delta_{i0}) \qquad (2)$$

TABLE III

CHEMICAL SHIFTS FOR FREE AND MICELLE-BOUND MELLITIN

Chemical shifts δ_{im} for melittin bound to micelle were measured with $1 \cdot 10^{-3}$—$4 \cdot 10^{-3}$ M melittin at p^2H 7.0 and 30°C. The concentration of the various detergents necessary to fully bind the melittin was 40—60 times the melittin concentration. For certain protons δ_{im} was directly observed whereas for others, where the chemical shifts are given in parenthesis, δ_{im} was obtained from extrapolation using measurements at low detergent concentrations (see text). For all extrapolated values, linear least-squares fitting of Eqn. 2 yielded standard deviations of 0.01 ppm or less. δ_{io} is the chemical shift of monomeric melittin in the absence of lipid at p^2H 3.0. Note that the chemical shift of the reference sodium 3-trimethylsilyl-[2,2,3,3-^{2}H]propionate varies by 0.019 ppm between p^2H 3.0 and p^2H 7.0 [31]. $\Delta\delta_{im}$ is the maximum difference in chemical shift between the spectra of melittin bound to five different detergents. $\delta_{io} - \delta_{im}$ is the average of the chemical shift changes observed when free melittin binds to the different detergents.

Resonance	Chemical shifts δ_{im} for melittin bound to micell of:					δ_{io}	$\Delta\delta_{im}$	$\delta_{io} - \delta_{im}$
	Decyl-dimethylamine oxide	Dodecyl-dimethyl-amine oxide	Dodecyl-(oxyethylene)$_{7,8}$-glycol	Dodecyl-phosphocholine *	L-α-Diheptanoyl phosphatidyl-choline			
Ala-4,15	1.540	1.530	1.544	1.555	(1.54)	1.361	0.03	—0.18
βCH_3	1.540	1.530	1.563	1.573	(1.55)	1.371	0.04	—0.18
Val-5,8 **	1.100	1.083	1.102	1.107 } 0.980	1.103	0.920	0.03	—0.18
γCH_3	1.148	1.142	1.159	1.160 } 1.004	1.139	0.920	0.02	—0.23
Thr-10,11	(1.32)	(1.32)	1.329	1.329	(1.32)	1.205	0.01	—0.12
γCH_3	(1.32)	(1.32)	1.329	1.343	(1.32)	1.210	0.02	—0.12
Ile-17 or 20 γCH_3 Trp-19	(0.97)	(0.99)	—	—	(0.99)	0.752	0.02	—0.23
C5-H	6.907	6.896	6.880	6.852	6.868	7.140	0.06	+0.24
C6-H	7.102	7.070	7.063	7.020	7.071	7.246	0.08	+0.18
C2-H	7.320	7.345	7.344	7.378	7.326	7.252	0.06	—0.09
C7-H	7.497	7.485	7.459	7.492	7.473	7.496	0.04	+0.02
C4-H	7.588	7.588	7.578	7.557	7.562	7.601	0.03	+0.03
Lys-7,	2.793, 2.841	2.839, 2.864	2.758, 2.853	2.571, 2.723	2.642, 2.799	2.944	0.27, 0.14	+0.23, +0.13
21,23	2.922	2.924	2.935	2.913	2.932	2.961	0.02	+0.04
ϵCH_3***	2.970	2.973	2.978	2.975	2.967	2.981	0.01	+0.02
Arg-22,24-	(3.13)	(3.15)	3.173	3.106	(3.14)	3.178	0.07	+0.04
δCH_2	—	(3.16)	3.173	3.179	(3.19)	3.117	0.03	—0.06
Gln-25,26 γCH_2	2.395	2.391	2.408	—	—	2.378	0.02	—0.02

* Measured in deuterated micelles [42].

** Only one γCH_3 of each valine was observable, except in the deuterated dodecylphosphocholine micelles.

*** In the micelles, two one-proton lines were observed for one of the lysine ϵ-CH_2's.

where X_m^i is the mol fraction of resonance i which experiences the detergent-bound situation with a corresponding chemical shift of δ_{im}. If for the melittin molecule as an entity only one detergent-bound state were populated, then at any given detergent concentration the same value of X_m^i would prevail for all resonances i.

For those melittin resonances which can be followed through the complete transition from free to micelle-bound melittin, the chemical shifts in the presence of saturating detergent concentrations can be measured. Using these chemical shifts as δ_{im} in Eqn. 2, X_m^i for the corresponding protons can be calculated for any given detergent concentration. Fig. 7 shows plots of X_m^i calculated on this basis as a function of decyldimethylamine oxide concentration for several melittin resonances. That the curves in Fig. 7 coincide for a variety of resonances arising from protons at quite different positions in the melittin molecule provides justification that assumption of a single detergent-bound state for melittin gives an adequate description of the titration shifts observed when melittin is titrated with decyldimethylamine oxide. In this case, Eqn. 2 shows that plots of $(\delta_i - \delta_{io})$ versus X_m determined from Fig. 7 should be linear, pass through the origin and have slope $(\delta_{im} - \delta_{io})$, which provides a ready means to obtain δ_{im} for resonances which are observable only over a limited range of detergent concentration. On the basis of Fig. 7 and corresponding data for the other detergents, a single detergent-bound state was assumed for melittin and plots of $(\delta_{im} - \delta_{io})$ versus X_m (Fig. 8) were used to obtain the extrapolated chemical shifts δ_{im} in Table III. That the experimental data in Fig. 8 conform reasonably well to linear plots passing through the origin provides further justification for the assumption of a single state for melittin bound to a given detergent.

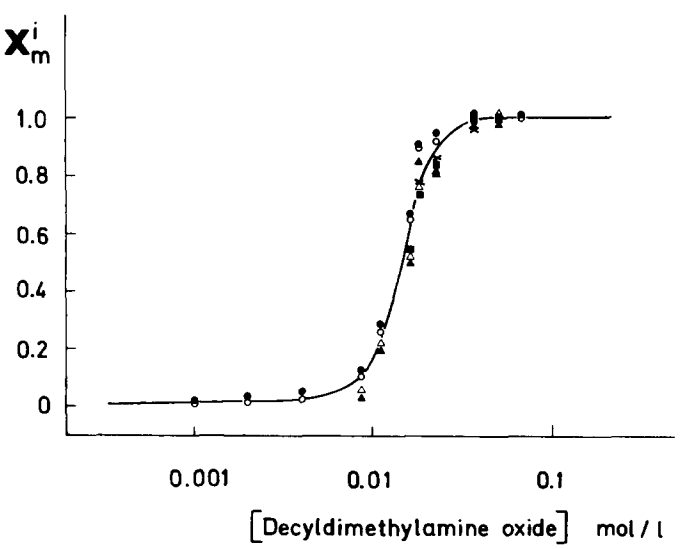


Fig. 7. Plots of X_m^i, the apparent fraction of detergent-bound melittin calculated from Eqn. 2 versus the concentration of decyldimethylamine oxide at p^2H 7.0 and 30°C. The melittin concentration was $1 \cdot 10^{-3}$ M. The symbols correspond to the resonances of: ●, Trp 19, H5; ○, Trp 19, H6; △, ▲, Ala 4 and 15, βCH_3; ■, ×, Val 5 and 8, γCH_3.

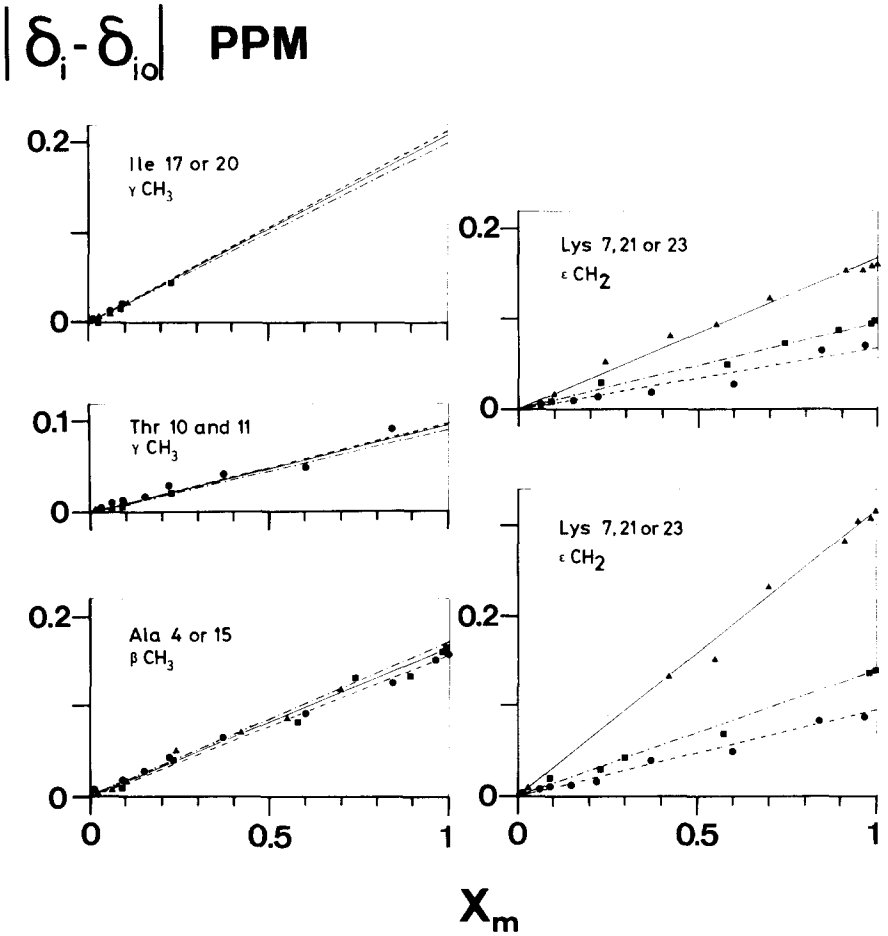


Fig. 8. Plots of $|\delta_i - \delta_{io}|$, the absolute value of the difference between the observed chemical shift and the chemical shift for free, monomeric melittin, versus X_m, the apparent fraction of melittin bound to detergent, for the detergents decyldimethylamine oxide (■—·—·■), dodecyldimethylamine oxide (●- - - - -●) and L-α-diheptanoyl phosphatidylcholine (▲———▲). Values of X_m for the various detergents were taken from plots analogous to that shown in Fig. 7 for decyldimethylamine oxide. The straight lines were obtained from linear least-squares fitting of the experimental points to Eqn. 2. The two plots for Lys ε-CH_2 correspond to two one-proton lines originating from the same lysine (see text).

When free, monomeric melittin was titrated with dodecylphosphocholine, a more complex situation than continuous titrations of the type shown in Fig. 6 was encountered. At low detergent concentrations two melittin spectra, one of which corresponded to free, monomeric melittin, were observed. The relative intensities of the two spectra varied continuously when the detergent to melittin ratio was increased from approximately 0.5 : 1 to 8 : 1, at which ratio the relative intensity of the monomer spectrum was very small. At detergent to melittin ratios greater than or equal to 8 : 1 a single melittin spectrum was observed and the chemical shifts varied continuously up to a detergent-to-melittin ratio greater than or equal to 40 : 1. At still higher detergent to melittin ratios the melittin spectrum was independent of detergent concentration. This behaviour excluded extrapolation with Eqn. 2 to obtain chemical

shifts and resonance assignments for melittin bound to dodecylphosphocholine micelles. These chemical shifts and resonance assignments (Table III) were therefore obtained from NMR studies of melittin bound to fully deuterated dodecylphosphocholine micelles [42].

Discussion

In the present study, important first results were that the light-scattering and ultracentrifugation experiments indicated melittin-micelle complexes of good homogeneity were obtainable at high detergent concentrations and that in the NMR experiments a high resolution ^{1}H NMR spectrum was observed for the micelle-bound polypeptide chain. It would thus appear that high resolution NMR studies of complexes formed between membrane proteins and detergent micelles could provide a viable method for obtaining conformational information for such proteins. In favourable cases the conformation of a micelle-bound protein might be closely related to the conformation which prevails under physiological conditions. Since a considerable number of membrane-bound enzymes have been solubilized in an active form by the use of detergents, micellar complexes may indeed be appropriate for a wide variety of membrane-bound proteins. In the systems studied here, the fluorescence and circular dichroism experiments provided direct evidence that the conformation of melittin bound to micelles or to phosphatidylcholine bilayers must be very similar. The major practical difficulty for ^{1}H NMR studies of the melittin-micelle complexes was that observation of resolved melittin resonances was limited by the intense detergent lines (Fig. 4C). The obvious solution to this problem is to fully deuterate the detergent molecules. This has been done and the quality of the high resolution ^{1}H NMR spectra of melittin bound to fully deuterated micelles was found to be comparable to the spectra of a polypeptide chain of similar size in solution [42].

In the structural interpretation of the high resolution ^{1}H NMR data obtained for micelle-bound polypeptide chains, certain novel aspects must be considered compared to the situation for peptides or proteins in aqueous solution. For non-aggregating peptides and proteins in solution, chemical shift differences relative to 'random coil' values [41] are necessarily a consequence of the spatial folding. For polypeptide-micelle complexes, both the environment provided by the hydrophobic interior or by the interfacial region of a detergent micelle as well as intermolecular peptide-peptide interactions in complexes containing more than one peptide chain may also influence chemical shifts. Thus even a 'random coil' polypeptide chain might show different chemical shifts in micellar complexes and in aqueous solution.

From these general considerations, it is apparent that identical NMR spectra might be obtained for polypeptide chains bound to micelles of different size or for micelles containing different numbers of peptide molecules if the peptides do not directly interact. Therefore, only limited information about the stoichiometry of melittin-detergent complexes could be obtained from NMR titrations of the type shown in Fig. 6. When an excess of dodecylphosphocholine was used, reliable information on the stoichiometry of the melittin-detergent complex was obtained by combining the NMR data with data from

a variety of other physical methods (Table II). From the fluorescence experiments shown in Fig. 1 it could be concluded that for the much higher melittin concentrations used for the NMR experiments, the equilibrium between free and micelle-bound melittin lay heavily on the side of micelle-bound melittin at detergent concentrations above the critical micelle concentration. Under these conditions, the detergent-to-melittin ratio at which the melittin NMR spectrum becomes independent of the detergent concentration represents a lower limit for the number of detergent molecules/melittin in the complex. For the detergents in Table III, the NMR spectrum of $1—4 \cdot 10^{-3}$ M melittin was independent of detergent concentration for detergent-to-melittin ratios of greater than or equal to 40 : 1—60 : 1, depending on the detergent, and in the presence of excess detergent the melittin NMR spectrum was characteristic of a single conformational state. For dodecylphosphocholine, where a stoichiometry of one melittin to 32 ± 10 detergent molecules was obtained by equilibrium ultracentrifugation in $H_2O/^2H_2O$ mixtures (Table II), the NMR data was thus useful to improve the accuracy of the estimate for the number of detergent molecules/complex. The stoichiometry of one melittin and approx. 40 detergent molecules/complex was also consistent with the apparent particle size determined from the observed diffusion constants (Table I).

The circular dichroism measurements (Fig. 2) imply that micelle-bound melittin adopts a markedly different conformation from that of monomeric melittin in aqueous solution. Major differences in the ^1H NMR parameters for free and micelle-bound melittin are also observed (Figs. 4—5, Table III) and appear to support this conclusion. For the complex formed between melittin and excess dodecylphosphocholine, where the stoichiometry has been extensively characterized (Tables I and II), a detailed analysis of the ^1H NMR spectrum of melittin bound to fully deuterated micelles (in progress in our laboratory) should provide data for a more complete comparison of the melittin monomer in solution and in micelles. A different approach for obtaining information on certain structural features of melittin-micelle complexes is used here, i.e. comparison of the NMR data for melittin bound to five different protonated detergent micelles (Table III).

The melittin sequence [43]

Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-
1 5 10 15

Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-NH_2
16 20 25

has a pronounced amphiphilic distribution of polar and non-polar amino acids. From residues 1 to 20 hydrophobic amino acids are predominant whereas residues 21—26 are polar and carry four positive changes. There is a striking correlation with the data in Table III which indicates that considerable conformational similarity exists for melittin bound to various types of micelles. Thus, the methyl groups of alanines 4 and 15, threonines 10 and 11, valines 5 and 8 and one of the isoleucines 17 or 20, all of which lie in the hydrophobic portion of the melittin amino acid sequence, show large, downfield changes in chemical shift when monomeric melittin binds to micelles. In comparison, the

differences in chemical shift between the various micelles are small for these resonances. This suggests that these residues, and therefore probably the entire hydrophobic portion of the melittin amino acid sequence, penetrate into the hydrophobic interior of a micelle to which melittin is bound. In contrast, the resonances of glutamines 25 and 26, arginines 22 and 24 and two of the lysines 7, 21 and 23 (Table III), which with the exception of lysine 7 all lie in the hydrophilic C-terminal portion of the melittin amino acid sequence, show only small shifts to either high or low field when melittin binds to micelles. For these resonances variation in chemical shifts amongst the various micelles are of comparable size to the changes observed on binding monomeric melittin to the micelles. This suggests that these residues are largely exterior to the micelle and have at most weak interactions with the lipid component. A yet different behaviour was observed for the indole ring resonances of tryptophan 19 and for the ϵ-methylene resonance of one of the lysine residues 7, 21 or 23, where large changes in chemical shift were observed both for binding monomeric melittin to micelles and between different types of micelles. A likely explanation seems to be that these residues are located at the interface between the hydrophobic interior and polar head group region of the micelles and are therefore sensitive to the structural differences between the head groups of the different detergents used (Table III). The lysine ϵ-methylene protons are particularly noteworthy, since for all of the detergents used their resonances were shifted to unusually high field and consisted of two one-proton lines separated by up to 50 Hz, which indicates that the side chain of this lysine must be held in a rigid conformation by the melittin-micelle interaction. Overall, the NMR spectra thus appear to reflect an amphiphilic three-dimensional structure for micelle-bound melittin in which the hydrophobic N-terminal sequence penetrates into the micelle interior, while the hydrophilic C-terminal sequence remains at the micelle surface and is probably not fixed in a rigid conformation.

Whereas in the presence of excess detergent the stoichiometry of the melittin-micelle complexes was quite well-defined, the NMR titration experiments indicated that complexes containing variable numbers of melittin and detergents molecules were formed at low detergent-to-melittin ratios. Under these conditions, NMR provided qualitative information about the nature of the melittin-detergent interaction and about conformational similarities between tetrameric and micelle-bound melittin.

For all the resonances shown in Table III except the C2-H, C4-H and C7-H lines of Trp-19 and for all detergents except dodecylphosphocholine, assumption that the NMR spectrum of detergent-bound melittin was always identical to the spectrum observed in the presence of excess detergent provided an adequate description of the chemical shift changes when free, monomeric melittin was titrated with detergent. However, at low detergent-to-melittin ratios, the binding equilibrium did not appear to involve one melittin molecule and one detergent micelle. Thus, ratios of total detergent concentration to the apparent concentration of bound melittin as low as approx. 15 : 1 were calculated from the NMR parameters at low detergent to melittin ratios. These ratios were considerably smaller than the minimum detergent to melittin ratio of complexes formed in the presence of excess detergent. This indicated that complexes containing more than one melittin/complex were formed at low

detergent concentrations. Since the C2-H, C4-H and C7-H resonances of the indole ring of tryptophan-19 were the only resonances which showed somewhat different titration behaviour, these results also indicated that for complexes with more than one melittin, peptide-peptide interactions had little influence on the melittin NMR spectrum. This suggests that in the presence of those detergents which showed continous titration behaviour, i.e. all those listed in Table III except dodecylphosphocholine, interactions between melittin molecules in the same complex were weak. At low dodecylphosphocholine-to-peptide ratios, where slow exchange between detergent-bound and free melittin was observed, complexes containing several melittin molecules and only a small number of detergent molecules appeared to be formed. In such complexes peptide-peptide interactions seem probable. These could then obviously be replaced by detergent-peptide interactions since melittin was monomeric in the presence of excess dodecylphosphocholine (Tables I and II).

Micelle-melittin complexes containing more than one melittin molecule were undoubtedly also formed when tetrameric melittin was titrated with detergent. Indeed, the NMR titration experiments indicated that such complexes were formed even at detergent concentrations below the critical micelle concentration by binding of detergent monomers to the melittin tetramer. These results appear to suggest that the amphiphilic environment provided by a detergent micelle can be replaced by peptide-peptide interactions in tetrameric melittin. The circular dichroism measurements (Fig. 2) suggest indeed similar conformations for tetrameric and micelle-bound melittin, whereas the ^{1}H NMR parameters of tetrameric and micelle-bound melittin show appreciable differences (Fig. 6). These may arise from differences in the polypeptide conformations and/or from the different environments provided by peptide-peptide contacts in tetrameric melittin and by peptide-detergent contacts in micelle-bound melittin. A more detailed comparison of the polypeptide conformations in tetrameric melittin and in micelle-bound melittin is in progress.

When considering the conditions under which melittin-detergent interactions were observed in the present experiments, a certain symmetry is evident. Thus, when both melittin and detergent were monomeric, no interactions were observed. On the other hand, monomeric melittin was found to bind to detergent micelles and detergent monomers interacted with tetrameric melittin aggregates. This suggests that the melittin-detergent interaction might be best described as the formation of mixed micelles by two different amphiphilic molecules, each of which can self-aggregate to form micelles at sufficiently high concentration. This interpretation is supported by the following observations for the melittin NMR spectrum in the presence of an excess of a variety of different detergents: (i) most of the melittin NMR spectrum was virtually identical in all detergents. (ii) The resonances from the hydrophobic and hydrophilic portions of melittin showed distinctive properties. (iii) For resonances from the hydrophilic portion of melittin, which showed some variation in chemical shift for different detergents, no systematic differences between nonionic and zwitterionic detergents were apparent. The conformation of micelle-bound melittin therefore appears to be largely a consequence of the location of melittin at a hydrophobic-hydrophilic boundary and not a result of strong binding of detergent monomers. This would be consistent with a mixed

micelle of melittin and detergent. This interpretation also provides an attractive rationale for formation of a melittin tetramer in which peptide-peptide interactions replace peptide-detergent interactions, for the stoichiometry of the complexes formed at low detergent to peptide ratios and for the observation that in the presence of excess detergent the melittin-micelle complex has a size close to that of micelles without bound melittin (Table II). In mixed micelles the major conformational difference between detergent and melittin molecules appears to be that the detergent molecules are flexible, whereas at least part of the melittin molecule assumes a more definite conformation. Consideration of possible relationships between the conformation of micelle-bound melittin and physiological functions must await completion of the more detailed analysis of the ^{1}H NMR spectrum of melittin bound to fully deuterated micelles [42].

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