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LIPID-PROTEIN INTERACTIONS: NMR STUDY OF MELITTIN AND ITS BINDING TO LYSOPHOSPHATIDYLCHOLINE

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Summary

Proton NMR of melittin differs according to the association state of the peptide in the monomer or tetramer. Melittin interacts with lysophosphatidylcholine micelles, whatever the association state of melittin; well resolved superimposed spectra from both components for all the lipid to peptide molar ratios are observed. Within the complexes, local mobility and fast exchange occurs. On binding concomitant shifts on Trp_{19} indole lines and on the aliphatic CH_2 protons of the lipids are detected. The lipid perturbation is maximum for methylene groups in a α and β of the ester bond, this could allow positionning of Trp_{19} in the hydrophobic core of the lipids.

Proton NMR spectroscopy is 'a priori' suitable for the study of both lipid and protein behaviour when these compounds are allowed to interact. However there has been some disappointment with the results so far obtained: (i) in some cases the interaction of peptides with lipid bilayers leads to partial or total disappearance of the high resolution spectra, this occurs for cyclic antibiotic peptides like alamethicin and valinomycin [1—3] or a small hormone such as glucagon [4]; (ii) in other cases the lipid spectra remained almost unchanged but the protein spectrum was undetectable despite the large amounts of protein added. This was the case for cytochrome b_5 [5] and for cytochrome *c*. Nevertheless for this last system accurate T_1 measurements and ^{13}C study allowed the authors to conclude that there was a weak decrease in local motions of the lipid molecules [6].

By studying lipoprotein assembly Hauser [7] and Atkinson et al. [8] were able to gain a new insight by NMR, since they obtained both the lipid and the aromatic part of the protein spectra and detected small shifts in the lipid protons induced by protein. Very recently, by extensive deuteration of

the lipids, the ^{1}H-NMR spectrum of the Trp residues of gramicidin A embedded in a lipid bilayer has been reported [9].

Here we report on the proton NMR spectra of melittin, a peculiar peptide from bee venom the sequence of which consists of a cluster of mainly hydrophobic residues (1—20), followed by a strongly basic C terminal peptide 21--26 [10]. By intrinsic fluorescence studies melittin was shown to interact with phospholipid bilayers and lysophosphatidylcholine micelles, the Trp_{19} being buried during formation of the complex [11,12]. Melittin is known to behave in solution as a tetramer [13]; however, in low ionic strength medium it dissociates and exists as a monomer of molecular weight about 2800 (Dufourcq, J., unpublished results).

Melittin used for this study was purchased from Sigma and from Serva. It was further purified according to Mollay et al. [14] in order to remove phospholipase contamination, and lyophilyzed in ammonium acetate buffer. Then, it was twice incubated and lyophilized in ^2H_2O provided by the Commissariat à l'Energie Atomique, Saclay, France, in order to remove exchangeable protons. Melittin concentrations used in the experiments ranged from 3 to 6 mM, the sample volume being 0.3 ml. p^2H was monitored inside the NMR tube by a combined electrode from Ingold no 405 M3; a p^2H value of 5.6 was used for both experiments at low and high ionic strength.

The proton NMR spectrum of melittin in ^2H_2O is reported in Fig. 1. It is sharp and most of the lines can be attributed unambiguously by comparison with chemical shifts of amino acids or unfolded proteins [15,16]. One can notice at low field the well resolved indole protons from Trp_{19}. Upfield from the broad CH_α resonance several well shaped lines are attributed to methylene groups of $Arg_{22,24}$, $Lys_{7,21,23}$, $Gln_{25,26}$. In the high field region resonances due to Leu, Ile and Val methyl protons are observed. The chemical shifts compare well with amino acid positions, so in water melittin probably has no significant secondary structure; this agrees with previous results from ORD [17] and the very recent conclusions of Dawson et al. [18].

On addition of NaCl to melittin in ^2H_2O, which leads to tetramer aggregation, pronounced changes occur in the NMR spectrum. Most of the lines are split and numerous new sharp lines appear in the spectrum. As an example, two peaks corresponding to Gln_{25} and Glu_{26} γCH_2 protons are resolved. The indole Trp_{19} lines are shifted and on the methyl region two peaks move up to higher values, 0.61 and 0.27 ppm, indicating that probably some Leu, Ile or Val residues are influenced by the Trp_{19} ring current. In the tetrameric state some local structure is then stabilized. This could be due to conformational changes and/or to intermolecular hydrophobic interactions. This has to be related to what happens for other amphipatic peptides such as glucagon [19,20].

Incremental additions of lysophosphatidylcholine micelles to melittin, or vice-versa, lead to composite spectra where the lipid and the protein lines are superimposed (Fig. 2). Most of the lines still derived from the lipid protons alone agree with the litterature [21]. However some resonance due to protein protons can be observed in windows of the lipid spectrum. The ϵCH_2 protons from Lys residues remain unaffected in the vicinity of the strong choline line which totally masks the Arg methylene resonance. The indole lines from Trp_{19} remain well resolved, but some peaks are shifted during binding.

In the lipid spectra all lines remain well resolved, no broadening occurs but some shifts are detected when melittin is added. These shifts plotted on Fig. 3 attain a maximum when the lipid to protein molar ratio is higher than ten; this agrees with fluorescence data [11,12]. The major effects are observed on the first methylene groups of the aliphatic chains (α and β from the ester group) which shifted 0.1 ppm upfield. The line coming from residual

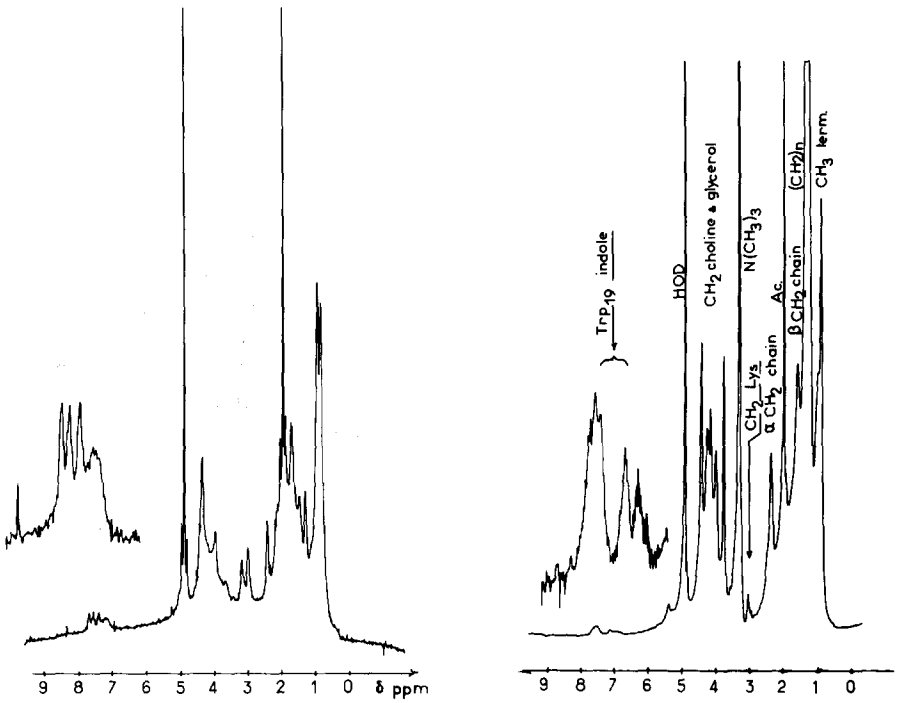

Fig. 1. 270 MHz ^{1}H-NMR spectra of melittin purified according to Mollay et al. [14], and twice lyophilized in ^2H_2O remove exchangeable protons. Fourrier transform spectra were obtained on a Brücker spectrometer, routinely 500 scans were collected at a repetition rate of 5 s. The sharp line at 1.93 ppm is due to residual acetate ions (Ac). Inserts: expanded aromatic region.

Fig. 2. 270 MHz ^{1}H-NMR spectrum of melittin-lysophosphatidylcholine complexes. Protein to lipid molar ratio is 0.1. Insert shows the expanded aromatic region. Lipid asignents according to Lee et al. [21] are indicated; the resolved lines only due to melittin protons are underlined.

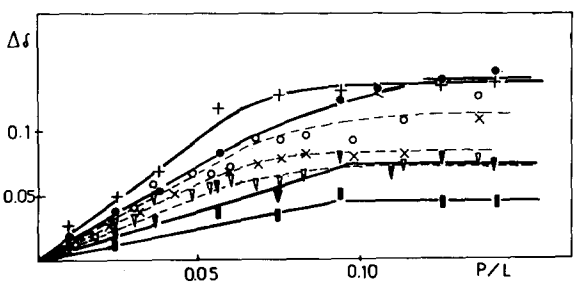

Fig. 3. Changes in chemical shifts induced by melittin interaction on lysophosphatidylcholine protons. P/L is the melittin to lipid molar ratio. (a) 0 M NaCl medium: o- - -o, α CH_2 from the ester group; x- - -x, β CH_2 from the ester group; ▽ - - - ▽, CH ethylenic. (b) 1.5 M NaCl medium: ●—●, α CH_2 from the ester group; +—+, β CH_2 from the ester group; ▼—▼, CH ethylenic; ■—■, CH_3 terminal.

ethylenic protons also shifts by 0.07 ppm. The main peak from unresolved $(CH_2)_n$ and the methyl terminal one are affected to a lesser extent 0.05 and 0.04 ppm. The lines due to the polar head group protons from the glycerol backbone to the choline methyl groups are not shifted at all.

Similar experiments have been carried out in 1.5 M NaCl in order to investigate the binding to lysophosphatidylcholine of melittin associated as a tetramer. The results obtained are shown in Fig. 3 and are very similar to those without added salt. The same lipid proton lines shifted almost to the same extent, the Trp_{19} indole positions are very close to those previously obtained. Furthermore, in the high field region, the upfield shifts of the methyl groups of melittin associated as a tetramer are no longer detected. So the binding to lipids occurs whatever the association state of melittin; moreover, the bound state of the peptide could be the same in both cases if it is supposed that Trp_{19} is a good indication of the structure.

Finally, it has to be emphasized that narrow lines are observed at average positions, whatever the lipid to protein molar ratio. Then, a fast exchange occurs between the free and bound states both for melittin and the lipids. The concomitant changes in the indole spectrum and the upfield shifts of methylene lipid protons can be interpreted by a ring current effect. So close contact must exist between Trp_{19} and the first methylene groups of the aliphatic chains. This conclusion, which agrees with the conclusions of fluorescence studies [11,12], is a new argument which reinforces the idea that the hydrophobic region of melittin is in contact with the aliphatic chains of the lipids, whereas the polar 21—26 region is in contact with the polar head group of the phospholipids.

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References

1 Hauser, H., Finer, E.G. and Chapman, D. (1970) J. Mol. Biol. 53, 419—433
2 Hsu, M. and Chan, S.I. (1973) Biochemistry 12, 3872—3876
3 Lau, A.L.Y. and Chan, S.I. (1974) Biochemistry 13, 4942—4946
4 Epand, R.M., Jones A.J.S. and Stayer, B. (1977) Biochemistry 16, 4360—4368
5 Dufourcq, J., Bernon, R. and Lussan, C. (1976) Biochim. Biophys. Acta 433, 252—263
6 Brown, L.B. and Wutrich, K. (1977) Biochim Biophys. Acta 468, 389—410
7 Hauser, H. (1975) FEBS Lett. 60, 71—75
8 Andrews, A.L., Atkinson, D., Baratt, M.D., Finer, E.G., Hauser, H., Henry, R., Leslie, R.B., Owers, N., Philipps, M.C. and Robertson, R.N. (1976) Eur. J. Biochem. 46, 549—563
9 Fergenson, G.W., Meers, P. and Kingsley, P.B. (1977) Biochim. Biophys. Acta 471, 487—491
10 Habermann, E. (1972) Science 177, 314—322
11 Mollay, C. and Kreil, G. (1973) Biochim. Biophys. Acta 316, 196—203
12 Dufourcq, J., Faucon, J.F. (1977) Biochim. Biophys. Acta 467, 1—11
13 Habermann, E. and Reiz, K.C. (1965) Biochim. Z. 343, 192—203
14 Mollay, C., Kreil, G. and Berger, H. (1976) Biochim Biophys. Acta 426, 317—324
15 MacDonald, C.C. and Philipps, W.D. (1969) J. Am. Chem. Soc. 91, 1513—1521
16 Roberts, C.J.K. and Jardetsky, O. (1970) Adv. Protein Chem. 24, 448
17 Jentsch, J. (1969) Z. Naturforsch. 246, 33—35
18 Dawson, C.R., Drake, A.F., Helliwell, J. and Hider, R.C. (1978) Biochim. Biophys. Acta 510, 75—86
19 Formisano, S., Johnson, M.L. and Edelhoch, H. (1978) Biochemistry 17, 1468—1473
20 Formisano, S., Johnson, M.L. and Edelhoch, H. (1978) Proc. Natl. Acad. Sci. U.S. 74, 3340—3344
21 Lee, A.G., Birdsall, N.J.M., Metcalfe, J.C. (1974) Methods in Membrane Biology (Korn, E.D., ed), Vol. 2, Plenum Press, New York