THE SELF-ASSOCIATION OF MELITTIN AND ITS BINDING TO LIPIDS

An intrinsic fluorescence polarization study

J. F. FAUCON, J. DUFOURCQ and C. LUSSAN
Centre de Recherche Paul Pascal, Domaine Universitaire, 33405 Talence, France

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1. Introduction

Intrinsic fluorescence has proved to be very useful for studying protein–protein or lipid–protein interactions [1–8]. Although these studies are mostly restricted to the analysis of intensity or wavelength changes in the emission spectra, polarization measurements can afford valuable information, as shown in the self-association of apo-lipoproteins [7,8] and the interaction of glucagon with lipids [5,6]. Here, intrinsic fluorescence polarization is applied to the study of the self-association of mellitin, and of its binding to lipids. Melittin is a small amphipathic peptide of 26 residues, extracted from bee venom, which is known to have a direct lytic activity on living cells [9–11]. It contains only one fluorescent residue, Trp_{19}, and its emission spectrum is very sensitive to interactions with lipids [1,3].

2. Materials and methods

Melittin was purchased from Serva, and was used without further purification. Glucagon and dipalmitoylphosphatidylcholine (DPPC) were supplied by Sigma, dipalmitoylphosphatidylglycerol (DPPG) by Serdary Res. Labs and phosphatidylserine (PS) by Lipid Products. Lysolecithin was prepared by the action of phospholipase A_2 on egg yolk lecithin, according to [12].

All the experiments were performed in a 20 mM phosphate buffer (pH 7.5) containing 1 mM EDTA to inactivate any trace of phospholipase A_2 which could contaminate the melittin sample [3,4,13]. The preparation of liposomes and the binding experiments were carried out as in [3,4].

Fluorescence polarization measurements were done with an apparatus built in the laboratory, following the scheme proposed in [14]. The studied samples were generally rather turbid, the binding of melittin inducing a breakdown of the vesicular lipid structure [11]. So, it has been controlled that turbidity does not perturb the polarization measurements either by varying the lipid concentration or by using fluorescence cuvettes of 0.5 cm pathlength.

3. Results

3.1. *Self-association of melittin*

Until now, it was generally recognized that melittin exists in solution in a tetrameric form [10,13,15]. However, results presented in fig.1a show that the behaviour of melittin is not so simple. An increase of the ionic strength leads to an enhancement of the degree of polarization P which reaches a plateau at ~1–2 M NaCl. Moreover, this effect depends on the melittin concentration, which is directly confirmed by the results in fig.1b: P increases regularly, at fixed ionic strength, when the concentration of melittin is enhanced.

This change of P can be attributed to a decrease of the rotation rate of the Trp residue and, since it occurs at constant viscosity and temperature, it has to be related to a change in the aggregation state of the peptide. Assuming that the complex is spherical and that the intramolecular rotation rate of Trp remains constant, Perrin's equation [16]:

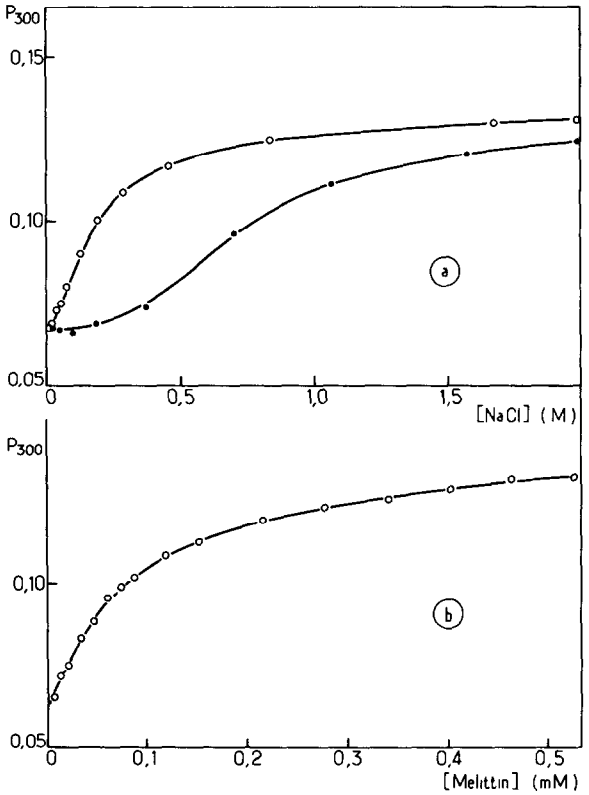

Fig 1a Effect of the NaCl concentration on the degree of polarization P of melittin at 20 μM (-•-) and 200 μM (-○-) Temp , 25°C Excitation wavelength, 300 nm Fig 1b Degree of polarization P as a function of the melittin concentration Phosphate buffer 20 mM (pH 7 5), EDTA 1 mM, NaCl 0 3 M Temp , 25°C Excitation wavelength, 300 nm

$$(P^{-1} - 1/3) = (P_0^{-1} - 1/3)(1 + RT\tau/\eta V)$$

can then be applied, and it is easy to show that the initial (V_i) and final (V_f) volumes of the fluorescent species are related to the corresponding P_i and P_f values by $(V_f/V_i) = (P_i^{-1} - P_0^{-1})/(P_f^{-1} - P_0^{-1})$ at constant temperature T, viscosity η and lifetime τ It has been verified that the total fluorescence intensity is not markedly changed upon increasing ionic strength, and so, τ can effectively be considered as constant P_0 has been measured using a solution of melittin in glycerol, and found equal to 0 294 when the excitation wavelength is 300 nm The initial P_i and final P_f values can be evaluated from the results of fig 1b and are found, respectively, equal to 0 065 and 0 145, which leads to $(V_f/V_i) \simeq 3\,4$ Thus, it seems that tetramers of melittin dissociate into monomers at low ionic strength and/or peptide concentration

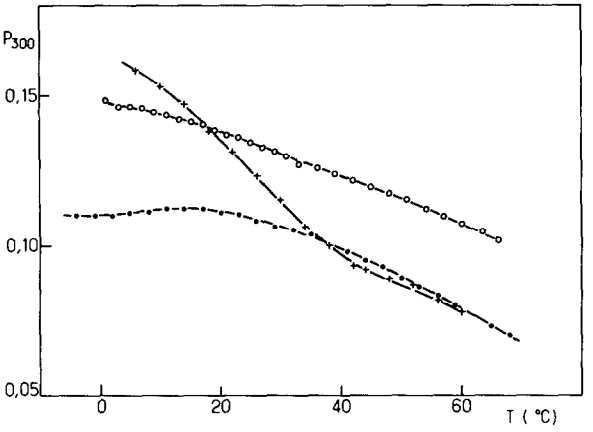

Fig 2 Temperature dependence of the degree of polarization P of melittin at 120 μM (-•-) and 583 μM (-○-), in phosphate buffer 20 mM (pH 7 5), EDTA 1 mM, NaCl 0 3 M Glucagon 64 μM (- + -) in K_2HPO_4 0 76 M (pH 10 6) Excitation wavelength, 300 nm

The degree of polarization of melittin is plotted in the fig 2 as a function of temperature At high NaCl (0 3 M) and peptide (580 μM) concentrations melittin is mainly in the tetrameric form, and P decreases monotonously from −4°C to 65°C On the contrary, at lower melittin concentration (120 μM), monomers and tetramers coexist in notable proportions (see fig 1b), then P slightly increases between −4°C and 25°C Such an anomalous variation has been observed in the case of apolipoproteins [7,8] and it can only be explained by assuming that, in this temperature range, the expected increase of the rotational mobility of Trp residues is compensated for by a displacement of the association equilibrium promoting the formation of tetramers For comparison, results obtained with glucagon, which is known to form a trimer in solution [17,18], are also plotted in the fig 2 In this case, P decreases very rapidly when temperature is raised, in agreement with previous results showing a 10^3-fold decrease of the association constant between 5°C and 50°C [19]

3 2 *Binding of melittin to phospholipids*

In the presence of phospholipids, an increase of P

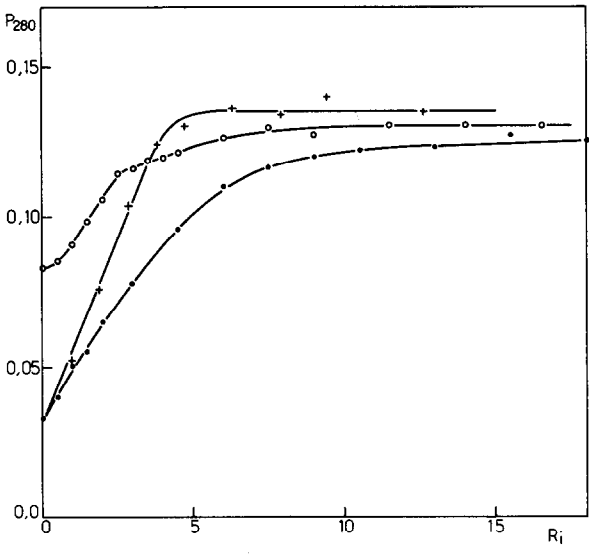

Fig.3. Degree of polarization P of melittin versus the lipid : protein molar ratio R_i: (- + -) phosphatidylserine and (-•-) lysolecithin in phosphate buffer 20 mM (pH 7.5), EDTA 1 mM; (-○-) lysolecithin in NaCl 2 M. Excitation wavelength, 280 nm.

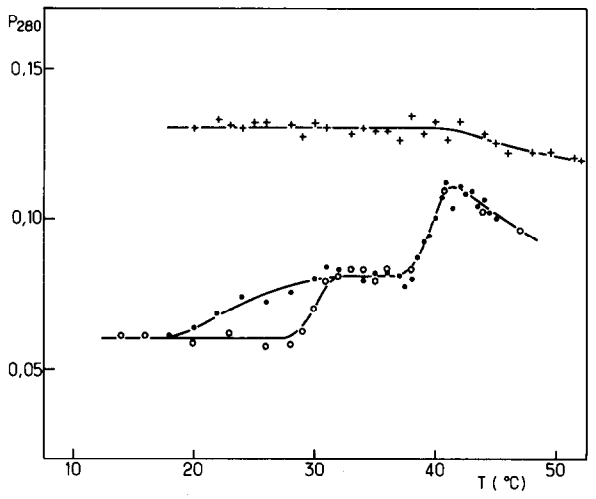

Fig.4. Temperature dependence of the degree of polarization P of melittin 20 μM in the presence of DPPG (R_i 48) (- + -) and DPPC (R_i 61). (-•-) Cooling curve; (-○-) heating curve. Excitation wavelength, 280 nm.

is observed, as in the case of glucagon [5,6], due to the formation of large size lipid–protein complexes (fig.3). As shown in [3], the stoichiometry of the complexes depends upon the net charge of the phospholipid: it is of 4–5 and ~10 lipid molecules/protein, respectively, in the case of negatively charged PS and zwitterionic lysolecithin. Binding occurs even in 2 M NaCl, which shows:

(i) That hydrophobic interactions are involved in the stabilization of the complex;

(ii) That tetramers of melittin may interact with lipids as well as monomers.

Moreover, although the shape of the binding curve is more complex in the case of tetramer, the P values obtained at the plateau are very close in both cases, which could indicate that the structures of the lipid–protein complexes are quite similar, in agreement with a recent NMR study [20].

The effect of the lipid fluidity has been investigated using zwitterionic DPPC and negatively charged DPPG, both lipids having a melting transition temperature (T_c) centered at 41°C. The binding of melittin was first performed above T_c, around 45°C, then the temperature dependence of P was recorded (fig.4). Only a very slight effect on P is observed at T_c in the case of the melittin–DPPG complex. On the contrary, in the case of DPPC, the changes of P centered around 41°C and 30°C lead to the unexpected conclusion that the rotational mobility of Trp residues increases when the lipid undergoes the liquid to gel transition. This could be due either to a vertical displacement of melittin with respect to the bilayer, or to the dissociation of the complex. It seems that a release of melittin in the solution is at least in part responsible for the observed phenomenon, since the precipitation of DPPC by centrifugation at 20°C allows the recovery of most of the melittin unbound in the supernatant. The anomalous variation of P around 30°C, which is probably related to the pretransition of DPPC, occurs very slowly as a function of time (several hours) upon decreasing temperature. This may explain the apparent discrepancy between heating and cooling curves, and why the release of melittin has not been previously detected [3,21,22].

4. Discussion

One of the main conclusions which arises from the above results is that intrinsic fluorescence polarization

allows us to obtain interesting and complementary information on the self-association as well as on the binding to lipids of melittin Concerning the first point, it is clear that melittin gives rise in solution to a monomer ⇆ tetramer equilibrium depending on the ionic strength and on the temperature of the medium This equilibrium has also been detected in the laboratory by many other techniques gel filtration, ultracentrifugation, light scattering (in preparation)

On the other hand, the above results show that melittin interacts with zwitterionic and negatively charged lipids in very different ways the physical state of the lipids effectively controls the binding to zwitterions, whereas it has practically no effect in the case of negatively charged lipids The partial reversibility of the interaction with DPPC indicates that the effect of lipid fluidity is not only kinetic, as proposed in [1,3] but it results in a drastic decrease in the affinity of melittin towards phosphatidylcholines in the gel state The behaviour of melittin is then opposite that of glucagon, which is known to interact preferentially with dimyristoylphosphatidylcholine below T_c [6] Finally, it has to be noticed that the pretransition of DPPC seems to play an important role in the dissociation of the complex, which means that the chain fluidity is not the only implicated parameter

Acknowledgements

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References

[1] Mollay, C and Kreil, G (1973) Biochim Biophys Acta 316, 196–203
[2] Dufourcq, J , Faucon, J F , Lussan, C and Bernon, R (1975) FEBS Lett 57, 112–116
[3] Dufourcq, J and Faucon, J F (1977) Biochim Biophys Acta 467, 1–11
[4] Dufourcq, J and Faucon, J F (1978) Biochemistry 17, 1170–1176
[5] Edelhoch, H (1976) in Biochemical Fluorescence (Chen, R F and Edelhoch, H eds) vol 2, pp 545–571, Marcel Dekker, New York
[6] Epand, R M , Jones, A J S and Schreier, S (1977) Biochim Biophys Acta 491, 296–304
[7] Gwynne, J , Palumbo, G , Osborne, J C , jr, Brewer, H B and Edelhoch, H (1975) Arch Biochem Biophys 170, 204–212
[8] Osborne, J C , jr, Palumbo, G , Brewer, H B and Edelhoch, H (1975) Biochemistry 14, 3741–3746
[9] Habermann, E and Jentsch, J (1967) Hoppe-Seyler's Z Physiol Chem 348, 37–50
[10] Habermann, E (1972) Science 177, 314–322
[11] Sessa, G , Freer, J H , Colacicco, G and Weissmann, G (1969) J Biol Chem 244, 3575–3582
[12] Kates, M (1975) in Techniques of Lipidology (Work, T S and Work, E eds) 2nd edn, North-Holland, Amsterdam
[13] Shipolini, R A , Callewaert, G R , Cottrell, R C , Doonan, S , Vernon, C A and Banks, B E C (1971) Eur J Biochem 20, 459–468
[14] Weber, G and Bablouzian, B (1966) J Biol Chem 241, 2558–2561
[15] Dawson, C R , Drake, A F , Helliwell, J and Hider, R C (1978) Biochim Biophys Acta 510, 75–86
[16] Perrin, F (1929) Ann Phys 12, 213–275
[17] Gratzer, W B and Beaven, G H (1969) J Biol Chem 244, 6675–6679
[18] Formisano, S , Johnson, M L and Edelhoch, H (1978) Biochemistry 27, 1468–1473
[19] Formisano, S , Johnson, M L and Edelhoch, H (1977) Proc Natl Acad Sci USA 74, 3340–3344
[20] De Bony, J , Dufourcq, J and Clin, B (1979) Biochim Biophys Acta 552, 531–533
[21] Mollay, C (1976) FEBS Lett 64, 65–68
[22] Verma, S P and Wallach, D F H (1976) Biochim Biophys Acta 426, 616–623