Structure–Immunogenicity Relationship of Melittin and Its N-Terminal Truncated Analogs†

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ABSTRACT: Melittin is an amphipathic 26-residue peptide from bee venom. We showed previously that, in the murine system, melittin has one major B-cell epitope in the hydrophilic region of residues 21–26 and one T-cell epitope in the hydrophobic midregion of 11–19. In this paper we compared the immunogenicity and the biophysical properties of a series of melittin analogs which differ by stepwise two-residue truncation in the N-terminus of residues 2–10. All analogs retain the B- and T-cell epitopes of melittin. However, the analogs which have more than two residues deleted at the N-terminus are nonimmunogenic for antibody responses although they are immunogenic for T-cell responses. The analogs were found to differ in their hemolytic activity, helical content, and oligomer formation in different solvents. These results support the hypothesis that the immunogenicity of melittin for antibody response is associated with its binding to cell membranes followed with oligomer formation but its immunogenicity for T-cell response is not.

The most common stinging insect allergy is caused by honeybees. Honeybee venom contains several proteins and peptides (Habermann, 1972). All bee-allergic patients have IgE antibodies specific for the bee venom proteins hyaluronidase and phospholipase. About one-third of patients have IgE specific for a venom peptide, melittin, but none has been reported to have IgE specific for the other venom peptides, apamin or MCD (Mackeler et al., 1972; King et al., 1976; Paull et al., 1977; Kemeny et al., 1983). The allergenicity of melittin is interesting as it is a small peptide of 26 residues while the other two peptides, apamin and MCD, of similar size of 18 and 22 residues, respectively, are not allergenic. Melittin comprises about 50% of dry venom weight, and it has cytolytic activity (Habermann, 1972); as well, it can form voltage-gated ion channels in cell membranes (Tosteson et al., 1990). Apamin and MCD comprise 2% each of venom weight and have neurotoxic and mast cell degranulating activities, respectively.

Melittin induces IgE and IgG responses in selected strains of mice irrespective of the adjuvant used (King et al., 1984; Fehlner et al., 1991b). Our previous studies with melittin analogs which varied in length and composition at the C-terminus suggested that its immunogenicity depends on its binding to cell membranes. Its T- and B-cell epitopes are mapped to the middle and the C-terminal regions of residues 10–19 and 20–26, respectively (Fehlner et al., 1991a; King et al., 1984). These regions correspond to the buried and the exposed portions of the molecule in one proposed model of melittin binding to the cell membrane, as described below.

Because of its distribution of polar and nonpolar amino acid residues (Figure 1), melittin has both linear sequence and helical amphipathicity. This peptide has a random coil conformation in dilute aqueous solution and it assumes α-helical conformation in crystals (Terwilliger & Eisenberg, 1982) and in aqueous solution at high peptide concentration or in hydrophobic environments like alcohols or lipid bilayers (Talbot et al., 1979). Melittin has a tetrameric structure in

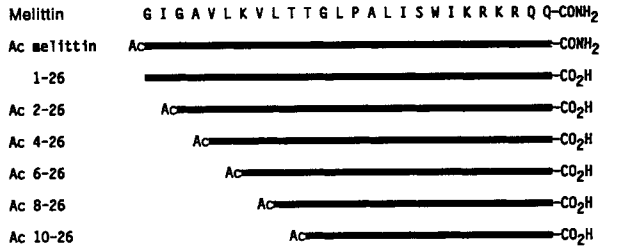

FIGURE 1: Amino acid sequences of melittin and its analogs.

crystals which is formed by criss-crossing a pair of two antiparallel monomers, so that the hydrophobic sides of the α-helix are removed from the aqueous phase and the cationic C-termini of the chains are on opposing ends to minimize charge repulsion.

Various models of melittin binding to cell membranes have been suggested to account for its cytolytic activity; melittin in monomer or oligomer form lies parallel and/or perpendicular to the lipid bilayer (cf. Dempsey, 1990). In one suggested model, the amphipathic helix cylinder of residues 1–20 of melittin traverses the lipid bilayer and the cationic C-terminal region of residues 21–26 is anchored on the surface of lipid bilayer by ionic attraction with the acidic polar lipid head groups (Vogel & Jahnig, 1986). The helix cylinders associate with each other to form a tetramer or higher oligomer with the hydrophobic face of the helix cylinder facing the lipid bilayer and the hydrophilic faces facing each other to form hydrophilic pores. Ion leakage occurs through the pores and the resulting osmotic change leads to cell lysis.

In this report we investigate the immunogenicity of a series of melittin analogs which differ at the N-terminus by stepwise two-residue truncation so that the role of the N-terminal residues 1–10 in antigen presentation can be assessed. The amino acid sequences of these melittin analogs are given in Figure 1. Melittin and synthetic peptide 1–26 both have a free α-amino group at their N-termini but they have respectively a carboxamide or a carboxyl group at their C-termini. Ac-melittin has both the N- and C-termini modified. The truncated synthetic analogs have acetylated α-amino groups at their N-termini and free carboxyl groups at their C-termini.

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MATERIALS AND METHODS

Melittin and Its Analogs. Melittin (Figure 1) was isolated from honey bee venom by gel filtration on Sephadex G50 in 0.05 M NH_4Ac buffer of pH 4.75 (King et al., 1976) followed by ion-exchange chromatography on CM-cellulose with a linear gradient of 0.02–0.30 M NH_4Ac buffer of pH 4.75 in 4 M urea (Maulet et al., 1980; Fehlner et al., 1991b). This allowed the resolution of the free and N^{α}-acetyl forms of melittin. Peptide 1–26 and the truncated analogs were prepared by the Rockefeller University Sequencing Facility with an Applied Biosystems 430A synthesizer (Foster City, CA). These analogs were purified in 7-mg amounts by reversed-phase chromatography on a 10- × 0.9-cm column of protein and peptide C18 Silica (The Separation Group, Hesperia, CA) with a linear 2-propanol gradient of 0.33%/mL in 0.1% trifluoroacetic acid at a flow rate of 60 mL/h. All peptides were characterized by amino acid analysis (within 5% of the expected composition) and by electrospray mass spectrometry (within 1 mass unit of the calculated molecular weight).

Biophysical Characterization of Peptides. Hemolysis assay was carried out as described (Fehlner et al., 1991b). Briefly, 100 μL of 4×10^8 murine red blood cells/mL were incubated at 37 °C in the presence of varying concentrations of test peptide in phosphate-buffered saline. After 30 min the cells were pelleted and the supernatants were diluted 1/100 with buffer for hemoglobin determination by absorbance at 415 nm. Phosphate-buffered saline contains 143 mM NaCl and 7.5 mM sodium phosphate (pH 7.2).

Circular dichroism (CD)[1] spectra were taken in cells of 1- and 5-mm optical path on an Aviv 62DS spectrometer, and the solvents used were phosphate-buffered saline or "in micelle", which is phosphate-buffered saline containing 10 mM hexadecylphosphorylcholine (R. Berchtold, Bern, Switzerland).

To determine the degree of noncovalent association of melittin or its analogs in different solvents, the peptides were cross-linked in the presence of 0.1% glutaraldehyde at ambient temperature for 30 min and then subjected to electrophoresis in a 12% polyacrylamide gel containing 0.4% sodium dodecyl sulfate and 8 M urea under standard conditions (Swank & Munkres, 1971). The presence of melittin peptides in gels was detected by Coomassie blue G-250 staining. Alternatively the peptides were electroblotted onto nitrocellulose paper for immunostaining by the catalyzed reporter deposition method (Bobrow et al., 1991).

Electroblotting was done in a Model TE 70 Semi-Phor semidry transfer unit (Hoeffer Scientific Instruments, San Francisco, CA) following the manufacturer's directions. After blotting, the nitrocellulose paper was kept for 0.5 h in a diluent buffer of 0.1 mg/mL bovine serum albumin, 0.5 mg/mL Tween 20, and 0.5 M NaCl in 0.05 M Tris-HCl buffer (pH 7.95). The paper was then immunostained by incubation in six successive steps with 1/400 diluted mouse serum specific for peptide 1–26 (described below), 10 μg/mL biotinylated rabbit anti-mouse IgG, 4 μg/mL avidin–horseradish peroxidase (Sigma, St. Louis, MO), 1/1000 diluted biotinyl tyramide solution in 0.01% H_2O_2 + 0.05 M Tris-HCl (pH 7.95) (DuPont Biotechnology, Boston, MA), 4 μg/mL avidin–horseradish peroxidase, and substrate. The substrate was a solution of 1.5 mg/mL 4-chloronaphthol and 0.01% H_2O_2 in 0.05 M Tris-HCl (pH 7.95). The incubation periods were 1 h each for steps 1–3, 0.5 h each for steps 4 and 5, and about 15 min for step 6. The paper was washed for 10 min in diluent buffer between the steps.

Immunization and Immunoassays. Groups of three or four female BALB/c mice at 8–10 weeks of age (The Jackson Laboratories, Bar Harbor, ME) were immunized intraperitoneally each with 0.2 mL of 10 nmol/mL peptide in 0.05 M phosphate buffer, pH 6.0, + 5 mg/mL alum at weeks 0, 2, 4, 6, 8, and 10. Sera were collected for antibody assay by retroorbital plexis puncture 1 week after each immunization. Or spleen cells were collected 9–10 days after two or four immunizations for proliferation assay in culture medium containing varying concentrations of stimulating peptide as described in our previous publication (Fehlner et al., 1991a). The culture medium consisted of RPMI 1640 (Gibco, Grand Island, NY), 1% normal mouse serum, 100 units/mL penicillin, 100 μg/mL streptomycin (Gibco), 10 mM HEPES buffer, pH 7.3 (Sigma Chemical Co., St. Louis, MO), and 54 μM 2-mercaptoethanol.

Melittin- or analog-specific IgGs were analyzed by solid-phase enzyme immunoassays (ELISA), and the amount of specific IgGs was estimated by reference to a monoclonal melittin-specific mouse IgG (King et al., 1984). Briefly, microtiter wells were coated overnight with 5 nmol/mL melittin or its analog in 0.05 M Tris-HCl (pH 8.0) and then the remaining reactive sites of wells were blocked with diluent buffer, the composition of which was given earlier. The wells were then incubated for 1-h periods in succession with varying dilutions of mouse sera or antibody to be tested, 10 μg/mL rabbit antibody specific for mouse IgGs, and 2 μg/mL sheep antibody specific for rabbit IgG conjugated with horseradish peroxidase. Finally the bound peroxidase was detected by A_{490} after incubation with a substrate solution of 16 mg/mL phenol, 0.5 mg/mL 4-aminoantipyrine, and 0.005% H_2O_2 in 0.1 M sodium phosphate (pH 7.0).

Peptide inhibition of enzyme immunoassay was done as described (King et al., 1984). The diluted sera were first incubated with varying concentrations of test peptide in diluent buffer and then analyzed by ELISA as given above. Serum dilution was chosen at which there was about 30% maximal binding of antibodies to solid-phase antigen.

Specific IgEs were analyzed by passive cutaneous anaphylaxis in rat (Ovary et al., 1975). Skin sites on the back of a shaved rat were subcutaneously sensitized with 0.1-mL aliquots of serially 5-fold diluted mouse sera. After 4 h, the rat was challenged intravenously with 1 mL of 1 mg/mL melittin or peptide 1–26 + 5 mg/mL Evans Blue in phosphate-buffered saline. Specific IgE titer represents the highest reciprocal dilution which gives a positive blue-colored skin reaction.

RESULTS

Immunogenicity of Melittin and Its Analogs for Antibody Responses. Groups of three to four BALB/c mice were immunized with six biweekly injections of peptide and alum. Sera were collected 1 week after each immunization for antibody analysis. In Table I are summarized the results obtained at weeks 7, 9, and 11. Only melittin and its acetyl derivatives peptides 1–26 and Ac 2–26 gave specific IgE and IgG responses, and the response to peptide Ac 2–26 is 1% or less of that to peptide 1–26. All other truncated analogs gave no response. As indicated in Table I, the results were obtained with duplicate sets of mice. The different results from duplicate sets of mice, e.g., groups B1 and B2 or C1 and C2, are not due to experimental error in antibody determination,

[1] Abbreviations: CD, circular dichroism; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin.

Table I: Antibody Responses to Melittin and Its Analogs

group[a]	immunogen	μg/mL specific IgG[b] week 7	week 9	week 11	titer of specific IgE,[c] week 7
A1	Ac-melittin	78	180	240	nd
A2	melittin	nd	nd	nd	nd (320)
B1	1–26	45	78	130	nd
B2	1–26	180	420	320	160 (40)
C1	Ac 2–26	0.06	0.07	0.5	nd
C2	Ac 2–26	0.08	1.5	3.0	<5
D1	Ac 4–26	<0.03	<0.03	<0.02	nd
D2	Ac 4–26	<0.01	<0.01	<0.01	<5
E1	Ac 6–26	<0.03	<0.03	<0.03	<5
E2	Ac 6–26	<0.01	<0.01	<0.01	<5
F1	Ac 8–26	<0.01	<0.01	<0.01	<5
F2	Ac 8–26	<0.01	<0.01	<0.01	<5
G1	Ac 10–26	<0.03	<0.03	<0.03	<5

[a] Groups of 2–4 mice were each immunized intraperitoneally with 2 nmol of peptide + 1 mg of alum at weeks 0, 2, 4, 6, 8, and 10. [b] Specific IgGs for group A and for groups B–G were measured by enzyme immunoassay on solid-phase melittin or peptide 1–26, respectively (nd signifies not determined). [c] Specific IgE titers were measured by passive cutaneous anaphylaxis on challenge with melittin (in parentheses) or with peptide 1–26.

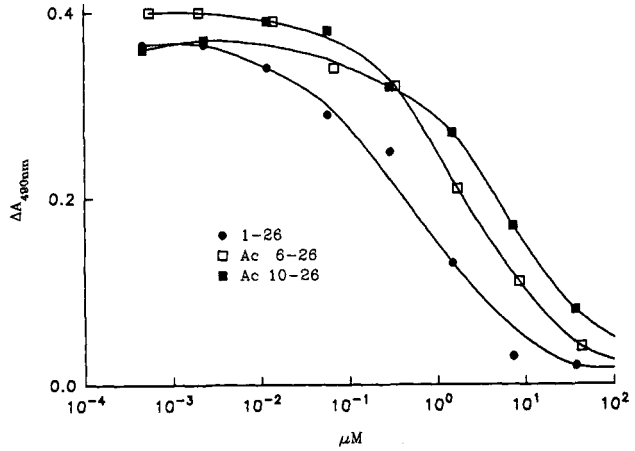

FIGURE 2: Inhibition of antibody binding to solid-phase peptide 1–26 by solution-phase peptides 1–26, Ac 6–26, or Ac 10–26. These results were obtained by ELISA with 1/20 000-diluted sera from mice after four biweekly immunizations of peptide 1–26.

which is <15%, and most likely they represent variations in mice which were obtained at different time intervals.

The specific IgE titers for group B2 mice in Table I were found to be 160 and 40 when challenged with peptide 1–26 and melittin, respectively. This difference probably reflects the antibody specificity as the group B2 mice were immunized with peptide 1–26. We have shown previously that melittin-specific IgGs bound melittin and peptide 1–26 with different affinities but peptide 1–26 specific IgGs did not show this difference (Fehlner et al., 1991b). Previous studies showed that IgG response to melittin or peptide 1–26 consisted of >90% IgG_1 isotype (Fehlner et al., 1991b).

Although truncated analogs shorter than peptide Ac 2–26 failed to elicit antibody response, they were shown to retain the B-cell epitope of peptide 1–26. This is demonstrated by inhibition analysis in Figure 2. Peptides 1–26, Ac 6–26, or Ac 10–26 inhibited the binding of peptide 1–26 specific antibodies to solid-phase peptides 1–26 with similar affinity. Similar findings were obtained with the other analogs; their results are not given. Specific IgGs for groups B–G in Table I were determined by ELISA on solid-phase peptide 1–26 and nearly the same values were obtained on solid-phase truncated peptides (results not shown). These findings also can be taken

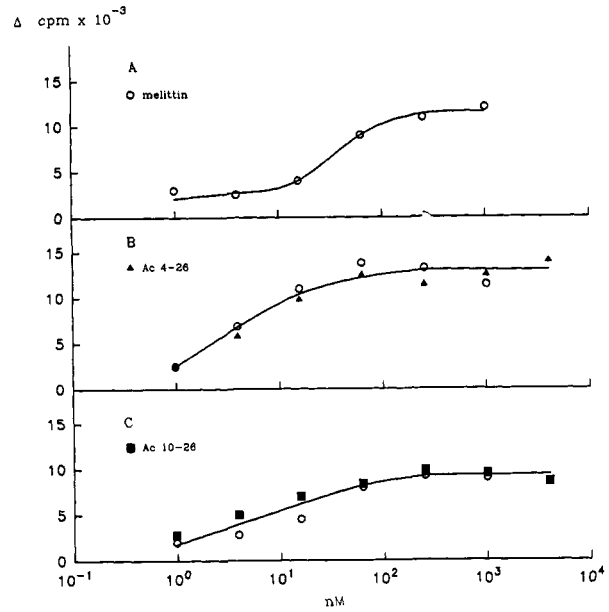

FIGURE 3: Proliferation assay of spleen cells from mice immunized with melittin, peptide Ac 4–26, or peptide Ac 10–26 (patterns A, B, and C, respectively). Spleen cells (2×10^5) were cultured with varying concentrations of peptide in 0.2 mL of medium, ^{3}H-thymidine was added on day 3, and the uptake of ^{3}H-thymidine was counted on day 4. Background proliferation was 4000 ± 500 cpm.

to demonstrate that the truncated peptides retain the B-cell epitope of peptide 1–26.

Immunogenicity of Melittin and Its Analogs for T Cell Responses. Mice were given four biweekly immunizations as described above, and 9–10 days after the last immunization their spleen cells were used to test T-cell responses by proliferation assay. The results obtained from mice immunized with melittin and peptides Ac 4–26 and Ac 10–26 are given in Figure 3. The same degree of proliferation was obtained on stimulation with the immunogen or melittin. Similar results were obtained with spleen cells specific for the other analogs (results not shown). Similar results were also obtained with mice which were given only two biweekly immunizations of the truncated analogs. There is one possible exception that cells from mice immunized twice with peptide Ac 10–26 gave about half the maximal proliferative response of that shown in Figure 3. The findings together indicate that the truncated analogs retain the T-cell epitope of melittin as well as its immunogenicity for T-cell responses.

Biophysical Studies of Melittin and Its Analogs. The membrane-binding activities of melittin and its analogs are compared by their lysis of mouse red blood cells. These results are given in Table II. Shortening of the N-terminal region of melittin led to a marked decrease in its lytic activity.

Melittin or Ac-melittin and its truncated analogs were found to have concentration-dependent circular dichroism spectra in phosphate-buffered saline. These spectra are not shown, but their mean residue ellipticity change at 222 nm as a function of concentration is given in Table II and Figure 4A. Compared to Ac-melittin, peptides 1–26, Ac 2–26, and Ac 4–26 show much less concentration-dependent ellipticity change. The concentration-dependent ellipticity change for melittin is known to be associated with oligomer formation (Talbot et al., 1979) as melittin has the α-helical conformation in its oligomer structure. Peptide 1–26 has a lower helical content than that of melittin, Ac-melittin, or peptide Ac 2–26. This difference is probably related to helix destabilization by its charged N- and C-terminal residues (Shoemaker et al., 1987).

Table II: Hemolytic Activities and Percent Helix of Melittin and Its Truncated Analogs

peptide	hemolysis[a] concn for 50% lysis (μM)	hemolysis[a] relative activity	$-\theta_{222} \times 10^{-4}$, % helix[b] PBS	$-\theta_{222} \times 10^{-4}$, % helix[b] micelle
melittin	4	1	1.12, 36	nd
Ac-melittin	4	1	1.02, 38	2.90, 82
1–26	5	8×10^{-1}	0.40, 18	2.34, 68
Ac 2–26	4	1	0.50, 21	2.40, 69
Ac 4–26	34	1.2×10^{-1}	0.37, 17	2.24, 65
Ac 6–26	210	1.9×10^{-2}	0.37, 17	2.06, 61
Ac 8–26	680	5.8×10^{-3}	0.35, 17	2.40, 69
Ac 10–26	>5000	$<8 \times 10^{-4}$	0.33, 16	2.30, 67

[a] Data for peptide 1–26 is taken from Fehnler et al. (1991a). [b] θ_{222} values are for 60 μM peptide in phosphate-buffered saline or in micelle which is 10 mM hexadecylphosphorylcholine; there is one exception that 200 μM melittin was used. Percent helix was estimated from θ_{222} using the formula $(\theta_{222} - \theta_{random})/(\theta_{helix} - \theta_{random})$, where θ_{helix} and θ_{random} represent θ_{222} values of −36 000 and 3000 deg cm^2/(dmol of residue) for helical and random coil peptides, respectively (Greenfield & Fasman, 1969).

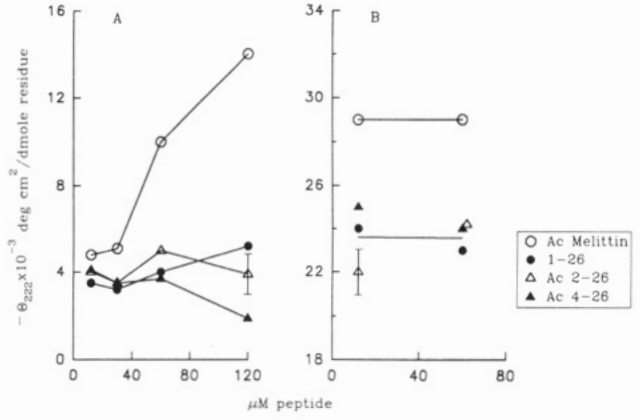

FIGURE 4: Circular dichroism data of melittin and analogs at 222 nm. Data were collected in phosphate-buffered saline (pattern A) or in phosphate-buffered saline containing 10 mM hexadecylphosphorylcholine (pattern B).

In contrast to the CD data in phosphate-buffered saline, Ac-melittin and its analogs in ethanol or in micelle were found to have CD spectra independent of their concentration in the range of 12–120 μM; the data in micelle are shown in Figure 4B. The CD spectra suggest that Ac-melittin or its analogs have high helical content in ethanol or in micelle as the spectra show the characteristic minima at about 210 and 220 nm of about equal magnitude. The CD spectra are not shown but their mean residue ellipticity values at 222 nm in micelle are given in Table II together with their estimated α-helical content. The data in ethanol are not given as they are the same as those in micelle within our experimental error of ±7%. The micelle used is a 10 mM solution of hexadecylphosphorylcholine (Lauterwein et al., 1979), and these studies were made to mimic the conditions of melittin binding to cell membrane.

To investigate the noncovalent self-association of melittin or its analogs in different solvents at pH 7, the samples were cross-linked by reaction with 0.1% glutaraldehyde and then subjected to gel electrophoresis. In Figure 5A are shown the patterns obtained with 250 μM peptide solutions in phosphate-buffered saline after staining with Coomassie blue dye. Only peptides 1–26 and Ac 2–26 (lanes 2 and 3 in Figure 5A) gave patterns similar to that of Ac-melittin (lane 1), and the data suggest relatively high concentrations of tetramers, pentamers, hexamers, and larger oligomers and low concentrations of trimers, dimers, and monomers. All other peptides gave patterns different from that of melittin. Peptides Ac 8–26

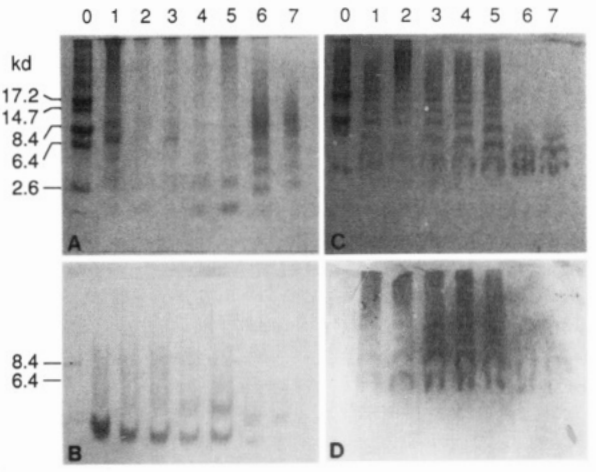

FIGURE 5: SDS–urea gel electrophoresis of melittin and analogs after cross-linking with 0.1% glutaraldehyde. In panels A and B, cross-linking was carried out with 250 μM peptide solutions in phosphate-buffered saline and in 60% ethanol containing 10 mM phosphate buffer, pH 7.1, respectively. In panels C and D, cross-linking was carried out respectively with 250 and 10 μM peptide solution in phosphate-buffered saline containing 10 mM dodecylphosphorylcholine. In panels A, B, and C, 4.4 nmol of each peptide mixture (about 12 μg) was applied and the gel was stained with Coomassie blue. In panel D, 0.18 nmol of each peptide mixture (about 0.5 μg) was applied and the gel was immunostained after electroblotting onto nitrocellulose paper. In all four patterns, lanes 0–7 contain respectively molecular weight standards, Ac melittin, and peptides 1–26, Ac 2–26, Ac 4–26, Ac 6–26, Ac 8–26, and Ac 10–26.

and Ac 10–26 (lanes 6 and 7) differ markedly from the other peptides in that they gave high concentrations of tetramers, pentamers, and hexamers and no large polymers near the origin of the gel.

In panels B and C of Figure 5 are shown respectively the cross-linking patterns for 250 μM peptide solutions in 60% ethanol containing 10 mM phosphate and in micelle after Coomassie blue staining, and in Figure 5D are shown the patterns for 10 μM peptide solutions in micelle as revealed by western blot. Comparison of Figure 5 panels B and C shows that extensive formation of oligomers only occurs in micelle and not in 60% ethanol for all peptides with the exception of peptides Ac 8–26 and Ac 10–26. Results similar to those shown in Figure 5B for 60% ethanol were also obtained in unbuffered absolute ethanol; they are not given. Comparison of Figure 5 panels C and D shows that the size distribution of oligomers depends on the peptide concentration in micelle. At 250 μM concentration in Figure 5C, peptides Ac 8–26 and Ac 10–26 (lanes 6 and 7) formed only trimers, dimers, and monomers, while melittin and the other analogs (lanes 1–5) all showed the presence of a series of oligomers of different sizes. The relative intensities of the bands suggest that peptides Ac 2–26, Ac 4–26, and Ac 6–26 show a lower extent of formation of oligomers larger than a tetramer than melittin or peptide 1–26 does. This is also seen in Figure 5D. At 10 μM concentration, both Ac-melittin and peptide 1–26 (lanes 1 and 2) appeared to form higher concentrations of oligomers larger than a tetramer than peptide Ac 2–26, Ac 4–26, or Ac 6–26 (lanes 3, 4, and 5) did. Very similar results were obtained with 6.7 μM peptide solutions (data not shown).

In Figure 5, the fastest moving band in lanes 1–7 is assigned as the monomeric peptide, and this was shown independently by tests of melittin without glutaraldehyde treatment. Melittin monomer migrated faster than expected for a peptide of its size. Comparison of Figure 5 panels A and C shows that the position of the monomer band is retarded by the presence of micelle. The melittin samples used in these experiments is

the N^{α}-acetyl derivative and similar results (not shown) were obtained with melittin.

DISCUSSION

Melittin analogs which are truncated in the N-terminal region retain the B- and T-cell epitopes of the full-length peptide. Yet they differ in their immunogenicity for antibody response but not for T-cell response. Removal of three amino acid residues from the N-terminus is sufficient to abolish the immunogenicity of melittin for antibody response. The findings suggest that the N-terminal region of residues 1–10 of melittin is required for B-cell response but not for T-cell response.

Cross-linking of membrane Igs by polyvalent antigen is believed to be important for activation of B cells so that it can lead to antibody secretion (DeFranco, 1987). Melittin has only one major B-cell epitope. Present biophysical studies suggest strongly that melittin at physiological concentrations can bind to cell membrane with subsequent oligomer formation while the truncated analogs show decreased binding and oligomer-forming activities. It is this difference which may account for the difference in immunogenicity for antibody response of melittin and its truncated analogs.

Melittin can form oligomers in aqueous solvent at concentrations of >30 μM as indicated by the CD data (Figure 4) and by the cross-linking experiment (Figure 5). However, this concentration is much higher than that which can be attained in a mouse during immunization. A maximal in vivo concentration of 0.4 μM is estimated on the assumption of an extravascular volume of 5 mL/mouse receiving 2 nmol of melittin.

Melittin can bind to cells with high affinity; the reported dissociation constant for melittin binding to red blood cells is 30 nM (Tosteson et al., 1985) and this is also indicated by the hemolytic data in Table II. The hemolytic activity of melittin has been suggested to be associated with its oligomer formation in the lipid bilayer (cf. Dempsey, 1990). With the exception of peptide Ac 2–26, the truncated peptides showed less than $^1/_{10}$ the hemolytic activity of melittin. The greatly reduced activities of the truncated analogs may reflect their decreased oligomer formation and/or that their helix segments are too short to traverse the length of the lipid bilayer of about 30 Å which requires an α-helical peptide of 20 residues.

The cross-linking experiments in Figure 5B–D indicate that melittin and peptide 1–26 each can associate to form oligomers in micelles of hexadecylphosphorylcholine but not in the hydrophobic solvent of ethanol. The truncated peptides Ac 2–26, Ac 4–26, and Ac 6–26 retain the property of self-association and they are of reduced tendency when compared to peptide 1–26 or melittin. But peptides Ac 8–26 and Ac 10–26 have lost this property.

Knoeppel et al. (1979) have reported that melittin showed increased helix content in micelles of deoxycholate or Brij when compared to melittin in aqueous buffer and that cross-linking of melittin in aqueous buffer led to predominant formation of tetramers but only monomer was observed in micelles of deoxycholate or Brij. Their findings together with ours suggest that the formation of melittin oligomers in micelles of hexadecylphosphorylcholine, or in lipid bilayers, depends not only on the peptide chain length and its helical conformation but also on the proper charge interaction of the positively charged C-terminus of melittin with the negatively charged phosphoryl groups of micelles.

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