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Aluminum-Induced Conformational Changes in Calmodulin Alter the Dynamics of Interaction with Melittin[1]

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Studies were undertaken to examine the impact of aluminum-induced structural changes in bovine brain calmodulin on the protein's interface region with melittin, a model for calmodulin's target enzymes. Both steady-state and time-dependent fluorescence characteristics of the single tryptophanyl residue of melittin were employed to derive information on aluminum-related changes in the fluorophore's microenvironment. In the presence of stoichiometric amounts of aluminum ions, calmodulin's target region with melittin appears to be more polar than that with aluminum absent. As a result, upon association of melittin with aluminum–calmodulin, the enhancement of helical arrays is less pronounced. The fluorophore's average microenvironment also is modified such that its apparent lifetime is shortened when aluminum is present. In the presence of aluminum ions, the solvation structure of calmodulin is possibly changed, which may be unfavorable for a proper fit between calmodulin and target proteins.

Calmodulin is an acidic, heat-stable protein which is pivotal in mediating a multitude of calcium-dependent physiological processes (1). Upon binding of calcium into specific regions, architectural events take place which are necessary for calmodulin's interaction with target proteins. Because melittin has a calmodulin affinity in the nanomolar range, and since it competes with target enzymes for calcium–calmodulin, this small amphiphilic polypeptide has been proposed as a model for investigating the interaction between calcium–calmodulin and its target proteins (2).

Binding of calcium produces specific structural rearrangements in calmodulin, and therefore the protein's conformation and its efficacy to interface with target proteins may be profoundly altered when toxic metal ions interfere with calmodulin. Indeed, upon application of stoichiometric amounts of solvated aluminum species to micromolar calmodulin solutions, the protein's helical content decreases concomitantly with an enhanced exposure of hydrophobic surface domains (3, 4) which, in turn, leads to the inhibition of 3',5'-cyclic nucleotide phosphodiesterase (3) and membrane-bound ATPase activity (5). Although aluminum ions (6), like calcium ions (7), strongly prefer oxygen-containing ligands, there exists evidence which suggests that aluminum ions bound to calmodulin do not displace calcium ions (8). Possible aluminum binding sites are carboxylic ligands at the protein's surface. Contrary to the aluminum ion, the trivalent terbium ion can displace calcium from its specific sites while maintaining the functional integrity of calmodulin (9, 10).

These aluminum-induced structural

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changes in calmodulin may constitute a molecular basis for aluminum toxicity (3, 4). This broadly defined syndrome occurs in man where aluminum has been implicated in neurological disorders and in osteomalacia-type diseases (11). In plants, aluminum toxicity represents a serious problem of global proportions because vast regions of the earth suffer from soil acidity which is favorable for aluminum mobilization in soil (12).

To assess further aluminum-induced lesions in calmodulin, we present in this article information on aluminum's impact on the interface between calmodulin and melittin. Since fast structural fluctuations appear to play a key role in protein dynamics and catalysis (13), it was our aim to study time-dependent processes in the nanosecond range in relation to aluminum-induced structural changes in calmodulin. Therefore, the fluorescence properties of melittin's single tryptophanyl residue were used as sensitive parameters to derive information on melittin's interface with calmodulin, in the presence and absence of aluminum. With aluminum present, the average microenvironment of the fluorophore is modified, probably resulting from rearrangement of water molecules in the protein's solvation shell.

METHODS AND MATERIALS

Materials. Affi-Gel phenothiazine, AG 1X-8, Chelex-100, and electrophoresis-grade acrylamide were purchased from Bio-Rad Labs (Richmond, CA). Bovine brain acetone powder, DEAE-Sephadex, Tris, Mes,[3] and Mops buffer, were obtained from Sigma Chemical Co. (St. Louis, MO). $AlCl_3$ $6H_2O$ was obtained from Mallinckrodt (St. Louis, MO). All other chemicals were of the highest quality available. All cuvettes and glass ware were acid-washed with concentrated nitric acid and rinsed with glass-distilled water, whereas plastic ware was treated with Chelex-100. Buffers were decontaminated of residual metals by passage through Chelex-100 columns.

Calmodulin. Bovine brain calmodulin was isolated by phenothiazine affinity chromatography as previously described (14). To enhance the purity of the isolated protein, modifications were incorporated into the isolation procedure (15). Calmodulin isolated in this manner was free from tryptophan-containing proteins as judged by fluorescence emission. The isolated calmodulin also activated 3',5'-cyclic nucleotide phosphodiesterase in a calcium-dependent fashion. The protein was further tested for purity with polyacrylamide gel electrophoresis and by an NMR spectrum at 250 MHz. Analysis for aluminum and calcium in the purified protein was performed by measuring the metal content on a polarized Zeeman Model 180-80 atomic absorption spectrophotometer (Hitachi, Tokyo, Japan). This analysis showed aluminum contamination to be minimal with an aluminum to calmodulin molar ratio of less than 0.1. Calcium content in the purified preparation was less than 0.6 calcium ions per calmodulin molecule. Metal analysis was further confirmed with use of a Jarrell-Ash Model 955 Atomcomp plasma emission spectrometer. Protein concentrations and ultraviolet absorbance scans of calmodulin were determined with a Perkin-Elmer Lambda-7 UV/VIS is spectrophotometer (Norwalk, CT). The molar extinction coefficient of bovine brain calmodulin at a wavelength of 277 nm was taken to be 3300 $M^{-1}cm^{-1}$ (16). Unless otherwise stated the buffer solutions used contained saturating concentrations of calcium and 0.2 N KCl to minimize possible errors from ionic strength effects (17).

Melittin. Melittin used throughout the experiments described was obtained from Serva Feinbiochemica (Heidelberg, FRG). Purity of the protein was determined by loading 90 μg of the commercial product on a 15% polyacrylamide gel. Even at these high loads, the melittin preparation migrated as a single band and showed no apparent contamination with phospholipase A_2. Melittin purchased from this company also has been found to be pure by independent investigation (18). To avoid aggregation of this basic and very hydrophobic protein, all measurements were conducted at concentrations of less than 50 μM melittin. Below this concentration the protein apparently does not adopt its tetrameric form (19). The molar extinction coefficient of melittin was taken to be 5470 $M^{-1}cm^{-1}$, at 280 nm (20).

Circular dichroism experiments. Circular dichroic spectra were obtained on a Jasco Model ORD/UV/CD-5 spectropolarimeter which has been modified by Sproul Scientific Instruments (Boulder Creek, CA). For the CD experiments quartz cuvettes with an optical path length of 1 cm were purchased from Beckman Instruments (Palo Alto, CA). The instrument was calibrated with a 10 μM solution of sperm whale myoglobin in aqueous solution at pH 7.0, for which the mean residue ellipticity, $[\Theta]_{222}$, is $-25{,}600$ deg cm^2 $dmol^{-1}$ (21). All spectra were recorded at room temperature between the wavelengths of 260 and 210 nm. Buffer solutions for these experiments contained 10

[3] Abbreviations used: CaM, calmodulin; CD, circular dichroism; ME, melittin; Mes, 4-morpholineethanesulfonic acid; Mops, 4-morpholinepropanesulfonic acid.

mM Tris adjusted to pH 6.5 with HCl, 0.2 N KCl. Solution volumes were 2 ml and calmodulin concentrations used were 10 μM. Observed ellipticities of the calmodulin/melittin complex were recorded which are related to the helical content of the protein (22).

Fluorescence studies. Fluorescence experiments were performed on a SLM Instruments Model 4800 spectrofluorometer (Urbana, IL). The instrument is interfaced with a Hewlett-Packard HP-85 desk top computer and plotter to aid in data acquisition and analysis. Quartz cuvettes used were as described above. Sample volumes of 2 ml were employed in all the experiments and dilution factors were minimized to 2% or less, apart from quenching studies. Polarization experiments involved the employment of Glan-Thompson calcite prism polarizers with high polarization extinction ratio. During the polarization and total intensity experiments the excitation wavelength was 295 nm, slit width was 8 nm, and emission was viewed through a Corning CSO-54 ultraviolet light cutoff filter.

Steady state fluorescence anisotropy, r, is defined as (23)

$$r = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp}),$$

where $I_{\parallel}$ and $I_{\perp}$ refer to the intensity of fluorescence emission measured parallel and perpendicular to the plane of the exciting beam, respectively.

Anisotropy is related to the fluorescence lifetime, τ, and the rotational relaxation time, ϕ, according to the relation

$$r = r_0(1 + \tau/\phi)^{-1},$$

where r_0 represents the anisotropy in the absence of rotational diffusion.

Fluorescence intensity experiments were performed by titrating aliquots of concentrated calmodulin solutions into 10 μM solutions of buffered melittin. Emission scans from 300 to 440 nm were recorded.

Fluorescent lifetimes were calculated from phase shift and demodulation data obtained with a Debye-Sears modulator (24). All fluorescence studies were performed on sample which had an optical density of less than 0.1 at 280 nm, thus scattering corrections were negligible.

The exciting beam was sinusoidally modulated at a frequency of 30 MHz. The modulating tank contained a 19% ethanol/81% water (v/v) mixture which was changed regularly to keep the ethanol/water ratio constant. 2,5-Diphenyl-1,3,4-oxadiazole (Sigma Chemicals), a commonly used laser dye, served as a mono-exponential lifetime standard (25). All lifetime experiments were conducted with the emission polarizer rotated to approximately 57°. During the lifetime experiments the excitation wavelength was 295 nm while emission was measured at 340 nm. Applying the techniques described above, our measurements of the fluorescent lifetime of *N*-acetyl-tryptophanamide in 0.1 M sodium phosphate, at pH 7.0, gave a value of 3.0 ± 0.1 ns, which is in close agreement with the reported value of 3.0 ns (26).

The collisional quencher acrylamide (26) was used to vary the fluorescent lifetime of tryptophan during the lifetime and quenching experiments. Stock solutions (8 M) of acrylamide contained 0.2 N KCl and were adjusted to pH 6.5 to maintain homogeneity of the quenching solution. Each data point reported is the average of 200 individual readings.

RESULTS

Circular dichroism. The observed ellipticity, at 222 nm, of the melittin-calmodulin complex is dependent upon the presence or absence of aluminum ions (Fig. 1). Upon titration of melittin onto a 10 μM solution of calmodulin, protein association occurs which is reflected by a biphasic plot. As indicated by a well-defined break in slope, a 1:1 complex is formed between melittin and calcium-calmodulin, consistent with previous reports (20). NMR studies of the interaction between calmodulin and melittin have clearly shown that the increased helicity associated with complex formation may be attributed to coiling in both calmodulin and its target (27, 28).

Upon association of aluminum with calcium-calmodulin, normal targeting is al-

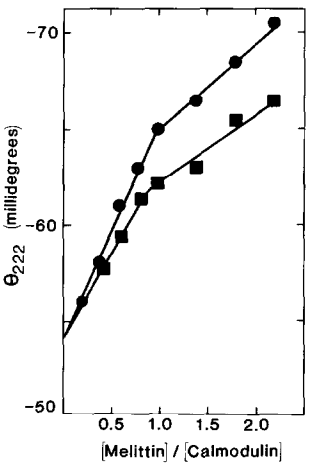

FIG. 1. Titration of 10 μM solutions of calmodulin (●) and aluminum-calmodulin (4:1) (■) by melittin as observed by ultravioleet circular dichroism. The buffer contained 10 mM Tris-HCl, pH 6.5, 0.2 N KCl, and 80 μM $CaCl_2 \cdot 2H_2O$. The abscissa indicates molar ratios. The contributions of calmodulin or aluminum-calmodulin to the ellipticity have been corrected for.

tered as evidenced by a reduced total α-helical content. In the unaltered complex, the mean ellipticity attains a value of about −65 mdeg at a molar ratio of unity as melittin is titrated onto calcium-saturated calmodulin. However, the association of aluminum with calmodulin, still under saturating calcium conditions, results in reduced helical increase in the overall complex. In the latter case helix induction is significantly less at an equimolar ratio of the two proteins (Fig. 1). Binding of aluminum to calmodulin thus results in structural alterations which impair melittin's association with the regulatory protein, and therefore less helices are formed in the complex.

Control experiments indicated that application of an eightfold excess of aluminum ions over melittin alone does not change the protein's CD spectrum. Since the binding affinity of calcium-calmodulin for melittin is reportedly in the nanomolar range (20), ellipticity changes arise from the melittin-calmodulin complex at the micromolar concentrations of both proteins used. We therefore assume that any direct aluminum-induced spectral changes originate from metal interaction with calmodulin rather than melittin.

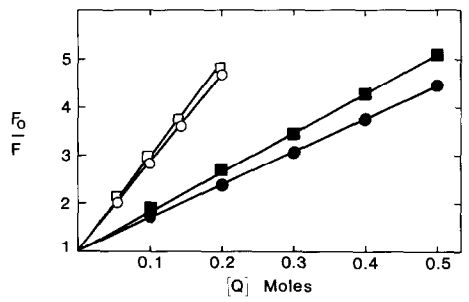

FIG. 2. Fluorescence quenching yield, F_0/F, of melittin's single tryptophanyl residue in the melittin-calmodulin complex. When melittin is associated with calmodulin (●), the fluorophore seems to be more sheltered from the acrylamide quencher, Q. The fluorophore is more accessible to the quenching molecule (■) when melittin associates with aluminum-calmodulin (4:1). (□) And (○) reflect fluorescence quenching of the tryptophanyl residue in free, monomeric melittin (10 μM), in the presence and absence of 40 μM $AlCl_3 \cdot 6H_2O$. The buffer contained 45 mM Mops-KOH, pH 6.5, 0.2 N KCl, 80 μM $CaCl_2 \cdot 2H_2O$. The calmodulin concentration was 10 μM. Excitation wavelength was 295 nm, emission was recorded at 340 nm. F_0 and F represent the fluorescence intensities in the absence and presence of quencher molecules.

TABLE I

FLUORESCENCE LIFETIMES, τ, BIMOLECULAR QUENCHING CONSTANTS, k_q, STERN-VOLMER QUENCHING CONSTANTS, K_D, ROTATIONAL CORRELATION TIMES, ϕ_A, OF MELITTIN'S TRYPTOPHANYL RESIDUE UPON ASSOCIATION WITH BOVINE BRAIN CALMODULIN, IN THE PRESENCE AND ABSENCE OF ALUMINUM[a]

Melittin-calmodulin	τ (ns)	$k_q\ 10^{-9}$ ($M^{-1}\ s^{-1}$)	K_D (M^{-1})	ϕ_A (ns)
Aluminum absent	2.84	1.20 ± 0.1	3.41 ± 0.05	8.42
Aluminum present	2.39	1.62 ± 0.1	3.88 ± 0.05	3.54

[a] All experiments were performed in the presence of calcium at a molar ratio of 8:1 for [calcium]/[calmodulin], at pH 6.5, 25°C. Aluminum was present at a molar ratio of 4:1 for [aluminum]/[calmodulin]. The uncertainty of lifetime measurements is approximately 0.1 ns.

Quenching of fluorescence. Acrylamide quenching of tryptophan fluorescence in the calmodulin/melittin complex is found to be altered if aluminum is associated with the calmodulin molecule (Fig. 2). The linearity of the plot suggests the existence of a single class of fluorophores accessible to the quenching molecules. Values for the bimolecular quenching constant, k_q, calculated for the quenching of melittin's tryptophanyl residue, when the protein is associated with the aluminum-calmodulin and native calmodulin, both in the presence of calcium, are listed in Table I. As opposed to melittin association with calcium-calmodulin, upon association with the aluminum-calcium-calmodulin complex, melittin's tryptophanyl residue is apparently more accessible to the quencher as evidenced by the somewhat higher value of k_q. That the quenching of tryptophan with acrylamide is a collisional rather than a static process becomes further evident from lifetime measurements which clearly indicate that the ratio τ_0/τ is greater than unity.

Fluorescence intensity experiments. Upon titration of 5-μl aliquots of calcium-satu-

rated calmodulin onto solutions of 10^{-5} M melittin, a dramatic increase in tryptophanyl fluorescence intensity was observed (Fig. 3). This increase in the fluorescent intensity, measured at 340 nm, was approximately 17%, compared with that in the absence of calcium-calmodulin. In addition, the fluorescence wavelength maximum shifted towards the blue, viz., from 355 nm, with no calmodulin present, to 345 nm, at a molar ratio of three calmodulin molecules per melittin. By contrast, when calmodulin, bound to aluminum at a molar ratio of 1:4, was titrated into a solution of melittin, no significant increase in fluorescence intensity was observed and the blue shift in the maximum emission wavelength was less, viz., from 355 nm to 348 (Fig. 3), in the presence of saturating calcium concentrations. Since tryptophan emission yields seem to be high in helical portions of a protein (29), the insignificant fluorescence enhancement is probably related to the lower helix content when melittin is associated with aluminum-calmodulin as compared with that of the complex in the absence of aluminum. The fluorescence maximum observed at 355 nm corresponds

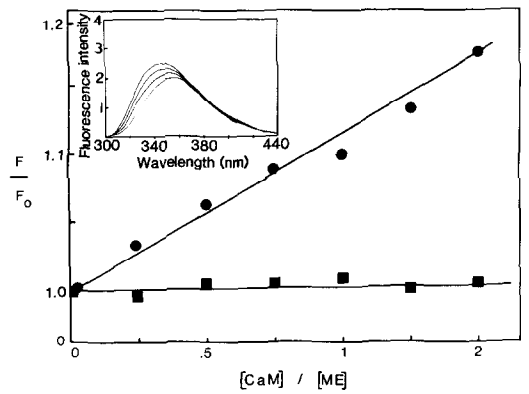

FIG. 3. Fluorescence intensity data of a 10 μM solution of melittin upon addition of concentrated aliquots of calmodulin; dilution effects could be ignored. Excitation wavelength was 295 nm. The buffer contained 45 mM Mops-KOH, pH 6.5, 0.2 N KCl, and 80 μM $CaCl_2 \cdot 2H_2O$. Titration of calcium calmodulin (●) and aluminum-damaged calcium calmodulin (■). Inset shows emission spectral blue-shift and intensity increase associated with subsequent titrations of calcium calmodulin.

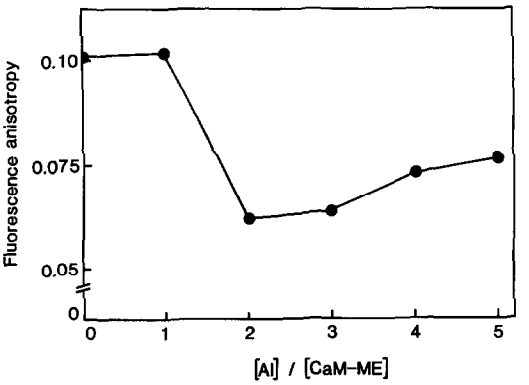

FIG. 4. Fluorescence polarization anisotropy of the melittin-calmodulin complex (9 μM) upon titration with aluminum. Excitation wavelength was 295 and emission was monitored at 340 nm through a Corning CS-054 filter. The buffer was 45 mM MOPS, 0.2 N KCl, pH 6.5, and 80 μM $CaCl_2 \cdot 2H_2O$. Error in polarization is about 10%.

to that expected for tryptophan completely exposed to water (30). Presumably the tryptophanyl residue of melittin is hindered from partitioning into the more hydrophobic areas of the complex when aluminum ions are bound to calmodulin. Alternatively, the diminished blue-shift in the fluorescence maximum of the tryptophanyl residue of melittin in association with aluminum-calmodulin may also arise from melittin being in a less rigid microenvironment (31), compared with the complex in the absence of aluminum ions.

Observation of the polarization anisotropy of the tryptophanyl residue in melittin, when the peptide is bound to calmodulin, gives further indication that aluminum ions alter the association process. Figure 4 shows the anisotropy of the calmodulin-melittin complex to be about 0.11 in the presence of saturating calcium concentrations. Subsequent titration of aluminum ions onto the complex results in a dramatic decrease in the anisotropy at a molar ratio of two aluminum ions per complex to a new value of about 0.06. Alteration in the lifetime of fluorescence, possibly due to modification of the hydration structure, or a dramatic increase in the rotational rate of the fluorophore, would be expected to cause such a decrease in the anisotropy.

Rotational correlation time. When melittin is bound to calmodulin, the rotational correlation time of the tryptophanyl residue may be calculated from results derived from measurements of fluorescence anisotropy vs fluorescence lifetime (Fig. 5). The respective data points define a slope when analyzed by linear regression. Extrapolating the slope to the ordinate of the graph, a value for the apparent limiting anisotropy, $r(0)$, can be derived. The slope is equal to $(r(0)\phi_A)^{-1}$, from which the apparent rotational correlation time, ϕ_A, can be determined (32).

The values of the apparent limiting anisotropy, $r(0)$, are smaller (Fig. 5) than the value of the limiting anisotropy of the tryptophan molecule, r_0, determined in the absence of rotation (32). In the presence of aluminum, the apparent limiting anisotropy, $r(0)$, is larger than that observed with aluminum absent (Table I). This increase suggests that the motional freedom of the tryptophanyl residue is somewhat restricted when melittin is associated with aluminum-calmodulin. Conceivably tryptophanyl residues are involved in hydrogen bonding (33). Moreover, the lifetime and the rotational correlation time of the tryptophanyl residue is smaller when melittin is associated with aluminum-calmodulin (Table I). Compared with the tryptophanyl residue in the absence of aluminum, a shortening of the fluorophore's lifetime and rotational correlation time may result from aluminum-induced changes in the fluorophore's average microenvironment. This notion is supported by our observations on the lack of increase in tryptophan fluorescence and the smaller blue-shift in the absorption spectrum when melittin is associated with aluminum-calmodulin as compared with corresponding results from experiments when aluminum was absent.

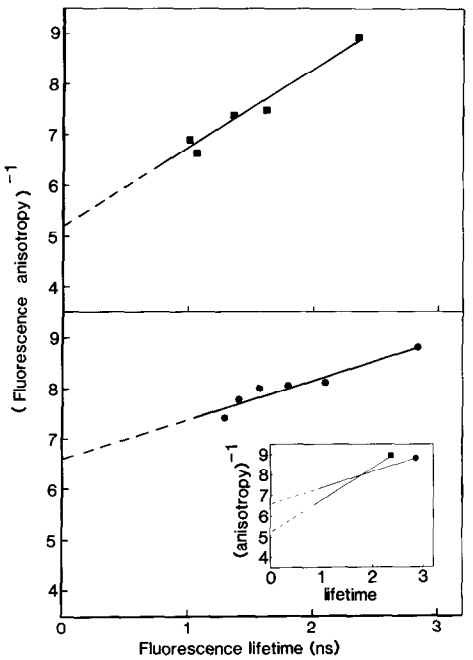

FIG. 5. Fluorescence anisotropy as a function of the lifetime of melittin's tryptophanyl residue upon association of melittin with aluminum-altered calmodulin (4:1) (top graph), or with equimolar calmodulin (bottom graph). Inset shows slope relationship between graphs. The protein concentration was 10 μM each. The buffer consisted of 45 mM Mops-KOH, pH 6.5, 0.2 N KCl, and 80 μM $CaCl_2 \cdot 2H_2O$. The excitation wavelength was 295 nm, anisotropic emission was observed at 340 nm on a SLM Model 4800 spectrofluorometer.

DISCUSSION

Binding of aluminum to calmodulin results in profound changes in the protein's helix content and its biochemical activities (3, 4). These aluminum-induced structural alterations in calmodulin have repercussions on the protein's capability to interact with target proteins such as melittin described in these studies. This small amphiphilic protein of molecular weight 2846 comprises 26 amino acid residues, 6 of which are positively charged at neutral pH and 10 are highly apolar (34). The association of basic melittin with acidic calmodulin probably involves electrostatic forces between charged groups (35). Additional stabilization of the melittin complex is provided by hydrophobic interactions. In the presence of calcium, with aluminum absent, calmodulin forms a mononuclear, high-affinity complex with melittin, with a dissociation constant in the nanomolar range (20). A nanomolar dissociation constant was also found for a highly basic peptide region which lies near the C-ter-

minal end of skeletal muscle myosin light chain kinase and which binds to calcium-calmodulin (36). As far as melittin's tryptophanyl residue is concerned, proton NMR experiments performed on the calmodulin/melittin complex indicated that the fluorophore is being shielded from the aqueous environment (28).

However, the type of interaction appears to be altered when melittin associates with aluminum-calmodulin as opposed to native calmodulin. In the presence of aluminum, helix induction in the complex is reduced presumably as a result of weaker hydrophobic interactions. This is supported by findings that a strong enhancement of melittin's helix content is brought about by interactions of melittin with hydrophobic structures such as membranes and proteins (20). Weaker hydrophobic interactions in the complex of melittin with aluminum-calmodulin are also indicated by the smaller blue-shift in the tryptophan maximum as opposed to that of the melittin-calmodulin complex, in the absence of aluminum. Furthermore, the observation of a shorter fluorescence lifetime and a lower fluorescence intensity are also consistent with the existence of a more polar microenvironment around the fluorophore in the presence of aluminum because tryptophanyl residues exposed to water generally display shorter lifetimes and smaller quantum yields (37). Apparently the blue-shift is not a result of a rigid microenvironment around the fluorophore.

The notion that the fluorophore is in a more polar microenvironment in the presence of aluminum is in accord with findings that aluminum-calmodulin has a more open structure as compared with the more compact structure of calmodulin (3, 4). For example, aluminum breaks helices, and aluminum-calmodulin migrates slower in electrophoresis studies. Aluminum-induced breakage of helices necessarily leads to a rearrangement of water molecules. The more open structure of aluminum-calmodulin presumably has a solvation structure different from that of calmodulin. The existence of a more open structure would be favorable for access of quenching molecules to melittin's tryptophanyl residue which is in accord with our observations. Furthermore, an increased presence of water molecules in aluminum-calmodulin would be less favorable for helix formation in melittin and there exists the possibility that these water molecules may participate in forming hydrogen bonds with the tryptophanyl residue. This is consistent with our interpretation of the enhanced value of $r(0)$ relative to that extrapolated from data obtained from experiments performed in the absence of aluminum.

In the more open structure of aluminum-calmodulin, as opposed to that of calmodulin, the rotational correlation time of melittin's fluorophore may become faster as a result of changes in its average microenvironment. It seems worth noting that a spin probe attached to aluminum-calmodulin is less immobilized than that covalently fixed to calmodulin in the absence of aluminum (38).

It is tempting to relate our results on the rotational correlation times to the three-dimensional structure of calmodulin (39). The calmodulin molecule has the shape of a dumbbell consisting of two globular lobes connected by a long exposed α-helix, where each lobe contains two calcium-binding domains. Let us further consider the overall rotational correlation time of a hydrated spherical molecule according to the Einstein-Stokes equation. For a combined molecular weight for calmodulin with melittin, M_r = 20,000, a partial specific volume of 0.73 cm^3/g, and a typical hydration of 0.2, a rotational correlation time of about 8.1 ns can be calculated. On the other hand, the rotational correlation time for the calmodulin/melittin complex, in the absence of aluminum, was found to be 8.42 ns. Using this simple model, melittin's tryptophanyl residue has little internal freedom, since the protein complex seems to rotate as a whole. This interpretation appears to be consistent with recent findings that mastoparan, a small peptide similar to melittin, binds to residues between sequence positions 73 and 106 of calmodulin (40). Such a location may be favorable for interlobe communication in the presence of proper targets. In the absence of target proteins,

calmodulin seems to have a fairly flexible structure (41).

Conducting experiments in the presence of aluminum ions, we measured a rotational correlation time of 3.54 ns (Table I). This value probably reflects enhanced segmental motions in the vicinity of the tryptophanyl residue in the protein matrix. While it might be intriguing to speculate that aluminum decouples in part interlobe communication, the experimental data base to support such a notion is currently not available.

Assuming the applicability of the Einstein-Stokes equation, free, hydrated melittin would have a correlation time of about 1.1 ns. However, in our experimental setup we cannot resolve correlation times which contribute to the average time and may in part arise from a subpopulation of unbound melittin. Attempts to determine binding constants from fluorescence data were unsuccessful because of the low protein concentrations needed for accurate measurements. Usage of melittin labeled with radioactive external tags, such as acetyl groups, would also lead to distorted estimates of binding parameters.

In summary, as deduced from measurements of steady-state and time-dependent processes of melittin interaction with aluminum-calmodulin, the latter structure and associated structural fluctuations differ appreciably from those of melittin-calmodulin. Since structural fluctuations play a crucial role in protein dynamics and catalysis (13), critical interactions are modified to such an extent in aluminum-calmodulin that a proper fit of calmodulin with target proteins cannot take place. This aluminum-induced mismatch, in turn, may be instrumental in malfunctions of calcium and calmodulin-dependent processes when aluminum ions enter the cell.

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