Membrane Channel Forming Polypeptides. Molecular Conformation and Mitochondrial Uncoupling Activity of Antiamoebin, an α-Aminoisoobutyric Acid Containing Peptide†

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ABSTRACT: The conformations of the 16-residue fungal peptide antiamoebin I (Ac-Phe-Aib-Aib-Aib-Iva-Gly-Leu-Aib-Aib-Hyp-Gln-d-Iva-Hyp-Aib-Pro-Phol) have been investigated in dimethyl sulfoxide solution by one- and two-dimensional NMR techniques. A substantial number of resonances in the 270-MHz 1H NMR spectrum have been assigned. Intramolecularly hydrogen-bonded (solvent inaccessible) NH groups have been identified by determining solvent and temperature dependence of NH chemical shifts and rates of hydrogen-deuterium exchange. Ten backbone NH groups are inaccessible to solvent, while three NH groups assigned to the Phe(1), Aib(2), and Aib(8) residues are exposed to solvent. Interresidue nuclear Overhauser effects are consistent with ψ values of ~120 ± 30° for Phe(1) and Leu(7). The NMR results, together with the stereochemical constraints imposed by the presence of α-aminoisobutyl, isovalyl, prolyl, and 4-hydroxyprolyl residues, favor a highly ordered structure. Two backbone conformations consistent with the data are considered. Antiamoebin is shown to be an effective uncoupler of oxidative phosphorylation in rat liver mitochondria, providing evidence for its membrane-modifying activity.

Membrane-modifying peptides of fungal origin, which are rich in α-aminoisobutyric acid (Aib), have been the focus of several recent investigations (Mathew & Balaram, 1983a; Jung et al., 1981). Representative sequences are listed in Figure 1. Alamethicin, a 20-residue polypeptide, which forms voltage-gated channels across lipid bilayer membranes (Mueller & Rudin, 1967; Boheim & Kolb, 1978; Hall et al., 1984), is the most widely studied member of this class of peptides (Mathew & Balaram, 1983; Jung et al., 1981; Fox & Richards, 1982; Hall et al., 1984). The Aib-containing fungal peptides are characterized by a remarkable microheterogeneity of the natural products and occur as complex mixtures of closely related sequence analogues (Balasubramanian et al., 1981; Rinehart et al., 1981; Brückner et al., 1984; Brückner & Przybylski, 1984; Przybylski et al., 1984). Acetylation of the amino-terminal and the presence of a C-terminal β-amino alcohol are common features of these sequences. The Aib-containing natural peptides may be broadly grouped into two classes: (i) the "long" sequences that contain 18–20 residues and lack hydroxyproline (Hyp), as exemplified by the alamethicins (Pandey et al., 1977a), suzukacillins (Katz et al., 1985), trichotoxins (Przybylski et al., 1984), hypelcins (Fujita et al., 1984), paracelsins (Przybylski et al., 1984), and trichorzianines (Bodo et al., 1985) and (ii) the "short" sequences that contain 15–16 residues and possess hydroxylated residues, particularly Hyp, as exemplified by the emerimicins (Pandey et al., 1977b), zervamicins (Rinehart et al., 1981), and antiamoebins (Pandey et al., 1977c).

While alamethicin, suzukacillin, and trichotoxin have been shown to form stable conductance states in artificial lipid bilayers (Boheim et al., 1976; Boheim et al., 1978; Hall et al., 1984), definitive data have not been reported for the shorter peptides. However, references to the pore-forming activities of the antiamoebins, zervamicins, and emerimicins have appeared in the literature [see footnotes in Pandey et al. (1977b,c) and Rinehart et al. (1981)]. There are no reports on detailed structural studies on the shorter peptides. Conformational analysis of the short sequences assumes importance in view of the presence of as many as three Pro or Hyp residues in the C-terminal segments of zervamicins, emerimicins, and antiamoebins. These should interrupt intramolecular hydrogen bonding and may result in structures distinctly different from those inferred for the alamethicin C-terminal (Fox & Richards, 1982; Mathew & Balaram, 1983a; Bosch et al., 1985a; Banerjee et al., 1983). We describe in this paper an NMR study of antiamoebin I that permits the development of secondary structural models for this sequence. We also establish the membrane-modifying activity of this peptide by demonstrating its effectiveness as a mitochondrial uncoupler.

Materials and Methods

Antiamoebin, a fungal peptide produced by the strains Emericellopsis poonensis Thirum., Emericellopsis synnemasticola Mathur and Thirum., and Cephalosporium pimprina Thirum., was the kind gift of Dr. N. Narasimhachari, Medical College of Virginia, Virginia Commonwealth University, and was originally isolated at Hindustan Antibiotics, Pune, India, as described earlier (Thirumalachar, 1968). Reverse-phase high-performance liquid chromatography (HPLC) analysis of the peptide was carried out on a Lichrosorb RP-18 column (4 × 250 mm, 10-μm particle size) with gradient elution (65–85% MeOH–H2O in 20 min, 85–95% MeOH–H2O in 5 min, flow 0.8 mL min⁻¹, detection 226 nm) on an LKB HPLC system. Purification of the peptide was effected by repetitive injections and collection of fractions using a Superrac fraction collector. For NMR studies, the peptide was used without HPLC purification (see Results and Discussion).

†H NMR spectra were recorded on a Bruker WH-270 FT-NMR spectrometer, equipped with an Aspect 2000 computer at the Sophisticated Instruments Facility, Indian Institute of Science, Bangalore. For correlated spectroscopy (COSY) (Figure 4), 512 free induction decays (FIDs), each of 24 accumulations, with 1-s relaxation delay were collected. The

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t1 domain was zero filled to 1K, while 1K data points were collected in the t2 domain resulting in a 1K × 1K data matrix. Phase-shifted sine bell window functions were applied prior to Fourier transformation on both domains. The spectral width in each dimension was 2500 Hz. Single channel detection with constant phase of pulses was used. In the difference nuclear Overhauser effect (NOE) experiments, the perturbed and normal spectra recorded sequentially (one on-resonance and one off-resonance) in different parts of the memory (8K of each) were obtained by low-power on-resonance saturation of a peak and by off-resonance shifting of the irradiation frequency, respectively. About 128 transients were accumulated with a relaxation delay of 3 s between transients to facilitate buildup of initial magnetization (Rao et al., 1983). Delineation of hydrogen-bonded NH groups was carried out as described earlier (Balaram, 1985; Kishore et al., 1985).

Peptide effects on respiration of rat liver mitochondria were monitored with a Hansatech oxygen electrode as described earlier (Das et al., 1985).

RESULTS AND DISCUSSION

Figure 2 shows an HPLC analysis of the sample of antiamoebin used in this study. A major component (~80%) is observed with a retention time of ~21 min. A significant second component (18%) is seen at ~20 min, while at least three minor components (1–2%) are also discernible. Four pure fractions with retention times of 20.4, 21.4, 23.7, and 24.7 min have been isolated by HPLC separation and shown to correspond to closely related polypeptides. The microheterogeneity of natural antiamoebin has been noted earlier (Pandey et al., 1977c; Rinehart et al., 1979). Sequence analogues of the antiamoebin I sequence, where the replacements of Pro for Hyp(13), Aib for Iva(5), and Ala for Gly(6) occur, have been characterized by mass spectrometry (Pandey et al., 1978; Stroh et al., 1985). The 270-MHz 1H NMR spectrum of the purified major component (retention time 21.7 min) was practically indistinguishable from that of unpurified antiamoebin. All NMR studies were therefore carried out without further purification of the antiamoebin sample. The 270-MHz 1H NMR spectrum of antiamoebin in (CD3)2SO is shown in Figure 3. The spectrum is fully consistent with the composition suggested for antiamoebin I (Figure 1, Pandey et al., 1977c). The amino acid analysis and 13C NMR spectrum of the peptide provide further confirmation (data not shown).

Assignment of Resonances. Figure 4 shows the two-dimensional correlated spectrum (Aue et al., 1976; Wider et al., 1984) of antiamoebin in (CD3)2SO. The five expected connectivities between the NH and C1H resonances [Phe(1), Gly(6), Leu(7), Gln(11), Phol(16)] are clearly seen. The Gly NH group is readily recognized by its triplet nature and its coupling to the resonance at δ 3.74 (C4H2), which in turn displays no further connectivity. The Gln spin system is characterized by the C6H2 (δ 1.818)–C4H2 (δ 2.187) connectivity, which permits identification of the Gln C6H (δ 4.17) and NH (δ 7.90) resonances. The Phe and Phol residues have overlapping C6H resonances in the region δ 2.55–3.00. The Phol C6H resonance is however observed at significantly higher field (δ 3.818) as compared to the Phe C6H group (δ 4.340). A similar chemical shift has been reported for Phol C6H in alamethicin (Martin & Williams, 1976). This resonance is also unambiguously identified by its connectivity to resonances at δ 3.2–3.5 (Phol C6H2), which in turn are coupled to a triplet OH resonance at δ 4.62. This hydroxyl proton resonance disappears on addition of D2O. The entire Leu spin system cannot be unambiguously traced in the COSY spectrum due to overlap of the C6H2 and C6H resonances (Nagayama & Wuthrich 1981; Davoust et al., 1983). However, the assignments of the C6H and NH protons are unequivocal, since all other residues with coupled C6H and NH resonances have been identified. The Hyp C6H and C6H resonances were assigned by virtue of their common connectivities to the C6H2 resonances, while the C6H NH resonances were also ambiguously observed at δ 5.18. The assignments are indicated in Figure 3, and the proton chemical shifts of unambiguously identified residues are summarized in Table I. An unambiguous assignment of the various singlet resonances to specific Aib/Iva residues is not possible at present. The singlet NH resonances at δ 6.78 and 7.29 correspond to the Gln carboxamide side-chain protons. A small geminal coupling between these protons is not detected in Figure 3, but is clearly observed as an off-diagonal cross peak in the COSY spectrum (Figure 4). In the low-field region, 15 NH resonances can be identified, due to 13 backbone and 2 side-chain NH groups. Resonances are labeled Sn, Dn, and Tn where S, D, and T denote singlets, doublets, and triplets, respectively, while the subscript n indicates the order of appearance from low field in (CD3)2SO (Figure 5).
FIGURE 3: 270-MHz $^1$H NMR spectrum of antiamoebin in dimethyl sulfoxide-$d_6$ (CD$_3$)$_2$SO (20 mg mL$^{-1}$). Assignments indicated were derived from COSY experiments (see Figure 4).

Table I: Proton Chemical Shifts for Specific Residues of Antiamoebin in (CD$_3$)$_2$SO

<table>
<thead>
<tr>
<th>residue</th>
<th>NH</th>
<th>$\delta$ (ppm)</th>
<th>chemical shifts, $\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe(1)</td>
<td></td>
<td>8.32 (D$_2$)</td>
<td>3.33, 2.82 (C$^3$H)</td>
</tr>
<tr>
<td>Gly(6)</td>
<td></td>
<td>7.98 (T$_3$)</td>
<td>3.64</td>
</tr>
<tr>
<td>Gln(11)</td>
<td></td>
<td>7.86 (D$_3$)</td>
<td>4.12, 1.88 (C$^2$H)</td>
</tr>
<tr>
<td>Leu(7)</td>
<td></td>
<td>7.07 (D$_3$)</td>
<td>4.03, 1.65 (–CONH)</td>
</tr>
<tr>
<td>Phol(16)</td>
<td></td>
<td>7.20 (D$_3$)</td>
<td>3.73, 3.00, 2.56 (C$^3$H)</td>
</tr>
</tbody>
</table>

$^a$ All Aib and Iva resonances cannot be assigned to specific residues in the sequence. The chemical shift values for these protons are therefore not tabulated. However, the Aib(2) NH and Aib(8) NH resonances have been identified by NOE connectivity to the preceding C$^3$H resonance (see text and Table II for $\delta$ values). $^b$ The assignment of the Hyp spin systems to specific residues in the sequence is not possible at the present.

Table II: NMR Parameters for Backbone NH Resonances in Antiamoebin

<table>
<thead>
<tr>
<th>resonance</th>
<th>$\delta$ (ppm/K)</th>
<th>$\delta$ (ppm/K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S$_1$</td>
<td>8.62</td>
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</tr>
<tr>
<td>D$_2$</td>
<td>8.32</td>
<td>0.0052</td>
</tr>
<tr>
<td>T$_3$</td>
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<td>0.0024</td>
</tr>
<tr>
<td>S$_4$</td>
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</tr>
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<td>D$_3$</td>
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<tr>
<td>S$_6$</td>
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<td>0.0049</td>
</tr>
<tr>
<td>S$_7$</td>
<td>7.75</td>
<td>0.0022</td>
</tr>
</tbody>
</table>

$^a$ All Aib and Iva resonances cannot be assigned to specific residues in the sequence. The chemical shift values for these protons are therefore not tabulated. However, the Aib(2) NH and Aib(8) NH resonances have been identified by NOE connectivity to the preceding C$^3$H resonance (see text and Table II for $\delta$ values). $^b$ The assignment of the Hyp spin systems to specific residues in the sequence is not possible at the present.

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Delineation of Hydrogen-Bonded NH Groups. The possible involvement of NH groups in intramolecular hydrogen bonding was probed with three criteria (Kessler, 1982; Balaram, 1985; Wuthrich, 1976): (i) temperature dependence of NH chemical shifts, (ii) solvent dependence of NH chemical shifts in CDCl$_3$–(CD$_3$)$_2$SO mixtures, and (iii) hydrogen–deuterium (H–D) exchange studies in (CD$_3$)$_2$SO–D$_2$O mixtures.

The temperature dependence of chemical shifts in (CD$_3$)$_2$SO is linear for all the NH groups in antiamoebin (Figure 6). The chemical shifts (293 K) and temperature coefficients ($d/dT$) are summarized in Table II. Ten backbone resonances exhibit low $d/dT$ values ($<$0.003 ppm/K) characteristic of solvent-shielded (intramolecularly hydrogen-bonded) NH groups. Three resonances [S$_1$, D$_2$ (Phe), and S$_6$] have high $d/dT$ values (>0.005 ppm/K) indicative of their exposure to solvent. The chemical shifts and temperature coefficients for NH resonances in (CD$_3$)$_2$SO–D$_2$O mixtures are illustrated in Figure 5. The solvent dependence of NH chemical shifts is summarized in Figure 8. An increase in the concentration of the strongly hydrogen-bond-accepting solvent, (CD$_3$)$_2$SO, results in significant downfield shifts of resonances S$_1$, D$_2$, and S$_6$ and the two Gln side-chain NH resonances. (Note that the chemical shift scale used for the side-chain NH resonances in Figure 8 is compressed relative to that used for the backbone NH protons.

The results summarized above establish that resonances S$_1$, D$_2$, and S$_6$ may be assigned to fully solvent exposed NH groups. The behavior of the remaining 10 backbone NH resonances is characteristic of solvent-shielded/intramolecularly hydrogen-bonded NH groups.

Nuclear Overhauser Effects. Nuclear Overhauser effects (NOEs) are particularly useful in identifying pairs of protons that are spatially proximate, with interproton distances in the range 2.2–3.0 Å (Bothner-By, 1979). The observation of
FIGURE 4: Contour plots of the COSY spectrum of antiamoebin in (CD$_3$)$_2$SO (20 mg mL$^{-1}$): (a) $\delta$ 9.00-3.60, (b) $\delta$ 5.00-0.5, (c) expanded region of the NH-$\text{C}^4\text{H}$ cross peaks, and (d) expanded region of the $\text{C}^4\text{H}$-$\text{C}^2\text{H}$ cross peaks. Specific residue assignments are indicated. The Phol $\text{C}^9\text{H}$-$\text{OH}$ cross peak, which is crucial to identification of this residue, is marked in spectrum b. Note that the Phol $\text{C}^9\text{H}_2$ protons lie very close to the water resonance.

FIGURE 5: Partial 270-MHz $^1\text{H}$ NMR of antiamoebin (NH and aromatic resonances) in CDCl$_3$-(CD$_3$)$_2$SO mixtures. $S_n$, $D_n$, and $T_n$ refer to singlet, doublet, and triplet NH resonances. The subscript $n$ refers to the order of appearance from low field in (CD$_3$)$_2$SO.

FIGURE 6: Temperature dependence of NH chemical shifts for antiamoebin in (CD$_3$)$_2$SO (notations as in Figure 5). Peptide concentration was 12.5 mg mL$^{-1}$.

interresidue NOEs ($\text{C}^4\text{H}$-$\text{N}^\alpha\text{H}$) in peptides is clearly diagnostic of specific peptide backbone conformations (Wüthrich et al., 1984; Shenderovich et al., 1984; Rao et al., 1983; Kishore et al., 1985; Balaram, 1985). All the NH and $\text{C}^4\text{H}$ resonances of antiamoebin were individually irradiated in a systematic series of difference NOE experiments. Limited irradiation power was used to saturate resonances in order to minimize nonselective effects. Only two distinct interresidue
FIGURE 7: Partial 270-MHz 'H NMR spectra in (CD$_3$)$_2$SO recorded at different time intervals after addition of D$_2$O (~10% v/v): (a) before D$_2$O addition and (b) 5 min, (c) 150 min, and (d) 75 h after D$_2$O addition. Peptide concentration was 12.5 mg mL$^{-1}$.

FIGURE 8: Solvent dependence of NH chemical shifts in antiamoebin as a function of (CD$_3$)$_2$SO concentration in CDCl$_3$-(CD$_3$)$_2$SO mixtures.

NOEs were observed, which are shown in Figure 9. A negative NOE of ~10.2% is observed on the Phe C$^\alpha$H peak, when the S$_6$ NH resonance is saturated. Irradiation of S$_6$NH results in a 16.4% NOE on the Leu C$^\alpha$H resonance. Partial saturation of closely overlapping NH resonances also occurs in this experiment. Nevertheless, the precise NOE connectivity of the S$_6$ NH and Leu C$^\alpha$H resonances was readily established by systematically varying the irradiating frequency about the S$_6$ NH chemical shift and monitoring the magnitude of the NOE.

FIGURE 9: (a) Partial 270-MHz 'H NMR spectrum of antiamoebin in (CD$_3$)$_2$SO; (b, c) difference NOE spectra (×16) obtained by saturation of specific NH resonances, (b) Aib(2) NH and (c) Aib(8) NH. The saturated peak appears as an intense negative signal in the difference spectrum.

Conformations of Antiamoebin I. The NMR results strongly suggest that antiamoebin I adopts highly folded solution conformations, in which ten backbone NH groups are largely inaccessible to the solvent. The formation of intramolecular 4→1 and 5→1 hydrogen bonds in Aib-containing peptides in the solid state and in solution is exceedingly well documented (Prasad & Balaram, 1984; Toniolo et al., 1983; Bosch et al., 1985a; Nagaraj & Balaram, 1981; Vijayakumar & Balaram, 1983). The solvent-shielded nature of the 10 backbone NH groups is therefore likely to be a consequence of their participation in intramolecular hydrogen bonding. The presence of several Aib/Iva residues in the antiamoebin I sequence imposes considerable stereochemical restrictions on the molecule. Aib residues are largely constrained to have conformations in the right- or left-handed 3$\theta/\alpha$-helical regions of conformational space (Prasad & Balaram, 1984), with $\phi$ ~ ±60 ± 20° and $\psi$ ~ ±30 ± 20° (IUPAC-IUB Commission on Biochemical Nomenclature, 1970). Similar conformational preferences have also been suggested for Iva residues from conformational energy calculations (Benedetti et al., 1984; Barone et al., 1985). The assignment of the three solvent exposed (non-hydrogen-bonded) NH groups to the Phe(1), Aib(2), and Aib(8) residues permits a hydrogen-bonding scheme to be proposed (Figure 10), which is fully consistent with the NMR data. The proposed scheme involves formation of ten 4→1 (C$_{10}$) hydrogen bonds. The involvement of the Phol NH group in an intramolecular hydrogen bond and its presence as the C-terminal residue in the Iva-Hyp-Aib-Pro-Phol sequence is consistent with both 4→1 and 5→1 hydrogen-bonding possibilities. This alternative is also considered in Figure 10. Such structures have been suggested earlier for oligopeptides having a Pro residue in the center of a helical segment (Rao et al., 1980). The formation of mixed 4→1 and 5→1 hydrogen bonds, with bifurcation involving interaction on the Leu C$^\alpha$H resonance. The maximum effect is indeed observed when the irradiating frequency is centered on the S$_6$ NH resonance. This NOE is also clearly observed in the reverse experiment, where a decrease in intensity of 11.8% is observed for the S$_6$ NH resonance on saturating Leu C$^\alpha$H. Some of these observations permit assignment of the S$_6$ and S$_8$ NH resonances to the Aib(2) and Aib(8) NH groups, respectively. It may be stressed that in peptides the only observable C$^\alpha$H-N$^\alpha$H NOEs are when $i = j$ (intraresidue) and $i + 1 = j$ (interresidue) (Wüthrich et al., 1984; Shenderovich et al., 1984).
Together with the observed NOE between Leu(7) C'H and Aib(8) NH protons, this supports a type of succeeding P-turn. The 2-6 segment of antiamoebin I values of 52.5 possibilities. Such interresidue NOEs are diagnostic of involvement of Aib(3) NH in an intramolecular hydrogen bond to crystallize HPLC-purified antiamoebin (Briickner et al., 1984). Attempts to crystallize HPLC-purified antiamoebin I are presently in progress, in order to establish the conformation in the solid state.

The detection of only two interresidue C<sub>α</sub>H-N<sub>1</sub>H NOEs, between, viz., Phe(1) C'<sub>α</sub>H ↔ Aib(2) NH and Leu(7) C'<sub>α</sub>H ↔ Aib(8) NH, further limits the backbone conformational possibilities. Such inter residue NOEs are diagnostic of ψ values of ~120 ± 30°, which result in C<sub>α</sub>H-N<sub>1</sub>H distances of ~2.5 Å (Shenderovich et al., 1984). Thus both Phe(1) and Leu(7) may be assigned α-helical conformation has been determined in crystals (Fox & Richards, 1982). Homooligopeptides of Aib (Shamala et al., 1978; Benedetti et al., 1982, 1985) and Aib-X repeating sequences (Francis et al., 1985) have been shown to adopt 3<sub>10</sub>-helical structures.

The solvent-shielded nature of the Aib(9) NH group suggests its involvement in an intramolecular hydrogen bond. Together with the observed NOE between Leu(7) C'H and Aib(8) NH protons, this supports a type II β-turn conformation at the Leu(7)-Aib(8) segment.

While the NMR results suggest that the C-terminal NH groups, Gln(11), Iva(12), Aib(14), and Phol(16), are all hydrogen bonded, the precise conformation of the 10-16 segment is unclear. The presence of the imino acids L-Hyp and L-Pro at positions 10, 13, and 15 necessarily restricts ϕ values to ~60 ± 15° at these residues. However, the lack of an amide hydrogen at these positions also interrupts regular helical hydrogen-bonding schemes. There are no crystal structures of large oligopeptides with such a distribution of imino acids. Theoretical studies of (Aib-Pro), sequences have suggested that Pro residues can be incorporated into 3<sub>10</sub>-helical structures with some distortions (Prasad & Balaram, 1982). Some evidence for regular helical conformations in an octapeptide with the sequence (Aib-Pro), has been reported (Venkatachala-pathi & Balaram, 1981). In these studies C<sub>α</sub> (3<sub>10</sub>-hydrogen-bonded) structures at Pro have also been considered.

In order to evaluate the influence of C-terminal conformational variations on the overall shape of antiamoebin I, ideal structures compatible with the NMR data were computer generated. Two structures were considered that differ only at Iva(12) and Hyp(13). In conformer I a fully right-handed C-terminal 3<sub>10</sub>-helical segment is considered, while in conformer II a C<sub>3</sub> structure is used at Hyp(13) and a left-handed 3<sub>10</sub>-helical conformation at Iva(12), to avoid poor intramolecular contacts. The backbone dihedral angles used are listed in Table III. Figure 11 shows a perspective view of the backbone conformations I and II. The two structures are distinctly different in overall shape, with I appearing particularly suitable for functioning as a membrane-spanning helical polypeptide. It is of interest to note that antiamoebin shows highly solvent-dependent CD spectra, suggesting a high degree of conformational variability (unpublished results). CD spectra distinct from those reported for the natural Aib-containing helical peptides, like alamethicin and suzukacillin, have been observed for antiamoebin (Brücker et al., 1984). Attempts to crystallize HPLC-purified antiamoebin I are presently in progress, in order to establish the conformation in the solid state.

**Mitochondrial Uncoupling Activity.** The proposed antiamoebin conformations consist of a helical amino-terminal...
nonapeptide, with subtle variations in handedness, and a C-terminal heptapeptide that is highly structured but not necessarily an ideal helix. Membrane channel forming activities have been rationalized with largely helical conformations, over the whole polypeptide chain, for alamethicin (Mathew & Balaram, 1983b; Boheim et al., 1983; Fox & Richards, 1982) although alternative structures have been suggested (Hall et al., 1984). In order to determine whether antiamoebin possesses membrane-modifying activities similar to alamethicin, we have examined the effect of the peptide on mitochondrial respiration in the intact state 4 mitochondrial membranes. Initial incubations of 2 mL of mitochondrial suspension in 2 mL of 2 mM HEPES containing D-(-)-mannitol (220 mM), sucrose (70 mM), EGTA (0.5 mM), MgCl₂ (2.5 mM), and KH₂PO₄ (2.5 mM), pH 7.4. Points 1, 2, and 3 indicate additions of succinate (7.5 mM), ADP (72 µM), and antiamoebin, respectively. The peptide solutions were prepared in ethanol and added volumes did not exceed 9 µL. Mitochondrial concentration in the assay mixture was 0.37 mg of protein mL⁻¹.

Channel formation, followed by dissipation of H⁺ and ionic gradients across the inner mitochondrial membrane, could result in the observed uncoupling (Mathew et al., 1981). However, a general membrane-perturbing effect accompanied by enhanced ionic permeability cannot be ruled out. In the case of alamethicin, a transition from uncoupling to inhibitory activity has been observed, with increasing concentration of phosphate in the assay medium. This has been attributed to the transport of phosphate ions through alamethicin channels (Das et al., 1985). Antiamoebin behaves purely as an uncoupler over the phosphate concentration range 2.5–100 mM. It remains to be established whether differences in membrane-modifying activities of alamethicin and antiamoebin can be correlated to secondary structure differences. Antiamoebin has also been shown to induce Ca²⁺ flux across liposomal membranes (M. K. Das, unpublished results), suggesting that peptide incorporation can indeed result in cation transport across lipid bilayers.

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