Structural chemistry and membrane modifying activity of the fungal polypeptides zervamicins, antiamoebins and efrapeptins

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Abstract - The fungal polypeptides zervamicins, antiamoebins and efrapeptins have been fractionated into several polypeptide components by HPLC. A zervamicin fraction lacking tryptophan has been characterized and shown to possess an N-terminal leucine residue. The conformations of zervamicin IIA and a synthetic analog in solution are compared with those determined for the related peptide, antiamoebin. The results are consistent with a completely helical structure for the apolar analog of zervamicin in chloroform, with partial unfolding in dimethylsulfoxide. A similar conformation has been determined for natural zervamicin IIB. A synthetic analog of efrapeptin forms a continuous helix in apolar solvents while, partial unfolding is seen in polar solvents. Natural zervamicin is an effective uncoupler of mitochondrial oxidative phosphorylation. Significant differences in membrane modifying activity are noted for the natural peptide and the synthetic apolar analog of zervamicin.


Fig. 1. Sequences of some Aib containing antibiotics
alamethicin composed of clusters of largely hydrophobic helices in lipid bilayers (ref. 1, 3), with voltage gating suggested to arise from interaction of the macroscopic helix dipole with the applied electric field (ref. 16, 17). This report summarises some studies on antiamoebins, zervamicins and efrapeptins.

**PEPTIDE ISOLATION AND PURIFICATION**

Zervamicins were isolated from cultures of emericellopsis salmosynnemata (strain CMI 58330) (ref. 18). An HPLC profile of a mixture of zervamicins is shown in Fig. 2. Peaks at 23-26 min. arise from acidic zervamicin components, which have been previously identified as possessing a single Glu residue at position 3 (ref. 8). The component at ~26 min. corresponds to the peptide zervamicin IC (ZIC). Peaks between 36-52 min. are all neutral peptides with the major components corresponding to the previously sequenced peptides, zervamicins IIA (ZIIA) and IIB (ZIIB), respectively. The peak at 57.4 min. is a peptide which lacks the amino terminal Trp residue, characteristic of the zervamicins sequenced earlier (ref. 8). The absence of Trp is clearly suggested by a comparison of the HPLC profiles obtained by detection at 226 nm and 280 nm (Fig. 2). This peptide (Z-L), has been shown to possess a Leu residue in place of Trp at the amino terminus and a tentative sequence is Ac-Leu-Ile-Gln-Iva-Ile-Thr-Aib-Leu-Aib-Hyp-Gln-Aib-Hyp-Aib-Pro-Phol. Supporting evidence for this assignment comes from the observation of a nuclear Overhauser effect (NOE) between the amino terminal blocking acetyl methyl protons and the NH proton of a Leu residue. The molecular mass, 1764 daltons, (FAB mass spectrum) is also consistent with this sequence assignment. Efrapeptins were isolated from cultures of tolypocladium inflatum niveum (strain IMI 202309) (ref. 19). The crude peptide mixture, inhibited oxidative phosphorylation in rat liver mitochondria. Fig. 3. shows an HPLC profile of the peptide mixture. The peaks were extremely broad and overlapping under a wide variety of conditions. Preliminary fractionation into components I-IV (Fig. 3) and analysis by mass spectrometry revealed that I has a major component with a molecular mass of 1619 and a minor component of 1605 daltons. Fractions II, III and IV have components with molecular masses of 1633, 1647, 1661 and 1675, respectively. The strongly hydrophobic and basic nature of the peptide limited further HPLC purification under the conditions used. The inset to Fig. 3 shows an HPLC profile obtained on a less hydrophobic C4 column. Work in progress is directed towards elucidating the sequence relationships of the efrapeptin components. Antiamoebins, from emericellopsis poonensis Thirum, were obtained as described elsewhere (ref. 11).
The Pro/Hyp rich C-terminal tail in the antiamoebins and zervamicins should lead to an interruption of the continuous intramolecular hydrogen bonding pattern expected in helical Ab peptide structures (ref. 13-15). In the case of efrapeptins the presence of piperolic acid (Pip), a proline homolog, and β-alanine (β-Ala) should lead to deviations from ideal helical conformations. An apolar synthetic analog of zervamicin IIA (Boc-Ile-Ala-Ile-Val-Ile-Pro-Ile-Pro-Leu-Pro-Val-Pro-Pro-Val-Pro-Pro-Phe-OMe) has been studied by 1H-NMR. Analysis of temperature dependence and solvent perturbation of NH chemical shifts in DMSO and chloroform solutions suggested that a large number of amide NH groups are inaccessible to solvent. In chloroform, with the exception of Trp (1) and Ile (2) NH groups, all the other NH groups are solvent shielded consistent with a completely helical conformation for the synthetic peptide. This is further supported by the observation of NH NH ↔ NH NOEs throughout the sequence, in chloroform. A partial unfolding of the structure at the amino terminus is observed in the strongly solvating medium DMSO, with the NH groups of Ala (3), Ab (4) and Ile (5) also being exposed to solvent. Interresidue NOEs of the type NH ↔ NH, observed for residues 1 to 4, suggest an extended conformation for this portion of the molecule, in DMSO. X-ray diffraction studies of crystals obtained from methanol-water mixtures revealed a completely helical structure in the solid-state (Fig. 4) with all the prolines being comfortably accommodated into a 3 helical structure, which has been termed as the "β-bend ribbon" (ref. 20). Natural zervamicin IIA has also been examined in detail by 2D NMR at 270 MHz and 500 MHz, in DMSO and methanol. Evidence for helical folding of residues 5 to 16 is obtained, with a partially unfolded amino terminus.

An NMR study of the major component of antiamoebin has indeed, also provided firm evidence for a largely helical conformation. NOE evidence supports a Type II β-turn at the N-terminus [Phe(1)-Aib(2)], a segment of left-handed helix followed by a Type II β-turn at Leu-(7)-Aib(8) and a right handed helix for residues 9 to 16 (ref. 11). A backbone conformation generated using these NMR derived parameters bears a remarkable resemblance to the crystal structure of the synthetic zervamicin analog (Fig. 4), although the precise torsional angles used are different. However, the Pro/Hyp rich C-terminus structures are almost identical. Structural information on the efrapeptins has been derived indirectly, using a synthetic 16-residue analog peptide sequence (Boc-Pro-Aib-Pro-Aib-Aib-Leu-β-Ala-Gly-Aib-Aib-Pro-Pro-Gly-Leu-Aib-Aib-OMe) in which the following replacements have been made; Pip to Pro, Iva to Aib. The unidentified C-terminal residue is absent. NMR analysis of this peptide in chloroform and DMSO solutions provides clear evidence for the solvent inaccessibility of all the amide protons except Aib (2) NH in CDC13. In DMSO, Gly (8), Aib (9) and Aib (15) NH groups are solvent exposed, in addition to Aib (2) NH. The chloroform structure is clearly a continuous helix in which both β-Ala (7) and Pro (11) are accommodated in the helix. The additional CH3 group in β-Ala is presumably accomodated within an eleven or fourteen membered hydrogen bonded ring as opposed to the ten or thirteen membered rings in ideal 3- or α-helical structures, respectively.

Fig. 4. (left) Molecular conformation of a synthetic apolar zervamicin analog, in crystals (ref. 20). (right) Backbone conformation of anti-amoebin I derived from NMR data (ref. 11).
The fragility of the structure in the vicinity of the central Leu-3-Ala-Gly region is evident in DMSO, where the Gly (8) and Gly (9) NH groups are solvent exposed. NMR studies on the hexapeptide Boc-L-Pip-Aib-Gly-Leu-Aib-Aib-OMe, (residues 12-16 of efrapeptin) support helical conformation similar to that observed for the peptide with Pro in the place of Pip. Crystal structures of model piperoyl peptides suggest that the conformational properties of Pip residues are fairly similar to those of proline (ref. 21).

MEMBRANE MODIFYING ACTIVITY

Both antiamoebin and zervamicin exhibit mitochondrial uncoupling activity suggesting their incorporation into the inner mitochondrial membrane. The synthetic, apolar zervamicin analog is appreciably less effective as an uncoupler. Similar behaviour was also observed in studies of peptide induced Ca++ fluxes across liposomal membranes. An interesting feature of the zervamicins, particularly the synthetic analog is the tendency to cause liposome aggregation as evidenced by a dramatic increase in Rayleigh scattering on addition of peptide to liposomes. Single channel conductance activity in planar bilayers has also been observed for zervamicins IIA, I-C and Z-L as well as the synthetic analog peptide. The life times of the channels formed by the synthetic apolar analog are dramatically shortened compared to those for the polar peptides (M.S.P. Sansom, personal communication). This is consistent with the involvement of polar side-chain functionalities in stabilizing channels formed by helix association in phospholipid bilayers.

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REFERENCES