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Item 19 - Abstract

The focus of this research is the interaction of a molecule at the lipid/water interface of a membrane with the external electric field, using peptaibols and the S4 segment of the sodium channel as model systems. Experiments are underway to assess the role of the molecular dipole in sensing the imposed molecular field; the role of α-helix-3_{10}-helix transitions in responding to the imposed molecular field as the transduction mechanism; and the role of the channel mouth in determining the ion selectivity associated with channel opening. Ac-(Lys(alamethicin 1-17))$_4$-NH_2, tetramethicin, which was prepared by coupling several small fragments to tetralysine while attached to a solid support, showed an effective concentration dependence 2.7 times that of alamethicin. Other oligomeric pores are in preparation using aza-crown ether scaffolds. This concentration dependence is dramatically different from the control, monomeric alamethicin 1-17 benzyl ester, which is severalfold less than alamethicin itself. This placed constraints on any model for the alamethicin channel. Analogs of emerimicin of different lengths have been prepared to probe the effect of helix length on pore stability. NMR has been used to show that a nonapeptide fragment of emerimicin changes its conformation to a predominately 3_{10}-helix from the alpha-helix seen in the crystal when dissolved in DMSO. This is consistent with a theoretical analysis of the forces stabilizing the two helical forms. Umbrella sampling of molecular dynamics simulations has shown that the activation energy barrier between the 3_{10}-helix and the alpha-helix is small, and that the relative stabilities of the two helical forms are a function of helix length. Solid state NMR techniques (REDOR) are being developed to allow the precise measurement of interatomic distances to determine conformations of peptaibol antibiotics in membranes. Integrations of the results from this multidisciplinary approach will provide the foundation for realistic models of voltage-gated synthetic pores.

"Molecular Mechanism of Voltage-Dependent Ion Channels"

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Principal Investigator

Contract N00014-90-J-1393
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ANNUAL REPORT
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Research Objectives

General

To gain an understanding of the interaction of a molecule at the lipid/water interface of a membrane with the external electric field, using peptaibols and the S4 segment of the sodium channel as a model system.

Specific

To assess the role of the molecular dipole in sensing the imposed molecular field; the role of α-helix-3_{10}-helix transitions in responding to the imposed molecular field as the transduction mechanism; and the role of the channel mouth in determining the ion selectivity associated with channel opening.

Progress (Year 1)

Significant progress has occurred in three areas, synthetic, experimental and theoretical.

Synthetic

Ac-(Lys(alamethicin 1-17))$_4$-NH_2, tetramethicin, which was prepared by coupling several small fragments to tetralysine while attached to a solid support, showed an effective concentration dependence 2.7 times that of alamethicin. This concentration dependence is dramatically different from the control monomeric alamethicin 1-17 benzyl ester, which is severalfold less than alamethicin itself. Because of difficulties in determining the absolute purity of tetramethicin, we decided to prepare the same material by a totally different synthetic route which was more conducive to monitoring purity. Dr. Karol Kociolek prepared the Boc-Lys(alamethicin 1-17) monomer in solution by fragment condensation. This monomer is being used in solid phase synthesis to prepare oligomers of this peptide. In this case, any synthetic problem will differ from the desired product by 18 amino acids, which should allow easy purification by size exclusion chromatography. A second area of progress has been in the resolution of α-methyl-Cys(S-benzyl) by Drs. Tom Leplawy and Urszula Slomczynska. We plan to use this compound to cross-link alamethicin sidechains. The availability of Boc-Lys(alamethicin 1-17) monomer will allow unambiguous interpretation of the position of the cross-link.

In an effort to determine the length dependence of activity, a series of emerimicin analogs has been prepared in which the emerimicin nonapeptide, Ac-Phe-MeA-MeA-MeA-Leu-Gly-Val-MeA-MeA-OBzl, has been extended by the dipeptide, Ala-MeA-OBzl, to yield the following peptides:

Ac-Phe-MeA-MeA-MeA-Leu-Gly-Val-MeA-MeA-Ala-MeA-OBzl (11)

Ac-Phe-MeA-MeA-MeA-Leu-Gly-Val-MeA-MeA-Ala-MeA-Ala-MeA-OBzl (13)

Ac-Phe-MeA-MeA-MeA-Leu-Gly-Val-MeA-MeA-Ala-MeA-Ala-MeA-Ala-MeA-OBzl (15)

These compounds will be sent for assay on bilayers when their characterization is complete.

In the past, synthetic difficulties associated with low yields in the couplings of α,α-dialkylamino acids have prevented the routine use of solid phase peptide synthesis in the preparation of peptaibol antibiotics. Recent developments in new coupling reagents (BOP, TBTU, etc.) have sufficiently improved the speed of reaction and yields in solution when using these sterically hindered residues that we intend to reinvestigate the use of solid phase synthesis in the preparation of analogs. Success would dramatically increase our synthetic capacity to explore structure-activity relations.

Experimental

On the experimental front, we continue to explore new solid state NMR techniques (REDOR) in collaboration with their developer, Dr. Jacob Schaeffer, which we believe will be applicable in liposomes. We demonstrated this approach on an emerimicin fragment using ^{15}N to perturb the ^{13}C label. To enhance the sensitivity, we have decided to use ^{19}F to perturb the ^{13}C label and to use an adjacent ^{15}N to allow selection of the label and elimination of natural abundance ^{13}C signals. To this end, the following new labelled emerimicin fragments have been prepared:

FCH_2CO-Phe-MeA-MeA-MeA-$^{13}C0$-^{15}N-Leu-Gly-Val-MeA-MeA-OBzl

FCH_2CO-Phe-MeA-MeA-MeA-Leu-Gly(C1,C2-^{13}C)-Val-MeA-MeA-OBzl

FCH_2CO-Phe-MeA-MeA-MeA-$^{13}C0$-Leu-Gly-Val-MeA-MeA-OBzl.

These peptides are being used to calibrate the REDOR approach.

Dr. Denise Beusen has completed the study of the conformation of emerimicin 1-9 benzyl ester in DMSO. Numerous technical problems had to be overcome because of the absence of α-protons in much of the sequence. Dr. Beusen employed systematic search procedures developed by our group to allow assignment of hydrogen bonding partners in an unambiguous way. The structure determined is predominately 3_{10}-helix, in contrast with the conformation in the crystal where this peptide shows α-helical hydrogen bonding. This result complements our theoretical results, indicating a length dependence on helical preference as well as a rationale for environmental selection of helical type. A paper describing this work is nearing completion.

Theoretical

On the theoretical front, we have investigated the transition between the 3_{10}- and α-helix by molecular dynamics. Using umbrella sampling and varying the stretch of the decapeptide, we have been able to show that the activation energy barrier between the two helical forms is relatively low compared with kT. This implies that hypotheses which consider a transition of helical forms are plausible. This is important for shorter peptaibols, such as emerimicin, in which the length of 15 residues is not sufficient to span the bilayer unless it assumes a 3_{10}-helix. Other studies on peptides of different numbers of residues suggest that a peptid· of 7 residues would show no preference beween an α- and 3_{10}-helix conformation in a hydrophobic environment with a shorter length favoring a 3_{10}-helix and a longer length favoring an α-helix. While 3_{10}-helices have been observed in protein crystal structures, they are all short (less than six residues in length). By providing a theoretical rationale for a facile transition between helix types, new mechanisms for other biological systems become plausible. For example, in the case of the insulin receptor, the extracellular receptor domain is connected to the tyrosine kinase domain by a single transmembrane helix. A single change in the hydrogen bonding scheme at the junction with the receptor domain on ligand binding could trigger a change in helix type and trigger a conformational change in the tyrosine kinase resulting in activation.

Work Plan (Year 2)

In year two, we will complete the synthesis and characterization of trimethicin, tetramethicin, pentamethicin and hexamethicin using the Boc-Lys(alamethicin 1-17) monomer prepared by Dr. Kociolek. We will incorporate resolved α-methyl-Cys(S-benzyl) into Boc-Lys(alamethicin 1-17) monomers and use these to prepare oligomers in which the monomers are cross-linked by disulfides as well as by the C-terminal oligoLys bridge. We will attempt to prepare cyclic oligomers of tetramethicin by synthesis on a benzyl ester support and cyclization of the oligoLys backbone between the N-terminal amine and the C-terminal carboxyl groups. If these compounds retain activity, then considerable constraints on the conformation of the active pore will have been established.

Other cyclic scaffolds will be used to build oligomers of peptaibols. We have derivatized aza-crown ethers by acylating the nitrogens with Boc-β-alanine. Peptide fragments will be added to the amino groups of the β-alanine to give a tetramer in the case of the aza-crown-4 system which is our model. By increasing the size of the ring and number of nitrogen sites, one can control both channel diameter as well as the size of the mouth.

We plan to study the conformation of emerimicin itself by NMR with several solvents of varying dielectric constants to confirm the ease of transition between 3_{10}- and α-helix. In addition, we will initiate studies of our labelled emerimicin fragments in various solvents in the frozen state for the same reasons.

Inventions (Year 1)

We are in the process of evaluating any commercial potential for the compounds prepared and the routes of synthesis. While no patents have been applied for, we believe that some should be in the future.

Publications and Reports (Year 1)

Beusen, D.D. and G.R. Marshall. The generation of three-dimensional structures from NMR-derived constraints. In: *Protein Structure and Engineering*, O. Jardetzky, Ed., Plenum, pp. 97-109 (1989).

Marshall, G.R., E.E. Hodgkin, D.A. Langs, G.D. Smith, J. Zabrocki and M.T. Leplawy. Factors governing helical preference of peptides containing

multiple α,α-dialkyl amino acids. *Proc. Natl. Acad. Sci. USA* 87:487-491 (1990).

Leplawy, M.T., K. Kociolek, A.S. Redlinski, U. Slomczynska, J. Zabrocki, J.B. Dunbar, Jr. and G.R. Marshall. Chemical-enzymatic synthesis of emerimicin IV and III, membrane-active pentadecapeptide antibiotics. In: *Proc. 11th Am. Peptide Symp.* J. Rivier and G.R. Marshall, Eds., ESCOM, Leiden, pp. 493-495 (1990).

Marsnall, G.R., D.D. Beusen, J.D. Clark, E.E. Hodgkin, J. Zabrocki and M.T. Leplawy. Helical preferences of peptides containing multiple α,α-dialkyl amino acids. In: *Proc. 11th Am. Peptide Symp.* J. Rivier and G.R. Marshall, Eds., ESCOM, Leiden, pp. 873-876 (1990).

Marshall, G.R., D.D. Beusen, K. Kociolek, A.S. Redlinski, M.T. Leplawy, Y. Pan and J. Schaefer. Determination of a precise interatomic distance in a helical peptide by REDOR NMR. In: *Proc. 11th Am. Peptide Symp.* J. Rivier and G.R. Marshall, Eds., ESCOM, Leiden, pp. 608-609 (1990); *J. Am. Chem. Soc.* 112:963-966 (1990).

Beusen, D.D., H. Iijima and G.R. Marshall. Structures from NMR distance constraints. *Biochem. Pharmacol.* 40:173-175 (1990).

Marshall, G.R., D.D. Beusen, J.D. Clark and E.E. Hodgkin. Helical transitions in peptides as possible gating mechanisms. Abstract, International Workshop on Transmembrane Electric Fields, Maryland, October 23-26, 1990.

Clark, J.D., D.D. Beusen, E.E. Hodgkin and G.R. Marshall. Helical transitions in peptides. Contribution for a volume to be dedicated to Prof. G.N. Ramachandran; to be published by the Indian Academy of Sciences, Bangalore, India (in press).

Hodgkin, E.E., J.D. Clark, K.R. Miller and G.R. Marshall. Conformational analysis and helical preferences of normal and α,α-dialkyl amino acids. *Biopolymers* (in press).

Slomczynska, U., J. Zabrocki, M.T. Leplawy and G.R. Marshall. Toward facilitated synthesis of peptaibols: Alamethicin via enzymatic segment condensation. In: *Peptides 1990*, E. Giralt and D. Andreu, Eds., ESCOM, Leiden (in press).

Leplawy, T., Jr., U. Slomczynska, M.T. Leplawy and G.R. Marshall. Peptides containing α,α-disubstituted amino acids. Experiments related to α-methylcysteine. In: *Peptides 1990*, E. Giralt and D. Andreu, Eds., ESCOM, Leiden (in press).

Beusen, D.D., W.C. Hutton, J.J. Kotyk, J. Zabrocki, M.T. Leplawy and G.R. Marshall. ^{13}C and ^{1}H resonance assignments and structural determination of an emerimicin peptide in DMSO. In: *Peptides 1990*, E. Giralt and D. Andreu, Eds., ESCOM, Leiden (in press).

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