

TOP SECRET

2

AD-A199 681

(U)

CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. SECURITY CLASSIFICATION (U)		1b. RESTRICTIVE MARKINGS NA	
2. SECURITY CLASSIFICATION AUTHORITY NA		3. DISTRIBUTION / AVAILABILITY OF REPORT Distribution Unlimited	
4. CLASSIFICATION / DOWNGRADING SCHEDULE NA		5. MONITORING ORGANIZATION REPORT NUMBER(S) NA	
6. DRIVING ORGANIZATION REPORT NUMBER(S) Rice University		7a. NAME OF MONITORING ORGANIZATION Office of Naval Research	
8a. NAME OF PERFORMING ORGANIZATION Rice University		7b. ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000	
8b. OFFICE SYMBOL (if applicable) NA		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-86-K-0087	
8c. ADDRESS (City, State, and ZIP Code) Physics Department P.O. Box 1982 Houston, TX 77251		10. SOURCE OF FUNDING NUMBERS	
8d. ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000		PROGRAM ELEMENT NO 61153N	TASK NO 4414704
11. TITLE (Include Security Classification) (U) Investigating the Structural Bases of Voltage-gating Model Channels by Using Perfectly Aligned Multilayer Samples.			
12. PERSONAL AUTHOR(S) Huang, Huey W.			
13a. TYPE OF REPORT Annual	13b. TIME COVERED FROM 10/87 TO 9/88	14. DATE OF REPORT (Year, Month, Day) 9/20/88	15. PAGE COUNT 4
16. SUPPLEMENTARY NOTATION			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD 08	GROUP	Alamethicin; Melittin; Gramicidin; Orientation Dependent Circular Dichroism; X-ray Diffraction; Neutron Scattering; Perfectly Aligned Multilayers	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) One-dimensional quasi-crystals of perfect multilayers, in which ion channels are uniformly oriented within parallel membranes, can be used to study the structural bases of channel conductivities. We have developed 1) the techniques for preparing such multilayer samples and 2) the spectroscopic methods (circular dichroism and x-ray diffraction) for extracting structural information from these samples. The sample variables include electric field, water content, ion concentrations, etc. We have observed conformation changes of alamethicin with water content, a result in favor of the barrel model (rather than the flip-flop model) for the channel. Our goal is to probe the conformation changes of the channels as we vary the sample variables, in order to elucidate the molecular mechanisms of voltage-gating.			
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION (U)	
22a. NAME OF RESPONSIBLE INDIVIDUAL Dr. Igor Vodyanoy		22b. TELEPHONE (Include Area Code) 202-696-4056	22c. OFFICE SYMBOL ONR

DD Form 1473, JUN 86

Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

DISTRIBUTION STATEMENT A

Approved for public release; Distribution Unlimited

88 9 26 90

Introduction

Because of the difficulty in making single crystals of membrane ion channels in their native forms (suitable for x-ray diffraction), there is a lack of structural information for understanding their molecular mechanisms. We believe that, under the circumstances, one-dimensional (1D) quasi-crystals of perfect multilayers, in which channels are uniformly oriented within parallel membranes, can be used to provide some of the much needed structural data. In the past few years, we have developed 1) the techniques for preparing such multilayer samples and 2) the spectroscopic methods for extracting structural information from these samples. Our goal is to investigate the conformation changes occurring in the channels when they are subject to electric field or variations in chemical conditions, in order to elucidate the molecular mechanisms of voltage-gating. A sensible approach to this complicated problem is to study simple model channels such as alamethicin and melittin first. However, it is important to point out that our method is applicable to natural proteins; for example, we have applied our method to study cytochrome b from yeast complex III in another research project. In the following, we review our objectives and the progress we made last year.

Objectives

1. Preparing multilayer samples of gramicidin, alamethicin and melittin, and experimenting the variations of their chemical conditions.
2. Circular dichroism (CD) of multilayer samples to study the orientations of the α -helical sections in the channels.
3. X-ray scattering of multilayer samples to study the ion binding sites.
4. Normal incident neutron scattering of multilayer samples to study the channel distributions in membrane.
5. Electric field studies of multilayer samples to create the voltage-gating condition in the channels.

Accomplishments

1. **Multilayer Samples** -- We have by now successfully produced perfect multilayers of lipid-peptide mixtures between two surfaces of fused silica, electrode (indium tin oxide) coated fused silica, mica and beryllium. Different substrata are used depending on the type of experiment. The thickness of multilayers can be varied between 1 and 100 μm . The sample variables include the peptide/lipid ratio, water content (15 to 40% of sample weight) and ion (e.g. Na^+ , K^+ , etc.) concentrations. The lipids used so far include dilauryl-, dimyristoyl-, dipalmitoyl-, and diphytanoyl-phosphatidylcholine (DLPC, DMPC, DPPC and DFhPC, respectively). These samples are free of smectic defects, transparent to light, and perfectly ordered in the direction perpendicular to the substrata surfaces (the mosaic spread $\sim 0^\circ$) (for details see Huang and Olah, 1987 and Olah and Huang, 1988a).

For	<input checked="" type="checkbox"/>
	<input type="checkbox"/>
	<input type="checkbox"/>
	<input type="checkbox"/>



By	Distribution/	
	Availability codes	
	Avail and/or	Special
Dist		
A-1		

2. CD -- Although a theory describing the dependence of CD on the orientation of an α -helix was known since the 1950's (Moffitt, 1956), experimentally it remained unproven until recently. The reasons are complicated and they are discussed in our recent papers (Olah and Huang, 1988a and 1988b). Because α -helices are somewhat flexible, only short (and hence straight) peptides can provide uniformly oriented α -helices. And in fact this condition has been achieved only in our perfectly aligned multilayer samples. We used the CD of alamethicin embedded in multilayers to prove the Moffitt theory and simultaneously established that the α -helical section of alamethicin was perpendicular to membrane under the condition we prepared the sample. A special technique was devised to measure CD of multilayers with light incident on the membranes at various tilted angles. Figure 1 shows an example of such measurement. The technique is very sensitive to the conformation changes of peptides. For example, Fig. 2 shows the changes of CD of alamethicin in DPhPC with hydration. As explained in the Fig. 2 caption, the results appear to be in favor of the barrel model (Hall, Vodyanoy, Balasubramanian and Marshall, 1984) rather than the dipole flip-flop model (Menestrina, Voges, Jung and Boheim, 1986) for the formation of the alamethicin channel. A more complete analysis is in progress.

3. 1-D X-ray Diffraction -- Since our multilayers are in fact one-dimensional (1-D), quasi-crystals (perfect ordering in the direction normal to the planes of membranes), their 1-D electron density profiles can be determined by x-ray diffraction. It is, however, not easy to unravel the peptide signals from the intense diffraction background due to lipid bilayers. Therefore, our effort has been concentrated on measuring the ion distribution profiles, including the locations of the ion binding sites in the channels, by using heavy metal ions such as cesium and thallium. Early on we had difficulties with the x-ray absorption by the materials used to support the multilayers. This problem has now been solved by using beryllium. The diffraction of thallium ions in the gramicidin channels has been measured. Twelve Bragg peaks were recorded, amounting to 2-3 Å resolution. The analysis, including solving the phase problem, is in progress.

4. Normal Incident Neutron Scattering -- This experiment was designed to measure the 2-D protein distribution in the plane of membrane, which contains information about the protein-membrane interactions (Huang, 1986). Other research groups have attempted similar measurements of 2-D protein distributions by using vesicular samples and always found their signals masked by that of vesicles (despite their efforts of index matching with deuteration). On the contrary, the normal incident neutron scattering of defect-free, pure lipid multilayers gave a flat background (no angular dependence). Therefore if a protein has a reasonable neutron scattering contrast against the lipid background, its distribution can be measured. For this purpose, either peptide or lipid needs to be fully deuterated. We have placed an order with Avanti Polar Lipids (Pelham, AL) to synthesize fully deuterated DLPC. Their schedule and the problems in both the Oak Ridge and the Brookhaven neutron facilities have delayed the progress of this experiment.

5. Electric Field -- We have successfully coated indium tin oxide on fused silica surfaces so that an electric field of up to 50 kv can be applied across a multilayer sample (Olah and Huang, 1988b). The coated electrode is thin enough that it does not interfere with the CD measurement of the sample. However, the joule heating and the electrode damage at the anode have been problems. We have reduced the sample conductivities by purifying the chemical components. We have also coated the electrode with thin silicon dioxide to prolong its life. After many experiments, we found that we could apply to our samples electric field of square steps, each 0.1 s on and 0.9 s off, for hours. This allows at least two types of experiments. 1) Our CD spectrometer (Jasco J-500A) allows signal averaging with 0.1 s point measurements. Thus we can measure the CD of ion channels in electric field. 2) With synchrotron radiation, subnanosecond-resolved diffraction measurement is now possible (Science 241, 295, 1988). Thus we can measure the x-ray diffraction of ion channels in electric field.

Publications

- H. W. Huang, "Deformation Free Energy of Bilayer Membrane and Its Effect on Gramicidin Channel Lifetime" *Biophys. J.* 50, 1061-1071 (1986).
- T. Y. Teng and H. W. Huang, "Hemoglobin and Myoglobin Embedded in Dry Polyvinyl Alcohol Film for X-ray Absorption Studies" *Biochem. Biophys. Acta* 874, 13-18 (1986).
- H. W. Huang and G. A. Olah, "Uniformly Oriented Gramicidin Channels Embedded in Thick Monodomain Lecithin Multilayers" *Biophys. J.* 51, 989-992 (1987).
- T. Y. Teng, H. W. Huang and G. A. Olah, "5K EXAFS and 40K 10-Second Resolved EXAFS Studies of Photolyzed Carboxymyoglobin" *Biochemistry* 26, 8066-8072 (1987).
- H. W. Huang, Book Review: "Accuracy in Molecular Processes" by T. B. L. Kirkwood, R. F. Rosenberger and D. J. Galas. *Am. Sci.* 76, 303 (1988).
- G. A. Olah and H. W. Huang, "Circular Dichroism of Oriented α -Helices I. Proof of the Exciton Theory" *J. Chem. Phys.* 89, 2531-2538 (1988).
- G. A. Olah and H. W. Huang, "Circular Dichroism of Oriented α -Helices II. Electric Field Oriented Polypeptides" *J. Chem. Phys.* Dec. (1988).

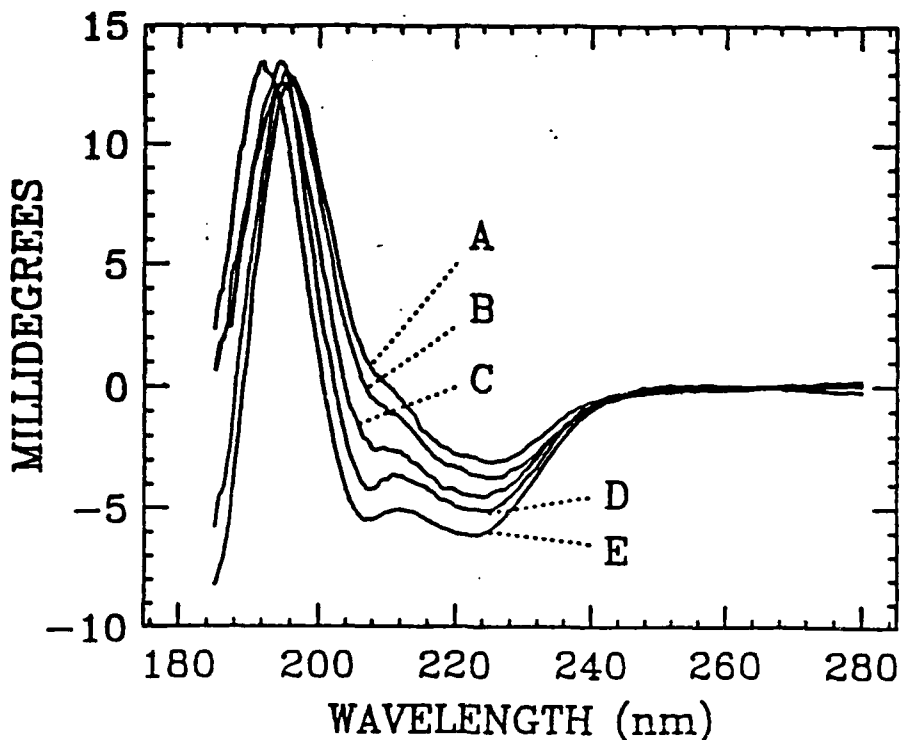


Fig. 1: Circular dichroism of oriented alamethicin embedded in DLPC multilayers at tilt angle (between the direction of light and the normal to the planes of bilayers) $\alpha = 0^\circ$ (A), 15° (B), 30° (C) and 45° (D). The amplitude at 208nm is proportional to $\sin^2\alpha$ as predicted by the Moffitt theory of α -helices; this is the first proof of the theory (Olah and Huang, 1988a).

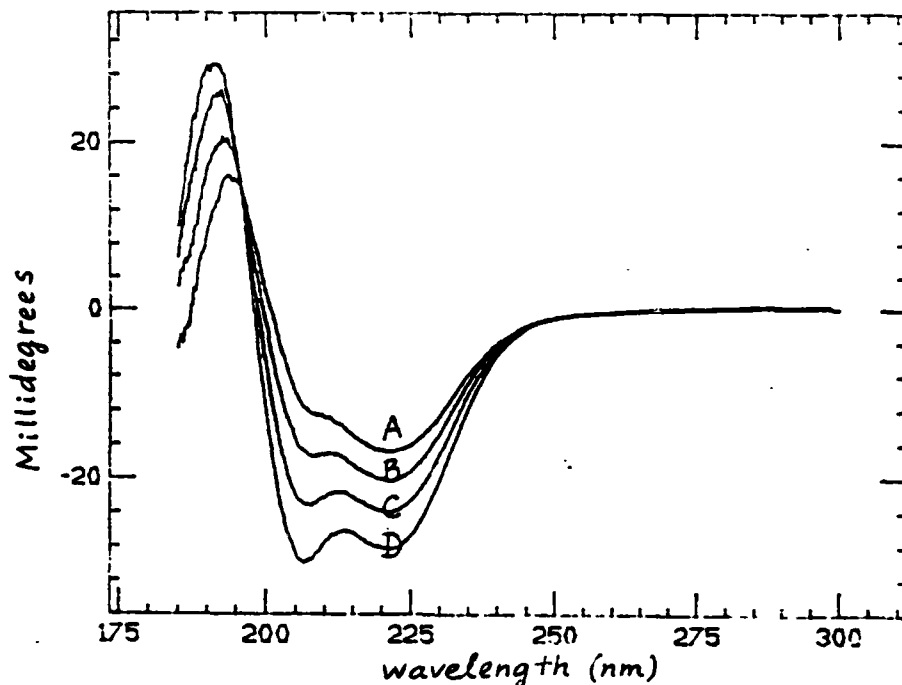


Fig. 2.: The changes of the circular dichroism of alamethicin embedded in DPhPC multilayers with the degree of hydration. Spectra were taken at normal incidence with the same sample by varying the equilibrating humidity: (A) 100% humidity; (D) 0% humidity; (B) and (C) in between. The results indicate that the insertion of α -helices into the membrane requires excessive water. This appears to be in favor of the barrel model (rather than dipole flip-flop model) for the formation of the alamethicin channel.

DISTRIBUTION LIST FOR REPORTS

May, 1972

ONR MEMBRANE ELECTROCHEMISTRY PROGRAM

Dr. Martin Blank
Department of Physiology
Columbia University College
of Physicians and Surgeons
630 W. 168th Street
New York, NY 10032

Dr. William E. Brownell
Department of Otolaryngology-HNS
Johns Hopkins University
School of Medicine
720 Rutland Avenue
Baltimore, MD 21205

Dr. Marco Colombini
Department of Zoology
University of Maryland
College Park, MD 20742

Dr. Michael A. Cusanovich
Department of Biochemistry
University of Arizona
Tucson, AZ 85721

Dr. D. W. Deamer
Department of Zoology
University of California
Davis, CA 95616

Dr. Edward A. Dratz
Department of Chemistry
Montana State University
Bozeman, MT 59717

Dr. Harvey M. Fishman
Department of Physiology and
Biophysics
University of Texas Medical Branch
Galveston, TX 77550

Dr. Sol M. Gruner
Department of Physics
Jadwin Hall
Princeton University
P. O. Box 708
Princeton, NJ 08544

Dr. Felix T. Hong
Department of Physiology
Wayne State University
540 E. Canfield Avenue
Detroit, MI 48201

Dr. Huey W. Huang
Department of Physics
Rice University
Houston, TX 77251

Dr. Israel R. Miller
Department of Membrane Research
The Weizmann Institute of Science
Rehovot 76100
ISRAEL

Dr. V. Adrian Parsegian
Laboratory of Chemical Biology,
NIADDK
Room 9N-307
Building 10
Bethesda, MD 20892

Dr. Davis S. Perlin
Department of Biochemistry
Public Health Research Institute
455 First Avenue
New York, NY 10016

Dr. H. Gilbert Smith
EG & G Mason Research Institute
57 Union Street
Worcester, MA 01608

Dr. Michael E. Starzak
Department of Chemistry
State University of New York
Binghamton, NY 13901

Dr. H. Ti Tien
Department of Physiology
Membrane Biophysics Laboratory
Michigan State University
East Lansing, MI 48824

Dr. Tian Y. Tsong
Department of Biological Chemistry
Johns Hopkins University
School of Medicine
725 N. Wolfe Street
Baltimore, MD 21205

Dr. Peter Vanysek
Department of Chemistry
Northern Illinois University
De Kalb, IL 60115

ONR MEMBRANE ELECTROCHEMISTRY PROGRAM

Dr. Howard Wachtel
Dept. of Electrical & Computer Eng.
University of Colorado
Campus Box 425
Boulder, CO 80309

Dr. James C. Weaver
Div. Health Sciences & Technology
Room 20A-128
Massachusetts Institute of Tech.
Cambridge, MA 02139

Dr. George S. Wilson
Department of Chemistry
University of Kansas
Lawrence, KS 66045

Annual Final and Technical Reports

ADMINISTRATORS

Dr. Igor Vodyanoy, Code 1141SB (2 copies)
Scientific Officer, Biophysics
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Dr. Robert J. Nowak, Code 1113ES
Scientific Officer, Electrochemical
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Administrator (2 copies) (Enclose DTIC Form 50)
Defense Technical Information Center
Building 5, Cameron Station
Alexandria, VA 22314

Program Manager
Biological/Human Factors Division
Code 125
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Administrative Contracting Officer
ONR Resident Representative
(address varies - obtain from contract or
your business office)

Program Manager Defense Technical
Support Technology Directorate
Office of Naval Technology, Code 223
800 N. Quincy Street
Arlington, VA 22217-5000

Annual and Final Reports Only (one copy each)

DoD ACTIVITIES

Commander
Chemical and Biological Sciences Division
Research Army Research Office, P. O. Box 1221
Research Triangle Park, NC 27709

Directorate of Life Sciences
Air Force Office of Scientific
Bolling Air Force Base Research
Washington, DC 20332

Head
Biomolecular Engineering Branch
Code 6190
Naval Research Laboratory
Washington, DC 20375

Final and Technical Reports Only

Director, Naval Research Laboratory (6 copies)
Attn: Technical Information Division, Code 2627
Washington, DC 20375