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MEMBRANE-MEMBRANE INTERACTIONS IN A LIPID-CONTAINING BACTERIOPHAGE SYSTEM

PROGRESS REPORT

Submitted by

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PROGRESS REPORT

The basic research efforts in our laboratory this past year have focussed on two aspects of the life cycle of PM2, a lipid-containing bacteriophage. The first of these concerns the initial interaction of PM2 with the outer membrane of its host cell, Pseudomonas BAL-31. The second concerns the assembly of PM2 in infected cells and the structural features of hydrophobic membrane perturbers that inhibit PM2 assembly.

Considerable emphasis has been placed on the potential use of hydrophobic membrane perturbers as antiviral agents. Since our initial discovery several years ago that butylated hydroxytoluene (BHT) inactivates lipid-containing viruses, including Herpes Simplex Virus, several other laboratories have shown that BHT is effective against a variety of animal viruses including Newcastle Disease Virus, cytomegalovirus, and semliki forest virus. We have concentrated on experiments designed to elucidate the mechanism of action of this class of agents and, in particular, the reasons why viruses are more sensitive to these compounds than are their host cells. Several studies related to the antiviral characteristics of hydrophobic membrane perturbers are outlined in this report.

Several projects of a general nature, advancing techniques and methods of analysis in our area of research, have been completed and are described below. One of these, the use of hydrophobic photosensitizers to specifically deliver damage to biological membranes, has opened up a new area of research which we hope to develop in the coming year.

A. Distribution of PM2 Receptors.

As outlined in last years renewal proposal, we have made use of a mutant of BAL-31, the host for PM2, to study the nature of PM2 receptors on the host cell surface. This mutant, designated RH12, is unable to

synthesize PM2 receptors at 32°, although it synthesizes normal amounts at 25°. The wild type cell synthesizes PM2 receptors equally well at both of these temperatures. Experiments were carried out in which cultures of RH12 previously grown at 25° for many generations were subsequently grown at 32° for one or more generations. The segregation of PM2 receptors during cell division was measured by determining the survival of cells to PM2 following growth at 32°. Our data suggest that PM2 receptors are non-randomly distributed on the cell surface, and may exist in patches which segregate as a unit during cell division. These data and ideas were presented at the Biophysical Society meeting in Atlanta (see abstract in Appendix I) and have been submitted for publication.

B. Effects of Adamantane Derivatives on PM2 Production.

Several years ago, we found that the membrane perturber adamantanone inhibits the assembly of PM2, even though it has no effect on growth of the host cell. This observation has more recently been interpreted in terms of a model for PM2 assembly in which specific phospholipid species undergo phase separation in the host cell membrane prior to their incorporation into the maturing virus (see Appendix II). Thus, evidence was presented that adamantanone may interfere with the phase separation and thereby prevent assembly of the viral lipid bilayer.

We have now extended these studies to a survey of the effects of adamantanone derivatives on PM2 stability and assembly. A summary is presented in the table below. Two results of considerable interest should be noted. First, the hydroxyl derivative is virucidal for PM2, causing a reversible inactivation of the virus. We have previously found that hydrophobic alcohols are potent inactivators of lipid-containing viruses and have

speculated that there might be a hydrogen bond involvement between the hydroxyl group and the ester bond in phospholipids. The second result of interest is that the acetic acid derivative is very effective in preventing PM2 production. This molecule is negatively charged at physiological pH, and its presence in the infected cell membrane may inhibit the aggregation of negatively charged phosphatidyl glycerol into pools and thereby inhibit PM2 assembly, as outlined in the model in Appendix II.

Table 1. Effects of Adamantane derivatives on the stability and assembly of PM2

Adamantane Derivative	Treatment with 2mM	
	% Survival	Burst Size
2-adamantanol	10	Not applicable
1-adamantamine	65	90
1-acetyladamantane	80	95
1-succinyladamantane	98	110
1-adamantane acetic acid	80	6

C. Hydrophobic Membrane Perturbers as Antiviral and Virucidal Agents.

Earlier, we found that a peak in virucidal activity for n-alkyl derivatives of BHT occurs for an alkyl chainlength of four carbons. The details of this work are now in press and are included as Appendix III. In the past year we have found that enveloped viruses are extremely sensitive to long-chain unsaturated monoglycerides and alcohols (see Appendix IV), with inactivation occurring in vitro at concentrations as low as 0.2 micromolar in some cases. In another study, retinal (vitamin A aldehyde) was shown to be very effective against HSV. A manuscript describing the antiviral characteristics of several vitamin A derivatives has been submitted for publication.

An important breakthrough in our understanding of the mechanism whereby membrane perturbers inactivate the enveloped bacterial virus ϕ6 came with the design and synthesis of a spin-labeled antiviral agent. This compound, designated BPN, is active against ϕ6, PM2, and HSV, and appears to act by a mechanism similar to that of BHT. When ϕ6 is treated with BPN or BHT a specific envelope protein is removed and the virus can no longer attach to its host. Spin label studies with BPN revealed that the molecule is localized in zones of comparatively free lipid in the host cell membrane, where it causes no apparent harmful effects. In ϕ6, however, the ESR data show that there are no such "free lipid" pools. This difference between the cell and viral membranes may explain the differential sensitivity of the virus to the agent. Our working hypothesis is that, in ϕ6, BPN interacts with "boundary lipids" that are in close contact with membrane proteins, that this weakens the forces that hold integral membrane proteins in place, and that the ϕ6 attachment protein is removed. These results were presented at the Biophysical Society meeting (see Appendix V) and are being submitted for journal publication.

D. Hydrophobic Photosensitizers.

Photodynamic dyes, combined with exposure to visible light, have been used in a number of studies with viruses and cells. The dyes that have most commonly been used are water soluble, have charged side groups, and often intercalate into DNA. We have initiated some studies with acridine, a hydrophobic photosensitizer that partitions into membrane structures. Acridine plus near ultraviolet light inactivate lipid-containing viruses through a singlet oxygen mechanism. Our evidence strongly suggests that damage to the viral membrane is the cause of inactivation. The details of this work were published in Photochemistry and Photobiology and are included as Appendix VI in this report.

E. Technique Development and Related Studies.

(1) A survey and summary of some of our previous work on the nature of cytoplasmic water appeared as a chapter in a book on that subject. Appendix VII is an outline of that article.

(2) A novel method for detecting metal ion chelation and complex formation, involving spin label linebroadening was developed this past year. Its greatest advantage is that it can be used in complex biological systems where the usual techniques for detecting chelation do not apply. This advancement was presented at the Biophysical Society meeting (see Appendix VIII) and a manuscript on the technique has been accepted for publication in Analytical Biochemistry.

(3) In collaboration with Dr. Thomas Coohill, who spent his sabbatical leave of absence in our laboratory, some experiments on the effects of ultraviolet radiation on the capacity of mammalian cells to support virus growth were carried out. A surprising observation was that cells irradiated with low doses of UV 24 - 80 hours prior to virus infection produce larger plaques than do unirradiated cells. An abstract of this work is given in Appendix IX and the details have been submitted for journal publication.

Personnel on Project*	% of Time
W. Snipes, Principal Investigator	10
N. DeLuca, Graduate Student	50
S. Wagner, Graduate Student	50
S. Y. Choo, Graduate Student	50
G. Keller, Research Aide	50
M. Murrer, Laboratory Helper	20
T. Coohill, Visiting Professor	20

* Other sources of funds were available for salary support of certain personnel.

APPENDICES

I. Non-Random Distribution of PM2 Receptors on the Host Cell Outer Membrane. (Abstract, manuscript submitted) Removed

II. Assembly of Viral Membranes. In Light Transducing Membranes, D. Deamer, Ed., Academic Press 1978. Removed

III. Hydrophobic Alcohols and Di-tert-butyl Phenols as Antiviral Agents. In Pharmacological Role of Lipids, J. Kabara, Ed. (In Press) Removed

IV. Extreme Sensitivity of Enveloped Viruses, including Herpes Simplex, to Long-Chain Unsaturated Monoglycerides and Alcohols. Antimicrob. Agents Chemother. 15:67 (1979).

V. Perturbation of Protein-Lipid Interactions in Bacteriophage ϕ6. (Abstract, manuscript in preparation.)

VI. Inactivation of Lipid-Containing Viruses by Hydrophobic Photosensitizers and Near-Ultraviolet Radiation. Photochemistry and Photobiology 29:785 (1979).

VII. Spin Label Studies on the Aquesous Cytoplasm. In The Aqueous Cytoplasm, A. Keith, Ed., Marcel Dekker, Inc., 1979.

VIII. A Spin Label Assay for Metal Complexation. (Abstract, manuscript in press in Analytical Biochemistry).

IX. Ultraviolet Radiation Affects Herpes Virus Plaque Development in Mammalian Cells (Abstract, manuscript submitted).