2
0
MASTER

COO-2311-29

MEMBRANE-MEMBRANE INTERACTIONS IN A LIPID-CONTAINING BACTERIOPHAGE SYSTEM

PROGRESS REPORT

Submitted by

Wallace Snipes, Principal Investigator

Biophysics Program
Department of Biochemistry and Biophysics
College of Science
The Pennsylvania State University
University Park, Pennsylvania 16802

Period Covered: October 1, 1977 to September 30, 1978

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

Prepared for the U. S. Department of Energy under Contract EY-76-S-02-2311

May, 1978

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

Reg

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

PROGRESS REPORT

Research in our laboratory for the past several years has focused on the properties of virus membranes and the dynamic aspects of virus membrane assembly. For most of these studies, the lipid-containing bacteriophage PM2 has been utilized. More recently, experiments have been carried out with the enveloped bacteriophage $\phi 6$ and the human pathogen herpes simplex virus. A fundamental observation, with potential clinical significance, was that virus membranes and membrane-associated processes are more susceptible to the effects of hydrophobic perturber molecules than are the membranes of cells. Thus, certain compounds such as butylated hydroxytoluene and dodecanol will inactivate enveloped viruses under conditions that are comparatively harmless to host cells. Other molecules, such as adamantanone, will inhibit the assembly of virus membranes at concentrations where host-cell growth is normal. The perturbation of virus membrane processes can be exploited both as a basic research tool and as a potential mechanism for controlling certain virus infections. We are continuing to investigate this selective effect on virus membranes, and some data relating to the structural requirements for maximum perturbation of virus membranes are included in this report. The accompanying renewal proposal describes several projects designed to explore the nature of the perturbations produced in virus membranes by hydrophobic agents.

Collaboration with other laboratories on related research has continued, and the results of some of these studies are also included in this report.

A. Dynamic Aspects of Virus Membrane Assembly

Sufficient data have now been accumulated on the infectious process of PM2 to form the basis of a model for its assembly. We have attempted to formulate a working hypothesis for PM2 based on what is known about its structure as well as the functional alterations it induces in infected cells. The main experimental observations that must be reconciled in such a model are the following:

- (1) Although the phospholipids that are incorporated in PM2 are cellular in origin, a selective process exists for their utilization. This is so because PM2 has approximately 65% phosphatidylglycerol (PG) and 35% phosphatidylethanolamine (PE), whereas the host cell has about 75% PE and 25% PG in its membrane.
- (2) The PM2 bilayer is asymmetric, with the negatively charged PG being located predominantly on the outer portion.
- (3) The external protein has a high isoelectric point, indicating that it is positively charged in the virion.
- (4) Calcium ions are strictly required for the assembly of PM2, but are not essential for its stability after assembly is complete. The mature virion contains no detectable calcium.
- (5) Membrane perturbers such as adamantanone, which fluidize the hydrocarbon zones, prevent PM2 assembly but do not disrupt the mature virion formed in the absence of perturber.

According to our model, pools of PG form in the plasma membrane of the host cell due to the electrostatic effects of calcium ions. Such phospholipid phase separations are known to occur in vesicles and several biological membranes. The virus core interacts with the cell plasma

membrane at a region rich in PE, this region being formed passively as a result of PG aggregation. Envelopment of the core proceeds by an infolding of the inner monolayer of the plasma membrane. Due to a specific interaction of PE with the core, a specific binding of PG to calcium ions, and the lateral mobility of each of these phospholipid species, the PM2 bilayer forms asymmetrically with PE interior and PG exterior. Finally, the positively charged coat protein displaces the calcium ions in its strong, specific interaction with PG, and the mature virus particle is complete. By this model, molecules such as adamantanone which increase the lateral motion of phospholipids would interfere with the separation of PG and PE into pools and would effectively prevent virus membrane assembly. It is unlikely that such pools are necessary for normal cellular membrane functions, explaining why the host cell grows normally in the presence of adamantanone or in the absence of high levels of calcium ions.

Full details of our model were presented at a joint Australian-American conference and will be published as a chapter in the proceedings of that conference (Academic Press). The abstract is included as Appendix I of this report. Descriptions of other aspects of membrane assembly for animal viruses are also described in that article and in a more general treatment of the dynamic properties of biological membranes (Appendix II).

B. Structure-Function Relationships in Membrane-Perturbing Antiviral Agents.

In an earlier study of the in vitro antiviral properties of long-chain alcohols, we observed a striking peak in antiviral activity for those compounds having chain lengths between 10 and 14 carbons. We have now evaluated dodecanol and a mixture of 80% dodecanol for their effectiveness against

herpes simplex virus (HSV) infections in mice. The latter preparation is particularly useful because it has a low melting point and remains liquid at room temperature. In our experiments, we found dodecanol and the mixture about equally effective for treating HSV skin infections. These agents show significant antiviral activity, but cause enough skin irritation in mice to be of concern.

The first compound that we discovered to have antiviral properties based on membrane perturbations was BHT. Because of the known safety of this material, we have characterized its effects on viruses in detail. We have now obtained a series of n-alkyl derivatives of BHT having hydrocarbon chains from 2 to 12 carbons long at the 4-position. In vitro studies with $\phi 6$ and HSV revealed that the most active derivative is the one with a 4-carbon chain, designated BH-4. The in vitro work emphasizes the importance of a proper balance between the polar and hydrophobic components of such molecules for optimal activity. BH-4 was also tested in vivo against HSV skin infections in hairless mice and found to be very effective. The details of our research on hydrophobic alcohols as antiviral agents are abstracted in Appendix III and will appear in a monograph on "Antimicrobial Effects of Lipids".

C. Related Studies Supported in Part by This Contract.

- (1) Diffusional Properties of Spin Labels. The spin label technique continues to provide us with fundamental knowledge about the dynamic properties of biological systems, and a certain amount of our effort goes into the development of new methods of analysis. Appendix IV describes such a study of the factors affecting translational diffusion of spin labels.

- (2) Spin- Reduction Assay for Lipoxidase. In a series of studies with lipoxidase, an enzyme that catalyzes the oxidation of double bonds in fatty acids and phospholipids, we discovered that spin label signals are reduced if the spin label is present in the reaction mixture. We have developed this observation into a quantitative assay for the enzyme. The unique usefulness of this assay is that it can be utilized in complex, light-scattering systems, such as phospholipid dispersions or cells, where the conventional spectroscopic assay cannot be used. This work appears in Appendix V.
- (3) Radiation Effects in Pseudomonas. A number of laboratories are actively studying the inducible repair system for radiation damage in Escherichia coli. An important question is whether this type of inducible repair is widespread in nature. We tested the organisms in everyday use in our laboratory for inducible radioresistance and for induced inhibition of DNA degradation. For Pseudomonas BAL-31 and Pseudomonas phaseolicola, strain HB10Y, neither inducible phenomenon was detected. These results are described in Appendix VI.
- (4) Resonance Energy Transfer Probes for Membrane Interactions. Our laboratory contributed in part to the development of a fluorescence enhancement assay for the intermixing of biological membranes. This was applied successfully to the fusion of cells induced by mutants of HSV. Appendix VII gives an account of this research.

Personnel on Project

% time

W. Snipes, Principal Investigator

10

J. Cupp, Graduate Student

50

P. Wanda, Graduate Student

50

G. Keller, Research Aide

50

M. Murrer, Laboratory Helper

25

APPENDICES

- I. Assembly of Viral Membranes
- II. Dynamic Aspects of Biological Membranes
- III. Hydrophobic Alcohols as Antiviral Agents
- IV. Factors Restricting Diffusion of Water-Soluble Spin Labels
- V. A Spin-Reduction Assay for Lipoxidase
- VI. Absence of Two Radiation-Induced Repair Processes in a Marine Pseudomonad
- VII. A Fluorescence Enhancement Assay of Cell Fusion

7
Preprints +
Reprints
Removed