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MEMBRANE-MEMBRANE INTERACTIONS IN A LIPID-CONTAINING BACTERIOPHAGE SYSTEM

PROGRESS REPORT

Submitted by

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DISCLAIMER

Abstract

Virus-cell interactions and the mechanism of viral entry have been the major focal points of this research. A method of analysis was perfected to investigate the entry process for herpes simplex virus. This technique makes use of a photosensitizing dye, FITC, that covalently binds to viral envelope proteins. Treated virions remain photosensitive until the envelope is shed during the process of infection. Our data strongly support an entry mechanism in which the viral envelope fuses with the cell plasma membrane. Other related projects have involved studies of the virucidal properties of retinoids, plaque development characteristics for viruses surviving treatment with membrane perturbers, and a "large plaque effect" that occurs when virus are plated on cells pretreated with UV light. In addition, we have characterized a new bacteriophage, investigated the interactions of divalent cations and proteins with phospholipid vesicles, extended our studies of the effects of hydrophobic photosensitizers on cell membranes, and used the spin-trapping technique to elucidate the reaction mechanism for an enzyme-like activity in soil extracts.

PROGRESS REPORT

Efforts in our laboratory this past year have focused on a number of different problems in membrane molecular biology, and several projects have been brought to conclusion. A major accomplishment was the use of fluorescein isothiocyanate (FITC) plus light to investigate the mechanism of entry of herpes simplex virus (HSV). Other studies with HSV were part of our continuing efforts to develop effective antiviral agents that act by perturbing viral membrane functions. Basic research on the dynamic and structural characteristics of membranes, involving electron spin resonance (ESR) studies with spin labels, remains an important part of our work, and new uses and applications of this methodology have developed. Additional projects completed this year include the characterization of a new bacterial virus and an investigation of the effects of acridine plus near-UV light on E. coli.

A. HSV Entry

For the most part, the mechanism whereby HSV enters its host cell at the time of infection is poorly understood. Electron microscopy has provided no convincing proof in support of either membrane fusion or endocytosis as the mechanism of entry. This technique is employed in experiments where the multiplicity of infection (MOI) is large, and it does not distinguish between infectious and non-infecting virus particles. We developed a new approach to study the early events in HSV infection using FITC plus light. This photosensitizing dye binds covalently to viral glycoproteins, and induces cross-linking reactions when the labeled virus are exposed to light. Thus, FITC-treated virions remain light-sensitive until the envelope is lost at some stage during entry. We carefully compared the kinetics for loss of light sensitivity of FITC-treated virus with the kinetics for loss of antibody sensitivity, and found them to be identical. This provides strong evidence for membrane fusion, rather than endocytosis, as the mechanism of entry for

infectious HSV. This work was published in the Proceedings of the National Academy of Science and appears as Appendix I of this report.

B. Virucidal Retinoids

In characterizing the virucidal activity of different classes of hydrophobic and amphipathic molecules, we found that retinoids are among the most potent virucidal agents in vitro. Retinal in particular is extremely active against HSV and shows activity against other viruses as well. Some of the results of this work were presented at the ASM conference on Current Chemotherapy and Infectious Disease, and the publication of these proceedings is given in Appendix II.

C. Plaque-size Assay for Antilipid Agents

We have contended for some time now that membrane perturbers may have some utility as a treatment for infections caused by enveloped viruses. Some of the obvious advantages of such agents, in contrast to drugs that interfere with viral DNA metabolism, are their lack of mutagenicity (and potential carcinogenicity) and their lesser interference with host-cell metabolism. Therefore, it is of general interest to us to have a way to quickly identify virucidal agents that may be considered antilipid rather than anti-DNA. One method that shows promise is the analysis of plaque sizes for viruses that survive the treatment. For HSV, we found that viruses which survive treatment with such agents as ether and butylated hydroxytoluene give normal plaque sizes, whereas the survivors of treatment with ultraviolet light or the chemical carcinogen AAAF give smaller plaques in comparison. This approach to the characterization of virucidal agents was published in the Biophysical Journal (see Appendix III).

D. Large-plaque Effect

An interesting observation was made in our studies of plaque development in HSV. We found that UV irradiation of the host cells, followed by a 2-day incubation, resulted in significantly larger plaques when these cells were used for plating HSV. This phenomenon, which occurs with both UV-irradiated and unirradiated virus, is referred to as the large-plaque effect. This initial discovery was reported in Photochemistry and Photobiology (see Appendix IV); additional studies are underway to further characterize this phenomenon.

E. Phospholipid-Divalent Cation Interactions

Last year we reported the development of a new method of analysis for detecting and quantifying the interactions of divalent cations with biological moieties. This technique was based on the fact that paramagnetic ions broaden spin-label absorption lines, and our discovery that the extent of broadening is reduced when the ion forms a chelate or complex with organic ligand sites. We completed a study this year in which this method was used to investigate interactions that occur between divalent cations and proteins at phospholipid vesicle surfaces. By competition analysis, several cations were ordered with regard to the strength of their binding to vesicles. Different proteins were found to compete to different degrees for surface binding sites. Some evidence was found for protein-induced aggregation of negative phospholipid species in the membrane bilayer. Appendix V gives a full account of the results of this study.

F. Characteristics of Bacteriophage Psp23la

In many of our studies on antiviral agents, we used the enveloped bacteriophage ø6 as a convenient model for detecting and characterizing virucidal activity. As a control virus, we also used the non-lipid-containing bacteriophage Psp23la (formerly designated ø23-1-a). This virus infects the same host cell as does ø6, but is insensitive to organic solvents and membrane perturbers.

Psp23la was provided to us by Anne Vidaver, but nothing was known about it except a few serological details. We undertook a thorough characterization of Psp23la in order to have a better understanding of its usefulness in our studies. This work was published in the Journal of Virology and is included as Appendix VI of this report.

G. Effects of Acridine plus Near-UV Light on *E. coli*

Acridine is a hydrophobic photosensitizer that partitions strongly into the organic phase of aqueous-organic two-phase systems. We found earlier that acridine plus near-UV light is extremely effective at inactivating lipid-containing viruses such as HSV and ø6 but is comparatively ineffective against non-lipid viruses. This year a study was completed of the effects of acridine plus near-UV light on E. coli. The damage resulting from this treatment appears to involve both cellular membranes and DNA. An interesting observation was that cells can recover from sublethal doses of acridine plus near-UV light, and this recovery most likely is due to the repair of damaged membranes (see Appendix VII). Further studies on this problem are in progress.

H. Spin-trapping of Soil Radicals

We continue to collaborate with other laboratories on projects where our spin-label technology can be of value. One project which was completed this year was a study of free radicals produced in a reaction mixture involving a soil extract. We use the spin-trapping method to stabilize and identify the radical intermediate involved. This work has significance with regard to the mechanisms whereby soil humus is formed by biological enzymic components. The publication of this work appears in Appendix VIII.

Personnel on Project:

	% time
W. Snipes, Principal Investigator	10
N. DeLuca, Graduate Student	50
S. Wagner, Graduate Student	25
K. Lang, Laboratory Helper	20
G. Fronko, Laboratory Helper	20
T. Schwartz, Laboratory Helper	20
D. Witters, Dishwasher	20
J. Sands, Visiting Professor	20

APPENDICES

I. Early events in herpes simplex virus type 1 infection: Photosensitivity of fluorescein isothiocyanate-treated virions. N. DeLuca, D. Bzik, S. Person and W. Snipes. Proc. Natl. Acad. Sci. 78:912, 1981.

II. Characterization of virucidal activities of retinoids. D. D. Auperin, A. Reinhardt, J. A. Sands, W. Snipes and W. D. Taylor. In: Current Chemotherapy and Infectious Diseases, Proc. of 11th ICC & 19th ICAAC Amer. Soc. Microbiology, 1368, 1980.

III. A comparison of herpes simplex virus plaque development after viral treatment with anti-DNA or antilipid agents. T. P. Coohill, M. Babich, W. D. Taylor and W. Snipes. Biophys. J. 30:517, 1980.

IV. Herpes simplex virus produces larger plaques when assayed on ultraviolet irradiated CV1 cells. T. P. Coohill, M. A. Babich, W. D. Taylor and W. Snipes. Photochem. Photobiol. 32:97, 1980.

V. Interaction of divalent cations and proteins with phospholipid vesicles. S. Wagner, A. Keith and W. Snipes. Biochim. Biophys. Acta 600:367, 1980.

VI. Characteristics of a new bacteriophage, Psp23la, infecting Pseudomonas phaseolicola HB10Y. W. D. Taylor, N. DeLuca, K. Vollherbst, T. Doman, T. d'Amato and W. Snipes. J. Virol. 35:918, 1980.

VII. Effects of acridine plus near ultraviolet light on Escherichia coli membranes and DNA in vivo. S. Wagner, W. D. Taylor, A. Keith and W. Snipes. Photochem. Photobiol. 32:771, 1980.

VIII. Electron spin resonance study of free radicals generated by a soil extract. J. M. Suflita, M. J. Loll, W. C. Snipes and J.-M. Bollag. Soil Sci. 131:145, 1981. (Preprint)

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