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This project was an investigation of surface-bound matrices designed to resemble lipid bilayer membranes and to maintain functions of incorporated receptor proteins. A detergent dialysis technique was adapted to assemble membrane structures onto several solid supports. The incorporation and function of two types of membrane receptor proteins was studied. These were the visual receptor, rhodopsin, which functions by activating a G-protein cascade, and the nicotinic acetylcholine receptor, which is a ligand-gated ion channel. Data obtained on the composition structure, and function of the surface-bound structures are consistent with the model of an anchored bilayer mimicking many of the attributes of a natural biomembrane.

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USE OF RECEPTOR SITES FOR GENERIC DETECTION OF CHEMICAL AGENTS AND TOXINS

Final Report

H. Gilbert Smith, Ph.D.

June 15, 1990

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Worcester, MA 01460

A. Statement of the Problem Studied.

Integral membrane proteins play a central role in biological transduction processes and are the sites of action for a wide variety of biologically active chemicals including hormones, neurotransmitters, odorants, and many drugs and toxins. Sensors based upon receptor functions have potential for the detection of chemical agents and toxins, but the problem of forming a viable interface between receptor proteins and electronic devices is a difficult one.

Model systems have contributed greatly to understanding the structure and function of biological membranes and this project was based upon a novel model system in which the components of a biological membrane are reassembled on a solid surface. This research may provide new tools for basic research into the fundamental processes of membrane function and will contribute to the knowledge base needed for the ultimate application of membrane processes in artificial sensory or signal transducing devices.

This project was an investigation of surface-bound matrices designed to resemble lipid bilayer membranes and to maintain functions of incorporated receptor proteins. A detergent dialysis technique was adapted to assemble membrane structures onto several solid supports. The incorporation and function of two types of membrane receptor proteins was studied. These were the visual receptor, rhodopsin, which functions by activating a G-protein cascade, and the nicotinic acetylcholine receptor, which is a ligand-gated ion channel. These biomimetic structures have the potential of allowing natural transduction elements to be linked both functionally and physically with electronic or optical devices.

B. Summary of Most Important Results.

Introduction.

This section highlights the key results of this project. More detailed information is given in the publications listed in section C., and in Progress Reports already submitted. Rhodopsin-containing disk membranes were isolated from bovine retinas (Smith & Litman, 1982), and acetylcholine receptors were isolated in membrane fragments from the electroplax of *Torpedo nobiliana* (Hazelbauer & Changeux, 1974).

Membrane Formation.

The approach to forming the surface-bound membranes was based upon the detergent dialysis technique which incorporates receptor proteins simultaneously

with the formation of a lipid bilayer. There is an extensive literature on using this technique to form model "reconstituted" membrane vesicles from purified proteins and lipids (Darszon, 1983; Hong & Hubbell, 1973; Jackson & Litman, 1982; Kagawa & Racker, 1971; Levitski, 1985; Madden, 1986; Tyminski et al., 1988).

We adapted this technique by conducting the membrane assembly in the presence of a solid substrate that had been "primed" by covalent attachment of long-chained alkyl groups. The substrates were reacted with organosilane reagents, such as octadecyltrichlorosilane or octadecyldimethylchlorosilane, to covalently attach a well-defined alkyl layer to the substrate surface (Murray, 1980; Sagiv, 1980). Surfaces amenable to this treatment include glass, metal oxides, silicon oxide, and silicon oxynitride. Substrates used in this project included small, 37 μm diameter glass beads, silicon oxide-coated silicon wafers, and other electrode materials.

The membrane structure was assembled by addition of a mixture of detergent-solubilized lipids and protein to the alkylsilanated surface and subsequent dialysis to remove the detergent. The dialysis was accomplished in a flow dialysis system which allowed careful control of the rate of detergent removal. After the dialysis was completed the substrates were washed to remove vesicles and other non-adherent materials, and were then analyzed for structure and function.

Structural Model.

Results obtained in this project suggest that the detergent dialysis approach forms a lipid bilayer membrane that is anchored to the substrate surface by the alkyl silane groups that are covalently attached to the surface. These alkyl chains appear to serve as initiation points for the reassembly of the protein and lipid into a membrane structure as the detergent is removed. Our model is that these chains serve as anchors inserted into the hydrophobic core of the membrane.

This project successfully incorporated rhodopsin and acetylcholine receptors into surface-bound structures. Data obtained on the composition, structure and function of those structures were consistent with the model of an anchored bilayer mimicking many of the attributes of a natural biomembrane.

Structure of Surface-Bound Membranes.

The compositions of membrane structures formed on 37 μm diameter glass microbeads were measured after the membrane deposition process and subsequent washing. Rhodopsin was determined by the absorption spectra of detergent extracts taken from the washed beads. These indicated that rhodopsin retained its structure and that the protein surface density was at least 10% of that of the natural disk membrane. In some experiments the protein composition approached that of the

natural membrane. The lipid composition was also consistent with a single bilayer surface structure.

Similar results were obtained for membranes incorporating acetylcholine receptors. The receptor density was measured by the binding of α-bungarotoxin to the membrane-coated beads. Here the surface density of active receptors was also about 10% of that of the natural membrane.

These results were supported by FTIR measurements. After membrane deposition by dialysis, the normalized intensity of the hydrocarbon peaks increased by a factor of two to three relative to the alkylsilanized substrate indicating the presence of lipid.

Electrical capacitance measurements of membranes formed on the SiO_2/Si electrodes provided additional information on the membrane structure. The capacitance values were determined from the quadrature component of the impedance at a bias potential chosen such that the p-silicon substrate was in the accumulation state and, therefore, conductive. Results with both rhodopsin and acetylcholine receptors were consistent with a bilayer structure. Assuming a 50 Å thick membrane with a dielectric constant of 3 yields a calculated surface coverage of greater than 64%. Conversely, assuming 100% surface coverage yields a calculated membrane thicknesses ranging from 33 Å to 65 Å.

The apparent surface coverage for the alkylsilane layers was less than for the complete membrane, which further supports the model of lipid being deposited around the alkyl chains that are covalently attached to the surface.

Function of Surface-Bound Membranes.

The techniques used to form the surface-bound membranes were specifically chosen to be appropriate for incorporating integral membrane proteins and retaining their functional attributes. The lipid matrix, although anchored to the substrate surface, appears to be in a bilayer configuration. It should, therefore, have properties like the interior of a natural membrane, and should accommodate the normal dynamic motions of receptor proteins.

As mentioned above rhodopsin incorporated into these structures retains its natural absorption spectra, and thus does not appear to be denatured. Similarly, the acetylcholine receptor retains its ability to bind α-bungarotoxin.

A more sensitive measure of the functional viability of rhodopsin is its ability to activate the natural G-protein cascade which results in cyclic-GMP hydrolysis

(Fung et al., 1981; Liebman & Pugh, 1981). The peripheral proteins transducin and phosphodiesterase are specifically activated by photoexcited rhodopsin to produce this photoactivity (Kühn, 1980). We isolated these peripheral proteins from natural, retinal rod outer segments and added them to a suspension of glass beads on which a surface membrane containing rhodopsin had been formed. We found that the reconstituted, surface-bound membranes containing rhodopsin were still competent to activate the G-protein cascade upon brief light exposure. The light-activated phosphodiesterase activity was about 50% of that measured for natural disk membranes under similar conditions.

In control experiments it was noted that bare glass surfaces strongly activate the phosphodiesterase activity even in the dark. This dark activity was eliminated upon formation of the surface-bound membranes. This provides another measure of surface coverage, and show that that the membranes formed by the dialysis procedure do not allow contact between the glass surface and the peripheral enzymes. This indicates that the membranes do not have significant numbers of protein-sized holes in them.

These results indicate that membranes formed by the detergent dialysis procedure have compositions similar to natural membranes and retain at least some of their functions. Detergent dialysis has been used to form reconstituted membranes with numerous receptor proteins, and the results obtained in this project indicate that the approach can be extended to assemble membranes on surfaces.

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C. List of Publications and Presentations.

Publications

N.W. Downer, J. Li, L.W. DeLuca, E.W. Pennimann & H.G. Smith, Surface-Bound Biomembranes Incorporating Receptors: Electrochemical and Structural Characterization. In final preparation for submission to *Biosensors* (1990).

H.G. Smith, J. Li, N.W. Downer & L.W. DeLuca, Surface-Bound Biomembrane Assemblies. *Proceedings of the IEEE/EMBS* **11,** 1329 (1989).

H. G. Smith, Surface-Bound Biomembrane Structures for use in Biosensors. *Proceedings for the Symposium on Agents of Biological Origins,* American Defense Preparedness Association and US Army CRDEC, Applied Physics Laboratory, Johns Hopkins University, March 1989, pp 44-52.

Presentations

N.W. Downer, J. Li & H.G. Smith, "Capacitive Devices Incorporating Integral Membrane Proteins", UCLA Symposia on Molecular and Cellular Biology of Biosensors and Bioprobes, Frisco, CO, Feb 3-8, 1990.

H.G. Smith, "Surface-Bound, Biomembrane Assemblies Incorporating Receptor Proteins for Biosensor Applications", Northeastern Section, American Chemical Society, Medicinal Chemistry Group, Boston College, Dec. 12 1989.

H.G. Smith, J. Li, N.W. Downer & L.W. DeLuca, "Surface-Bound Biomembrane Assemblies", 11th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Seattle, Washington, Nov. 9-12, 1989.

H.G. Smith, N.W. Downer & J. Li, "Surface-Bound, Biomembrane Assemblies Incorporating Receptor Proteins for Biosensor Applications", Symposium on Biosensors, SUNY Buffalo, Buffalo, NY, Nov. 13, 1989.

H. G. Smith, "Surface-Bound Biomembrane Structures for use in Biosensors", 4th ARO Neurosciences Workshop, May 2, 1989.

H. G. Smith, "Surface-Bound Biomembrane Structures for use in Biosensors", American Defense Preparedness Association Symposium on Agents of Biological Origins, Applied Physics Laboratory, Johns Hopkins University, March 22, 1989.

D. List of Scientific Personnel.

This contract provided partial support for the following scientific personnel:

H. Gilbert Smith, PhD – Principal Investigator
Nancy W. Downer, PhD – Receptor Biochemistry
Jianguo Li, PhD – Electrochemistry
Leslie W. Deluca – Technician
Elizabeth M. Millar – Technician
Kristina M. Curci – Intern