This report summarizes the results of theoretical and experimental investigations of the role of charged surfaces on membrane processes. The focus on electrical double layer processes of the channels involved in ion transport and excitation leads to a relation between the ion selectivity and the rate of channel opening. Recent studies show that similar electrochemical processes may be occurring in biosynthetic structures and causing the changes seen in the proteins of cells exposed to electromagnetic signals.
SURFACE PROCESSES IN MEMBRANE TRANSPORT

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1. STATEMENT OF THE PROBLEM STUDIED

The effect of molecular charge on protein aggregation reactions provides an approach to understanding the opening/closing reactions of voltage gated channels. The surface charge also affects the electrochemical gradients across membranes during transients and the ionic fluxes. Using theoretical and experimental approaches, we have studied the role of charged surfaces in membrane processes.

2. SUMMARY OF THE MOST IMPORTANT RESULTS

2.1 - The relation between surface charge density and protein aggregation has been verified experimentally for the hemoglobin tetramer $\rightarrow$ dimer reaction. This provides a basis for understanding the energetics of membrane channel opening/closing reactions, and their relation to charging.

2.2 - The ion selectivity of voltage gated channels appears to be related to the gating current. Since the electrochemical gradients across the channels vary with the time after electrical stimulation, the effective gradient depends upon the gating current, which sets the time when the channels open.

2.3 - The same processes that lead to the opening/closing of channels in membranes apparently occur in biosynthetic structures under the influence of imposed electromagnetic fields and lead to changes in the patterns of transcription and translation.

3. DESCRIPTION OF RESEARCH

3.1 - MEMBRANE CHANNEL PROCESSES

The fundamental membrane processes of living cells, e.g. generation of ion gradients, sensory transduction, conduction of impulses, energy transduction, are electrochemical in nature and involve ion movement through specialized protein assemblies called channels. There are structural similarities between channels that suggest a common design and mode of operation. We have studied protein aggregation/disaggregation reactions as a basis for channel function and to establish an electrochemical link between electrical stimuli and channel properties.

We have measured osmotically the disaggregation of hemoglobin tetramers into dimers as a function of pH, and have calculated the equilibrium constant. In the alkaline range, the equilibrium constant varies with the pH, and its logarithm is a linear function of the charge on the molecule. Since aggregation-disaggregation reactions appear to involve changes at the interfaces of the subunits rather than in the internal structure, the total free energy change in such reactions can be evaluated in terms of the surface free energy change (i.e., calculated from the area and surface charge density). This assumption leads to the correct prediction of the disaggregation constant.

The surface free energy model of oligomer association appears to be useful as a model for the opening and closing of oligomeric channel structures in membranes, and we have used it to describe the voltage gated channels of excitable membranes, in conjunction with a model of the ionic processes in the electrical double layer regions at charged surfaces. The Surface Compartment Model (SCM) of ion flow across the channels of natural membranes emphasizes the role of electrical double layers in ion transport and is derived from first principles. When the SCM is applied to the membrane of an excitable cell, (e.g., the squid axon), one can calculate voltage clamp currents that are similar to those observed in the sodium and potassium channels of excitable membranes. The
difference in the selectivity of the two types of ion channels appears to be
determined by the difference in gating current, and is in line with measurements
on the sodium and potassium channels of squid axon. These results indicate that
there is a kinetic basis for the selectivity of voltage gated channels and
suggest that other types of channels may operate by related mechanisms. In
summary, our calculations show that: (1) the ionic currents in excitable
membranes can be described by electrodiffusion theory, (2) the selectivity of an
ionic channel is due to the kinetics of channel opening, and (3) the transient
ion concentration changes due to oscillating electric fields can stimulate ion
pump enzymes.

3.2 - ELECTROCHEMICAL EFFECTS IN BIOSYNTHETIC STRUCTURES
Recent electrochemical approaches to the mechanisms of oligomeric protein
disaggregation and membrane channel opening have been successful in accounting
for ion flows during nerve excitation (Blank - BBA 906:277-294, 1987). Since the
electrochemical mechanisms are general, they should also operate during the
electrical stimulation of transcription and translation in cells. To consider
these processes we have adapted the electrochemical model to the interaction of
two anionic surfaces with adsorbed counterions in an alternating electric field.
The two charged surfaces could represent two DNA's capable of synthesizing mRNA
when activated, or an mRNA on a ribosome capable of synthesizing polypeptides.
We have examined published data for both RNA and polypeptide synthesis and find
that the distributions of molecular weights have the predicted dependence on the
frequency of the external electromagnetic field, in support of the proposed
mechanism. The electrochemical model predicts other properties (e.g. the
properties of new proteins not found in the absence of stimulation) that are
being studied further. Because of its generality, it may also provide a
rationale for the effects of endogenous electrical stimulation, such as occurs
during nerve excitation.

4. LIST OF PUBLICATIONS
4.1 - Blank, M.
Ionic Processes at Membrane Surfaces: The Role of Electrical Double Layers in
Electrically Stimulated Ion Transport. In: "Mechanistic Approaches to the
Interaction of Electric and Electromagnetic Fields with Living Systems". Edited
4.2 - Blank, M.
Influence of Surface Charge on Oligomeric Reactions as a Basis for Channel
Dynamics. In: "Mechanistic Approaches to the Interaction of Electric and
Electromagnetic Fields with Living Systems". Edited by M. Blank and E. Findl.
4.3 - Blank, M. and Soo, L.
Surface Free Energy as the Potential in Oligomeric Equilibria: Prediction of
Hemoglobin Disaggregation Constant. Bioelectrochemistry and Bioenergetics,
4.4 - Blank, M. and Goodman, R.
An Electrochemical Model for the Stimulation of Biosynthesis by External Electric
Fields. Bioelectrochemistry and Bioenergetics, in press.

5. SCIENTIFIC PERSONNEL
5.1 - Martin Blank, Ph.D.
5.2 - Lily Soo, Ph.D.