1-Anilino-8-Naphthalenesulfonate: A Fluorescent Probe of Ion and Ionophore Transport Kinetics and Trans-Membrane Asymmetry

Duncan H. Haynes and Philip Simkowitz

Department of Pharmacology, University of Miami Medical School, Miami, Florida 33152

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Summary. The kinetics of the transport of the 1-anilino-8-naphthalenesulfonate (ANS-, an anionic fluorescent probe of the membrane surface) across phospholipid vesicle membranes have been studied using a stopped-flow rapid kinetic technique. The method has been used to gain detailed information about the mechanism of transport of this probe and to study ionophore-mediated cation transport across the membrane. The technique has also been exploited to study differences between the inside and outside surfaces of vesicles containing phosphatidyl choline (PC).

The following is a summary of the major conclusions of this study. (a) Binding of ANS- on the outside surface occurs within times shorter than 100 μsec while permeation occurs in the time range 5–100 sec. (b) Net transport of ANS- occurs with cotransport of alkali cations. (c) The transport rate is maximal in the region of the crystalline to liquid-crystalline phase transition, and the increase correlates with changes in the degree of aggregation of the vesicles. (d) Incorporation of phosphatidic acid (PA), phosphatidyl ethanolamine (PE) or cholesterol into PC membranes decreases the rate of ANS- transport. (e) Neutral ionophores (I) of the valinomycin type increase ANS- permeability in the presence of alkali cations (M+) by a mechanism involving the transport of a ternary I-M+-ANS- complex. The equilibrium constants for formation of these complexes and their rate constants for their permeation are presented. The maximal turnover number for ANS- transport by valinomycin in dimyristoyl PC vesicles at 35°C was 46 per sec. (f) The partitioning of the ionophore between the aqueous and membrane phases and the rate of transfer of an ionophore from one membrane have been determined in kinetic experiments. (g) A method is described for the detection of I-M+ complexes on the membrane surface by their enhancement effects on ANS- fluorescence at temperature below the phase transition temperature on “monolayer” vesicles. The apparent stability constants for several I-M+ complexes are given. (h) Analysis of the effect of ionic strength on the ANS- binding to the inside outside surfaces indicates that the electrostatic surface potential (at fixed ionic strength and surface change) is larger for the inside surface than for the outside surface. (i) Analysis of the dependence of the maximal ANS- binding for the inside and outside surfaces of vesicles made from PC and a variable mole fraction of PA, PE or cholesterol indicate that the latter three are located preferentially on the inside surface.
Fluorescent probe methods have become increasingly important to the biophysical study of membranes. The fluorescence methods enjoy a several order of magnitude advantage in sensitivity over nuclear magnetic resonance (NMR) methods. Such sensitivity will be necessary for the study of specific biological reactions such as the high-affinity binding to receptors. Many fluorescent probes exhibit an extreme sensitivity to the polarity of the environment as well as reporting molecular motion offering an important advantage over electron spin resonance (ESR), probes whose primary sensitivity is to molecular motion. A final advantage of the fluorescent probe technique is illustrated in the present communication. Fluorescent probes can be used in conjunction with rapid kinetic methods to obtain information about the rates of binding processes, membrane structural changes and, membrane transport.

The most widely-used fluorescent probe to date is 1-anilino-8-naphthalenesulfonate (ANS−). This anionic probe of the membrane surface shows an inverse relationship between its fluorescent quantum yield and solvent polarity and is thus a very sensitive probe for hydrophobic binding sites. The present communication is the third in a series of four papers dealing with the behavior of ANS− fluorescence in phospholipid vesicles. In the two previous studies, we showed that ANS− fluorescence can be used to gain information about structure changes (Haynes & Staerk, 1974) and ion binding and interaction (Haynes, 1974) in phospholipid vesicles. Specifically, we showed that ANS− can bind between four polar head groups of phosphatidyl choline (PC) and that changes in the number of these binding sites in vesicles made from mixed phospholipids can be analyzed to determine whether the lipids are randomly mixed on the microscopic scale. This was found to be the case for mixtures of phosphatidic acid (PA−), dimyristoyl phosphatidyl ethanolamine (PE), and cholesterol with dimyristoyl PC. We have also shown that the binding equilibrium of ANS− is influenced by the electrostatic surface potential, \( \psi_0 \), of the membrane and that the probe can be used to register ion binding on the membrane surface (Haynes, 1974).

In the present communication, we use the stopped-flow rapid mixing technique to measure the rate of transport of ANS− across the vesicle membrane and to distinguish between binding on the inside and outside surfaces. In two of our previous studies we reported quantitative data for monolayer vesicles, ca. 500 Å spheres of organic solvent covered with a monolayer of phospholipid (Träuble & Grell, 1971). We observed quantitatively similar data for bilayer vesicles but did not report their behavior in detail because we were unable to distinguish between effects on the