Water Permeation Measured by Solvent Isotope Effects

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In the following sections methods to measure the passive water permeation across biological membranes will be discussed together with some results obtained. The systems considered always consist of vesicles surrounded by lipid bilayers or single cells surrounded by a plasma-membrane. Thus the membrane separates the aqueous intracellular from the extracellular compartments. If $\tau$ is the mean lifetime (= $1/k_{\text{ex}}$ with $k_{\text{ex}}$ the exchange relaxation rate) of water-molecules in the intracellular compartment the permeability coefficient, $P_d$, is given by $P_d = V/A \cdot 1/\tau$, where $V/A$ is the volume to surface ratio of the vesicles or cells. With the exception of rather specialized cases (e.g. epithelial cells (Parisi et al. 1985)) the barrier for the transfer of water molecules across lipid bilayers or plasma-membranes is rather low and, in particular, water channels are usually absent.

Methodology.

The non-invasive methods to measure the water permeation across membranes can be divided into transient and stationary methods. The latter measures the hopping frequency of water molecules across the membranes in question. Usually Mn$^{2+}$ is added to the extracellular compartment at mM concentrations and the $^1$H or $^{17}$O NMR relaxation rates of water are measured. The comparison with the Mn-free system allows the deduction of the hopping frequency and at known volume to surface ratio the permeability coefficient $P_d$ can be determined (e.g. Lipschitz-Farber & Degani 1980).

On the basis of transient experiments either the diffusive ($P_d$) or the osmotic ($P_f$) water permeability coefficient is determined. We will focus here on techniques in the absence of any net-flux. These methods lead to the determination of $P_d$. The osmotic permeation is obtained from induced volume changes of the vesicular or cellular particles in flux experiments brought about by changes of osmolalities. The net-flux always causes a swelling or shrinking and therefore changes of the membrane area in parallel with changes of the membrane packing. Variations of the bilayer area up to 25% are observed without breaking of the membrane (Hantz et al. 1986). Thus the two permeability coefficients, $P_d$ and $P_f$, are not necessarily equal because they refer to different states of the membrane.

All transient methods observe the time-dependent decrease of an initially enforced asymmetry of the isotopic solvent composition. Experiments with radioactive tracers use only low tracer concentrations of $^3$HHO in the extracellular space. In mixing type experiments the radioactivity passing into the intracellular space is determined according to the pioneering work of Solomon and co-workers (Paganelli & Solomon 1957). If $D_2O$ is used usually half of the extracellular solvent is rapidly replaced by $D_2O$ and the $D_2O/H_2O$ exchange of the intracellular solvent is observed. These experiments are performed in an excess of extracellular solvent so that the
isotopic composition within the extracellular medium stays constant during the equilibration process of D₂O/H₂O across the membranes. Under this condition the exchange-kinetics becomes a first order process (Lawaczeck 1979, 1984). So far only those differences of H₂O and D₂O are considered which are manifested in the optical detection of the two isotopically different solvents. The influence of other physical differences of H₂O and D₂O are neglected to a first approximation, and identical chemical potentials for the two isotopic solvent species are assumed.

D₂O and H₂O become "visible" on the basis of direct and indirect effects. The direct effects are: i. the different scattering cross section for neutrons, ii. the different fluorescence quantum yields of some chromophores in H₂O and D₂O, and with iv. the isotopic effect of the scattering cross section of the particles in question. The difference of the neutron scattering cross section of H₂O and D₂O served to observe the water permeation across stacks of lipid bilayers in costly experiments (Franks & Lieb 1980). On the basis of the effects ii. to iv. methods were developed in Würzburg to measure the H₂O/D₂O exchange and thus to determine the water permeation across membranes (Lawaczeck 1979, 1984; Engelbert & Lawaczeck 1985a, b; Pitterich & Lawaczeck 1985). Usually equal volumes of a suspension containing the lipid vesicles or cells in H₂O are mixed with the deuterated buffer in a stopped-flow apparatus. Following the short mixing period, the exchange of H₂O/D₂O is registered either by the D₂O-sensitive fluorescence of reporter-molecules or by the turbidity of the solution. The turbidity measurements are complemented by measurements of the light scattering. The detection technique based on the absorption in the IR and NIR regions is not suited for stopped-flow experiments but allows the direct observations of H₂O/D₂O exchange processes.

For the fluorescence technique D₂O-sensitive reporter molecules have to be intracellularly encapsulated prior to the mixing experiment. The reporter molecules are selected with respect to large solvent isotope effects of the fluorescence quantum yield and low permeation rates across the membranes. Water-soluble indole- and naphthylamine-derivatives are suitable for that purpose (Lawaczeck 1979). The method has the disadvantage of the encapsulation- and removal-procedures of extracellular chromophores. The light scattering technique is experimentally simpler but is theoretically more involved. Large hollow spheres (no point scatterers) are considered and Mie's theory is used to calculate the scattering cross section (Engelbert & Lawaczeck 1985a, b). The light scattering technique is based on the differences of the refraction indices of H₂O (n_D 1.33299) and D₂O (n_D 1.32844). For large particles surrounded by a membrane (hollow spheres) the three indices of refraction for the extracellular and intracellular aqueous compartments and for the membrane contribute to the scattering process. From these the index of refraction of the intracellular medium changes with time and exponentially approaches the new steady state where the isotopic composition in both aqueous media is equal. To a first approximation the scattering cross section reflects the same exponential time dependence. Thus the exchange rates are easily deduced from the time dependent registration of the turbidity of the solution or the light scattering.

The NIR region seems to be especially promising for the direct observation of the D₂O/H₂O exchange as NIR-sensors and video-cameras with high sensitivity in the spectral range from visible light to about 1800 nm are available. We have focussed