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# Determination of solute reflection coefficients in kidney brush-border membrane vesicles by light scattering: influence of the refractive index

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**Solute reflection coefficients σ of cell membrane vesicles or liposomes are commonly determined by comparison of the water flow induced by a gradient of the studied solute and that of a reference molecule using light scattering techniques. However, variations in scattered light which are mainly related to change in vesicle volume are also influenced by the refractive index of the surrounding medium. Therefore comparing kinetics of vesicle shrinkage induced by hyperosmotic solutions which have different refractive indexes might lead to an under or over estimation of σ. We determined $\sigma_{NaCl}$ in rat kidney brush-border membrane vesicles by two different approaches using mannitol, a poorly permeant molecule, as reference. (1) The refractive index of the hyperosmotic NaCl solution was adjusted to that of mannitol by addition of polyvinyl pyrrolidone ($M_r$ 40000), without a significant increase in osmolality. Thereby the change in scattered light intensity induced by osmotic vesicle shrinkage due to gradients of NaCl and mannitol were comparable and led to a $\sigma_{NaCl}$ value close to one instead of the previously published value of 0.53. (2) The reflection coefficient was calculated from the lifetime of vesicle shrinkage which is not refractive index-dependent. Again $\sigma_{NaCl}$ was not different from one. These results suggest that the water proteic pathways found in the luminal membrane of proximal tubules are not shared by salts.**

The reflection coefficient σ of a solute for a given membrane is an index of whether this solute and water have common pathways and to what extent they interact. As defined by Staverman [1] and Kedem and Katchalsky [2], a reflection coefficient equal to one indicates separate pathways whereas $0 < \sigma < 1$ reveals solute-solvent interactions. σ is generally determined by comparison of water fluxes induced by different solutes for a given osmotic gradient. Low-permeability molecules, for which σ is assumed to be one, are used as reference.

Water movements in liposomes and cell membrane vesicles, are often studied by light scattering techniques. The vesicles are rapidly mixed with an hyper- or hypo-osmotic solution in a stopped-flow apparatus; the change in transmitted or scattered light induced by swelling or shrinkage of the vesicles is monitored with time. Water flux is calculated from fits of the experimental curves and from the vesicle size.

With this experimental approach, the previously reported σ values of epithelial cell membranes for various salts and solutes were mainly lower than one. In rat intestine brush-border membrane Van Heeswijk and Van Os [3] found $\sigma_{NaCl} = 0.59$ and $\sigma_{KCl} = 0.64$. In basolateral membrane vesicles from rabbit kidney Verkman and Ives [4] obtained $\sigma_{urea}$ ranging from 0.5 to 1.0 depending on the imposed gradient. Kasai et al. [5] have evidence for a $\sigma_{KCl}$ between 0.55 and 0.59 in sarcoplasmic reticulum vesicles. In rat brush-border membrane we also found reflection coefficients for NaCl and KCl close to 0.55 [6]. However, at similar osmotic gradients, solutions may have different refractive indexes which in turn modify the observed volume dependence of scattered light. In this report we present data which show how changes in solute composition do alter the intensity of transmitted light through vesicle suspensions and the consequences for reflection coefficient determinations.

Rat kidney brush-border membrane vesicles were prepared by calcium precipitation methods from male Sprague-Dawley rats weighing 280–320 g and sealed in a buffer containing 50 mM sucrose, 10 mM Tris-Hepes

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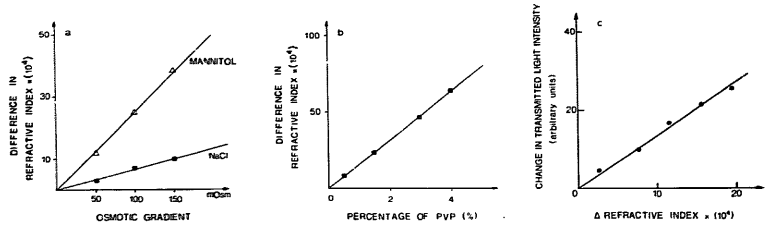


Fig. 1. (a) Relationship between refractive index and osmolalities of mannitol and NaCl solutions. Mannitol (△) or NaCl (■) were added to the control buffer in order to obtain a gradient of 50, 100 and 150 mosmol/kg water. At 23°C the control buffer's refractive index was 1.3355. (b) Influence of polyvinyl pyrolidone (PVP) on refractive index of control buffer solution. Different amounts of PVP ($M_r$ 40000) were added to the control buffer. (c) Changes in transmitted light intensity across a vesicle suspension as a function of refractive index. Different amounts of PVP were added to modify the refractive index of the solution with minimal alteration in osmolality.

(pH 7.4) and 0.01% $NaN_3$. The osmotic pressure of this control buffer was close to 75 mosmol/kg water and its refractive index measured at 23°C was 1.3355. The presence of vesicles at a concentration of 0.2 mg protein per ml does not change the refractive index of the buffer solution.

Hyperosmotic solutions were obtained by addition of mannitol or NaCl to the control buffer. The relationship between their respective osmolality and refractive index in the observation chamber are given Fig. 1a. The influence of such changes in refractive index on transmitted light through the vesicle suspension was studied in the following way. The refractive index of the control buffer solution was adjusted to the index of the hyperosmotic mannitol and NaCl solutions by addition of polyvinyl-pyrrolidone (PVP) (molecular weight 40000) which barely modified the final osmotic pressure (Fig. 1b). Measurements of the transmitted light through vesicles suspended in buffer solutions contained the different amounts of PVP clearly indicated that, independently of vesicle size, an increase of refractive index leads to a proportional increase in transmitted light intensity (Fig. 1c).

This was confirmed by our stopped-flow experiments: when mixing the vesicles with a hyperosmotic solution the induced change in transmitted light, $\Delta I$, was smaller with NaCl than with mannitol, as predicted by the difference in refractive index [6]. In contrast, when NaCl solutions reached the same refractive index as mannitol by addition of PVP, the kinetics of vesicle shrinkage were similar for both solutions thus indicating a reflection coefficient for NaCl close to one (Fig. 2).

Although the refractive index modifies the amplitude of the change in transmitted light and thereby the initial slope, it does not interact with the lifetime of vesicle shrinkage. Since for a given osmotic gradient, the induced water flux $J_V$ is directly proportional to the rate constant $k$ of the exponential curve describing the vesicle shrinkage [7], the reflection coefficient $\sigma$ is equal to:

$$\sigma = \frac{J_v\ (\text{NaCl})}{J_v\ (\text{mannitol})} = \frac{k\ (\text{NaCl})}{k\ (\text{mannitol})}$$

In our previously published light transmission experiments [6], irrespective of the osmotic probe (sucrose, mannitol, NaCl or KCl) the rate constants of the exponential fits of the experimental curves were not statis-

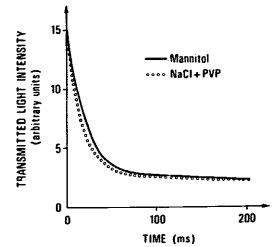


Fig. 2. Representative experiment of changes in transmitted light intensity as a function of time. Rat kidney brush-border membrane vesicles were mixed with isoosmolar amounts of mannitol or NaCl + PVP. Final osmotic gradient was 150 mosmol/kg water. PVP ($M_r$ 40000) was added to NaCl to equalize the refractive index of mannitol and NaCl solutions. Under these conditions changes in transmitted light intensity were similar whatever the osmotic probe. Assuming a reflection coefficient of 1 for the poorly permeant molecule mannitol, the similarity of the two kinetics of vesicle shrinkage suggests that the reflection coefficient of kidney brush-border membrane for NaCl is close to 1.

TABLE I

*Kinetic parameters of vesicle shrinkage*

Values are means of four experiments. Rat kidney brush-border membrane vesicle suspension was mixed in a stopped-flow apparatus with a hyperosmotic solution of NaCl or mannitol to reach an osmotic gradient of 50 mosmol/kg water in the optical cell. The experimental curves of change in light scattering with time were analyzed with a double-exponential fit between 0 and 1 s. Assuming the water flux out of the vesicles to be proportional to the rate constant of the exponential $k$ [7], the reflection coefficient of NaCl was calculated as: $\sigma = k$ (NaCl)/$k$ (mannitol), for each component. Although the mean reflection coefficient for NaCl was not statistically different from 1, the present data cannot excluded the possibility of $0.90 < \sigma_{NaCl} < 1.00$. All values are means ± S.E.

| | Mannitol | NaCl |
|---|---|---|
| Life time (ms) | | |
| fast component | 14.8 ± 2.0 | 15.3 ± 0.9 |
| slow component | 78.4 ± 2.5 | 78.0 ± 1.5 |
| Rate constant $k$ ($s^{-1}$) | | |
| fast component | 48.8 ± 5.3 | 45.7 ± 2.7 |
| slow component | 8.9 ± 0.3 | 8.9 ± 0.2 |
| Reflection coefficient $\sigma$ | | |
| fast component | reference | 0.96 ± 0.07 |
| slow component | reference | 0.98 ± 0.04 |

tically different suggesting that $\sigma_{NaCl}$ and $\sigma_{KCl}$ were close to one.

These results were confirmed by similar experiments performed in light scattering, with the same buffer to which mannitol or NaCl was added to reach a final osmotic gradient of 50 mosmol/kg water. A temperature controlled Bio-Logic SFM2 stopped-flow apparatus was used ($T = 23°C$). Three to five runs were stored and averaged in each series. The experimental curves were best fitted by a double-exponential function. The presence of a fast and a slow component could correspond to two populations of different size and comparable permeability or on the contrary to populations of same size but with different permeabilities. In the absence of a clear bimodal size distribution of rat kidney brush-border membrane vesicles [7,8], the two components of the exponential were attributed to differences in vesicle osmotic permeability. The corresponding $P_f$ values were 91 ± 4 μm/s and 500 ± 51 μm/s ($n = 4$), comparable to those found by Van Heeswijk and Van Os [8]. Rate constants of the exponential vesicle shrinkage and their corresponding lifetimes are given in Table I. The kinetic parameters obtained with NaCl were the same as that with mannitol, indicating that the induced water fluxes are similar for the two osmotic probes. The $\sigma_{NaCl}$ values calculated from the ratio of the rate constants $k$ of each exponential were not statistically different from one.

We conclude from these experiments that refractive indexes of the hyperosmotic solutions to which the vesicles are mixed have an influence on scattered light and thus on the determination of the reflection coefficient. When this phenomenon is taken into account, and assuming a $\sigma_{mannitol}$ value of 1, the data indicate that in the kidney brush-border membrane the reflection coefficient for NaCl is close to 1, suggesting that the water proteic pathways found in this cell membrane are not shared by salts.

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