The Apparent Diffusion of Water, Ions, and Small Molecules in the *Xenopus* Oocyte is Consistent with Brownian Displacement

Jonathan V. Sehy[1], Joseph J. H. Ackerman[2,3,4] Jeffrey J. Neil[4,5,6]

[1] Program in Molecular Cell Biology, Washington University, St. Louis, MO; [2] Department of Chemistry, Washington University, St. Louis, MO; Departments of Internal Medicine[3], Radiology[4], Neurology[5], and Pediatrics[6], Washington University School of Medicine, St. Louis, MO, USA

Introduction The incoherent displacement motion of water in living tissues is of considerable interest because of the widespread use of diffusion-weighted MR imaging, for which image contrast is based on the water apparent diffusion coefficient (ADC). The ADC reflects a measurement of incoherent water displacement over time that can be affected by many factors. One intriguing question about water displacement in the intracellular space regards the relative roles of passive, thermally driven (Brownian) diffusion versus more active processes such as cytoplasmic streaming. One mechanism suggested for the decrease in intracellular water ADC with stroke is that the decrease is a consequence of energy failure and cessation of cytoplasmic streaming (1). Cell extracts from the *Xenopus* oocyte have previously been used as a model system in which to study some of the specific processes that can be more generally referred to as cytoplasmic streaming (2,3).

In this study, the ADC values for water, as well as other molecules and ions of varying hydrodynamic radii (R_{HD}), are measured in the intracellular space of the oocyte. If intracellular motion is purely Brownian in nature, then the ADC values of these various species should vary with hydrodynamic radii according to the Stokes-Einstein relationship. Marked deviations from this relationship would indicate a significant role for some size-independent displacement motion such as cytoplasmic streaming.

Theory The Stokes-Einstein equation relates the diffusion coefficient (D) of a probe molecule to the fluid-phase viscosity (η) of the solution as:

$$D = \frac{kT}{6\pi R_{HD} \cdot \eta} \qquad [1]$$

where k is Boltzmann's constant and T is absolute temperature. Note that this equation is valid for the special case where the probe particle (the probe molecule plus associated solvent molecules, if any) is a sphere. This relationship is often used in the diffusion analysis of particles and molecules that are not pure spheres, in which case R_{HD} describes an effective radius.

For many applications of the Stejskal-Tanner diffusion experiment, motion cannot be assumed to be exclusively due to Brownian (i.e., random, thermal) displacement. At the same time, impediments to Brownian displacement may include more than just the fluid-phase viscosity. Therefore, we model the ADC as:

$$ADC = \frac{kT}{6\pi R_{HD} \cdot \eta_{app}} + B \qquad [2]$$

where B is a factor that represents all size-independent motion (e.g., streaming), and η_{app} represents the apparent viscosity, which may reflect the combined effects of barriers to motion, intermolecular binding, and fluid phase viscosity (4). Combining these two equations, we get:

$$ADC = \left(\frac{\eta}{\eta_{app}}\right) D_{free} + B \qquad [3]$$

where η is equal to 0.93 cP for pure water at 23° C.

Materials and Methods Data were obtained at 4.7 T and 23° C from oocytes isolated from *Xenopus laevis*. Intracellular water ADCs were determined from high resolution diffusion-weighted 3D spin-echo imaging. The intracellular ADCs of $^{133}Cs^+$, $^{23}Na^+$, 2-[^{19}F]luorodeoxyglucose-6-phosphate (2FDG-6P), and AT[^{31}P] were measured by MR spectroscopy of the relevant nuclei. Intracellular ADCs of tetramethylammonium (TMA^+) and polyethylene glycols (PEGs) of several molecular weights were measured by ^{1}H MR spectroscopy. Intracellular $^{133}Cs^+$ and $^{23}Na^+$ signals were resolved from extracellular signals using the shift reagent $TmDOTP^{5-}$. Other compounds, excluding ATP, were introduced into oocytes by microinjection. Free diffusion coefficients of probe species were measured in dilute solution. All diffusion experiments were conducted with a diffusion time of 8.6 ms.

Results Figure 1 shows the dependence of intracellular ADC on D_{free} for probe species. The calculated intracellular apparent viscosity was 2.07 ± 0.09 cP.

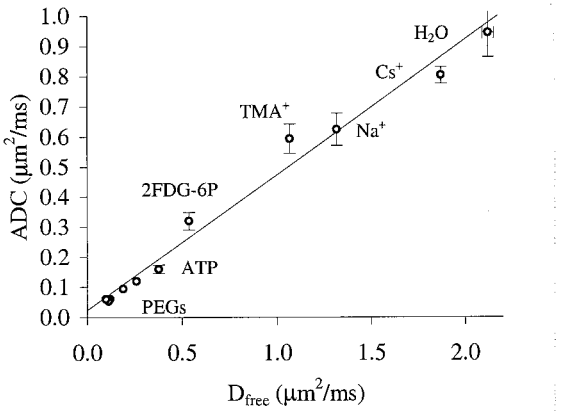

Fig. 1. Plot of intracellular ADC versus D_{free} for several small molecules and ions. The data were well fit (R^2 = .983) by least squares to a straight line with slope of 0.45 ± 0.02 and y-intercept of 0.02 ± 0.02 μm²/ms.

Discussion Results from figure 1 suggest that motion in the *Xenopus* oocyte is consistent with Brownian displacement with little or no role for size-independent motion such as cell streaming. If these results hold true for brain, a cessation of cell streaming (or other ATP-driven motion) should have little effect on the tissue water ADC.

References

1. Norris. *NMR Biomed*; 14:77-93, 2001.
2. Rogers, et al. *J Cell Biol*; 146:1265-1276, 1999.
3. Reinsh and Karsenti. *Curr Biol*; 7:211-214, 1997.
4. Kao, et al. *J Cell Biol*; 120:175-84, 1993.