Low Viscosity in the Aqueous Domain of Cell Cytoplasm Measured by Picosecond Polarization Microfluorimetry

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Abstract. Information about the rheological characteristics of the aqueous cytoplasm can be provided by analysis of the rotational motion of small polar molecules introduced into the cell. To determine fluid-phase cytoplasmic viscosity in intact cells, a polarization microscope was constructed for measurement of picosecond anisotropy decay of fluorescent probes in the cell cytoplasm. We found that the rotational correlation time (τc) of the probes, 2,7-bis-(2-carboxyethyl)-5-(and-6-)carboxyfluorescein (BCECF), 6-carboxyfluorescein, and 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) provided a direct measure of fluid-phase cytoplasmic viscosity that was independent of probe binding. In quiescent Swiss 3T3 fibroblasts, τc values were 20–40% longer than those in water, indicating that the fluid-phase cytoplasm is only 1.2–1.4 times as viscous as water. The activation energy of fluid-phase cytoplasmic viscosity was 4 kcal/mol, which is similar to that of water. Fluid-phase cytoplasmic viscosity was altered by <10% upon addition of sucrose to decrease cell volume, cytochalasin B to disrupt cell cytoskeleton, and vasopressin to activate phospholipase C. Nucleoplasmic and peripheral cytoplasmic viscosities were not different. Our results establish a novel method to measure fluid-phase cytoplasmic viscosity, and indicate that fluid-phase cytoplasmic viscosity in fibroblasts is similar to that of free water.

The structure of cell cytoplasm has been a topic of major interest in cell biology (Fulton, 1982; Stossel, 1982; Taylor and Fechheimer, 1982; Porter, 1984). The view is now widely accepted that the cell cytoplasm is not a homogeneous solution but a gel-like structure composed of complex networks of actin filaments, microtubules, and intermediate filaments (Luby-Phelps et al., 1988). The aqueous domain of the cytoplasm that occupies the space between the cytoskeletal network contains macromolecules, and small organic and inorganic solutes (Horowitz and Miller, 1984; Bridgman and Reese, 1984; Paine, 1984). Many of the metabolic and enzymatic activities of living cells occur in the aqueous cytoplasm. Although the cytoskeletal network has been studied extensively, little information is available about characteristics of the aqueous domain of cell cytoplasm.

The physical state, or specifically the rheological characteristics of the aqueous domain of the cytoplasm, influences a number of intracellular dynamic processes, including solute transport, diffusion-limited enzyme kinetics, and cell motility. Current investigations on cell membrane transport and enzyme kinetics are based on the assumption that the cytoplasmic fluid has a physical state similar to that of aqueous solutions in vitro, so that thermodynamic information obtained in vitro can be applied to the biological activities in cell cytoplasm. In contrast, primarily based on proton nuclear magnetic resonance studies, the existence of "organized water" has been proposed (Clegg, 1984a; Parsegian and Rau, 1984). If the majority of cell water is organized by cytoplasmic macromolecules, thermodynamic information obtained in vitro would not be applicable to the cell (Clegg, 1984b). Because the rheological characteristics of organized water are probably very different from those of normal water (Keith et al., 1977a), the presence of organized water would have significant effects on intracellular dynamic processes.

The rheological characteristics of the aqueous domain of cell cytoplasm have been studied by several biophysical methods. However, because of methodological uncertainties, reported values of cytoplasmic viscosity vary in a range from 2 cP to >100 cP. One approach to measure cytoplasmic viscosity was the direct observation of the displacement of microinjected small magnetic particles and macromolecules in the cell (Valberg and Albertini, 1985; Dembo and Harlow, 1986). Values obtained by this method are higher than fluid-phase cytoplasmic viscosity because of mechanical barriers imposed by the meshlike structure of the cytoskeletal network. Another method was electron spin resonance (ESR). From the translational diffusion of spin label probes, fluid-phase cytoplasmic viscosity values of 2–8 cP have been cal-

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**Materials and Methods**

**Chemicals**

2,7-Bis(2-carboxyethyl)-5-(and-6-) carboxyfluorescein-acetoxymethyl ester, 688 \( \mu \)M, BCECF-AM, BCECF acid (520 \( \mu \)M), 6-carboxyfluorescein, 376 \( \mu \)M (6-CF), and 8-hydroxypropene-1,3,6-trisulfonic acid, 524 \( \mu \)M (HPTS) were purchased from Molecular Probes Inc. (Junction City, OR). Other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

**Cell Culture**

Swiss 3T3 fibroblasts (American Type Culture Collection No. CL-101; Rockville, MD) were grown on 18-mm round glass coverslips in DME-H21 supplemented with 5% FBS, penicillin (100 U/ml) and streptomycin (100 \( \mu \)g/ml). Cells were maintained at 37°C in a 5% CO\(_2\)/95% air incubator.

Exponentially growing cells or quiescent cells grown to confluence were studied. For fluorescence measurements, cells were perfused continuously in buffer containing (in millimoles): 0.7 CaCl\(_2\), 1.1 MgCl\(_2\), 2.7 KCl, 1.5 KH\(_2\)PO\(_4\), 138 NaCl, 8.1 NaHPO\(_4\) (buffer A) in a 200-\( \mu \)l perfusion chamber in which the cell free surface of the glass coverslip made contact with the immersion objectives (Chao et al., 1989). Experiments were performed at 24°C unless otherwise specified. To activate phospholipase C, 20 nM vasopressin was added to the perfusion solution. Cell volume was decreased by addition of sucrose. The cell cytoskeleton was disrupted by incubation of cells with 5 \( \mu \)g/ml cytochalasin B for 5 h.

**Labeling Protocol**

For BCECF labeling, fibroblasts were incubated with buffer A containing 5 \( \mu \)M BCECF-AM for 15 min at 37°C. For 6-CF labeling, cells were incubated with 10 mM 6-CF, pH 6, for 15 min. 6-CF is relatively permeable to plasma membranes at pH 6, and is entrapped in cell cytoplasm at pH >7. 6-CF staining was stable for >30 min. For HPTS studies, cells were incubated with buffer A containing 10 mM HPTS for 15 min for uptake by fluid-phase endocytosis. The cells were then washed and incubated with buffer A for 2 h at 37°C. HPTS remained entrapped in the endocytic vesicles for <30 min and was released into the cytoplasm by diffusion (Straubinger et al., 1990).

**Fluorescence Confocal Imaging System**

Fluorescently labeled fibroblasts were viewed using an epifluorescence microscope (E. Leitz, Rochleigh, NJ) with a coaxial-confocal attachment (Technical Instrument Co., San Francisco, CA). The excitation source consisted of a 100-W Hg-arc lamp and 490 ± 5-nm six-cavity interference filter [Omega Optical, Inc., Brattleboro, VT]. Light was reflected onto the perfusion bath by a 510-nm dichroic mirror. A 40× quartz objective (Leitz, N.A. 0.65, glycerol immersion, 0.35-mm working distance) was used. Emitted light was filtered by KV500 and GG530 cut-on filters and focused onto a variable gain, microchannel plate image intensifier (Videoscope International, Washington DC), and imaged with a solid-state CCD camera (Cohu Inc., San Diego, CA) operating at fixed gain. The output of the CCD camera was digitized with a frame grabber (DT2861; Data Translation, Marlboro, MA) and an auxiliary processing board (DT2858) in an 80287 computer.

**Time-resolved Fluorescence Measurements**

Picosecond anisotropy decay of cytoplasmic fluorophores was measured by Fourier transform, multiharmonic fluorimetry by interfacing a fluorimeter (SLM Instruments, Inc., Urbana, IL) to a Nikon inverted epifluorescence microscope (Fig. 1; Verkman et al., 1991). Cells were excited with polarized light at 443 nm from a 25-nW He-Cd laser (Liconix, Sunnyvale, CA) or a 514-nm argon laser (Coherent, Santa Clara, CA). Emitted light was focused onto a series of four dichroic mirrors, each at angles of ±45°, which were used to separate the excitation beam from the emission beam. The emission beam was then focused onto the photomultiplier tubes, which were mounted in a 16-channel detector module (Hamamatsu, Bridgewater, NJ). The system was interfaced to a computer (Pentium, Sun Microsystems, CA) using a National Instruments data acquisition card (Model 1800, Austin, TX) and a National Instruments data acquisition card (Model 1800, Austin, TX) and a National Instruments data acquisition card (Model 1800, Austin, TX) and a National Instruments data acquisition card (Model 1800, Austin, TX).
at 488 nm from a 4-W Argon laser (Coherent Inc., Palo Alto, CA). Light was modulated to form brief (1–2 ns) pulses by a Pockel's cell. 3% of the laser light was reflected onto a glass fiber optic cable. The laser beam was reflected onto the cell chamber by a 1.5-mm square front surface mirror or a 510-nm fused silica dichroic mirror. Values of modulation amplitude ratios obtained with the dichroic mirror were corrected by a G-factor as described elsewhere (Fushimi et al., 1990). A 40× quartz objective and 20× objective (NA 0.75, 0.17-mm working distance) were used in anisotropy measurements; these objectives gave no measurable depolarizing effect (Axelrod, 1989). By use of lenses in the excitation path, the beam could be focused/defocused onto a <0.6-μm area in a single cell or onto one or more whole cells. Emitted light was filtered by serial KVS00 and GG530 barrier filters and detected by a photomultiplier with an analyzing polarizer that could be rotated by 90°. The validity of the time-resolved anisotropy system was tested in every set of experiments by measuring solutions of known time-resolved anisotropy. Calibration solutions consisted of 20-μM BCECF in buffer A and in a 10% glycerol solution.

Differential phase angles and modulation amplitudes, representing the time-resolved motion of cytoplasmic fluorophores, were measured at 30–80 different modulation frequencies (4–280 MHz) in parallel by multiharmonic cross-correlation detection. Parameters for rotational models were fitted from differential phase angles and modulation amplitudes by a weighted nonlinear least squares procedure (Calafut et al., 1989). In studies of the kinetic response of cytoplasmic viscosity to various maneuvers, the complete multifrequency analysis was performed every 147 ms.

**Results**

Fig. 2 shows a bright field (left) and confocal fluorescence micrograph (right) of fibroblasts labeled with BCECF. The arrow in the brightfield micrograph points to the focused laser beam. The fluorescence micrograph shows very little nonuniformity in the staining. Staining was also uniform in other focal planes and was not different after 30 min. Similar images were obtained for staining of fibroblasts with 6-CF and HPTS.

The viscosities determined from steady-state anisotropy and lifetime are upper limits to the true cytoplasmic viscosity because of the uncertain extent of fluorophore binding to cytoplasmic components. If fluorophore depolarizing rotations are blunted due to binding, then steady-state anisotropy is higher than that expected for an equivalent cytoplasmic viscosity in the absence of fluorophore binding. For an anisotropically rotating fluorophore, more than one rotational correlation time is required to describe the decay of anisotropy with time, r(t),

\[ r(t) = r_e \sum f_i \exp \left(-t/t_{c_i}\right) \]

where ro is the maximum anisotropy in the absence of depolarizing rotations (0.39 for BCECF), and fi is the fractional component of anisotropy loss corresponding to rotational correlation time tci. Theoretically, the fastest component of the rotational motion should describe the rotational motion of the unbound fluorophore according to the Stokes-
The approximate independence of $f_1$ on glycerol concentration indicates that fraction of bound fluorophore does not depend upon solution viscosity. These results validate the interpretation of $t_{1c}$ in terms of fluid-phase viscosity.

Additional anisotropy decay studies were carried out to show that $t_{1c}$ for BCECF does not depend upon pH or excitation wavelength. At low pH ($< \approx 6.3$) BCECF is protonated, whereas at high pH ($>\approx 6.3$) BCECF has multiple negative carboxyl groups. $t_{1c}$ and $f_1$ were measured in droplets containing $10 \mu$M BCECF in perfusion buffer titrated to pH 5.5 and 8.0, using an excitation wavelength of 443 nm (He-Cd laser) or 488 nm (argon laser). In the absence of glycerol, $t_{1c}$ was $267 \pm 12$ ps (pH 5.5, mean $\pm$ SD for five measurements) and $257 \pm 8$ ps (pH 8.0) at 443 nm, and $263 \pm 11$ (pH 5.5) and $249 \pm 8$ (pH 8.0) at 488 nm. In the presence of 32% glycerol, $t_{1c}$ was $596 \pm 25$ ps (pH 5.5) and $649 \pm 20$ ps (pH 8.0) at 443 nm, and $620 \pm 22$ (pH 5.5) and $668 \pm 21$ (pH 8.0) at 488 nm. Therefore, the rotational correlation times do not depend upon the extent of BCECF protonation and therefore does not depend upon intracellular pH or excitation wavelength. The $(2.4 \pm 0.1)$-fold increase in $t_{1c}$ for a 2.5-fold increase in viscosity (0–32% glycerol) in Fig. 3 (right) provides further support for the interpretation of $t_{1c}$ in terms of fluid-phase viscosity.

Anisotropy decay of BCECF, 6-CF, and HPTS were measured in Swiss 3T3 fibroblasts. A typical phase modulation plot for BCECF is shown in Fig. 4. To evaluate the sensitivity of fitted parameters to viscosity values, curves corresponding to viscosities of 2.0 and 0.8 times that of water are shown. Rotational parameters and calculated viscosities are summarized in Table I. Viscosities calculated from $t_{1c}$ values of the three fluorophores show that fluid phase cytoplasmic viscosity is very low, only 20–40% more viscous than free water. Fluid-phase cytoplasmic viscosity values determined from the three fluorophores were in good agreement, providing strong support for the validity of our method. $f_1$ and $t_{1c}$ values were different for these fluorophores indicating different intracellular binding properties, as predicted, because of differences in fluorophore structure and polarity. Although control experiments showed a significant amount of the fluorophore binding in low concentration albumin solutions (Fig. 3, right), $f_1$ values in Table I indicate that only 20–40% of the fluorophores were bound in the cytoplasm.

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**Table I. Cytoplasmic Viscosity in Swiss 3T3 Fibroblasts**

| Viscometer | $t_{1c}$ | $f_1$ | $t_1$ | $\eta/\eta_0$ |
|------------|---------|-------|-------|--------------|
| BCECF      | $n = 15$| 294 ± 7| 0.78 ± 0.01 | 3.4 ± 0.2 | 1.22 ± 0.03 |
| 6-CF       | $n = 14$| 180 ± 20| 0.65 ± 0.01 | 4.5 ± 0.2 | 1.37 ± 0.17 |
| HPTS       | $n = 5$ | 181 ± 12| 0.59 ± 0.01 | 5.1 ± 0.3 | 1.42 ± 0.09 |

Rotational parameters of the time-resolved anisotropy decay of BCECF, 6-CF, and HPTS in fibroblasts are given. Phase modulation data were fitted to a two component anisotropic rotational model; $t_{1c}$ and $t_0$ are shorter and longer rotational correlation times; and $f_1$ is the fractional anisotropy loss corresponding to $t_{1c}$. Fluid-phase cytoplasmic viscosity relative to that of water, $\eta/\eta_0$, was calculated from $t_{1c}$. Lifetime values ($t_1$) were obtained by phase modulation lifetime analysis. Values are mean ± SEM for 5–10 separate cells. Experiments were performed at 24°C.
was weakly temperature dependent. The activation energy (4.3 ± 0.2 kcal/mol) was 4.3 ± 0.2 kcal/mol, not different from that of free water (4.1 kcal/mol).

The fluid-phase cytoplasmic viscosity of fibroblasts was compared in a series of different conditions (Fig. 5). To examine the effect of cell growth on fluid-phase cytoplasmic viscosity, anisotropy decay of BCECF was measured in quiescent confluent cells and exponentially growing, nonmitotic cells. No significant difference in fluid-phase cytoplasmic viscosity was observed. The rotational correlation time was weakly temperature dependent. The activation energy for fluid-phase cytoplasmic viscosity determined from three to five measurements at different temperatures (13–32°C) was 4.3 ± 0.2 kcal/mol, not different from that of free water (4.1 kcal/mol).

The spatial distribution of fluid-phase cytoplasmic viscosity was related directly to the fluid-phase viscosity. As a reference for interpretation of fluid-phase cytoplasmic viscosity values, measurements of anisotropy decay of BCECF were made in 3–10% solutions of small molecules and macromolecules (Table II). Bulk viscosities of these solutions were determined by a Cannon-Fenske Viscometer (Fisher Scientific Co., Pittsburgh, PA). Table II shows that the rotational correlation time increases in parallel with bulk viscosity only in solutions containing small molecules but not in solutions containing macromolecules, indicating that fluorophore rotation provides an accurate measure of fluid-phase viscosity but not of bulk viscosity.

**Table II. Fluid-Phase Viscosity and Bulk Viscosity of Solutions**

|            | $t_0$ (BCECF rotation) | $\eta/\eta_{H_2O}$ (viscometer) |
|------------|-------------------------|----------------------------------|
| PBS        | 255 ± 5                 | 1.05 ± 0.02                      | 1.05 ± 0.01                     |
| 10% Alanine| 301 ± 12                | 1.24 ± 0.05                      | 1.22 ± 0.02                     |
| 10% Glycerol| 318 ± 10               | 1.31 ± 0.04                      | 1.29 ± 0.02                     |
| 10% Sucrose| 328 ± 12                | 1.35 ± 0.05                      | 1.38 ± 0.02                     |
| 10% Dextran| 281 ± 19                | 1.16 ± 0.08                      | 7.58 ± 0.05                     |
| 3% Globin  | 320 ± 17                | 1.32 ± 0.07                      | 1.56 ± 0.05                     |
| 3% ALP     | 252 ± 15                | 1.04 ± 0.06                      | 1.62 ± 0.04                     |
| 10% $\gamma$-Globulin| 261 ± 5              | 1.08 ± 0.02                      | 1.30 ± 0.03                     |
| 5% Mucin   | 268 ± 12                | 1.10 ± 0.05                      | 1.50 ± 0.05                     |
| 3% Muscle extract| 254 ± 10             | 1.05 ± 0.04                      | 1.33 ± 0.04                     |

Fluid-phase viscosities of protein and dextran solutions were calculated from $t_0$ values of BCECF rotational analysis. Bulk viscosities were measured by a Cannon-Fenske viscometer. All chemicals are obtained from Sigma Chemical Co. ALP, alkaline phosphatase. Data are mean ± SD for three measurements.
cosity significantly, and that fluid-phase viscosity of cytoplasm and nucleoplasm do not differ.

The time resolved fluorescence anisotropy method provided a quantitative value for viscosity of macromolecule-containing solutions like cytoplasm. The rotational correlation time of small polar fluorophores was directly proportional to viscosity as set by glycerol addition (Fig. 3). In the presence of albumin or polylysine, apparent fluorophore rotational motion was described well by a two-component model for anisotropic rotation. The fraction of anisotropy decay due to the faster rotational component decreased as the concentration of either BSA or polylysine increased. The shorter correlation time represented the rotation of unbound fluorophore whereas the longer correlation time represented the rotation of the protein-bound fluorophores, whose rotation was blunted and altered by binding (Lakowicz, 1983). Importantly, the relationship between the faster rotational correlation time and fluid-phase viscosity was similar, for all of the fluorophores used in this study, even in the presence of significant protein binding.

As shown in Fig. 2, cells labeled with BCECF showed quite uniform staining in the cytoplasm and the nucleoplasm. There was no evidence of fluorophore compartmentation 30 min after labeling. It is assumed that the submicroscopic distribution of fluorophores that do not bind to cytoplasmic structures is reasonably uniform. Diffusion of unbound fluorophore is not restricted geometrically since the size of these fluorophores, 10-12 Å, is much smaller than that of the cytoskeletal mesh (200-400 Å; Luby-Phelps et al., 1986). From the polar and hydrophilic characteristics, the unbound fluorophore molecules are expected to diffuse in the fluid-phase cytoplasm as small ionic and nonionic solutes.

Our value of fluid-phase cytoplasmic viscosity is significantly lower than the values estimated by ESR, FRAP, and steady-state anisotropy. In some ESR studies (Keith et al., 1977a; Schobert and Marsh, 1982; Mastro et al., 1984) and FRAP studies (Wojcieszyn et al., 1981; Salmon et al., 1984; Luby-Phelps et al., 1986), cytoplasmic viscosity values estimated from the diffusional movement of probes were generally higher than fluid-phase viscosity because of the non-Newtonian characteristics of cytoplasmic fluid. Mechanical barriers imposed by the cytoplasmic macromolecules and nonspecific low affinity binding of the probes to cytoplasmic structures alter probe diffusional motion (Luby-Phelps et al., 1988). In other ESR studies (Lepock et al., 1983; Mastro and Keith, 1984) and steady-state anisotropy studies (Lindmo and Steen, 1977; Hashimoto and Shinozaki, 1988; Dix and Verkman, 1990), cytoplasmic viscosity values estimated from probe rotation were too large because of probe binding. As shown in the present study, small, polar fluorophores do bind to cytoplasmic components appreciably. Contrary to the previous methods, time-resolved analysis of anisotropy provides information about the rotational movement of unbound fluorophores, a quantity that is related directly to fluid-phase cytoplasmic viscosity in a complex macromolecule solution.

Low fluid-phase cytoplasmic viscosity suggests that the diffusional movement of small solutes in cytoplasm is similar to that in a dilute aqueous solution. However, the bulk viscosity of aqueous cytoplasm is probably higher because of the presence of cytoplasmic macromolecules. As shown in Table II, small solutes increase the fluorophore rotational correlation time in parallel with bulk viscosity, whereas macromolecules increase bulk viscosity with minimal effect on fluorophore rotation. In aqueous cytoplasm, diffusion of small molecules is mainly limited by fluid-phase viscosity, whereas diffusion of macromolecules is limited by the interaction with other macromolecules and the cytoskeletal mesh (Mastro and Keith, 1984; Luby-Phelps et al., 1988). Therefore, our finding of low fluid-phase cytoplasmic viscosity indicates that intracellular dynamic processes involving small solutes are similar to those in free solution.

Similarly, the low fluid-phase cytoplasmic viscosity and the activation energy of ~4 kcal/mol suggest that the physical state of water molecules in cytoplasm is similar to that of free water. The physical state of cell water and the possibility of organized cell water have been debated. Based on nuclear magnetic resonance studies showing that the rotational movement of 10-50% of cell water molecules is slower than that in free solution, it was suggested that a significant fraction of cell water is "bound" or "organized" by macromolecules (Cameron et al., 1988). Although the rheological characteristics of organized water have not been defined clearly, our findings that the rheological characteristics of cell water are comparable to those of free water are not consistent with extensive organization of cell water. (The rotational correlation time of BCECF measured in ice was >15 times longer than that in free water. Therefore, the rotational motion of fluorophores is probably sensitive to physical state or organization of water.) However, there are several cautions in the assessment of water organization by measurement of probe rotation. First, if the fluorophore probes were selectively excluded from organized water, our measurement would not provide information about the physical characteristics of organized water. However, because the distribution of small polar fluorophores would be similar to that for small ions and solutes, fluorophore inaccessible regions in the cell might be biologically inactive. Second, even if fluorophores do diffuse into organized water, the rotational movement of fluorophore in organized water might be very slow and indistinguishable from the rotational movement of bound fluorophores. In this case, the $f_l$ values in Table I indicate that at most 20% of water molecules are organized.

Our findings that viscosity of fluid-phase cytoplasm is not different from that of nucleoplasm, and not modified by major cytoskeletal alterations, suggest little influence of the cytoskeletal network on the rheological characteristics of the fluid-phase cytoplasm. In previous studies, small changes of ESR probe movement by actin filament disruption were observed (Mastro and Keith, 1984); however, these changes might be caused by alteration of probe binding to cytoplasmic structures. Interestingly, whereas nucleoplasm has little filamentous structure and different solute composition compared to cytoplasm (Horowitz and Miller, 1984; He et al., 1990), it was found that fluid-phase viscosity of nucleoplasm and cytoplasm are similar.

In conclusion, we showed that fluid-phase cytoplasm and nucleoplasm in fibroblasts is only 1.2-1.4 times as viscous as free water and not influenced by major alterations in the cytoskeleton. The present study establishes an optical method to measure fluid phase cytoplasmic viscosity in selected regions of single cells. Our findings suggest that the majority of cell water is not organized and has physical properties similar to those of free water.
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References

Axelrod, D. 1989. Fluorescence polarization microscopy. Methods Cell Biol. 30:333–352.

Bridgman, P. C., and T. S. Reese. 1984. The structure of cytoplasm in directly frozen cultured cells. I. Filamentous meshworks and the cytoplasmic ground substance. J. Cell Biol. 99:1655–1668.

Cameron, I. L., G. D. Fullerton, and N. K. R. Smith. 1988. Influence of cytomatrix proteins on water and on ions in cells. Scanning Microsc. 2:275–286.

Calafut, T. M., J. A. Dix, and A. S. Verkman. 1989. Fluorescence depolarization of cis- and trans-parinaric acids in artificial and red cell membranes resolved by a double hindered rotational model. Biochemistry. 28:5051–5058.

Chao, A. C., J. A. Dix, M. Sellers, and A. S. Verkman. 1989. Fluorescence measurement of chloride transport in monolayer cultured cells: mechanisms of chloride transport in fibroblasts. Biophys. J. 56:1070–1081.

Clegg, J. S. 1984a. Intracellular water and the cytomatrix: some methods of study and current views. J. Cell Biol. 99(1 Pt. 2):167s–171s.

Clegg, J. S. 1984b. Properties and metabolism of the aqueous cytoplasm and its boundaries. Am. J. Physiol. 246, R133–R151.

Cooper, J. A. 1987. Effects of cytochalasin and phalloidin on actin. J. Cell Biol. 105:1473–1478.

Dembo, M., and F. Harlow. 1986. Cell motion, contractile networks, and the physics of interpenetrating reactive flow. Biophys. J. 50:109–121.

Dix, J. A., and A. S. Verkman. 1990. Mapping of fluorescence anisotropy in living cells by ratio imaging. Application to cytoplasmic viscosity. Biophys. J. 57:231–240.

Fulton, A. B. 1982. How crowded is the cytoplasm? Cell. 30:345–347.

Fushimi, K., J. A. Dix, and A. S. Verkman. 1990. Cell membrane fluidity in the intact cytoplasm proximal tubule measured by orientation-independent fluorescence anisotropy imaging. Biophys. J. 57:241–254.

Hashimoto, H., and N. Shinozaki. 1988. Measurement of cytoplasmic viscosity by fluorescence polarization in phytohemagglutinin-stimulated and unstimulated human peripheral lymphocytes. J. Histochem. Cytochem. 36:609–613.

He, D., J. A. Nickerson, and S. Pennan. 1990. Core filaments of the nuclear matrix. J. Cell Biol. 110:569–580.

Horowitz, S. B., and D. S. Miller. 1984. Solvent properties of ground substance studied by cryomicrodissection and intracellular reference-phase techniques. J. Cell Biol. 99(1 Pt. 2):172s–179s.

Keith, A. D. 1973. Viscosity of cellular protoplasm. Science (Wash. DC). 183:666–668.

Keith, A. D., W. Snipes, and D. Chapman. 1977a. Spin-label studies on the aqueous regions of phospholipid multilayers. Biochemistry. 16:634–641.

Keith, A. D., W. Snipes, R. J. Mehilhorn, and T. Gunter. 1977b. Factors restricting diffusion of water soluble spin labels. Biophys. J. 19:205–218.

Lakowicz, J. R. 1983. Time-dependent decays of fluorescence anisotropy. In Principles of Fluorescence Spectroscopy. Plenum Press, New York. 156–185.

Lepock, J. R., K. H. Cheng, S. D. Campbell, and J. Kruuv. 1983. Rotational diffusion of tempone in the cytoplasm of Chinese hamster lung cells. Bioophys. J. 44:405–412.

Lindmo, T., and H. B. Steen. 1977. Flow cytometric measurement of the polarization of fluorescence from intracellular fluorescein in mammalian cells. Biophys. J. 18:173–187.

Luby-Phelps, K., D. L. Taylor, and F. Lanni. 1986. Probing the structure of cytoplasm. J. Cell Biol. 102:2015–2022.

Luby-Phelps, K., F. Lanni, and D. L. Taylor. 1988. The submicroscopic properties of cytoplasm as a determinant of cellular function. Annu. Rev. Biophys. Chem. 17:369–396.

Mastro, A. M., and A. D. Keith. 1984. Diffusion in the aqueous compartment. J. Cell Biol. 99(1 Pt. 2):180s–187s.

Mastro, A. M., M. A. Babich, W. D. Taylor, and A. D. Keith. 1984. Diffusion of a small molecule in the cytoplasm of mammalian cells. Proc. Natl. Acad. Sci. USA. 81:3414–3418.

Paine, P. L. 1984. Diffusive and nondiffusive proteins in vivo. J. Cell Biol. 99(1 Pt. 2):188s–195s.

Parsegian, V. A., and D. C. Rau. 1984. Water near intracellular surface. J. Cell Biol. 99(1 Pt. 2):196s–200s.

Porter, K. R. 1984. The cytomatrix: A short history of its study. J. Cell Biol. 99(1 Pt. 2):3s–12s.

Salmon, E. D., W. M. Saxton, R. J. Leslie, M. L. Karow, and J. R. McIntosh. 1984. Diffusion coefficient of fluorescein-labeled tubulin in the cytoplasm of embryonic cells of a sea urchin: video image analysis of fluorescence redistribution after photobleaching. J. Cell Biol. 99:2157–2164.

Schoeber, B., and D. Marsh. 1982. Spin label studies on osmotically induced changes in the aqueous cytoplasm of phaeodactylum tricornutum. Biochim. Biophys. Acta. 725:87–95.

Stossel, T. P. 1982. The structure of cortical cytoplasm. Phil. Trans. R. Soc. Lond. B Biol. Sci. 299:275–289.

Straubinger, R. M., D. Papahadjopoulos, and K. Hong. 1990. Endocytosis and intracellular fate of liposomes using pyranine as a probe. Biochemistry. 29:4929–4939.

Taylor, D. L., and M. Fechheimer. 1982. Cytoplasmic structure and contractility: The solution-contraction coupling hypothesis. Phil. Trans. R. Soc. Lond. B Biol. Sci. 299:185–197.

Valbeug, P. A., and D. F. Albertini. 1985. Cytoplasmic motions, rheology and structure probed by a novel magnetic particle method. J. Cell Biol. 101:130–140.

Verkman, A. S., M. Armijo, and K. Fushimi. 1991. Construction and evaluation of a frequency-domain epifluorescence microscope for lifetime and anisotropy decay measurements in subcellular domains. Biophys. Chem. In press.

Wojcieszyn, J. W., R. A. Schlegel, E. S. Wu, and K. A. Jacobson. 1981. Diffusion of injected macromolecules within the cytoplasm of living cells. Proc. Natl. Acad. Sci. USA. 78:4407–4410.