Thurnhofer, H., & Hauser, H. (1990b) *Biochim. Biophys. Acta 1024*, 249–262.
Thurnhofer, H., Schnabel, J., Betz, M., Lipka, G., Pidgeon, C., & Hauser, H. (1991) *Biochim. Biophys. Acta 1064*, 275–286.
Venien, C., & Le Grimellec, C. (1988) *Biochim. Biophys. Acta 942*, 159–168.
Verkleij, A. J., Zwaal, R. F. A., Roelofsen, B., Comfurius, P., Kastelijn, A., & van Deenen, L. L. M. (1973) *Biochim. Biophys. Acta 323*, 178–193.
Zachowsky, A., & Devaux, P. F. (1990) *Experientia 46*, 644–656.
Zwaal, R. F. A., Roelofsen, B., Comfurius, P., & van Deenen, L. L. M. (1975) *Biochim. Biophys. Acta 406*, 83–96.

Picosecond Rotation of Small Polar Fluorophores in the Cytosol of Sea Urchin Eggs†

N. Periasamy, M. Armijo, and A. S. Verkman*
Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, California 94143-0532

Received June 12, 1991; Revised Manuscript Received September 19, 1991

ABSTRACT: A new fluorescence method to measure viscosity in cell cytosol [Fushimi, K., & Verkman, A. S. (1991) *J. Cell Biol. 112*, 719–725] has been applied to determine fluid-phase viscosity in sea urchin eggs. Freshly harvested eggs from *Lytechinus pictus* were loaded with the dyes 2,7-bis(2-carboxyethyl)-5-(and-6-)carboxyfluorescein (BCECF), 6-carboxyfluorescein (6CF), fluorescein, or calcein. Fluorescence lifetimes and anisotropy decay were measured in single eggs by multiharmonic, frequency-domain microfluorometry using a 1–2-μm focused laser spot and 25× air objective. In calibration solutions consisting of glycerol in pH 8 buffered sea water, probe lifetime was single exponential and probe rotation was isotropic with a single correlation time which increased linearly with viscosity in the range 1–3.6 cP. In eggs at 22 °C, there were single lifetimes (in nanoseconds) of 3.6 (BCECF), 3.4 (6CF), 3.2 (fluorescein), and 3.3 (calcein). Probe rotation in eggs had two components, a fast component (in picoseconds, mean ± SE, 10–18 eggs) of 568 ± 39 (BCECF), 311 ± 21 (6CF), 313 ± 15 (fluorescein), and 516 ± 44 (calcein) and a slow component of 10–40 ns. The fractional amplitude of the fast component, corresponding to unbound dye, was 0.72–0.81. Apparent viscosities of fluid-phase cytoplasm (centipoises) given by the four different probes were in good agreement: 2.3 ± 0.2 (BCECF), 2.1 ± 0.1 (6CF), 2.5 ± 0.1 (fluorescein), and 2.3 ± 0.2 (calcein). The viscosity in cytosol of sea urchin eggs (2.1–2.5 cP) is thus relatively low, yet significantly greater than that of water (1 cP) or cytosol in cultured fibroblasts (1.2–1.4 cP). Taken together with previous results, the increased viscosity in egg cytosol may be a consequence of the high concentration of small organic osmolytes required to maintain cell volume.

Cell cytoplasm is a complex compartment consisting of water, dissolved solutes, and organized macromolecular structures and organelles (Fulton, 1982; Stossel, 1982; Porter, 1984; Gershon et al., 1985; Luby-Phelps et al., 1986). The rotational and translational dynamics of solute molecules depend upon the viscous properties of the cellular environment as well as upon solute geometry and specific interactions with intracellular components. A particularly important quantity is the microviscosity of "fluid-phase" cytoplasm, defined as the viscosity detected by a very small probe molecule which does not interact with intracellular macromolecular and lipid structures. Fluid-phase cytoplasmic viscosity is an important determinant for the kinetics of diffusion-limited transport and enzymatic processes and for the energetics of cell motility (Taylor & Fechheimer, 1982; Clegg, 1984b; Cameron et al., 1988; Luby-Phelps et al., 1988).

A number of approaches have been applied to estimate fluid-phase viscosity in cell cytoplasm. Although there is evidence that cytoplasm is a viscous gellike compartment [for reviews, see Keith (1973) and Porter (1984)], more recent studies of the motions of small molecules suggest that cytoplasm has many properties of simple aqueous solutions. Studies of probe translational motion in cell cytoplasm by fluorescence recovery after photobleaching and electron spin resonance methods gave viscosities as low as 2–6 cP when probe size was extrapolated to zero (Luby-Phelps et al., 1986; Salmon et al., 1984; Wojcieszyn et al., 1981); measurements of probe translational diffusion would overestimate fluid-phase viscosity because of probe binding to and collisions with intracellular structures and the existence of intracellular barriers such as cytoskeleton and organelles (Keith et al., 1977b). Probe rotation would be influenced to a lesser extent by physical barriers; however, probe binding could strongly hinder rotational mobility. Electron spin resonance studies of the rotation of small spin-labeled probes gave viscosities of 2 to >50 cP (Keith et al., 1977a; Lepock et al., 1983; Mastro & Keith, 1984). Because fluid-phase viscosity would be overestimated by each of the measurements above, it was predicted that the actual fluid-phase viscosity of cytosol, at least in some cells, is <2 cP.

We introduced an approach recently to estimate fluid-phase viscosity in cell cytosol on the basis of the picosecond rotational

† This work was supported by Grants DK39354, DK17054, HL42368, and DK35124 from the National Institutes of Health and a grant from the National Cystic Fibrosis Foundation. Dr. Verkman is an Established Investigator of the American Heart Association.
* Address correspondence to this author.

0006-2960/91/0430-11836$02.50/0

characteristics of small polar fluorophores measured by frequency-domain microfluorometry (Fushimi & Verkman, 1991). The fraction of free (unbound) and bound probe in cytosol was determined by a multicomponent analysis of time-resolved probe rotation; the rotational correlation time of free probe provided a measure of fluid-phase viscosity. In Swiss 3T3 fibroblasts, fluid-phase viscosity was very low, 1.2–1.4 cP, and had an activation energy of 4 kcal/mol. It was concluded that the physical characteristics of the fluid-phase cytoplasmic compartment were similar to those of free water. Similar viscosities of <1.5 cP were measured for other cultured mammalian cells and cells in intact kidney epithelia (K. Fushimi and A. S. Verkman, unpublished data).

The purpose of our study was to apply the picosecond microscopy approach to measure fluid-phase cytoplasmic viscosity in sea urchin eggs. The sea urchin egg was selected as a model cell for several reasons. The egg from the species *Lytechinus pictus* is optically transparent and large (80 μm) compared to cultured cells, facilitating time-resolved fluorescence measurements and subsequent anisotropy mapping studies using confocal microfluorometry. There is extensive literature on the activation mechanisms of sea urchin eggs that are associated with large changes in surface membrane morphology and intracellular signaling (Johnson & Epel, 1975, 1976; Wolf et al., 1981; Salmon et al., 1984; Rakow & Shen, 1990; Chandler, 1991). Most importantly, we wished to determine whether the surprisingly low viscosity measured in mammalian cells was shared by the urchin egg, a cell thought classically to have a relatively viscous cytoplasm. Experiments were carried out using four different intracellular fluorescent probes to examine whether the derived viscosity depended upon probe identity. It was found that the fluid-phase viscosity in sea urchin eggs determined by the four probes was relatively low at 2.1–2.5 cP, yet significantly greater than that of water or mammalian cell cytosol.

Methods

Egg Isolation and Fluorescence Labeling. Sea urchins (*L. pictus*) were purchased from Pacific Biomarine Laboratories (Venice, CA) and maintained in a sea water aquarium at 16 °C. Ovulation of gametes was induced by intracoelomic injection of 0.5 M KCl (Johnson & Epel, 1975). Extracellular jelly was removed by swirling for 1 min in acidified sea water at pH 5 followed by three to four rinses in filtered sea water at pH 8. The temperature was maintained at 20–22 °C throughout the experiments.

The fluorophores 2,7-bis(2-carboxyethyl)-5-(and-6-)-carboxyfluorescein acetoxymethyl ester (BCECF-AM), 6-carboxyfluorescein diacetate (6CF-DA), calcein acetoxymethyl ester (calcein-AM), and fluorescein were purchased from Molecular Probes (Junction City, OR). Eggs were labeled by a 5–10-min incubation with 5 μM BCECF-AM, 6CF-DA, or calcein-AM in filtered sea water (pH 8) at 22 °C, or by a 5–10-min incubation with 1 mM fluorescein in sea water titrated to pH 6 with HCl. Extracellular fluorophore was removed by rinsing in sea water at pH 8. Experiments were carried out within 10 min after labeling, at which time no fluorophore leakage was observed.

Time-Resolved Fluorescence Microscopy Experiments. Fluorescence measurements were carried out by parallel acquisition frequency-domain fluorometry (Verkman et al., 1991). The excitation source was a 4-W CW Ar laser operating at 488 nm. Light was impulse modulated by a Pockel's cell and components of an SLM48000 multiharmonic fluorometer (SLM Instruments, Urbana, IL) to give a 4–7-MHz train of 1–2-ns pulses of polarized light. Approximately 4% of the light was reflected by a cover glass onto a reference photomultiplier by a fiberoptic cable, and the remaining light was reflected from a 510-nm dichroic mirror for sample excitation through a 25× long working distance air objective (Leitz, numerical aperture 0.35). The sample was positioned on the stage of a Nikon Diaphot inverted epifluorescence microscope. There were no "high numerical aperture" depolarizing effects with this objective (Axelrod, 1989). The beam diameter in the focal plane could be adjusted between ~1 and 100 μm by inclusion of a biconcave lens 5 cm proximal to the dichroic mirror.

Emitted fluorescence was collected by the objective lens and passed through the dichroic mirror, serial KV508 and GG530 barrier filters (Schott Glass, Duryea, PA), and a Glan-Thompson calcite polarizer that could be rotated by 90°. Fluorescence was detected by a selected R3896 photomultiplier (Hamamatsu) whose gain was modulated for multiharmonic cross-correlation mapping of high frequency (4–210 MHz) phase and modulation data to low frequency (6.25–187.5 Hz) data. The low frequency data was continuously acquired by direct analog-to-digital conversion, signal averaging, and subsequent Fourier transform into the frequency domain.

Fluorescence lifetimes were determined from phase and modulation values using fluorescein in 0.1 N NaOH as a reference (lifetime 4.0 ns). The Glan-Thompson polarizer was positioned at an angle of 54.7° from the parallel orientation (parallel to the plane of polarization of reflected excitation light) to eliminate effects of fluorophore polarization. Anisotropy decay was determined by differential polarization in which differential phase angles and modulation amplitude ratios were determined by comparing data obtained for parallel and perpendicular orientations of the polarizer. Differential modulation amplitudes were corrected for the instrument *G*-factor as described previously (Dix & Verkman, 1990; Fushimi et al., 1990). Generally, five to eight pairs (parallel and perpendicular) of data acquisitions were obtained, each averaged over 7.5 s. Median phase and modulation values and standard deviations determined for the paired data were used for weighted nonlinear least-squares fitting of lifetimes and rotational parameters (Calafut et al., 1989). In urchin eggs, the time-resolved anisotropy, $r(t)$, was described by two rotational correlation times (τ_{1c} and τ_{2c}) and a fractional anisotropy loss (f_1) as given by

$$r(t) = r_0(f_1 e^{-t/\tau_{1c}} + [1 - f_1]e^{-t/\tau_{2c}}) \quad (1)$$

where r_0 is the anisotropy in the absence of depolarizing rotations.

Results

The first set of studies were carried out to examine the lifetime and rotational properties of the fluorophores in aqueous solutions of varying viscosity (η). Solutions consisted of 15 μM BCECF, 6CF, calcein, or fluorescein in filtered sea water (pH 8) containing varying concentrations of glycerol to give bulk viscosities of 1.2–3.6 cP as determined by a Cannon-Fenske viscometer. Samples were placed between 0.1-mm glass cover slips to give a thickness of ~50 μm. Lifetime measurements were made comparatively using fluorescein in 0.1 N NaOH as a reference. A single fluorescence lifetime was determined in each sample as summarized in Table I. There was no effect of viscosity on fluorophore lifetime.

Time-resolved anisotropy was determined by comparing phase and modulation values for parallel and perpendicular orientations of the emission polarizer. The polarizer was aligned prior to experiments by replacing the dichroic mirror

Table I: Fluorophore Properties in Sea Water–Glycerol Solutions[a]

	τ_f (ns)	τ_c (ps)	τ_c/η (ps/cP)
BCECF	3.94	281 ± 7	247 ± 4
6CF	3.90	157 ± 6	155 ± 3
calcein	3.85	254 ± 9	227 ± 9
fluorescein	4.00	142 ± 8	101 ± 3

[a] τ_f values represent lifetimes and τ_c values are the rotational correlation times. Solutions consisted of 15 μM fluorophore in mixtures of sea water and glycerol as described in the text. τ_f and τ_c (mean ± SE, five determinations) are given in sea water in the absence of glycerol. The slopes of rotational correlation time vs solution viscosity plots (τ_c/η, see Figure 4) are given.

and barrier filter by a partially silvered mirror and placing a glass cover slip on the microscope stage to reflect ~4% of incident light. The ratio of parallel-to-perpendicular intensities was >300. The polarizer alignment and intensity ratio were not sensitive to beam diameter, focus, and objective (for numerical aperture <0.7).

Figure 1 shows differential phase angles and modulation ratios for BCECF in the sea water–glycerol solutions. The data showed little variation and were well-determined to modulation frequencies of ~150 MHz. The phase and modulation values were quite sensitive to viscosity. The phase-modulation values at all viscosities were fitted well by a model for unhindered isotropic rotation with a single rotational correlation time, $r(t) = r_0 e^{-t/\tau_c}$. Similar sets of data were obtained for 6CF, calcein, and fluorescein. For each fluorophore in sea water–glycerol solutions, the rotational correlation time increased linearly with viscosity as given in Figure 4. Table I summarizes rotational correlation times (τ_c) and the slopes (τ_c/η) for the correlation time vs viscosity plots. In control experiments, rotational correlation times measured by fluorescence microscopy using the 25× objective were not different from those measured by cuvette fluorometry, indicating absence of significant objective depolarization. In addition, rotational correlation times did not depend upon laser beam diameter in the focal plane (1–100 μm), objective focus, sample thickness (5–100 μm), and fluorophore concentration (10 μM–1 mM).

Sea urchin eggs were loaded with each of the four fluorophores and washed to remove external dye. Eggs were stained uniformly without extracellular fluorescence as observed by confocal fluorescence microscopy (K2 BIO; Technical Instruments, San Francisco, CA). Eggs were studied within 10 min after loading to minimize loss of viability.

Figure 2 shows phase-modulation plots for fluorophore lifetimes in sea urchin eggs. Background signal with the egg moved out of the laser beam was <2% of the signal with the egg in place. The data fitted well to a single-lifetime component as summarized in Table II. Fluorophore lifetimes in eggs were slightly lower than those in the sea water–glycerol standards. In control studies, fluorophore lifetimes were independent of intracellular concentration (1 μM rather than 5 μM loading solution), indicating the absence of fluorescence self-quenching. In addition, the lifetime did not depend upon intracellular pH in the range 6.5–8 when cytosolic pH was set

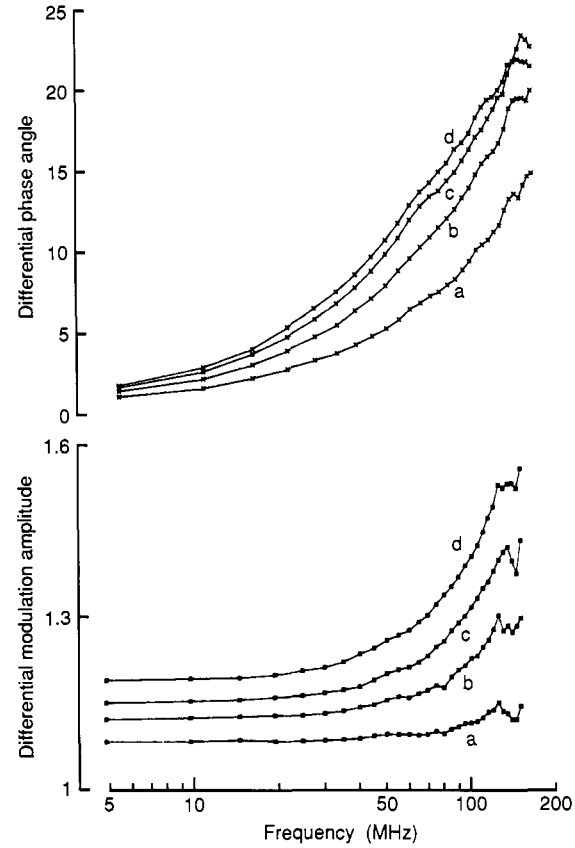

FIGURE 1: Anisotropy decay of 15 μM BCECF in sea water containing 0–40% (w/w) glycerol to give bulk viscosities (cP) of 1.20 (a), 2.07 (b), 2.98 (c), and 3.60 (d). Differential phase angles (top) and modulation amplitude ratios (bottom) are shown for five to eight pairs of measurements (see Methods). Data were fitted to an isotropic rotation model; fitted rotational correlation times are summarized in Table I and Figure 4.

by the replacement of 200 mM NaCl by 200 mM KCl and the addition of 2 μM nigericin (K/H-exchange ionophore). The lack of ground-state lifetime heterogeneity for the four fluorophores was important for the subsequent analysis of fluorophore rotation.

Representative time-resolved anisotropy decay studies are shown in Figure 3. Rotation could not be described by a single correlation time. The most simple time-resolved anisotropy model which fitted the data well was a two-correlation time model (eq 1) containing the three parameters, τ_{1c}, τ_{2c}, and f_1. Fitted rotational parameters for a series of measurements are summarized in Table II. The shorter correlation time, τ_{1c}, accounted for the majority of depolarization for each of the fluorophores (f_1 = 0.72–0.81). In control experiments, the rotational parameters were not dependent upon the size of the laser spot (1–80 μm) or the intracellular pH (6.5–8). There was no systematic difference in the fitted parameters when the laser beam was focused at the center or periphery of the egg.

The shorter rotational correlation time, τ_{1c}, was assumed to represent rotation of unbound fluorophore in fluid-phase

Table II: Fluorophore Properties in Cytosol of Sea Urchin Eggs[a]

	n	τ_f (ns)	n	τ_{1c} (ps)	τ_{2c} (ns)	f_1	η (cP)
BCECF	13	3.24 ± 0.06	15	568 ± 39	22 ± 3	0.72 ± 0.01	2.3 ± 0.2
6CF	7	3.60 ± 0.08	10	311 ± 21	14 ± 4	0.74 ± 0.02	2.1 ± 0.1
calcein	8	3.25 ± 0.10	12	516 ± 44	35 ± 12	0.81 ± 0.02	2.3 ± 0.2
fluorescein	6	3.4 ± 0.2	18	313 ± 15	19 ± 2	0.80 ± 0.01	2.5 ± 0.1

[a] Lifetimes (τ_f), rotational correlation times (τ_{1c} and τ_{2c}), and the fractional anisotropy loss associated with τ_{1c} (f_1) (mean ± SE for n eggs) were measured in sea urchin eggs loaded with the indicated fluorophores as described under Methods. Fluid-phase viscosity relative to water (η) is given as determined by comparison of τ_{1c} with rotational correlation times of sea water–glycerol calibration solutions (see Figure 4).

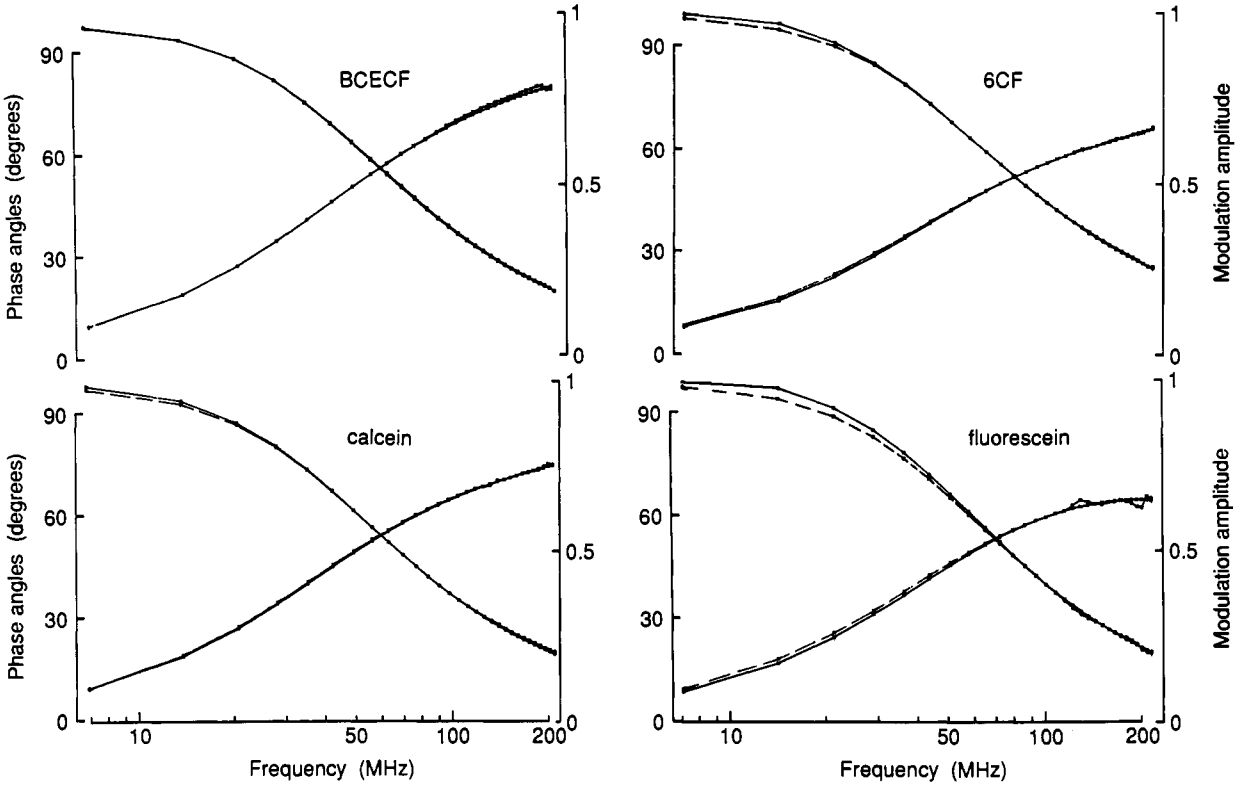

FIGURE 2: Lifetimes of BCECF, 6CF, calcein, and fluorescein in sea urchin eggs measured by phase-modulation fluorometry. Phase angles and modulation ratios were referenced to fluorescein in 0.1 N NaOH as described under Methods. The emission polarizer was positioned at the magic angle to eliminate depolarization effects. Data were fitted to single-component lifetimes as summarized in Table II. Phase angles (increasing ordinate) and modulation amplitudes (decreasing ordinate) are shown along with fitted curves (dashed).

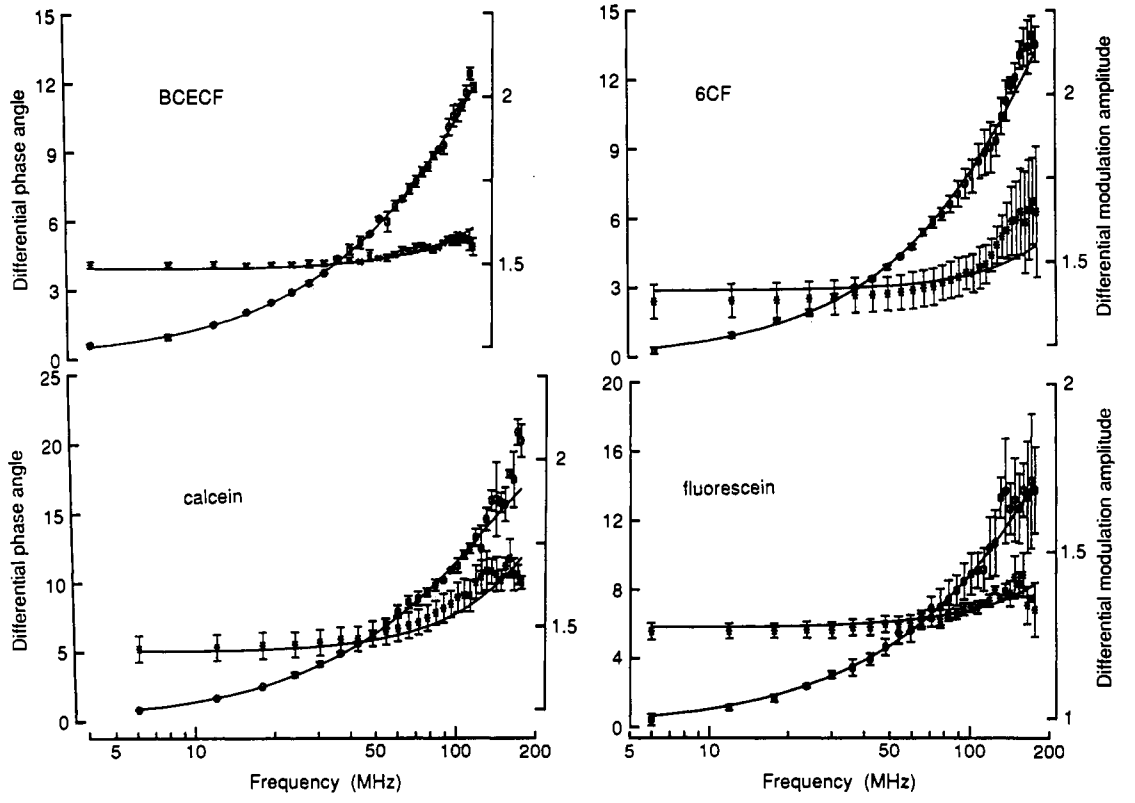

FIGURE 3: Anisotropy decay of fluorophores in sea urchin eggs. Differential phase angles (O) and modulation amplitude ratios (×) (mean ± SD, five to eight measurement pairs) for sea urchin eggs loaded with the indicated fluorophore. Data were fitted to the anisotropy decay model given in eq 1. These data are representative of a large series as summarized in Table II.

cytosol (Fushimi & Verkman, 1991; and see Discussion). The τ_{1c} values in eggs, together with the results for calibration solutions, are given in Figure 4. Interpolated fluid-phase viscosities are projected on the abscissa as shown by the stippled area; viscosities are summarized in Table II. Importantly, the fluid-phase viscosities in sea urchin egg cytosol determined by the four independent fluorophores were in good agreement.

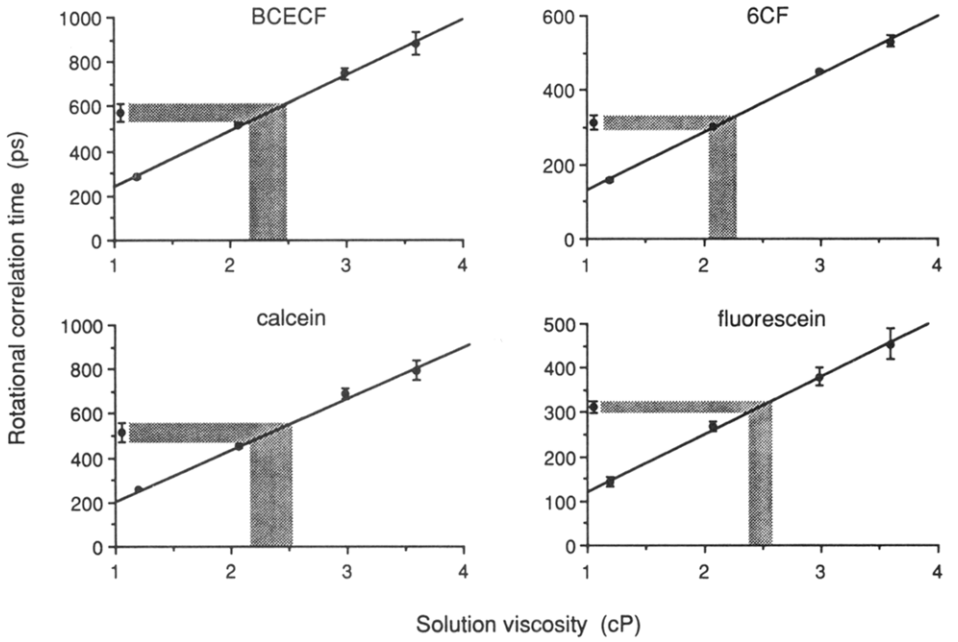

FIGURE 4: Fluid-phase viscosity of sea urchin egg cytosol measured by rotational correlation times of BCECF, 6CF, calcein, and fluorescein. Rotational correlation times (mean ± SE, $n = 5$) for fluorophores in sea water–glycerol are plotted from anisotropy decay experiments as in Figure 1. Correlation times and fitted line slopes are given in Table I. The shorter rotational correlation times (τ_{1c}, mean ± SE) for fluorophores in sea urchin eggs are shown on the ordinate along with the projected interpolated viscosity (stippled area). Results are summarized in Table II.

Discussion

The goal of our study was to measure the picosecond rotational dynamics of four small polar fluorophores in fluid-phase cytosol in sea urchin eggs. The data indicated a single fluorescence lifetime for each fluorophore and an anisotropy decay described by two rotational correlation times. The fluid-phase cytoplasmic viscosities determined from the shorter rotational correlation times and standard calibration curves gave averaged viscosities of 2.3, 2.1, 2.3, and 2.5 cP for the four different probes. The internal consistency of the derived viscosities provides support for the methodology and assumptions made in the data analysis. The absolute fluid-phase viscosity of urchin egg cytosol has significant biological implications as described below.

The fluorophores used for labeling of urchin eggs were selected for their small size, high polarity, noninvasive egg loading and slow leakage, single-exponential fluorescence lifetime, and bright fluorescence when excited at 488 nm. Additionally, the rotation of each fluorophore in aqueous solutions was described well by an isotropic rotational model with a single correlation time. The rotation remained isotropic when solution viscosity was increased from 1 cP to >8 cP by the addition of glycerol or sucrose (high viscosity data not shown); the rotational correlation time increased linearly with viscosity as predicted by the Stokes–Einstein relation.

Two correlation times were required to describe fluorophore rotation in eggs. The shorter correlation time was in the range 300–600 ps and accounted for 72–81% of the depolarization. The longer correlation time, which accounted for 19–28% of the depolarization, was >10 ns and was not well-determined. It was assumed that the shorter correlation time described rotation of unbound fluorophore whereas the longer correlation time described rotation of bound fluorophore. This assumption was validated in a previous study in which rotational correlation times were determined in aqueous buffers containing protein to cause fluorophore binding and glycerol to increase solution viscosity (Fushimi & Verkman, 1991). It was found that even in the presence of >90% fluorophore binding, the shorter rotational correlation time gave an accurate value for viscosity which was independent of the extent of binding. The majority of fluorophores in the cytosolic compartment of urchin eggs are unbound, facilitating the determination of the faster correlation time. We cannot formally exclude the possibility that the shorter correlation time provides an overestimate of fluid-phase viscosity because of extensive binding of fluorophores to small intracellular solutes. We believe that this possibility is unlikely for the following reasons: (a) similar viscosities in urchin egg cytosol were determined by the four different fluorophores; (b) viscosity was remarkably lower in cytosol in mammalian cells, which presumably contains similar small solutes; and (c) we have not been able to identify a solute which would bind these fluorophores extensively, increase rotational correlation time by ~2-fold, and not increase macroscopic viscosity.

The average fluid-phase viscosity in egg cytosol determined by the rotational mobility of the four fluorophores was 2.1–2.5 cP, significantly greater than that of water (1 cP) and mammalian cell cytosol (1.2–1.5 cP). The mean value of 2.3 cP is nevertheless quite low and may be important for diffusion-controlled enzymatic processes in egg cytosol. Sea urchin eggs respond rapidly to fertilization by intracellular alkalinization and calcium elevation and marked morphological changes in the surface membrane (Johnson & Epel, 1976; Wolf et al., 1981; Rakow & Shen, 1990; Chandler, 1991). In addition, fertilization induces brisk exocytosis of cortical granules (Fishkind et al., 1990; Jackson & Modern, 1990). A very high fluid-phase viscosity in the cytosolic compartment could impede this complex cellular response which involves second messengers, soluble proteins, and vesicular trafficking.

Although 2.3 cP is a relatively low viscosity, it is interesting to consider the nature of the cytosolic component(s) responsible for the difference between 2.3 cP and the viscosity of a dilute aqueous solution of ions and small solutes (~1 cP). It was shown previously that the microviscosity sensed by picosecond fluorophore rotations could be different from bulk viscosity (as measured by a Cannon-Fenske viscometer), even for homogeneous solutions (Fushimi & Verkman, 1991). Whereas rotational microviscosity and bulk viscosity were nearly

equivalent for aqueous solutions of small solutes including amino acids, glycerol, and sucrose, rotational microviscosity was increased relatively little when bulk viscosity was increased by addition of larger proteins and dextrans. A rotational microviscosity of 2.3 cP would be measured in an aqueous solution containing 24% (w/w) sucrose or 25% glucose. The osmolalities of these solutions would be 1014 mOsm (sucrose) and 1900 mOsm (glucose), considerably greater than the osmolality of mammalian cells (~300 mOsm) but comparable to the osmolality of sea water (~1070 mOsm; CRC Handbook) and cells of marine invertebrates (1000–1400 mOsm; Yancey et al., 1982). Sea water contains (in millimolar concentrations) 457 Na, 10 K, 10 Ca, 56 Mg, and 536 Cl, whereas urchin egg cytosol contains 40 Na, 220 K, 8 Ca, 2 Mg, and 115 Cl (Jaffe et al., 1978; Steinhart & Epel, 1974; Shen, 1983). From these values, the osmolar gap in urchin egg cytosol is ~700 mOsm. Thus, the 2.3-cP viscosity may be related to the presence of large amounts of small organic osmolytes (e.g., amino acids, polyhydric alcohols, and methylamines; Yancey et al., 1982) in the cytosol of marine animal cells that are not present in mammalian cells grown in 300 mOsm media. It would be interesting to compare the viscosity of kidney papilla cells grown in isotonic and hyperosmolar media. The dominant organic osmolytes in these cells have been identified to be sorbitol, inositol, glycerophosphocholine, and betaine (Garcia-Perez & Burg, 1991).

In summary, picosecond anisotropy decay data for four small fluorophores indicate that fluid-phase viscosity in sea urchin egg cytosol is 2.1–2.5 cP. This low viscosity would facilitate the complex cellular events which accompany egg fertilization. This viscosity is higher than that of water or mammalian cell cytosol which may result from the presence of small organic osmolytes which are required to maintain egg volume in hyperosmolar sea water.

References

Axelrod, D. (1989) *Methods Cell Biol. 30*, 333–352.

Calafut, T. M., Dix, J. A., & Verkman, A. S. (1989) *Biochemistry 28*, 5051–5058.

Cameron, I. L., Fullerton, G. D., & Smith, N. K. R. (1988) *Scanning Microsc. 2*, 275–288.

Chandler, D. E. (1991) *J. Electron Microsc. Tech. 17*, 268–293.

Clegg, J. S. (1984a) *J. Cell Biol. 99*, 167s–171s.

Clegg, J. S. (1984b) *Am. J. Physiol. 246*, R133–R151.

Dix, J. A., & Verkman, A. S. (1990) *Biophys. J. 57*, 231–240.

Fishkind, D. J., Bonder, E. M., & Begg, D. A. (1990) *Dev. Biol. 142*, 439–452.

Fulton, A. B. (1982) *Cell 30*, 345–347.

Fushimi, K., & Verkman, A. S. (1991) *J. Cell Biol. 112*, 719–725.

Fushimi, K., Dix, J. A., & Verkman, A. S. (1990) *Biophys. J. 57*, 241–254.

Garcia-Perez, A., & Burg, M. B. (1991) *J. Membr. Biol. 119*, 1–13.

Gershon, N. D., Porter, K. R., & Trus, B. L. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 5030–5034.

Jackson, R. C., & Modern, P. A. (1990) *J. Membr. Biol. 115*, 83–93.

Jaffe, L. A., & Robinson, K. R. (1978) *Dev. Biol. 62*, 215–228.

Johnson, J. D., & Epel, D. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4474–4478.

Johnson, J. D., & Epel, D. (1976) *Nature 262*, 661–664.

Keith, A. D. (1973) *Science 183*, 666–668.

Keith, A. D., Snipes, W., & Chapman, D. (1977) *Biochemistry 16*, 634–641.

Keith, A. D., Snipes, W., Mehlhorn, R. J., & Gunter, T. (1977a) *Biophys. J. 19*, 205–218.

Lepock, J. R., Cheng, K. H., Campbell, S. D., & Kruuv, J. (1983) *Biophys. J. 44*, 405–412.

Luby-Phelps, K., Taylor, D. L., & Lanni, F. (1986) *J. Cell Biol. 102*, 2015–2022.

Luby-Phelps, K., Lanni, F., & Taylor, D. L. (1988) *Annu. Rev. Biophys. Biophys. Chem. 17*, 369–396.

Mastro, A. M., & Keith, A. D. (1984) *J. Cell Biol. 99*, 180s–187s.

Porter, K. R. (1984) *J. Cell Biol. 99*, 3s–12s.

Rakow, T. L., & Shen, S. S. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 9285–9289.

Salmon, E. D., Saxton, W. M., Leslie, R. J., Karow, M. L., & McIntosh, J. R. (1984) *J. Cell Biol. 99*, 2157–2164.

Shen, S. S. (1983) in *Mechanism and Control of Animal Fertilization* (Hartmann, J. F., Ed.) pp 213–267, Academic Press, New York.

Steinhardt, R. A., & Epel, D. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 1915–1919.

Stossel, T. P. (1982) *Philos. Trans. R. Soc. London 299*, 275–289.

Taylor, D. L., & Fechheimer, M. (1982) *Philos. Trans. R. Soc. London 299*, 185–197.

Verkman, A. S., Armijo, M., & Fushimi, K. (1991) *Biophys. Chem. 40*, 117–125.

Wojcieszyn, J. W., Schlegel, R. A., Wu, E. S., & Jacobson, K. A. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4407–4410.

Wolf, D. E., Kinsey, W., Lennarz, W., & Edidin, M. (1981) *Dev. Biol. 81*, 133–138.

Yancey, P. H., Clark, M. H., Hand, S. C., Bowlus, R. D., & Somero, G. N. (1982) *Science 217*, 1214–1222.