Diffusion in the Aqueous Compartment

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ABSTRACT Measurements of diffusion of molecules in cells can provide information about cytoplasmic viscosity and structure. In a series of studies electron-spin resonance was used to measure the diffusion of a small spin label in the aqueous cytoplasm of mammalian cells. Translational and rotational motion were determined from the same spectra. Based on measurements made in model systems, it was hypothesized that calculations of the apparent viscosity of the cytoplasm from both rotational and translational motion would distinguish between the effects of viscosity and structure on diffusion. The diffusion constant measured in several cell lines averaged 3.3×10^{-6} cm^2/s. It was greater in growing cells and in cells treated with cytochalasin B than in quiescent cells. The viscosity of the cytoplasm calculated from the translational diffusion constant or the rotational correlation time was 2.0–3.0 centipoise, about two to three times that of the spin label in water. Therefore, over the dimensions measured by the technique, 50–100 Å, solvent viscosity appears to be the major determinant of particle movement in cells under physiologic conditions. However, when cells were subjected to hypertonic conditions, the translational motion of the spin label decreased threefold, whereas the rotational motion changed by <20%. These data suggest that the decrease in cell volume under hypertonic conditions is accompanied by an increase in cytoplasmic barriers and a decrease in the space between existing cytoplasmic components without a significant increase in viscosity in the aqueous phase. In addition, a comparison of reported diffusion values of a variety of molecules in water and in cells indicates that cytoplasmic structure plays an important role in the diffusion of proteins such as bovine serum albumin.

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Commencement of the study of the structure of cell cytoplasm was nearly coincident with the discovery of living, moving cells. Descriptions of the cytoplasm over the years have ranged from "gelatinous mass" to "spongelike network" (for a review of the early history of cell biology see reference 1). Descriptions depended on the cell type being studied as well as on the sophistication of the available tools. The picture has changed as the techniques for viewing the architecture have improved. The cytoplasm, the structureless ground substance between the organelles, has been found not to be structureless at all. Components are numerous and complex. Various fibrous bundles such as actin filaments, microtubules, and intermediate filaments actually make the cytoplasm appear to be crowded (2). Superimposed on, or part of, these fibers is a microtrabecular lattice (3, 4) viewed as a network of interconnected fibers serving as an organizing framework for many enzymes and other molecules in the cytoplasm.

The study of the architecture of cells has been paralleled by studies of metabolism and functional responses in the cell. Metabolic pathways, responses to hormones and growth factors, growth, and cell division all must be accommodated by an organized but flexible system. Thus, the complex and ordered cytoplasm revealed by transmission and high-voltage electron microscopy needs to be considered in serious attempts to describe metabolic responses and functions. Models of cytoplasm that attempt to reconcile information about structure and function must also explain how things move in the cytoplasm.

Movement, particularly cytoplasmic streaming, was one of the earliest functions to be observed. Simple descriptions of streaming and movement of organelles were the forerunners of various studies of movement in the cytoplasm. Results of such studies may provide information about diffusion, the apparent viscosity of the cytoplasm, and the contribution of structure to movement.

One common approach to the measurement of movement

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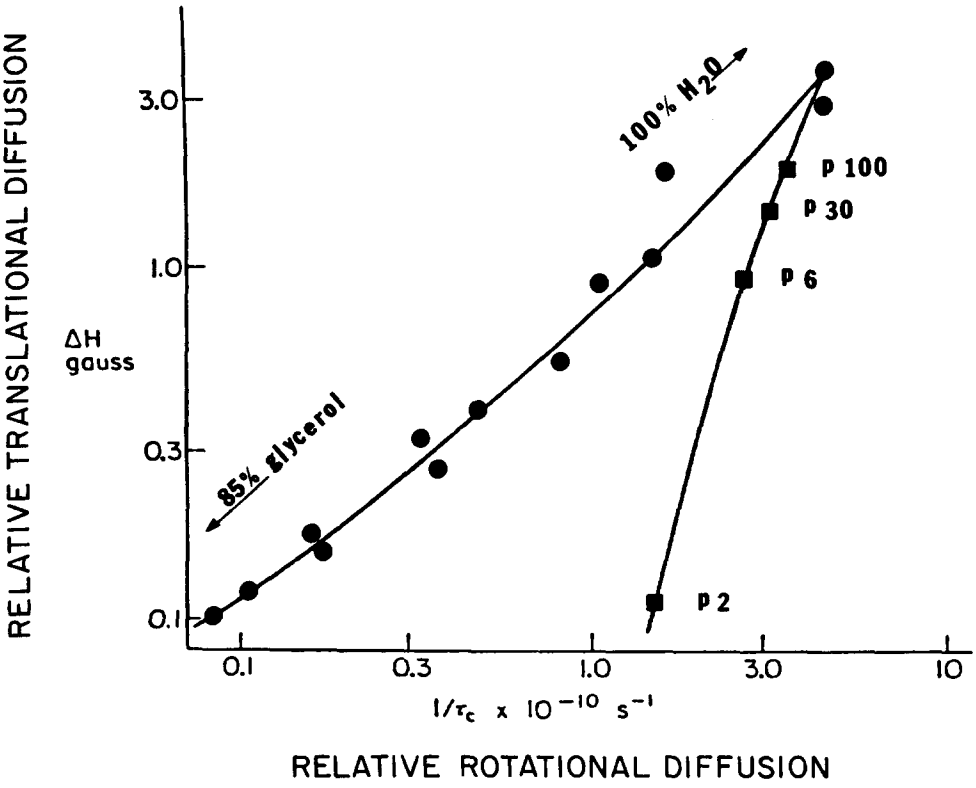

FIGURE 1 Comparison of the effects of barriers vs. solvent viscosity on the translational and rotational diffusion of a spin label. The spin label tempone was dissolved in mixtures of water and concentrations of glycerol increasing to 85% (vol/vol) (circles), or it was dissolved in water and trapped in polyacrylamide beads (Bio-Rad Laboratories, Richmond, CA, Bio-Gel P, 100 mesh) of decreasing pore size: P100, P30, P6, P2 (squares). In the glycerol and water mixtures the translational and diffusional motion were slowed about equally as the viscosity of the solution increased. In contrast, when the barriers in the beads inhibited the translational diffusion as much as 30-fold (P100), compared to diffusion in bulk water, the rotational correlation time changed less than threefold (adapted from reference 17).

in the cytoplasm has been to follow large probes phagocytized by cells or introduced into them by microinjection. For example, Crick and Hughes (5) examined the cytoplasm of chicken fibroblasts that had phagocytized iron filings. Several groups (6–10) have used radiolabeled molecules of a variety of sizes to measure diffusion in large cells, such as oocytes, muscle fibers, and axons. More recently the techniques of microinjection and fluorescence recovery after photobleaching (FRP)[1] have been combined to estimate the translational diffusion of proteins such as bovine serum albumin (BSA), immunoglobulin (IgG), and actin in cell cytoplasm (11, 12). All of these techniques give a measure of translational diffusion at the "macro" level, i.e., micrometers to millimeters. One cannot tell by these methods whether limitations on diffusion are due to local cytoplasmic viscosity or to barriers in the cytoplasm. The former could be due to a protein-rich water phase and the latter to a cytoplasmic network of fibers.

On the other hand, electron-spin resonance (ESR) techniques have been used to measure the rotational diffusion of small probes (i.e., <1,000 M_r) in cell cytoplasm (13, 13a). The rotational correlation time (τ_c) of the spin label is closely related to the cytoplasmic viscosity within a few angstroms of the spin probe. In addition, the translational diffusion of these molecules can also be calculated from the same ESR spectra. It had been shown (13a–16) with a model system of spin label in polyacrylamide beads that the presence of a barrier can affect translational motion more than rotational motion; the apparent viscosity (η) calculated from the translational diffusion constant, D, is greater than that calculated from τ_c (Fig. 1).

In this study we used an ESR technique to measure the translational diffusion constant of a small, water-soluble spin label molecule in mammalian cells. In addition, we compared the rotational with the translational diffusion to try to detect the effect of barriers over distances of ~50–100 Å. These dimensions are based on the average distribution of the molecules at the spin label concentrations used. By comparing these data with those presented in the literature for the D of other molecules in aqueous solutions and in cells, we also have estimated the relative roles of solvent viscosity and cytoplasmic barriers in controlling movement in the cytoplasm.

MATERIALS AND METHODS

Cells: Swiss 3T3 cells and their SV40-transformed 3T3 counterparts were used for most of the experiments. Two clones of BALB/c 3T3 and SV40-transformed BALB/c 3T3, a clone of methylcholanthrene (MCA)-transformed BALB/c 3T3, cells and baby hamster kidney (BHK) cells were also tested. Stock cultures were maintained at low cell density in Dulbecco's modified Eagle's medium, 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (10 mg/ml). Cells were grown on 100-mm plastic dishes and kept at 37°C in a humidified incubator in 5% CO_2 and 95% air. To obtain quiescent cultures, 3T3 cells were grown to confluency in medium with 5% serum. These cultures showed little evidence of cell division when examined microscopically. 3T3 cells in the G_1 phase of the cell cycle were obtained from quiescent cultures by changing the medium to one containing 10% serum. These cells were tested 12–16 h after medium change. Some cells were used while in exponential growth phase. These are called "growing cells." SV40-transformed cells were plated to obtain cultures of the same density as the untransformed cells on the day of the experiment. Transformed cell cultures always contained growing cells.

Spin Labeling Procedure: The technique for labeling of cells with spin label has been described (15). In brief, cells were gently scraped from plates, centrifuged (300 *g*, 6 min) to remove the culture medium, and resuspended in fresh medium to a concentration of between 3 and 10 × 10^6 cells/

[1] *Abbreviations used in this paper:* BHK, baby hamster kidney; BSA, bovine serum albumin; D, diffusion constant; ESR, electron-spin resonance; FRP, fluorescence recovery after photobleaching; MCA, methylcholanthrene; PCAOL, 2,2,5,5-tetramethyl-3-methanolpyrroline-*N*-oxyl; tempone, 2,2,5,5-tetramethylpiperidine-*N*-oxyl; η, viscosity; τ_c, rotational correlation time.

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ml. One-milliliter aliquots were transferred to 12 × 75-mm glass tubes and centrifuged, and the supernatant was discarded. Approximately 40 μl of medium remained with the cells. The cell pellet was gently vortexed and the spin label, 2,2,5,5-tetramethyl-3-methanolpyrroline-*N*-oxyl (PCAOL), and H_2O were added. $NiCl_2$ was then added. Where indicated, the spin label deuterated 2,2,6,6-tetramethylpiperidine-*N*-oxyl (tempone) was used. The cell suspension was drawn into a 100-μl capillary glass pipette (Corning Glass Works, Science Products Div., Corning, NY), and the end of the tube opposite the cell suspension was sealed with a flame. The tube was cooled and centrifuged (No. 5 on an International Equipment Co. [Needham Heights, MA] clinical centrifuge) for 1 min to pellet the cells. The supernatant solution was withdrawn with a capillary pipette, and the sample was placed in the chamber of the ESR spectrometer (JEOL, USA Electron Optics, Peabody, MA, model JES-ME) for analysis. One sample at a time was processed. $NiCl_2$ was used to quench extracellular spin label signal. This treatment was not toxic to the cells (15). Measurements were taken at four concentrations of spin label between 3 and 40 mM. Dilutions of spin label were made into the cell suspension solution. The actual values were calculated from the volume of each sample. Based on the average distribution of spin label over this range of concentration, spin label interactions can be measured within distances of ~50–100 Å.

The change in midfield line width (ΔH) of the ESR signal was plotted as a function of spin label concentration. The D for PCAOL was determined from the slope of this line using the equation $D = (K\Delta H)/M$.

ΔH is the line width component contributed by the spin label concentration, and is calculated from $\Delta H_m - \Delta H_{min}$, where ΔH_m is the line width at a given molar concentration of spin label and ΔH_{min} is the minimum line width of a very dilute spin label concentration; M is the molarity of the spin label and K is a constant of proportionality relating spin label collision frequency with molar concentration (16). Measurements were made in water and in cells. τ_c was calculated from the same spectra at the lowest spin label concentration, 1 mM in water and 3–4 mM in cells.

Reagents: The spin label molecules were synthesized in our laboratory (17). Other compounds were reagent grade.

RESULTS

To measure changes in line broadening, PCAOL was added to cells at several concentrations. ESR signals were recorded and line broadening was measured at each concentration. τ_c was calculated from the same spectra at the lowest spin label concentration.

The change in line broadening (ΔH) with increasing concentration of spin label was less in 3T3 cells than in water (Fig. 2). This D for PCAOL was about twofold lower in the cells compared with its value in water. An average D of $3.9 \pm 0.2 \times 10^{-6}$ cm²/s was calculated for PCAOL in G_1 cells. For the quiescent cells this value was $3.4 \pm 0.4 \times 10^{-6}$ cm²/s. The change in ΔH represents movement over ~50–100 Å at the concentrations of PCAOL tested. τ_c was calculated to be about 0.9×10^{-10} s, which was ~2.5 times higher in cells than in water, corresponding to a 2.5-fold decrease in the rotational diffusion constant.

The same experiment was repeated with several different lines of cells in quiescent or growing states. Cells transformed with SV40 virus or MCA were also tested. The average value of D for PCAOL in all of the cells tested was calculated to be 3.3×10^{-6} cm²/s (Table I). In general, this value did not vary greatly among the different clones nor with various changes in growth parameters. More specifically, however, when quiescent (G_0 phase) Swiss 3T3 cells were compared with Swiss 3T3 cells in G_1 phase in the same experiment, there was a small but reproducible difference in D of PCAOL between the two cultures (Fig. 2). We saw this difference in five separate experiments. D of PCAOL in the G_1 cells was always greater than in quiescent cells.

The SV40-transformed Swiss 3T3 cells showed little difference in diffusion parameters on average compared with their nontransformed counterparts, although in paired experiments differences in D were seen. The change in D between the normal 3T3 and their transformed counterparts varied, depending on the particular cell lines being compared (15).

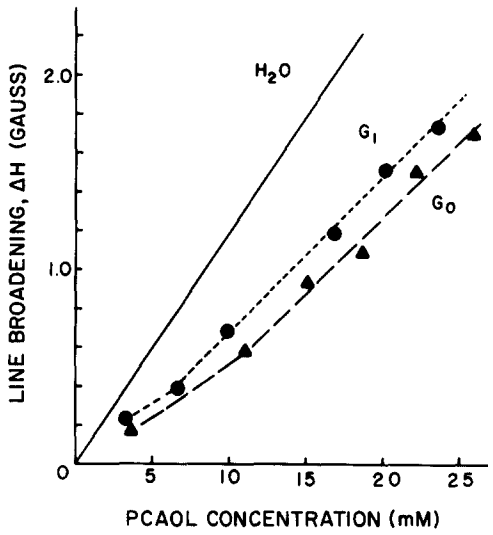

FIGURE 2 Diffusion of PCAOL in 3T3 cells. Quiescent or serum-stimulated cultures of 3T3 cells were labeled with PCAOL at the concentrations shown. The results of one experiment are shown. This experiment was repeated five times with similar results. The line broadening (ΔH) of PCAOL in water is given as a reference. (▲) PCAOL in quiescent G_0 cells. $D = 3.4 \times 10^{-6}$ cm²/s; $\tau_c = 0.97 \times 10^{-10}$ s. (●) PCAOL in serum-stimulated G_1 cells. $D = 3.9 \times 10^{-6}$ cm²/s; $\tau_c = 0.90 \times 10^{-10}$ s. (—) PCAOL in water. $D = 6.4\ 10^{-6}$ cm²/s; $\tau_c = 0.36 \times 10^{-10}$ s.

We also labeled Swiss 3T3 cells grown on solid plastic beads (Biosilon, Nunc, Roskilde, Denmark). These nonporous beads do not sequester the free spin label, and the cells can be labeled while attached to the bead. We found the D for PCAOL to be approximately the same as that for the same cells cultured in plastic dishes and released by scraping (Table I).

BALB/c 3T3 cells, whether quiescent, growing, or transformed by MCA or by an SV40 virus, all showed approximately the same values of D for PCAOL (Table I).

Several experiments were performed to determine whether the movement of spin label could be varied by changing the cytoplasmic structure. In different experiments, cultures of quiescent 3T3 cells were treated with trypsin (0.25%), colcemid (1×10^{-6} M), or vinblastin (1×10^{-6} M). There was no consistent change in ΔH with these three treatments (data not shown). However, treatment of cells with cytochalasin B caused an increase of ~20% in the translational and rotational diffusion of PCAOL compared with untreated cultures (Fig. 3). Similar results were seen when SV40 3T3 and BHK cells were treated in the same way. There was a consistent increase in the diffusion of the spin label in the cytochalasin B-treated cells that was not seen in the untreated control cells in the same experiment.

In general, the apparent viscosities of the aqueous cytoplasm calculated from D and from τ_c were similar (Table II, *A*). This correlation between η calculated from D or from τ_c was seen when the spin label was dissolved in a series of aqueous sucrose solutions of increasing viscosity. In contrast, when spin label was trapped inside polyacrylamide beads (Fig. 1 and Table II, *A*), the translational motion was slower than the rotational motion and the apparent viscosities reflected this effect.

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TABLE I

Diffusion of PCAOL in Several Mammalian Cell Lines

Cell line	Growth state	N*	Diffusion constant	Viscosity‡
			$cm^2/s \times 10^6$	*centipoise*
Swiss 3T3	Quiescent	13	3.4 ± 0.4	2.0
Swiss 3T3	Serum stimulated	5	3.4 ± 0.5	2.0
Swiss SV40 3T3	Growing	7	3.2 ± 0.4	2.1
Swiss 3T3 (beads)	Growing	4	3.8 ± 1.9	1.8
BALB/c 3T3§	Quiescent	3	3.4 ± 0.2	2.0
BALB/c SV40	Growing	3	3.2 ± 0.4	2.1
BALB/c MCA 3T3§	Growing	1	3.5 ± 0.3	1.9
BALB/c 3T3‖	Quiescent	1	3.3 ± 0.3	2.1
BALB/c SV40 3T3‖	Growing	1	2.2 ± 1.3	3.1
BHK	Growing	5	3.6 ± 0.3	1.8

PCAOL was added to cells and D was calculated from the line broadening as described in Materials and Methods and in the legend to Fig. 2. At least four concentrations of spin labels were used in each experiment. The value shown is the average D ± SD calculated for the number of experiments indicated. The τ_c for all the cells was $\sim 0.90 \times 10^{-10}$ s.

* Number of experiments.

‡ Calculated from Stokes-Einstein equation: $D = kT/(6\pi r\eta \cdot f/f_0)$.

§ Clone of BALB/c; gift from Dr. R. Scott, University of Minnesota.

‖ Clone of BALB/c; gift from Dr. P. Beall, Baylor University.

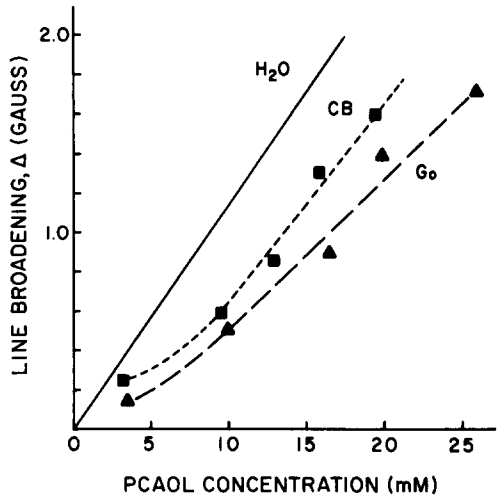

FIGURE 3 Diffusion of PCAOL in cells treated with cytochalasin B. Quiescent cultures of Swiss 3T3 cells were treated with cytochalasin B (5 μg/ml in 0.4% DMSO) for 5 h at 37°C before being prepared for spin labeling. DMSO alone had no effect of ΔH. (▲) Untreated. (■) Cytochalasin B. (—) Water.

However, a differential change in translational and rotational motion was seen when cells were subjected to hypertonic salt concentrations (Fig. 4 and Table II, *B*). As the volume of cells in the highest salt solution decreased by a factor of about 2, the translational diffusion constant decreased fourfold. The finding that D decreased more than the volume decreased is correlated with the fact that the aqueous volume changed while the volume of the solid matter of the cell remained constant. In contrast to the change in D, τ_c decreased by only ~20% compared with that of cells under isotonic conditions. When the apparent viscosities calculated from D and from τ_c were plotted as a function of cell volume, this difference was clear (Fig. 4). The apparent viscosity of cytoplasm calculated from translational diffusion increased fourfold when the volume decreased, whereas the apparent viscosity calculated from the rotational motion changed little. If, as the cell volume decreases, the water-rich compartments also become dehydrated, one would expect an increased concentration in the possible components of those compartments such as proteins, ions, and other small metabolites. This increase would be expected to change the viscosity dramatically (see reference 19 for a discussion). The fact that the viscosity based on τ_c changed little with the change in volume suggests that the longer range translational motion represented by D was impeded by barriers rather than by an increase in the fluid viscosity of the cytoplasm as the cell volume decreased.

TABLE II

Apparent Viscosities of Solutions Determined from ESR Spectra

Solute	$\eta(\tau_c)$	$\eta(D)$	$\eta D/\eta\tau_c$
A			
Water	1.1	1.1	1.0
10% Sucrose	1.3	1.5	1.2
40% Sucrose	4.1	3.9	1.0
Water in P300 beads	1.1	2.7	2.5
Cells, 3T3 (isosmotic)	2.6	2.2	0.8
B			
Cells, BHK, 300 mosmol	3.4	2.9	0.8
Cells, BHK, 550 mosmol	4.0	10.0	2.5

The viscosities calculated from the rotational correlation time τ_c and from the translational diffusion coefficient D were compared. Viscosity, η, is given in centipoise. (*A*) PCAOL was used as the spin label. (*B*) Deuterated tempone was used, and the values were normalized to those of PCAOL.

For comparison, we also measured D for PCAOL in aqueous solutions of 10% protein. D decreased by 7–20% compared with that in water alone (Table III).

DISCUSSION

ESR was used to measure the diffusion of a small molecule in the aqueous cytoplasm of several mammalian cell lines. The D for PCAOL, the spin label probe, was about 3.3×10^{-6} cm^2/s in the cells at room temperature. This diffusion constant is about one-half that determined for PCAOL in bulk water measured by ESR. One can interpret the decrease in diffusion constant as an increase in the viscosity of cell cytoplasm compared with water. From a consideration of the diffusion of PCAOL in aqueous sucrose solutions (14), the viscosity of cell cytoplasm corresponds roughly to that of a solution of 15% sucrose, ~2.0 centipoise. Calculation of viscosity from measurements of τ_c of PCAOL in the cells supports this interpretation (Table II).

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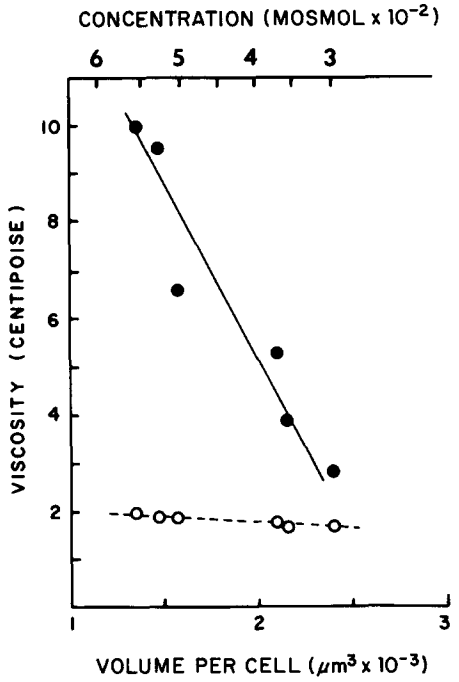

FIGURE 4 Apparent viscosities of cells under hypertonic conditions. BHK cells were labeled with deuterated tempone. $NiCl_2$ (75 mM) and increasing concentrations of KCl were added to increase the hypertonicity of the medium. The osmotic pressure ranged from 290 to 550 mosmol. Spin label was used at 10 mM to measure D and at 1 mM to measure τ_c. The τ_c for deuterated tempone is smaller than that for PCAOL, but the values of each are proportional in cells compared with water. At 290 mosmol, $D = 2.3 \times 10^{-6}$ cm^2/s; $\tau_c = 0.57 \times 10^{-10}$ s. At 550 mosmols, $D = 6.7 \times 10^{-6}$ cm^2/s; $\tau_c = 0.68 \times 10^{-10}$ s. Volumes of cells were calculated from the data of Raaphorst and Kruv (18). Viscosities were calculated from the Stokes-Einstein equation [$D = kT/(6\pi r\eta \cdot f/f_0)$]. (●) η calculated from D. (○) η calculated from τ_c.

TABLE III

Diffusion of PCAOL in Aqueous Solutions of Protein

Protein	$M_r \times 10^{-3}$	Diffusion constant	Viscosity
		$(cm^2/s) \times 10^{-6}$	*centipoise*
None		6.4 ± 0	1.1
Cytochrome C	12.4	6.0 ± 0.2	1.1
RNAse	13.7	5.6 ± 0.6	1.2
Lysozyme	14.4	5.6 ± 0.4	1.2
Bovine serum albumin	68.0	5.4 ± 0.5	1.3

PCAOL was added to solutions of proteins (10% wt/vol) in water. PCAOL was used at 14, 20, 27, and 33 mM final concentration. ESR signals were taken and diffusion was calculated from the ΔH values at each concentration of spin label. The average D ± SD is given.

However, the cytoplasm is not a homogenous sucrose solution. It contains organelles, proteins, ions, etc., which can make it appear to be very crowded (2). There is no evidence to suggest that the signal of PCAOL is due to its adhering to these structures. For example, if one assumes that the protein content of cytoplasm is similar to that of serum, ~7% (see reference 11), one can ask whether that protein accounts for the apparent increase in cytoplasmic viscosity. We found this not to be the case (Table III). In aqueous solutions of 10% protein, the D of PCAOL was changed by only 7–20% compared with that in water. Furthermore, in previous studies we found that PCAOL did not bind to DNA or to synthetic polymers (13a–16).

There is a great deal of debate as to the organization of the structures in the cytoplasm and to what extent water is present in that structure in a free or bound state. We have also considered whether PCAOL preferentially localized in the free or bound water. In a model system using phospholipid multilayers we determined that the water-soluble spin label, tempone, partitioned nonpreferentially between bound and free water (20). The spin label exchanged freely between the two kinds of water, but because the exchange rate is longer than the spin lattice relaxation time, two distinct signals could be seen, one from spin label in bound water and one in free. In a system containing both, the spectrum is an arithmetic mean of the two but is dominated by the signal from the free water because of the more narrow line widths. In the mammalian cell the shape and intensity of the signal indicate that the spin label is predominantly in free water. Furthermore, based on the intensity of the signal for a given spin label concentration, under both isotonic and hypertonic conditions, a calculation of the volume of this free water domain suggests that it is ~90% of the water in the cell.

Another possible explanation for the lowered D of PCAOL in cells is that structural barriers limit movement. Evidence for such barriers is plentiful from electron microscope studies. In a model system, we used the ESR technique to detect barriers and to distinguish between the effects of barriers and increased viscosity. When spin label was added to beads of various pore sizes, the translational movement of the spin label was slowed considerably as the pore spaces became smaller (Fig. 1). The rotational motion was largely unaffected (13a). Therefore, if barriers alone limited the movement of PCAOL in cytoplasm the rotational parameter τ_c, should be relatively unchanged compared with that of water, whereas D should decrease. This was not the case. Both D and τ_c changed by about the same amount. The viscosity calculated from either measurement is ~2–3 centipoise. Therefore, there is no evidence to support the idea that PCAOL movement is blocked by barriers in cells under normal physiologic conditions. Over the dimensions detectable with 3–40 mM PCAOL, ~50–100 Å, solvent viscosity appears to be the most important determinant of movement. On the other hand, changes in the diffusion of PCAOL caused by treatment with cytochalasin B indicated that microstructure plays some role. In this case, treatment with cytochalasin B causes an apparent decrease in solvent viscosity, perhaps through depolymerization of actin.

The strongest evidence that cytoplasmic structural components can play a role in diffusion was seen when cells were subjected to hypertonic conditions (Fig. 4). As cell volume decreased, the translational diffusion constant decreased, corresponding to an apparent increase in the viscosity of the cytoplasm. However, the rotational diffusion changed much less in the same cells. This differential effect on D vs. τ_c is similar to that seen when spin label is sequestered in porous beads and is most likely due to the presence of physical barriers to diffusion. The spaces between cytoplasmic structural barriers would become smaller as the cells decreased in volume. Porter and colleagues (4, 21) have reported that the lattice spacing of the cytoplasmic matrix, as seen by high-voltage electron microscopy, decreases under hypertonic conditions. Theoretical calculations of the intermicrotrabecular spacing under hypertonic conditions suggest that these would

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not alone account for the barriers. For example, if the lattice spacings decrease in size in relation to the decrease in the aqueous compartment, ~75% for a 50% decrease in cell volume (22), the interlattice spaces would still exceed 500 Å, too large to be detected by this concentration of spin label. However, as the aqueous volume decreases the concentration of protein and other structural elements increases, leading to a greater number of associations and more compact configurations (2). The data reported here suggest that under hypertonic conditions barriers appear with spacings in the range of 100 Å. Schobert and Marsh (23) have also reported decreases in cell volume, apparent increases in cytoplasmic viscosity, and an increase in cytoplasmic density when algae are exposed to hypertonic conditions. Mansell and Clegg (22), using conventional electron microscopy, found a similar compression of the cytoplasmic ground substance in L cell fibroblasts.

A better indication of the role of barriers in cytoplasmic diffusion in cells under physiologic conditions can be drawn from a comparison of the movement of PCAOL with those of other molecules, both in aqueous solutions and in cells (see reference 24) (Table IV). The diffusion of molecules of various sizes, ranging from sorbitol (182 M_r) to apoferritin (467,000 M_r), has been investigated in various cells by a variety of techniques. The techniques all allow measurement of translational diffusion. The distance over which diffusion was measured has ranged from micrometers for low temperature autoradiography and for FRP to millimeters for dye-diffusion methods.

The D of the molecules in aqueous solutions varies approximately inversely with the size of the molecules as predicted on the basis of diffusion theory (Table IV). In spite of the variety of experimental methods and cell systems, this general relationship holds true for the small molecules and the dextran spheres in the cytoplasm. D in the cytoplasm is about two to five times smaller than that in water.

The same relationship between molecular radius and diffusion constant does not hold true for the larger proteins, such as actin, ovalbumin, BSA, IgG, vinculin, α-actinin, and apoferritin. This deviation is not surprising for actin because the measurements were taken under conditions under which actin was largely immobile in the cells (12). D is about 160-fold less than in water. BSA and IgG also had much slower diffusion constants in the fibroblasts, although they were reported to be >90% mobile. D for BSA is roughly 100-fold less than in water, whereas that for IgG is about 50-fold less. In these cases, the mobile fraction of molecules was determined from the fractional recovery of fluorescence after photobleaching.

Vinculin and α-actinin were estimated by FRP measurements to be ~75–80% mobile in the interfibrillary domain of the cytoplasm. They were less free than BSA and IgG and also had a lower D. Ovalbumin and apoferritin, molecules of 45,000 and 467,000 M_r, respectively, had Ds similar to that of BSA and IgG in fibroblasts.

Using the Stokes-Einstein equation [$D = kT/(6\pi r\eta \cdot f/f_0)$], one can calculate the apparent viscosity (η) of each molecule in water and in cytoplasm. A plot of η vs. radius shows that all the molecules in water experience a viscosity of ~1 centipoise (Fig. 5). The viscosity of cytoplasm appears to be between 2 and 6 centipoise for the small molecules and dextrans (Fig. 5). However, the viscosity of mammalian cell cytoplasm would appear to be ~60–80 centipoise for the larger molecules BSA and IgG. The other large proteins appear to be in solutions of similarly high viscosity, ranging from 27

TABLE IV

Diffusion Constants of Various Molecules in Cytoplasm

Compound	M_r	Radius	Diffusion constant		D_w/D_c	Viscosity		Cells	References
			D_w (H_2O)	D_c (Cells)		η_w (H_2O)	η_c (Cells)		
		Å	(cm^2/s) × 10^{-7}			centipoise			
PCAOL	170	3.2	64	33	1.9	1.1	2.1	Mouse fibroblasts	13a
Sorbitol	182	2.5*	94	50	1.9	0.9	1.7	Barnacle muscle fibers	9
Methylene blue	320	3.7*	40	15	2.6	1.5	4.0	Squid axons	10
Sucrose	324	4.4	52‡	20	2.6	1.0	2.5	Frog oocytes	6
Eosin	648	6.0*	40	8.0	5.0	0.9	4.5	Squid axons	10
Dextran	3,600	12.0	18	3.5	5.0	1.0	5.2	Frog oocytes	8
Inulin	5,500	13.0§	15	3.0	5.0	1.1	5.6	Frog oocytes	7
Dextran	10,000	23.3	9.2	2.5	3.7	1.0	3.7	Frog oocytes	8
Dextran	24,000	35.5	6.3	1.5	4.2	1.0	4.1	Frog oocytes	8
Actin	43,000	23.2§	5.3‖	0.03	167.0	1.1	179.0	Chicken gizzard fibroblasts	12
Ovalbumin	45,000	23.8§	8.9	0.34	26.2	1.0	26.7	Mouse "macrophagelike" cells	25
				3.8	2.3	1.0	2.4	Amebae	
BSA	68,000	36.0	6.9	0.10	71.0 >	0.9	61.0	Human fibroblasts	11
				0.06	111.0 >		101.0	Chicken gizzard fibroblasts	12
				4.0	1.7	1.0	1.5	Amebae	25
IgG	153,000	35.0§	4.0‖	0.09	43.5 >	1.1	50.0	Human fibroblasts	11
				0.06	66.7 >		75.0	Chicken gizzard fibroblasts	12
Vinculin	130,000	33.3§	—	0.03	—	—	217.0	Chicken gizzard fibroblasts	26
α-Actinin	200,000	78.2	2.7	0.03	90.0	1.0	93.0	Chicken gizzard fibroblasts	26, 27
Apoferritin	467,000	51.7§	3.6‖	0.10	36.0	1.2	42.1	Human fibroblasts	¶

* Calculated from structure.
‡ CRC Handbook of Chemistry and Physics.
§ Calculated for a sphere of equivalent volume.
‖ CRC Handbook of Biochemistry and Molecular Biology.
¶ Dr. K. Jacobson, University of North Carolina, personal communication.

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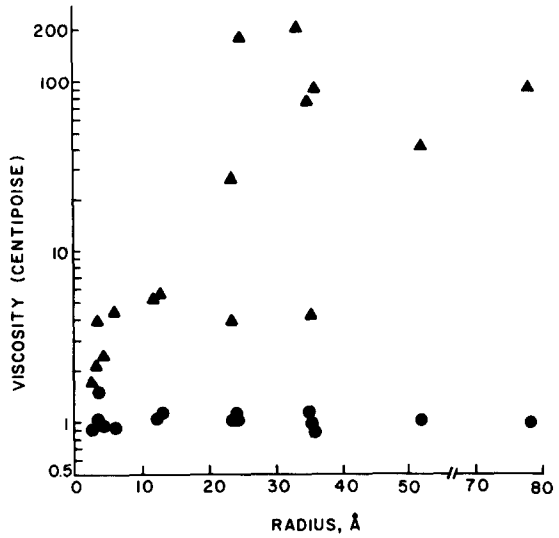

FIGURE 5 Comparison of D in water and cytoplasm for molecules of various sizes (see Table IV). The data for ovalbumin and BSA in amebae are not plotted. (●) Water. (▲) Cytoplasm.

centipoise for ovalbumin to >200 centipoise for vinculin. The apparent viscosity for the cytoplasm with respect to actin is nearly 200 centipoise, as might be expected considering its association properties (12). Vinculin may also be associated with other cytoplasmic elements. In general, however, the movements of proteins are slowed to a much greater extent than would be expected on the basis of cytoplasmic viscosity alone.

Two explanations for this effect are that the proteins encounter barriers in the cytoplasm or that they bind with low affinity to cytoplasmic structures. BSA is particularly known for its nonspecific binding. In fact, BSA diffuses at the same rate as apoferritin, another globular protein of a much greater molecular weight. If the binding were reversible with rate constants of the same order as the rate of diffusion, the effect of continual binding and release would be seen as an apparent decrease in the diffusion constant. This "chromatographic" effect was described by Horowitz et al. (28) for the movement of cysteamine phosphate in oocyte cytoplasm. Gershon et al. (29) also concluded on the basis of theoretical considerations of cytomatrix spacing, that BSA injected into cells binds to cytoplasmic proteins. In either case, barriers or binding or a combination would lead to the mistaken conclusion that relatively large proteins such as BSA, IgG, apoferritin, etc., were in a more viscous solution.

The largest dextran molecule tested has about the same radius as BSA, yet it has a much higher D than BSA (Table IV). The same is true for intermediate-sized dextran (radius 28.3) and ovalbumin (radius 23.8). If barriers were responsible for the low D of BSA and ovalbumin in cells, then it would be expected that dextran would encounter the same barriers. However, there are differences between the molecules and the methods used to measure D. Dextran is a carbohydrate polymer chosen purposely for its lack of binding (8). Albumins are proteins known to bind specifically and nonspecifically to other molecules. Furthermore, the movement of dextran was measured in frog oocytes by low-temperature autoradiography, whereas the movements of BSA and ovalbumin were measured in fibroblasts by FRP. It should also be noted that when the diffusion of BSA was measured by FRP in amebae (25) it moved much more rapidly than when measured by the same technique in human fibroblasts (11, 12) (Table IV). The same effect was also seen when the diffusion of ovalbumin was measured in amebae and in a macrophage cell line by FRP by the same investigators (25). In amebae the diffusion of ovalbumin was more than 10 times faster than in the mammalian cell line. Thus, there may well be major differences in the cytoplasmic structure of amebae that limit direct comparisons with mammalian cells.

Knowing the rotational diffusion constants for larger proteins in cells would be helpful in distinguishing between changes in viscosity and the presence of barriers, and the development of a spin label technique that would allow the use of concentrations of label ranging from 1×10^{-8} M to 1×10^{-7} M in cells would make it possible to estimate barriers spaced ~100–1,000 nm apart. This is the estimated distance between microtrabeculae in cells (3, 4), a possible network of barriers to diffusion in the cell cytoplasm.

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