

# How Crowded Is the Cytoplasm?

## Minireviews

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All models of the cytoplasm must lie within the constraints set by physical chemistry. Those constraints are unexpectedly narrow. I will use known physical chemistry and recent experimental results to examine and refine the available models of the cytoplasm. Surprisingly, a good place to begin is by considering the properties of protein crystals.

In a crystal of protein, approximately 40% of the weight is water or solvent. The proteins within the crystal are loosely packed, making only a few contacts with each other, and the spaces between them are occupied by solvent. The solvent in those spaces is present in two forms; about half of it is water of hydration, strongly adsorbed to the protein. The remaining water resembles bulk water in its physical and chemical properties. Few crystals contain as little as 20% solvent; some crystals contain more than 90% solvent by weight (Cohen et al., *CSHS* 37, 287-297, 1972). Protein crystals can therefore form with less than 10% protein by weight. That crystals can be a useful model for considering physical chemistry in the cytoplasm becomes clear when the protein content of many cells is considered. Muscle cells contain approximately 23% protein by weight; red blood cells contain about 35% protein by weight; and in general actively growing cells contain between 17% and 26% protein by weight (Loewy and Siekevitz, *Cell Structure and Function*, Holt, Rinehart & Winston, 1969). Therefore, the volume occupied by protein in our models should more closely approximate crystals than dilute solutions, in which proteins are 0.1% or less.

One property of protein crystals is that water is present within them in two phases, simply because water adsorbs onto protein with great avidity. It might be expected that since cells contain so much protein, they would contain two phases of water as well. This has been shown experimentally in two different systems by quite different methods. They complement each other well because in one a high degree of precision was available, and in the other entirely physiological conditions could be maintained throughout.

The two phases of water in isolated mitochondria were studied by Garlid (in *Cell-Associated Water*, Drost-Hansen and Clegg, eds., Academic Press, pp. 293-362, 1979), who measured the distribution of permeant uncharged solutes at different mitochondrial volumes. The solutes distribute between two phases of water; one is osmotically inactive and of more or less constant volume, and the other phase is osmotically active and behaves in its solvent properties entirely like bulk water. The osmotically inactive compartment differs from bulk water in its solvent prop-

erties, so that some solutes are excluded and some are preferentially dissolved. The volume of the osmotically inactive compartment can be accounted for by water of hydration, as Garlid pointed out.

Paine and Horwitz (*Biophys. J.* 25, 33-62, 1979) injected frog oocytes with a droplet of gelatin to create a reference phase. After equilibration the cell was frozen, and the gelatin reference phase was dissected out to measure the solvent properties within oocytes. They also found two compartments of water, one of which resembles bulk water in its solvent properties and a second that excludes some solutes such as sucrose. One consequence of these two phases is that the activities within the bulk water phase of the cytoplasm are higher than the concentrations in the cytoplasm overall for the solutes excluded from the other phase. These higher activities have been confirmed directly by ion-selective electrodes. Again, the compartment of water in the oocyte that behaves unlike bulk water is quantitatively accounted for by the water of hydration, although there is no direct proof of the identity of the two.

Water of hydration is not immobile water like ice, but it differs in many properties from those of bulk water. It has a higher heat capacity and is generally more ordered than bulk water. It has reduced mobility compared with bulk water, and it may be a better or worse solvent for particular solutes than bulk water; thus the presence within cells of two significant phases of water has to be considered in any model. In particular, the water of hydration is the water that proteins see. Rupley (*ACS Symp.* 127, 111-132, 1980) has shown that it is water of hydration that is required for enzyme activity. Lysozyme is enzymatically active as soon as it is fully hydrated; it is fully active before there is even a completed monolayer of bulk water around it. Water of hydration also coats other macromolecular components of the cell, such as carbohydrates, nucleic acids and the lipids of membranes. Thus its volume in the cell depends on those components as well.

Since cells contain two phases of water, it becomes of great interest to ask what the relative importance of those two phases is in the life of the cell. This question has been explored in great detail for an organism that changes in hydration physiologically. Clegg has examined the brine shrimp *Artemia* and the dependence of various metabolic processes on the hydration of the cell (reviewed by Clegg, *J. Exp. Zool.* 215, 303-313, 1981). He has shown that at approximately 35% hydration (that is, 0.35 g water/g protein), the cells resume metabolism of small molecules, synthesize lipids and amino acids and fix CO<sub>2</sub> into organic molecules. This water content is roughly that of the water of hydration. There is almost certainly no bulk water phase present, and that has been confirmed by studies with nuclear magnetic resonance carried out by Clegg et al. (*Arch. Biochem. Biophys.* 210, 517-524,

1981). At slightly higher levels of hydration, approximately 70%–80% hydration, the cells resume the synthesis of RNA and protein, and intermediary metabolism proceeds as quickly as in fully hydrated cells. This hydration is only slightly greater than that of many crystals, and it is difficult to conceive of how more than a small degree of relative mobility among the protein molecules is possible. There are no further qualitative changes in the metabolism of these cells up to the full hydration level of the cell. The cells display these metabolic activities independent of the route by which the degree of hydration is obtained: whether by rehydration of dry cells or dehydration of wet cells.

Some might suppose that this is a peculiar property of *Artemia*, since it is an organism that lives in salt lakes. However, Clegg and Mansell (*Cryobiol.*, in press) have extended these studies to mammalian cells. In mouse L cells that have been reduced to half their volume by sorbitol dehydration, the transit from glucose to CO<sub>2</sub> through glycolysis and respiration is virtually independent of cell volume down to about 60% reduction in cell volume. In addition, in these dehydrated cells the qualitative production of small metabolites, determined by chromatography, is unaffected. The cells are fully viable after this treatment and show no persistent consequences upon rehydration.

These results from *Artemia* and mammalian cells can be accounted for by either of two explanations. Either the size variations of the bulk water phase are accompanied by exquisitely balanced reciprocal variations in enzymatic activities with changes in concentration; or the precise volume of the bulk water phase is of minor importance because the major site of regulation is in fact in the second phase, the phase generated by water of hydration. The results do not by themselves exclude either one. However, they do exclude the possibility that the cell is exquisitely sensitive to reductions in the size of the bulk water phase.

Another aspect of the crowded interior of the cytoplasm has startling consequences. Protein solutions that contain high concentrations of proteins do not behave like ideal solutions. Minton (*Biopolymers* 20, 2093–2120, 1981) has shown by extremely elegant and detailed mathematics that the volume occupied by proteins affects the activity of the other proteins in solution, in effect by "crowding" them into a smaller space, with less freedom of movement. Thus the presence of 10% or more protein in a solution will tend to drive several reactions. It will tend to force proteins into compact configurations and out of extended ones. It will favor self-association and hetero-associations that would be less frequent in dilute solutions. And finally, it can affect enzyme catalytic activities if either the configuration or the association of the protein is driven by the high concentration of proteins. These effects can show 50 to 100 fold excesses over the

values predicted from dilute solutions. That this "excluded volume" effect is not only theoretical but borne out by experience has also been shown by Minton and Wilf. The presence of 10%–20% of any other protein in the solution will drive myoglobin into dimers (Minton and Wilf, *BBA* 670, 316–322, 1981). In the presence of similar amounts of other proteins, the enzyme glyceraldehyde-3-phosphate dehydrogenase will form less active tetramers (Minton and Wilf, *Biochemistry* 20, 4821–4826, 1981).

Thus the actual enzyme activity in the cytoplasm is a function not only of which enzyme is there but also of the concentration of all the other proteins. This is probably the explanation for the conflicting results that have been seen in the search for a glycolytic particle, a complex of the glycolytic enzymes. DeDuve (in *Structure and Function of Oxidation-Reduction Enzymes*, Åkeson and Ehrenberg, eds., pp. 715–728, 1972) saw little or no association between the glycolytic enzymes, while Clarke and Masters (*BBA* 381, 37–46, 1975) reported fairly extensive associations of these enzymes. The major difference between the two experiments was that DeDuve used dilute solutions of enzymes, while Clarke and Masters studied the enzymes at physiological concentrations of protein. Since these levels of protein are well within the range for which Minton (op. cit.) predicts nonideal behavior, it is more likely that the results of Clarke and Masters represent the state of the enzymes in the cell.

All of these considerations suggest that the cell is a very crowded place. Such crowding could be non-specific and simply due to the density of proteins, or it could be specific and maintained by selection. That in fact the crowding is for the most part selected for and quite specific is suggested by a recent observation by McConkey (*PNAS* 79, 3236–3240, 1982). McConkey used high-resolution two-dimensional gel electrophoresis to show that at least half of the polypeptides from hamster cells and human cells are indistinguishable in isoelectric point and molecular weight. This is far less variation than would be predicted from the soluble proteins in the Atlas of Protein Sequence and Structure. Neither is the similarity a consequence of the multiplicity of proteins, since *Escherichia coli* and HeLa polypeptides are at least 98% distinguishable in this system. McConkey suggested that polypeptides with highly conserved isoelectric points and molecular weight form an extensive, if delicate, structure that demands the conservation of these properties for the formation of what he calls the quinary structure. Certainly many highly conserved proteins, such as the histones and actins, are in fact present in structures for much of their lifetime. McConkey's observations suggest that perhaps as many as half of the polypeptides in the cell participate in comparably specific structures.

These complex and extensive structures may well include enzymes, as well as those proteins present in

the structure solely for structural purposes. Brady and Lasek (Cell 23, 515-523, 1981) have suggested that the enzymes enolase and creatine phosphokinase are transported on the guinea pig axoplasmic matrix as members of the slow component b. These enzymes move down the axon at a rate of 2 mm/day with a discrete front, shared with the rest of slow component b. This is not the behavior that would be expected for proteins moving purely by diffusion.

Porter and McNiven (Cell 29, 23-32, 1982) have shown an even more extensive degree of structural integration in the cytoplasm, exhibited during the cyclical migration of pigment granules in chromatophores. These pigment granules move in and out radially with the appropriate stimulus. In each cycle, the granules return to the same place with an accuracy of 1-4  $\mu\text{m}$ . This minor amount of displacement is not compatible with a model of the cytoplasm in which cytoplasmic material has been dissolved and reannealed with each cycle. In fact, the detailed observations show that at no time are these pigment granules moving as if they were free in bulk water. At rest, they saltate gently, showing movement faster than that of Brownian motion; during migration toward the center, they move steadily without reversals, and during out-migration they saltate in a net outward direction. Thus at no time do they display the motion expected if they were floating freely. Since they are evidently attached at all times, and they return repeatedly to one location, the structure to which they are attached must preserve its topological connections and most of its metric properties throughout these cycles.

With so much evidence pointing toward an extensive and persistently crosslinked structure in the cytoplasm, is there any evidence that confirms the presence of genuinely soluble proteins? The answer to this seems to depend on the cell examined. Lasek (in *The Biology of Microtubules*, Sakai, ed., Academic Press, in press) was able to determine what fraction of the glycolytic enzymes in the axoplasm of the squid giant axon behave as soluble monomers at any given time. Between 60% and 70% of a given enzyme was free to diffuse out of the isolated axoplasm. Thus at any one moment approximately one third of the enzymes were immobilized on the matrix and the other two thirds were freely diffusing. This sharply contrasts with what Paine saw in oocytes. Less than 20% of the

protein, exclusive of yolk, was free to diffuse (calculated from Paine, in *The Nuclear Envelope and the Nuclear Matrix*, Maul, ed., Alan R. Liss, 1982, pp. 75-83). By examining the proteins contained in the reference phase, he determined the soluble proteins at equilibrium. Those proteins, in total, were less than one fifth of the cellular non-yolk protein, and the pattern of proteins in the reference phase showed many differences from the total cytoplasm, suggesting that many cytoplasmic proteins do not diffuse at all.

The conflict between these two results is partially explained by the different cells examined. Perhaps more important, though, are Minton's (op. cit.) observations on the effects of excluded volume. In the axon, protein is about 2% of the weight of the axoplasm. In contrast, in the oocytes the cytoplasm is between 30% and 40% protein by weight, excluding the protein of the yolk. Thus, in the axon, the proteins would be expected to behave ideally for the most part, while in the oocytes the protein content would cause protein behavior to be highly nonideal and would favor associations between different proteins. As mentioned before, the protein contents of most cells is about halfway between these two, but that is still in the range in which Minton predicts highly nonideal behavior.

These different strands of evidence support a model of the cytoplasm that is compact and only a few times more open than a crystal. This compact cytoplasm has long been reflected in the dense and complex images seen in electron micrographs. The complex latticework with persistent elements in it is held together partly by high-affinity interactions and partly by the nonideal behavior of proteins under physiological conditions. The latticework itself is thinly coated with a phase of water that behaves unlike bulk water, but that nevertheless may be the phase of water most important in normal metabolic activities. In the cytoplasm, some proteins clearly exist free in solution. In many cells, however, that solution is not adequately modeled by a dilute solution of proteins. Equally clearly, some proteins are more reasonably described as participating in a crystal, an extensive, three-dimensional, persistent structure in which the relative position of the proteins is maintained. The challenge that lies ahead is to frame more experiments that determine how particular proteins behave in the highly "nonideal" interior of the cell.