Water near Intracellular Surfaces

V. ADRIAN PARSEGIAN and DONALD C. RAU
National Institutes of Health, Bethesda, MD 20205

ABSTRACT In this paper we make the following points:
Water is perturbed within several angstroms of the surfaces of soluble molecules.
Removal of this water can require significant amounts of work, seen as an exponentially varying "hydration force" with respect to molecular separation.
The favorable and specific attractions that occur in molecular assembly or in ligand binding imply that the specific association between the molecular surfaces is stronger than the association of those surfaces with water.
The specificity of biochemical association is not simply a matter of protein-protein interaction but also of competing protein-water interactions.
Small structural changes in molecular surfaces can evoke large changes in the contact energy of hydrated surfaces; surface hydration and the energetics of water displacement are a likely mechanism for the contact specificity of intracellular associations integrating the cell matrix.

There is an unspoken theme that runs through this supplement. It is the idea that the structures composing the cytomatrix can form, dissolve, and reform spontaneously from otherwise invisible constituents. No one says here that these constituents are precipitously synthesized when the trabecular matrix (or microtubule or spindle fiber or actin filament or whatever) appears. Rather, it is assumed that, before the aggregate appears, the pieces are waiting to come together. After all, many structures simply disappear when the cell is cooled and reappear when warmed. Where else can they be going but into some invisible pool from which they then emerge again?

This implicit theme of spontaneous assembly brings questions of cellular integration down to questions of molecular assembly, questions that cytologists tend to shun but which, in our opinion, are central to any firm view of cellular organization. To think about assembly at the molecular level one must bring the scale of one's thinking down by a factor of 10^4 from the micrometer or 10-micrometer scale of cellular structures to the scale of angstroms or nanometers that characterizes the molecular surface.

We have recently learned how to measure forces between macromolecules in their last 15 Å of separation as they are brought into contact. What we have learned from these measurements disproves most of what we have all been taught about interactions between large molecules. It also provides some very strong hints about what must be going on when molecules come together spontaneously, as they apparently do inside a cell.

The salient result of our measurements is that forces between molecules or between membranes are what we have come to call "hydration forces." By this we mean that water, rather than being merely a medium through which macromolecular interactions are transmitted, is in fact itself a chemical species interacting with a molecular surface. It is part of the equation of energy balance. When two macromolecules come together, water on their surfaces must leave. A tautology, perhaps, but one that reminds us of factors that are often ignored.

To a first approximation, this is similar to what people have always had in mind when talking about "hydrophobic" forces or the "hydrophobic effect." These traditional models differ from what we discuss here in that actual force measurements show (*a*) much longer range, (*b*) repulsion as well as attraction, (*c*) the action of polar rather than nonpolar surfaces, and (*d*) the likelihood of much greater molecular specificity than previously expected.

Any water-soluble molecule is hydrated by water that is attracted to its surface. Unless another body is more strongly attracted to that particular surface, it takes work to remove the water. Several years ago, in measurements of forces between bilayer membranes, we found that the hydration shell can extend for several molecular layers. The repulsive forces that are encountered in removing this boundary water between two like bodies are thus long range. They are also quite strong and grow exponentially with a characteristic constant of 2.5 to 3.0 Å.

A second feature of these hydration forces is that they can

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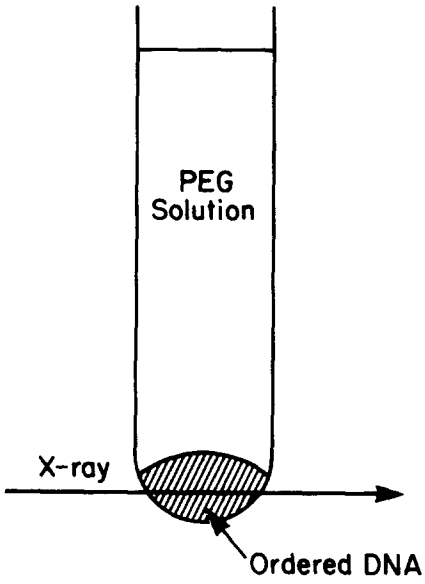

FIGURE 1 Scheme of osmotic stress measurement. Controlled vapor pressure or direct pressure through a semipermeable membrane is also used (10, 15, 24).

be attractive. Complementary molecular surfaces appear to form water bridges that can hold molecules together at distances on the order of 10 Å. We have seen this recently with DNA double helices. The conditions for attraction are highly specific, but the forces are as strong as the repulsive hydration forces when conditions are right. The specificity of design may allow proteins or nucleic acids to undergo spontaneous directed assembly through controlled hydration.

MATERIALS AND METHODS

The ways in which we measure intermembrane or intermolecular forces are described elsewhere (6, 7, 15, 24, 25). A schematic picture of how we do it will suggest a way to connect these measurements to cellular processes. We watch the material—protein, lipid, or nucleic acid—in a state of osmotic stress in which it is subject to the osmotic pressure of a reference solution (Fig. 1) of some inert polymer such as dextran, polyvinylpyrrolidone (PVP),[1] or polyethylene glycol (PEG). Using x-ray diffraction, we can see what structural changes occur when bodies are brought together after removal of water by the stressing solution.

RESULTS

Phospholipid Bilayer Membranes

Electrically neutral, but zwitterionic, bilayer membranes automatically stack up to create multilayers of bilayers alternating with water. Osmotic stress pushes the membranes together and also causes them to thicken as the cross-sectional area of the constituent molecules decreases to accommodate the loss of water. Fig. 2 shows a typical force-vs.-separation curve for one particular neutral phospholipid. The repulsion encountered while pushing in from 30-Å separation grows exponentially. With a characteristic length of 2.7 Å it reaches pressures of several hundred atmospheres as the bilayers near contact. The energies corresponding to the work of such forces approach 10–15 kcal/mol of lipid molecule (14).

Forces between electrically charged bilayers look virtually the same as those shown in Fig. 2 when the distances between bilayers are less than 20–30 Å (2, 8, 9, 12). At greater distances, electrostatic repulsion can overwhelm the van der Waals attraction that forms the multilayer from individual bilayers.

The behavior portrayed in Fig. 2 also is seen in distilled water as well as in concentrated salt solutions. Since in distilled water there is nothing but water coming out of the space between bilayers, we have no choice but to see this repulsion as a work of removal of water from between bilayer surfaces. The force changes only if something else displaces water from the surface, and that displacement occurs only if the new species is more strongly attracted to the surface than is water. Conditions for such displacement are highly specific, but when they are met—for example, by Ca ions reacting with bilayers of phosphatidylserine (11, 22)—the resulting energy of attraction holding together the collapsed bilayers is of the same tens-of-kilocalorie magnitude previously encountered in repulsion (14).

DNA Double Helices

Forces between parallel DNA double helices are surprisingly similar to those between bilayer membranes just described. To make the required measurements, we use the well-known polymer condensation of DNA wherein PEG (or dextran or PVP) added to a DNA solution causes the DNA double helices to form an ordered hexagonal array of parallel molecules. We measured the lattice spacing and polymer osmotic pressure (25). The distance between molecular surfaces is the interaxial distance minus the 20-Å molecular diameter.

Fig. 3 gives one example of the interaction in 0.5 M NaCl solution with and without the "condensing agent" spermidine. In the absence of any such agent and at interaxial distances of 35 Å or less, the force grows exponentially with a characteristic constant of ~3 Å. The interaction bears little resemblance to the electrostatic double layer force except at very large distances in media of low ionic strength. The 3-Å decay is seen in media of univalent or divalent cations and over a very wide range of ionic strengths. (For more details, see reference 25.) Force curves in different ionic solutions are parallel but displaced from one another; the coefficients of the forces differ while their exponential rate of decay is the same.

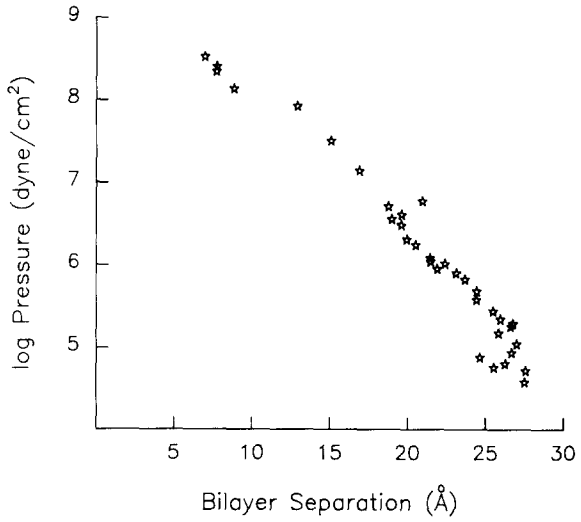

FIGURE 2 A typical interbilayer force-vs.-separation curve; dilaurylphosphatidylcholine bilayers in distilled water. The exponential decay constant is 2.7 Å (data from reference 10).

[1] *Abbreviations used in this paper:* HbS, deoxy sickle-cell hemoglobin; PEG, polyethylene glycol; PVP, polyvinylpyrrolidone.

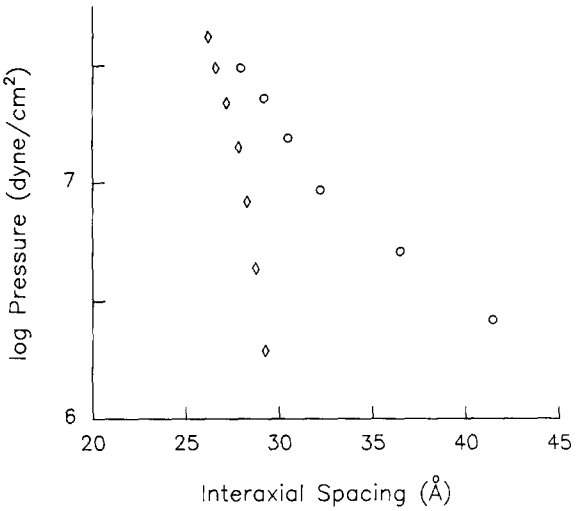

FIGURE 3 Pressure on a hexagonal array of DNA double helices: upper curve, in 0.5 M NaCl; lower curve, 0.5 M NaCl and 0.01 M spermidine Cl.

A most dramatic effect occurs, though, in the presence of one of the trivalent or multivalent "condensing agents," spermidine, spermine, cobalt-hexamine, etc., that are located inside the major groove of the double helix. These agents cause the DNA molecules to form ordered hexagonal arrays without the action of condensing external polymers, but the array shows interaxial spacings of 28–32 Å. The molecules sit stably at separations of 8–12 Å, with only ionic solution between them. The lower curve in Fig. 3 shows a typical repulsive force encountered when the molecules are pressed closer together. Rather than the 3-Å exponential seen previously, the rate of change is a constant of ~1.5 Å.

To understand this behavior and to learn how to transfer it to other situations, we have benefited greatly from a theory proposed by Marcelja and co-workers (4, 5, 13) on solvent-mediated interactions. In a highly oversimplified version of that theory one may think of the first layer of water at a surface being perturbed by its interaction with that surface. (Marcelja uses the language of an order parameter or of a polarization and speaks of a surface polarization, P_S.) The next layer of water is perturbed not directly by the intruding surface but only indirectly by the neighboring layer of water. The influence of the surface then extends by a series of nearest-neighbor interactions (e.g., dipole-dipole interactions [26, 27]) characteristic of the medium, while the magnitude of the perturbation reflects properties of the molecular or membrane surface.

If that perturbation is such that it lowers the energy of the water molecules near the surface, and if two like surfaces are brought near each other, the surfaces must repel. It is easy to imagine that like surfaces will orient their respective water molecules so that the two surfaces are "back-to-back" and repelling each other. In the language of the original elegant formalism, the repulsive force between like planar parallel surfaces varies as $P_S^2/\sinh^2(d/21) \rightarrow P_S^2\, e^{-d/l}$, where d is the separation between surfaces and l the decay distance characteristic of the solvent. Different surfaces will have different polarizations, P_S, but the decay of the force will be the same sinh or exponential function.

The DNA data (25) suggest that different ions binding to a surface will confer different coefficients that reflect the combined hydration properties of the ion and the original surface. Lipids with different polar groups (specifically phosphatidylcholine and phosphatidylethanolamine show different force coefficients but similar rates of decay [10]).

Hydration forces analogous to those described here have been seen between mica surfaces made polar by the adsorption of cations (17–19). There, the decay rates of forces vary with ionic type, presumably because of ion desorption or displacement from the mica surface. Under some conditions, the forces in that system can be convincingly interpreted to be oscillatory as discrete layers are removed from the hard, smooth mica surfaces (20, 21). Stable multiple spacings between clay particles in lamellar arrays observed many decades ago (1) may now be attributed to oscillatory forces.

But what if the surfaces are unlike? Then one can imagine water molecules of the same orientation reaching across the space between them, since water molecules orient themselves in opposite ways with respect to each surface. The theory predicts an attractive force of the form $A/\cosh^2(d/21) \rightarrow A\, e^{-(d/l)}$, where the coefficient A again reflects the perturbing, polarizing, strength of the two surfaces. As these surfaces come toward contact, this attraction approaches a constant value. Unlike the repulsive force, it does not diverge.

Interacting surfaces composed of attractive and repulsive areas can feel net attraction only if A is greater than R. At sufficiently small distances, though, the repulsive component is expected to dominate.

Double helical DNA exposed to trivalent cations will precipitate from solution to form ordered arrays of parallel molecules that maintain a finite separation of 6–10 Å of water (J. Schellman, unpublished observation [1980]; Rau, D. C., and V. A. Parsegian, unpublished observation [1983]). We have pushed together the molecules from the minimum energy position (e.g., as shown in Fig. 3) to measure the difference in contending attractive and repulsive forces. We find a characteristic length of 1.6 Å. One may verify by expansion of the cosh and sinh functions that this decay rate is precisely what is to be expected from the combination of attractive and repulsive hydration forces.

Because the perturbing surfaces lower the entropy of the intervening water, net hydration attraction is expected to increase with temperature. It is in fact observed that trivalent cationic condensing agents are more potent at higher temperatures. It would not surprise us to find that many of the temperature-enhanced associations traditionally ascribed to "hydrophobic bonding" were in fact due to hydration forces.

Negatively charged bilayer membranes sometimes bind divalent ions to create membrane surfaces that fall together, leaving little or no water between them (11, 22). We suspect that there, too, there is an attractive hydration force acting between bilayers.

DISCUSSION

It is probably annoying to most cell biologists to have to think about water. So many peculiar properties have been ascribed to cell water that one must surely be suspicious. All of molecular biology seems to assume that the interesting molecules are the nonaqueous, specifically synthesized cellular constituents rather than the medium in which synthesis and assembly are going on. We do not really disagree with this view but would point out that each of these constituents must maintain its own interaction with its surroundings, an interaction that must change when two large molecules come

together to assemble into organelles, or when a water-soluble agonist finds its particular receptor, or when a substrate finds its enzyme. We suggest that at some stage in the study of cellular processes we must recognize the competition between water and other species coming near the molecular surface.

It is important to remember that, unlike the interior of membranes, the cytoplasm is an aqueous compartment. Proteins associate because they find it energetically more favorable to touch each other than to be in contact with the water that would otherwise surround them. If the association is permanent, one is tempted to label the molecular surface as "hydrophobic." More often, the association is temporary as well as highly specific. In those cases one must recognize that contact depends on peculiar details of the particular interacting species. It is probably unwise to think then about such contact in terms equivalent to that occurring between chains of hydrocarbon. Although we speak of surfaces as "hydrophilic" or "hydrophobic," it is only in the past few years that we have learned that the solvent is perturbed several molecular layers away from these surfaces. It is apparently the interaction of boundary water layers that determines whether two surfaces repel or attract each other. Either attraction or repulsion can dominate at separations greater than 10 Å.

Hydration energies are large. It costs on the order of 100 ergs/cm^2 to push together two bilayer membranes (16). Translated into more familiar language, this is on the order of 10–15 kcal/mol required to push together two square surfaces 10 Å on a side. These are large energies when one thinks about protein binding and stable association. (It might help to recall, too, that one gets about 7 kcal/mol out of high-energy phosphate bonds and that it costs about 87 kcal/mol to break a carbon-carbon bond.) Attractive forces can be as large. For example, phosphatidylserine bilayers in Ca^{++} solution will spontaneously come together with net energies on the order of 10 or more kcal/mol per 10-Å-square patch.

The sign of the net force depends, it turns out, on fairly small differences in the structure of interacting surfaces. Such small differences are all that is needed for specificity. If our imaginary 10-Å-square patch is the surface of a peptide ligand, one may imagine that small changes in structure can make one particular peptide stick strongly while near relatives cannot. The energies are there. Again, nature's trick is to create arrays of surface charges that interact more strongly with each other, that fit better with each other, than with the intervening water, or to create arrays that cooperate to polarize a few intervening water molecules to form a stable arrangement. Small changes, such as would occur upon ion binding or small conformational changes, can spoil the specific fit that is required for molecular recognition.

For the most part, boundary water should act little differently from normal water as a medium for diffusion. Seen as a perturbation per molecule of water, boundary water deviates only slightly from normal water. The large forces encountered between large molecules occur because many water molecules are simultaneously displaced. Small diffusing solutes displace relatively few water molecules. They move through most boundary water as though it were normal water. Hence, diffusive probes are insufficiently sensitive to the perturbations that govern the interaction and assembly of large molecules (14).

One can cite several examples in which the properties of boundary water control molecular interaction and several other situations in which the control of water activity influences molecular association. One is the interaction of DNA double helices (25; Rau and Parsegian, unpublished observation). It does not look like the electrostatic repulsion expected from earlier thinking but is clearly a hydration force. Most important to the cytoplasmic focus of this journal supplement is the fact that the distances at which this interaction is measured are exactly the distances at which DNA is seen to pack in many bacteriophages, wherein, we must now recognize, the DNA is under a pressure of several atmospheres.

We have also measured the work of packing hemoglobin, both normal and deoxy sickle-cell hemoglobin (HbS) in gels and in solution (23; Prouty, M. S., et al., manuscript in preparation). Up to a critical pressure, all hemoglobins look alike and reflect the work of packing finite spheres derived by Minton and Ross. Then HbS undergoes a sudden condensation to a "gel" state which, with higher pressures, condenses further. These "higher pressures" of roughly one-half an atmosphere are typical of the cellular interior. The condensation shows that normal internal cytoplasmic stress can trigger assembly of correctly designed species.

Examination of the contacting faces of protein dimers or tetramers (28) reveals that those combinations whose component monomers are water soluble have contact faces that are studded with polar groups and do not make the oily "hydrophobic" contacts characteristic of more permanent combinations. Our inference is that these reversible associations, which are what one expects in the cytoplasm, achieve their controlled association by exploiting the properties of boundary water.

Cells, through active transport and controlled leakage, maintain a strong osmotic stress on their contents. How many cellular organelles would be affected if that stress were removed? What happens, for example, when the cell membrane is perforated with a channel-forming material such as the nystatin used by Freedman and Hoffman (3) to swell red blood cells? Any effect of membrane leakage would show an immediate connection between activities in the cytoplasmic matrix activity at the cell membrane mediated by the controlled activity of cell water.

One reason people have been stuck for so long in their attempts to understand muscle contraction might be their failure to see the making and unmaking of protein-protein contacts that accompany the sliding of contraction as due to controlled changes in hydration of the cross-bridge surface. The newfound ubiquity of contractile proteins near cell membranes will amount only to a cataloging exercise until we have a more compelling idea of how contractile proteins actually generate physical forces.

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