Biochem. J. (1987) 248, 615–617 (Printed in Great Britain)

A role for water in cell structure

John G. WATTERSON
Department of Biochemistry, Monash University, Clayton, Vic. 3168, Australia

The question of a role for water in biochemical and cellular events is ignored by most workers (apart from its obvious role in hydrolysis reactions, which is not under discussion here). But much recent research has pointed to the importance of physical, as well as biochemical, processes of the cell, which focus attention on such straightforward elementary questions as position and relationship in space of cell components. In this communication these questions are examined in terms of a new model of water structure. A radically new feature of this model is that water clusters have long-term rather than flickering existence and are as large as the macromolecular components of the cell. These properties allow the clusters and other components to pack together spatially so giving rise to integrated, large-scale, subcellular structures.

WATER STRUCTURE AND CELL ARCHITECTURE

In this paper a new picture of the cell is presented in which four of its most important components, namely lipid membranes, nucleic acids, proteins and water, are seen as fitting together spatially to make an overall integrated structure in a much more precise way than is usually imagined. At present, workers are inclined to picture the cell, as revealed by their description of its architecture, in analogy to man-made buildings, with walls to demarcate separate compartments as well as strong beams and uprights in the form of the cytoskeleton to maintain overall cohesion and shape. The solvent content of the compartments (which may be as much as 85% of total volume, as in brain grey matter) is viewed as a structureless, space-filling, background medium in which biochemical events occur, in which the same way as we tacitly accept the air which fills our rooms and corridors. But, in the new picture, water itself, even in the absence of biological material, is able to form clusters or ordered aggregates with the sizes and shapes required to build the cell. Thus water clusters can also take the form of a wall or a support beam, as seen in the shapes illustrated in Fig. 1. Put another way: the underlying factors determining the dimensions of the structural units of the cell can be traced to structural forms intrinsic to the solvent.

Studies on cell water so far have led to the conclusion that different types of water exist in cells, variously described as bound, hydration, vicinal and bulk water. The traditional, and at the moment majority, view claims that most, being 95% or more, is identical with bulk water, so fitting the role of the space-filling medium. However, as pointed out by others [1], these conclusions are based mainly on interpretations of results from dielectric-relaxation and n.m.r. techniques, which monitor the average rotational motion of single molecules and do not probe long-term collective processes of solvent interactions that underlie long-range effects. For example, extensive studies with these techniques in similar systems, such as clay, polymer and protein gels, reveal that here also water differs little, if at all, from bulk water. Yet, viewed from a larger perspective, the solvent in these systems is totally immobilized, a state which is very pertinent to biological systems. The ideas presented hereunder concern this latter question of how water takes part collectively in the mutual interaction on the macromolecular scale.

In the recently published wave model of liquid structure [2], ordered clusters do not just flicker on and off, appearing and disappearing at random, but travel as a wave through the medium. These clusters are instantaneous aggregates held together by co-operative intermolecular bonds, such as the hydrogen bond, which is known to be very strong in water. We can most readily depict the size and shape of these formations when the motion sets up stationary waves. In the simplest situation, i.e. within the unbounded bulk medium of a pure liquid, such stationary waves form an array of cubic clusters separated from one another by the nodal planes that are the faces of the cubes. Although made up of individual molecules, the clusters themselves are able to act as independent entities and take part in processes occurring in liquid media [3]. One such process is osmosis, and an examination of their role in the mechanism underlying this phenomenon reveals that the volume of a cluster equals that occupied by a molecule of perfect gas. This means that, for water at room temperature and pressure, the basic cubic cluster depicted in Fig. 1(*a*) has an edge of about 3.3 nm, i.e. 11 molecules long, contains more than 1000 molecules, and has a molecular mass of more than 20000 Da.

The addition of solutes causes these basic cubic building blocks to adopt other forms, because the presence of an unlike neighbour places constraints on the intermolecular bonding of the solvent molecules [2]. This can be readily illustrated by imagining what occurs within the wave motion on introducing an extended two-dimensional solid surface into the otherwise uniform medium. If the molecules closest to this surface, i.e. the first hydration monolayer, are restricted in their motion, the foreign surface will force a nodal plane in the wave motion to be positioned at the interface. Then a two-dimensional, layer-shaped, co-operatively interconnected solvent aggregate (Fig. 1*c*) would form in contact with the solute surface. Similarly, a cluster that is highly extended in only one dimension can be formed next to a filament solute parallel to its axis (Fig. 1*b*). In this case we can imagine that, in those regions of the cell containing arrays of parallel protein filaments, the medium is constrained to form parallel elongated solvent clusters

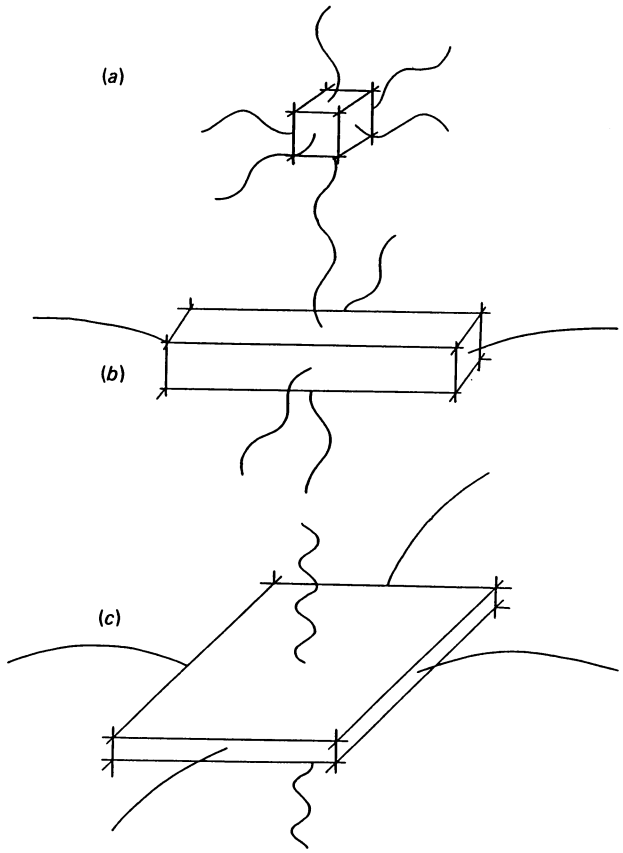

Fig. 1. Illustration of clusters in liquid water formed by stationary structure waves travelling in three perpendicular directions, x, y and z

The sketched waves are diagrammatic only and are included to show the wave form that shapes the neighbouring cluster in each direction. The real form of the wave is given by an expression of the type $\sin(x)\cdot\sin(y)\cdot\sin(z)$.

packed in columns together with the filaments, building an overall integrated structure.

The existence of hydration forces has been recognized for some time by many workers in those fields of research covered by the colloid sciences. Over the past decade, these forces have been the subject of ever-increasing interest, especially in water. Their underlying cause is still disputed, but the fact that they are observed at clay, organic and biological surfaces gives the impression that they are a property expressed by the solvent. Considering this lack of dependence on the chemical nature of the solute surface, it is astounding how often the distance of 3 nm is found to be their range of influence [4]. In other words, many surfaces of various chemical composition are able to promote a force in the solvent medium that can repel other solutes over a distance of at least 3 nm. For example, one fundamental observation made with biological systems is that bilayers of common lipids at room temperature and pressure pack together at an interlayer distance of around 3 nm [5], so that between the bilayers is sandwiched a layer of water just 3 nm thick. It is surely no coincidence that the hydrocarbon region of the bilayer is itself about 3 nm across, giving the repeat distance through the alternating layers of lipid and water of 6–7 nm. Now we have an overall picture of a stack of two-dimensional building blocks of solute and solvent fitting together. And, moreover, the length of the lipid hydrocarbon chain is such that the bilayers pack naturally within the underlying water structure, where the repeat distance is predetermined at around 3 nm.

In systems where hydration forces are operating, high pressures exceeding several atmospheres are needed to bring solute particles into contact. How then do proteins, which in their function are required to make contact, manage to approach each other? The accepted theory on this point states that such interactions are specific, which means that there are definite amino acid side chains on the opposing protein surfaces that are responsible for successful interparticle association. Now, hydration forces exist at, for example, metal, mineral, liquid emulsion, solid synthetic latex, random polymer chain as well as biological surfaces. In view of this wide range of possibilities for surface physics and chemistry, it is not reasonable to propose that simply switching some supposedly crucial side groups will convert strong long-range repulsion into specific attraction. Of course, once contact has been made the nature of the side chains determines whether a specific interaction can take place. But before biochemical reactions can proceed the protein partners must first be able to approach each other. A good illustration of this problem is the ability of the actin filament to bind a variety of diverse proteins. It has been reported that there may be as many as 60 physiologically relevant partners for actin [6], so that one must propose the very unlikely situation, namely, that there are also approximately as many distinct binding determinants, each somehow uniquely composed out of the side groups available on its surface. A completely different, and very much simpler, explanation is supplied by the model of structured water of hydration. The actin filament is 6–7 nm across, and so with these dimensions fits snugly together with solvent clusters. This is seem immediately from the powerful long-range effect the filament has on water structure overall, i.e. its ability to cause gellation, even at a protein content as low as 0.1 %. This means the very presence of the filament is able to lock regions of the bulk water somehow into position so that they can no longer flow relative to one another. This state is readily interpreted in terms of the cluster model. The filament is the immobile architectural beam along which the building blocks are packed together, and, since their spatial dimensions fit those of the filament, their arrangement also becomes immobilized. This statement should not be taken as a peculiarity of the model presented here. On the contrary, much of the recent research in the broad field of colloid science implicates the solvent in gellation. For instance, rheological studies on actin and tubulin gels as well as cytoplasm indicate that these systems resemble smectic liquid crystals in their behaviour, which can be interpreted in terms of large extended anisotropic regions [7]. That these workers found such systems can be produced even at a protein content below 1 % without the need for interfilament cross-linking is very compelling evidence for an active role of the solvent in forming these solid-like phases, and supports the picture of the protein filament surrounded by solvent packed in an orderly, as opposed to fluid random, way. The approach of another protein molecule must displace this intervening hydration water, or, in other words, must replace the water clusters. Thus these other proteins must

themselves have the required geometric dimensions, so that the solvent building blocks are replaced as entities and not removed by being simply disrupted and squeezed out. In this way the region remains filled with spatially compatible units and our building is still constructed intact. It has been estimated that there may be as much as 50% of total cell water associated with the cytoskeleton if the hydration layer is of the order of 3 nm thick [8]. Values of this magnitude strongly implicate water in the role played by this ubiquitous protein framework.

The idea of 'required geometric dimensions' for the interacting proteins is not just a convenient phrase. It is well known that soluble proteins smaller than about 20000 Da need in general constraints to keep their native structure, whereas larger proteins fold spontaneously in solution under native conditions to yield a stable, remarkably persistent, conformation. Even larger proteins (whose tertiary structure is available) are known to be subdivided into domains with sizes in the range 15000–25000 Da. For example, the structure of the actin molecule reveals two domains of about 20000 Da each [9]. Of course the term 'domain' is used loosely in discussing protein structure, e.g. the 50000 Da Fab fragments of the immunoglobulins contain the 'variable domains', but each fragment can be divided both enzymically and structurally into two equally sized stable domains, one of which possesses the variable amino acid sequences. In the model presented here it is no coincidence, but a requirement, that this basic domain size corresponds to that of the basic unit in water structure. This requirement ensures that soluble proteins and water fit together constructively. Furthermore, the underlying compatibility between water and protein structures not only allows, but promotes, co-operative functioning (J. G. Watterson, unpublished work).

Another example of structural matching is the association of DNA- and RNA-binding proteins with these nucleic acid polymers. A full turn of the double-helix stretches a distance of just 2.8 to 3.6 nm, depending on whether it adopts the A or the B conformation [10]. It is perhaps of no great significance to say that the binding proteins must possess domains that pack with the helix, but it is no platitude to predict, as does this model, that these domains will be found to fit segments of the helix in simple ratio to the length of its pitch, say half or full turns, and, further, that the binding of protein complexes occurs in simple integers of this basic dimension. As with protein–protein association, the binding process must be accompanied by the reciprocal replacement, unit for unit, of water clusters of the same dimensions, all clearly compatible with the helical intervals. In this way we do not need special energetic mechanisms to remove the gelled water of hydration for which polynucleotides are long known to have a very high affinity.

Membrane-bound proteins also possess similar spatial requirements, so that the membrane as a whole is tailored to its aqueous environment. In the first place, the thickness of the bilayer itself is compatible with layer-shaped water clusters, as pointed out above. Now, to this basic arrangement we add proteins that must have both hydrophilic and hydrophobic regions with dimensions suiting the sizes of the construction units in these co-existing adjacent media. For example, some membrane-bound proteins that possess enzymic ATPase function show a stalk in electron micrographs separating the soluble enzymic portion of the molecule from the region inserted in the membrane. This stalk is about 3 nm long [11], a fact that is readily interpretable in terms of the present model. It means that the soluble portion must be positioned at a set distance away from the membrane–water interface beyond the hydration layer, i.e. beyond the solvent region within which strong hydration forces operate. Another example is supplied by the proteins of the plasma membrane, most of which are known to be glycosylated, projecting their oligosaccharide chains outward from the cell surface. These chains are commonly branched, but in the overwhelming number of cases the linear stretch of any such chain is just six to nine sugar residues long [12]. In an extended conformation these chains span a distance of around 3 nm, and so would just reach through the hydration layer. We usually picture these proteins as anchored within the membrane, but are they anchored in the layer of ordered hydration water as well? So here we have, again, a larger structural assembly than normally depicted, which involves water in a fundamental way in addition to the other components. Considering the long-known influence of soluble polysaccharides on the physical state of water, one must surely think it highly likely that this layer of water adjacent to the cell surface also plays a role in the function of the cell membrane. This is one example of the general proposal that, in all the cellular assemblies where water participates as an integral component in their construction, it is also co-operating as an active participant in their function.

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Received 4 August 1987/22 September 1987; accepted 29 September 1987