PHYSICAL CHEMISTRY

Water's response to the fear of water

Spectroscopic analysis reveals that, at low temperatures, hydrophobic molecules dissolved in water strengthen the hydrogen bonding between nearby water molecules. But at high temperatures, the reverse can be true. SEE LETTER P.582

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Water-repellent molecules are said to be hydrophobic — which, literally translated, means they have a fear of water. But unlike human phobias, the effects of the molecular fear of water are difficult to predict. For example, if a nanometre-scale hydrophobic ball is inserted into liquid water, one might expect it to break up the hydrogen bonds between water molecules, thus strongly increasing disorder. The actual outcome, however, is much more complex, and so the effects of hydrophobic solutes on the structure of their hydration shells — the layers of water molecules that surround the solutes — have been a topic of debate for almost 70 years. On page 582 of this issue, Davis *et al.*[1] present an invaluable contribution to the discussion by showing that hydrophobic groups in molecules in fact enhance the ordering of the surrounding hydrogen-bond network of water.

The debate about the effect of hydrophobic molecular groups on water goes back to the work of Frank and Evans[2] in the 1940s. They discovered that the hydration of such groups is associated with a decrease of entropy. In addition, they observed that the heat capacity of the resulting solutions becomes anomalously high. Both these effects suggest that a hydrophobic solute induces a strong ordering of the surrounding water. On the basis of these findings, Frank and Evans formulated the 'iceberg' model for hydrophobic hydration[2]. In this model, hydrophobic solute molecules create ice-like 'clathrate' structures in the surrounding water. The high local order of these structures could explain the observed entropy decreases associated with the hydration of hydrophobic molecules, whereas 'melting' of ice-like hydration shells could account for the large increases in heat capacity.

During the subsequent decades, the iceberg model has been severely challenged, in particular by neutron-diffraction studies. In these studies, the distance between water molecules near hydrophobic solutes was found to be quite similar to that in bulk liquid water, thereby casting serious doubts on the existence of any local ice-like structures[3,4]. Moreover, theoretical studies have shown that the thermodynamics of hydrophobic-molecule hydration do not require the surrounding water to be different from bulk water — the observed entropy decrease can be explained as the effect of the exclusion of water from the volume taken up by the molecule[5]. The water around a solute can maintain the same amount of hydrogen bonding as in the bulk liquid, at least for small hydrophobic solutes (less than 1 nanometre in diameter), in the same way that one can create a hole in a loosely woven fabric without breaking the threads.

Davis and colleagues' experiments shed fresh light on the structure of water surrounding hydrophobic solutes, using a spectroscopic method called Raman multivariate curve resolution. This method allows spectral features associated with the vibrational modes of water molecules hydrating the solute to be selectively extracted. Specifically, the authors obtained the spectra of water molecules that were locally hydrating alcohol molecules, for a variety of alcohols bearing hydrophobic groups of different lengths. By focusing on the spectral features corresponding to vibrations of water's hydroxyl (OH) groups, they obtained information about the strength and the distribution of hydrogen bonds around the alcohols' hydrophobic groups. The authors observed that the hydrogen-bond network in this region is significantly enhanced — that is, more ordered than in bulk liquid water.

How should one envisage this enhanced hydrogen-bond structure? It cannot be truly ice-like, because this would contradict the previous neutron-scattering data. The picture that emerges is that the methyl (CH_3) and methylene (CH_2) groups of the alcohols' hydrophobic groups form ideal templates around which the water network can fold, leading to locally enhanced, tetrahedral order. This network will consist of ridges approximately 0.3 nm in height (the intermolecular distance of the water molecules), with ridge angles of 104.5° (the intramolecular bond angle of the water molecule), as is generally the case for hydrogen-bond networks in water.

Davis *et al.* further found that the enhanced hydrogen-bond structure vanishes when the temperature of the solution is increased. For instance, they observed that the hydrogen bonding of the hydration shell of *n*-pentanol ($C_5H_{11}OH$) at 60 °C is similar to that of bulk

50 Years Ago

During September 1961, immediately following the resumption of nuclear testing, when there was widespread public concern that the amount of iodine-131 in human thyroids might reach significant proportions, it was decided to make regular measurements of thyroid radioactivity on a few members of the staff at the Radiological Protection Service … During the peak period a total of 20 members of the staff were measured and the average amount of iodine-131 in their thyroids was 0.25 mμc … the average amount of iodine-131 actually measured in the thyroids of the group could be predicted from a knowledge of the iodine uptake into the thyroid and the known concentration of iodine-131 in milk during this period. This adds weight to the belief, now widely accepted, that fresh milk is the principal vehicle whereby iodine-131 from nuclear test explosions enters humans.
From *Nature* 24 November 1962

100 Years Ago

The Vulgate Version of the French Arthurian Romances. Edited from manuscripts in the British Museum by H. Oskar Sommer — These sumptuous volumes are priceless gifts to the world of scholarship by the Carnegie Institution of Washington … In his introduction the editor gives an outline of his studies of the vulgate cycle, as the French version of the Arthurian prose-romances is called … The core of the typical tale of the conception and birth of an illustrious child of unknown father and a king's wife or daughter appears in the Welsh and Irish versions as something separate from any moral considerations … We must come down to the vulgate cycle to find in such legends the element of sin.
From *Nature* 21 November 1912

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water. This finding is in line with the original ideas of Frank and Evans: the ordered water structures surrounding hydrophobic groups 'melt' upon an increase in temperature.

The enhanced hydrogen-bond structure of the hydration shell and its disappearance upon heating have counterparts in the shells' dynamics. The molecular reorientation of water primarily proceeds through the transient formation of defects in its hydrogen-bond network — particularly the formation of bifurcated hydrogen bonds[6], in which a single hydrogen atom contributes to two hydrogen bonds. The fact that water is excluded from the volume filled by a solute reduces the rate of formation of defects in hydration shells[7], an effect that is amplified by the enhancement of the hydrogen-bond network. So, water molecules hydrating a hydrophobic group should undergo slower reorientations than those in the bulk, as is indeed observed in spectroscopic studies[8–10]. As temperature increases, the enhanced hydrogen-bond structure gradually vanishes, which means that the density of weak and/or defective hydrogen bonds also increases. On heating, therefore, the orientational dynamics of water in hydration shells ought to speed up more than the dynamics of bulk water. Again, this effect has been observed[8–10].

Davis *et al.* report another interesting effect for alcohols that have hydrophobic chains longer than 1 nm — at temperatures above 80 °C, the water surrounding the hydrophobic chains acquires a structure that is less ordered than bulk liquid water at the same temperature. This phenomenon has been predicted by theory[5], and brings us back to the effect of inserting a hydrophobic ball into water: for a large ball with a diameter greater than 1 nm, the curvature of its surface does not fit into the three-dimensional arrangement of the hydrogen bonds of water, and so causes hydrogen bonds near the ball's surface to break up[5]. There is thus a striking difference between small hydrophobic structures of about 0.5 nm in diameter, which enhance the surrounding hydrogen-bond network, and large hydrophobic structures greater than 1 nm in diameter, which break the hydrogen bonds of nearby water molecules.

These findings contribute to our understanding of the hydrophobic interaction — the tendency of hydrophobic groups to cluster together in liquid water. The hydrophobic interaction is one of the most important driving forces in nature, and is key to processes such as protein folding and the self-assembly of lipid membranes. The present study shows that the interaction of a solute with surrounding water is more than just the sum of local chemical interactions; the water's structure strongly depends on how well the solute fits into the water network. The structure of water surrounding a biomolecular solute will thus be the result of a complex interplay of the sizes and relative positions of the solute's hydrophobic and hydrophilic regions. It is to be hoped that future studies will shed light on these combined effects, and thereby pave the way to a full understanding of the conformational dynamics and aggregation of biomolecules in liquid water. ■

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REPRODUCTIVE BIOLOGY

Stem cells bear eggs

Researchers have coaxed cultured embryonic stem cells to develop into eggs that then give rise to normal offspring. The discovery should help to decode the molecular basis of gamete formation and might lead to treatments for infertility.

SIHEM CHELOUFI & KONRAD HOCHEDLINGER

Unlike testes, ovaries do not make gametes throughout life: female mammals are born with a limited supply of oocytes (eggs), which progressively decrease in number until the menopause. This biological barrier has complicated the study both of oocyte development in mammals and of the mechanisms underlying female infertility. Reporting in the latest issue of *Science*, Hayashi *et al.*[1] have induced embryonic stem cells to develop into mature oocytes that, when fertilized, give rise to apparently normal, fertile animals. Together with another report[2] by the same group on the generation of functional sperm cells from embryonic stem cells, these findings provide the first efficient and accessible protocols to recapitulate male and female gametogenesis from cultured stem cells.

Pluripotency denotes the capacity of cells to give rise to essentially all cell types of the body, including oocytes and sperm. Pluripotent cells normally reside in the early embryo, before and shortly after it implants in the uterus wall. The pluripotent state can be stably captured *in vitro* through the culture of pre-implantation-stage embryos called blastocysts to generate embryonic stem (ES) cells. Moreover, induced pluripotent stem (iPS) cells can be derived from differentiated cell types by introducing a cocktail of transcription factors[3]. Despite their different origins, iPS cells are highly similar to ES cells at molecular and functional levels.

Because of the developmental plasticity of ES and iPS cells, and the ease with which they can be derived from both mice and humans, stem-cell researchers have focused much of their attention on understanding how to instruct the cells to become therapeutically relevant, differentiated cell types *in vitro*. Indeed, scientists have been able to produce nerve cells, heart-muscle cells and gut cells, among others, from cultured pluripotent cells. However, it has been notoriously difficult to derive functional gametes from ES cells[4]. This is not surprising, because germ-cell maturation is a complex process of cellular, chromosomal and molecular changes that endow mature sperm and oocytes with the unique potential to give rise to an entire organism after fertilization.

In an experimental tour de force, Hayashi and co-workers[1] generated oocytes from ES and iPS cells by devising a multi-step procedure that mimics the events occurring in developing embryos (Fig. 1). Shortly after implantation, early gamete precursors, called primordial germ cells (PGCs), are specified within a layer of advanced-stage pluripotent cells called the epiblast, which is poised for differentiation[5]. In a first step, the authors identified a combination of growth factors that could efficiently generate epiblast-like cells from ES cells. The epiblast-like cells were then exposed to the growth factors that induce germ-cell formation *in vivo*, and so gave rise to PGC-like cells *in vitro*.

To reset the genome of germ cells for the next generation, PGCs have to lose certain genome-wide chemical modifications that control gene activity[5]. These epigenetic marks include DNA methylation of imprinted genes, which are expressed in a sex-specific manner from the maternal or paternal chromosomes, and have crucial roles in fetal growth. In developing oocytes and sperm, these marks are

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