

Water, Water Everywhere

Subtly shaping protein structure and function

Surreptitiously converting dead matter into oil and coal

By ELIZABETH PENNISI



For a substance essential to life, water seems remarkably commonplace: colorless, odorless, tasteless, always there. Though scientists frequently use it as a solvent and study it extensively

as a molecule in its own right, they often fail to recognize that water also functions as an agent of chemical change and molecular modification in living systems.

"Water is so obvious that we almost dismiss it," admits John A. Tainer, a crystallographer at the Scripps Research Institute in La Jolla, Calif. "It's taken for granted."

Yet Tainer and other researchers now argue that water is no innocent bystander in the chemistry of life. True, it seems insignificant when stacked up against many of the larger, more complex molecules within a cell. But just as a swarm of gnats can drive even quite large animals to distraction, water, too, may exert a powerful effect in its environment.

In fact, water molecules don't flit aimlessly about a protein, they strongly influence its three-dimensional structure.

"Water isn't just a solvent that the proteins float around in; it has a chemical role," explains Leslie A. Kuhn at Scripps. "Water molecules are really important in catalysis and in binding." Consequently, she and others think water deserves more consideration by scientists developing drugs designed to interact with proteins.

"Water is crucial to protein folding and protein function," says David Eisenberg, a crystallographer at the University of California, Los Angeles. "But finding ways to describe the interaction between proteins and water is an elusive goal."

This dynamic interaction arises from water's polarity. A water molecule's relatively large oxygen atom draws electrons from its two hydrogen atoms, imparting positive and negative charges to the ends of the molecule. This polarity enables water



just as everyone knows that oil and water don't mix, scientists know that organic reactions don't work well in aqueous solutions.

But to a small group of scientists studying how oil and coal form from carbon-rich decayed plants and algae, aqueous organic chemistry — reactions of carbon-based compounds in hot water — represents a better way of thinking about how the Earth created those vast underground energy reserves.

"We're promoting the idea that water is important in natural organic reactions," says Michael D. Lewan, a geochemist with the U.S. Geological Survey (USGS) in Denver.

In addition, sustained investigation into how hot water affects organic materials may lead to more efficient and environmentally friendly processes. Someday, water may aid in making — and recycling or cleaning up — plastics and other petroleum-based products.

Once again, this commonplace substance turns out to have some uncommon attributes. "We just take water for granted," says Theodore P. Goldstein, an organic chemist at Mobile Research & Development Corp. in Princeton, N.J. "We don't think its properties can change."

Little did he and others realize how mutable water can be.

Until now, scientists thought that coal forms when dying plants in soggy marshes get buried, creating a peat that simmers in this soupy environment. If no oxygen is present, chemical events slowly change peat, first into lignite and then, millions of

years later, into bituminous coal. If temperatures climb high enough, anthracite coal forms.

Oil formation was viewed similarly. Dead marine microorganisms sink to the seafloor, then become buried by silt



Watts Peterson

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to dissolve a wide array of substances in high concentrations.

Polarity also gives water the power to mold complex molecules. Often, such molecules possess both polar and nonpolar parts. The polar parts interact well with water, while the nonpolar, or hydrophobic, parts seek to avoid water. Thus, just as oil droplets mixed in water will merge into larger droplets, nonpolar parts also tend to cluster. They are, in a sense, "squeezed" together by the water around them, because water molecules prefer to associate with one another rather than with these nonpolar entities.

The polarity of water molecules also prompts them to align with their negative ends close to the positive ends of other molecules, and vice versa. Once aligned, weak interactions called hydrogen bonds tend to maintain this formation. Water's hydrogen bonds interact with many substances, including some of the amino acids that make up proteins. In this way, water affects protein structure.



Like other researchers, Kuhn, Tainer, and their colleagues Michael A. Siani and Elizabeth D. Getzoff at Scripps want to learn more about protein structure and water's influence on it. They cannot see these molecular gnats directly, so they have developed new ways to analyze X-ray diffraction data. "They [have created] a new computational tool that will be one of a whole group of tools that modern protein chemists will use," Eisenberg predicts.

The Scripps group studied data from 56 proteins whose crystal structures had been delineated by other scientists. The proteins' various amino acids — especially their side chains (SN: 1/30/93, p.72) — give shape to a protein surface. This topography of grooves, bulges, and bumps determines what target molecules link with each protein.

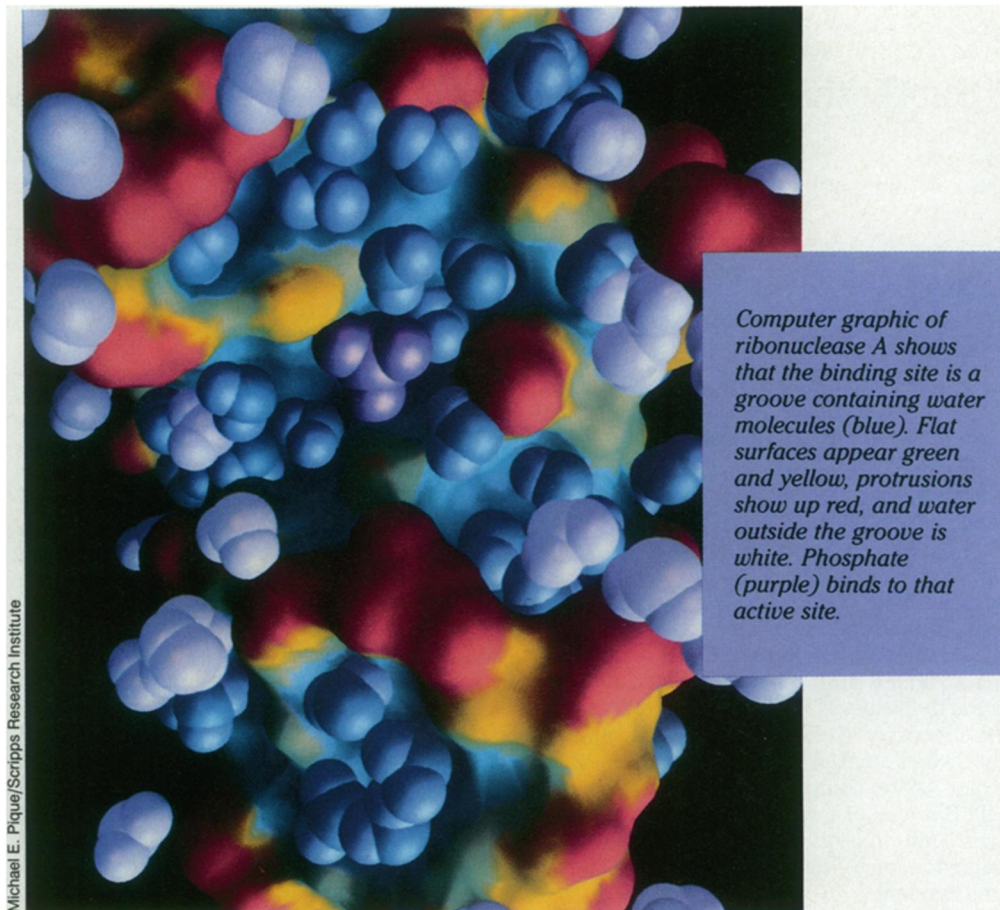
Water influences that landscape, especially when it snuggles up against the protein surface.

As proteins crystallize, their crystals trap water — in fact, water accounts for 27 to 77 percent of the crystals' volume. The Scripps researchers focused just on the

10,837 water molecules — from 56 to 690 per protein — that had nestled close enough to proteins to show up in the X-ray diffraction data every time. These are the water molecules most relevant to protein activity, Kuhn explains.

The Scripps scientists developed special computer-implemented algorithms for analyzing the interactions between bound water and a protein surface. With

point on the protein surface, using that point as the center of the sphere. It then counts the number of protein atoms in that sphere and derives the atomic density around the center point. Low densities occur at bumps or protrusions; deep grooves exhibit high atomic densities, says Kuhn. The researchers can then translate these numerical data into a map of surface topography.



Computer graphic of ribonuclease A shows that the binding site is a groove containing water molecules (blue). Flat surfaces appear green and yellow, protrusions show up red, and water outside the groove is white. Phosphate (purple) binds to that active site.

Michael E. Pique/Scripps Research Institute

one set of equations, they determine whether a molecule — water, in particular — can touch a protein surface at any given point, Kuhn explains. The second set takes a more global look at the protein and evaluates how tightly atoms pack at a particular spot.

The first algorithm works by placing an imaginary sphere at each point on a protein's surface, then expanding that sphere until it rubs up against another part of the protein. If that point happens to exist in a groove, then the sphere can swell no bigger than the width of the groove. But at the tops of bumps, the sphere can expand quite a bit, says Kuhn. This analysis generates a map of places where water can fit on the protein surface.

The second algorithm evaluates how crowded atoms are. The computer simulates an imaginary sphere around each



Basically, a protein looks like a globule, some areas of which are convoluted with deep grooves wide enough for one water molecule. The researchers found about three times as many water molecules on surfaces with deep grooves as elsewhere on the protein. Those grooves took up a quarter of the protein's surface area yet held onto half of all the water molecules on that surface, the scientists report in the Nov. 5, 1992 JOURNAL OF MOLECULAR BIOLOGY.

Other research indicates that water molecules hop in and out of the proteins' grooves at an incredible pace: more than 1 million times in a thousandth of a second. Nevertheless, water molecules in the grooves may form chains or networks

that extend throughout the protein surface. There, they can influence the fine-scale structure and function of the protein.

"The water molecules are stabilizing the surface topography," Tainer explains.

A protein's kinks and folds arise because of the particular sequence of its chain of amino-acid building blocks. Without water between them, two folds forming a groove might keep bending until they closed the groove. Thus, water keeps the grooves — and all the amino acids inside — accessible to target molecules. "And [grooves are] where the action is," Tainer adds.

Scientists know that side chains influence the water affinities of amino acids. Very polar side chains such as aspartic acid, glutamic acid, lysine, arginine, and to a lesser extent histidine seek a closeness with water. Leucine, isoleucine, and valine tend to avoid water molecules. But the Scripps results indicate that these affinities dominate only in grooves.

Inside grooves, water molecules seem quite picky about the amino-acid side chains they settle near. Negatively charged side chains of aspartate, for example, attracted an average of 9.3 water molecules per square nanometer, whereas valine's side chain lured fewer than one water molecule per square nanometer. Asparagine, serine, and glutamine, whose side chains both offer and

accept hydrogen bonds, bind more water than amino acids with positive side chains but fewer than those with negative charges.

Outside the grooves, however, those differences fade. "They all act similarly," says Kuhn. Aspartate kept about two water molecules nearby; valine still had slightly fewer than one. "Everyone else thinks that hydrophobicity is hydrophobicity, but it actually depends on the [protein] surface shape," Kuhn adds.

Another unexpected finding, says Tainer, is that peptide bonds, the backbone of the amino-acid chains, also interact with water molecules in the grooves — as much as or even more than the most water-loving side chains.

These relationships hold no matter how big the protein. Indeed, larger proteins contain more grooves and more water — and that water resides in the grooves, says Kuhn.



She and her colleagues have analyzed the water positions in similar enzymes found in very dissimilar organisms, including the superoxide dismutase enzymes found in yeast, cows, and humans. "Our preliminary data show that the water in grooves is conserved [across species]," says Tainer. Such a finding adds weight to the notion that the grooves play important roles in how a protein functions.

The grooves offer drug designers at-

tractive targets, or docking sites, for new therapeutic molecules, says the Scripps team. Often, scientists examine new drug structures by simulating the molecule with a computer. The Scripps results indicate that these simulations should include water molecules in key places, such as grooves; otherwise, the shape and chemistry will not be right, Tainer notes.

The presence of water may affect how quickly a protein can bind a drug, because the drug may first have to shoulder the water aside. Consequently, the binding part of the drug may need a stronger affinity for the protein's groove than water has. Or it may require an ability to attach to the protein with water still in the groove. Finally, the Scripps results indicate that the protein's chemistry may vary along its surface. "It's common knowledge that binding usually occurs in deep clefts or grooves," says Kuhn. "What people haven't known is that the chemistry of water is so different in the grooves. If water is different, then you'd expect that of other molecules, too."

Thus, the Scripps findings hint that more needs to be learned about the grooves in proteins and about how water, particularly water in grooves, alters the chemical preferences of proteins and thus their binding by other substances. Such understanding, says Eisenberg, "is the essence of designing new drugs." □



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washing out of a river. If enough silt piles up, it creates a geologic Dutch oven, in which high temperature and pressure cause the organic debris to condense. A source rock — oil shale — forms. In its pores, chemical processes continue until oil oozes forth. The key requirement is getting this “oven” hot enough for a long enough time — or so everyone thought.

These explanations did not satisfy Andrew Kaldor, a researcher at Exxon Research and Engineering Co. in Annandale, N.J. He realized that ideas about oil and coal formation had evolved many years ago and had not really been updated to include new chemical and biological knowledge.

So Kaldor and Exxon organic chemist Michael Siskin decided to reexamine these ideas by first determining the chemical composition of organic materials in source-rock shales — an awesome job given the complex and highly variable nature of this starting material and the cascade of molecular transformations that occurs in forming oil and coal.

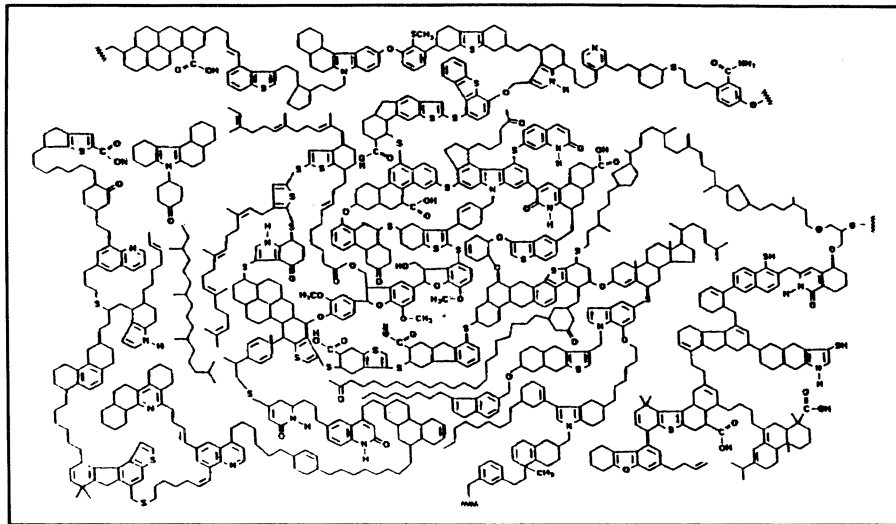
In both, plant matter decays into a potpourri of molecules that, depending on the conditions at hand, break up and clump in any number of ways. Carbon atoms get rearranged into assorted rings and chains to create a complex, interlocking network. Hydrogen atoms join, leave, and sometimes rejoin this network, as do other elements such as oxygen, nitrogen, or sulfur, eventually forming giant, insoluble macromolecules. “It’s everything that winds up in the sediments,” notes Goldstein.

Few chemists would even know how to begin teasing out the right combination of hydrocarbons to create oil or coal, but somehow nature manages to break these giant molecules in just the right places.



To understand this process better, the Exxon group collected samples of oil shale from different parts of the world. The samples included a series from oil shale under the North Sea, where rocks in different locations exhibit different degrees of transformation. Siskin then placed the samples into a pressurized reaction vessel and heated them individually to temperatures ranging from 570°C to 750°C. These hotter-than-natural conditions sped up the transformation from a geologic time frame of millions of years to one measured in days and hours.

Over the course of about two years, these and other tests helped the scientists piece together the locations of various atoms and side groups in representative molecular structures and in the intermediate products created in the



Siskin/Exxon

A summation of several years' work, this molecular structure is a statistical representation of the atoms and side groups in the organic matter that breaks down to form oil. Some of the linkages require water to disintegrate.

transformation from molecular glob to oil.

“Knowing the structures at different stages of maturation can help us see the pathways,” notes Siskin. He and Kaldor began to realize that heat alone could not break some of the bonds between these atoms.

To examine this inconsistency further, the scientists decided to look at “model” molecules — simple, commercially available hydrocarbon compounds that stood in for the organic matter in source rock or coal. Each represents a class of organic material — esters, amides, alkanes, for example — found in nature. The researchers wanted to see what conditions dissolved the linkages in these molecules. The experiments confirmed their suspicions.

“A lot of the molecules present in the structures we had developed were thermally not reactive,” says Siskin. Certain groups of atoms key to holding the macromolecules together just would not fall apart, no matter how hot the chamber got.

The results perplexed the Exxon scientists, because they knew these groups of atoms disintegrated naturally — and at lower temperatures than those used in the experiments. Also, coal formed under similar conditions. They began to eye water for answers.

“We knew that oil forms in an aqueous environment,” recalls Kaldor. “One natural question is, ‘Is the water really benign, or does it play another role?’”

As early as 1979, Lewan, then working for an oil company, and his colleagues had demonstrated that they could simulate oil formation in the laboratory, but only if they added water to their system. Without water, “the products were seldom like that in natural crude oil,” recalls Lewan. He and his colleagues then tried adding water to their source-rock samples. When they opened up their reactor at the end of the experiment, “we found a beautiful

layer of oil on top of the water,” he adds. He called this conversion hydrous pyrolysis.

Work at Exxon had also suggested aqueous influences on organic reactions. One group had discovered that water and carbon monoxide can enhance the liquefaction of certain types of coal. A different research team in Canada began using a mix of hot water and steam to increase the amount of heavy oil recovered from reservoirs buried under Cold Lake in Alberta. Also, Alan R. Katritzky, an organic chemist from the University of Florida in Gainesville, had decided to wet down some of his reactions to test whether water could help remove sulfur, oxygen, and nitrogen contaminants from ringed hydrocarbon compounds.



In 1985, Katritzky teamed up with Siskin to investigate water's role more intensively. “The folklore would have it that organic molecules will not react with water,” says Siskin. But the two researchers realized they could make water more amenable to organic materials by putting it under pressure and heating it up. As water molecules get hotter, they become less polar and so are more likely to interact with nonpolar organic molecules. At 300°C, water acts like the organic solvent acetone at room temperature, Siskin adds.

The hot water molecules also tend to break apart, splitting into positive hydrogen and negative hydroxyl (OH⁻) components. They become acidic and basic — and therefore much more reactive. Also, keeping the water under pressure ensures that it remains liquid; as such, “it can act as a solvent, it can act as a catalyst, and it can act as a reagent,” Siskin says.

Siskin and Katritzky started adding

water to their reaction vessels and experimenting with different combinations of heat, water, brine (salt water), minerals, and clay – conditions that might exist deep within Earth's crust. They discovered that high temperatures cause an organic molecule to break into fragments – and so does water and brine, sometimes more effectively.

"In these model systems, the results are that water is not always benign," Kaldor says. Some classes of organic molecules proved very susceptible to water's influence. In fact, water sometimes causes organic material to disintegrate into fragments that then transform into oil's hydrocarbons more readily than heat-induced fragments do.

The results indicate that hot water becomes a catalyst for a series of ionic reactions – creating a second pathway for the cascade of molecular transformations that leads to oil. The acidic and basic nature of hot water – rather than heat – drives this cascade.

For example, water may function first as a base, nibbling away at certain linkages in the organic material. As new molecular fragments build up and modify the reaction environment, water can change its catalytic nature. It can then act as an acid, accelerating different reactions. The resulting products attack parts of the remaining molecules, further speeding the breakdown. Siskin and Kartzky described these processes in the Oct. 11, 1991 *SCIENCE*.

"What we have learned is that ionic chemistry predominates in most cases and opens up pathways not accessible by the thermal route," Kaldor says.

These results bolster findings obtained by Lewan and complement research in hydrous pyrolysis by providing some details about the chemistry that could be occurring during these transformations. "It opens up the idea that you can't focus on one aspect, the organic aspect, of oil formation," says Goldstein. "You've got to focus on the chemistry of the whole system."

Lewan, too, has continued his investigations into the role of water. From his perspective, hydrous pyrolysis takes place because small amounts of water trapped in pores in source rock become awash in organic molecules, not because organic material dissolves in aqueous solution. Like Siskin, he runs his experiments at higher temperatures than exist naturally in order to speed up the process. Some of Lewan's ideas about the details of water's role differ from those of the Exxon group. Nevertheless, these and other results are building a convincing body of evidence.

"Over the past 10 years within the organic geochemistry community, it's been very controversial whether water is important," says Lewan. "I think what we'll argue over the next 10 years is *how* it's important."



Whatever the actual mechanism, the fact that water plays a role could wreak havoc on established ideas about oil formation. The results suggest that oil can mature faster than previously thought, says Kaldor. Consequently, not only do the ideas buck tradition, but, if right, they will require the revision of time parameters in computer programs now used to predict locations of new reserves. For that reason alone he expects the oil-exploration community to accept these ideas slowly.

"We have not convinced Exxon geoscientists that the model is correct," Kaldor says.

"But these experiments are making believers out of them," Lewan adds, citing his work as well as that of Exxon.

To help convince their colleagues that such revision is warranted, Kaldor and Siskin are seeking ways to verify their ideas. "You can't do real-time experiments," Kaldor says, "so you have to begin to look for clues in the natural environment." Those clues could be molecules that form only through water-initiated ionic reactions, for example. "But we don't have that yet," he says.

Meanwhile, the concepts that have come out of this work may have broad impact. "We started this whole quest with just an interest in oil," Kaldor says. "But it really has paid off in a generic way in natural processing. It enables people to begin to do things they wouldn't be able to do before."

Already, Exxon foresees the possibility of using hot water to introduce more hydrogen into coal – to make it more amenable to liquefaction and to reduce the cost of this process. Also, hot water and steam might help add hydrogen to low-quality oil deposits, improving and loosening the oil from pores in source rock so it will move easily to the surface.

Hot-water chemistry promises to aid other chemical processes as well. For example, it could increase the efficiency of the production of isopropyl alcohol by providing a way for a waste product, an ether, to be converted into more alcohol. Hot-water processing also offers ways to break down petroleum-based materials that might otherwise contaminate the environment. Even the U.S. Army has expressed interest in aqueous organic chemistry – as a way to destroy chemical warfare agents. In other instances, the use of hot water may eliminate the need for other catalysts that prove difficult to dispose of safely. "It opens up an entire area of synthetic chemistry," Goldstein says.

"We're at the stage where it's becoming more and more routine," adds Kaldor. "My guess is that in the next few years, we'll see commercial applications." □

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