Targeting a metabolic sensor. Many compounds that activate AMPK were first identified as active components of herbal medicines. Several (purple) probably act on mitochondria to increase concentrations of AMP and ADP, which bind the γ subunit. The small molecule A769662 and salicylate (blue), the active derivative of aspirin, activate AMPK through a mechanism involving the AMPK β subunit (blue).

metformin, all of which are proposed to lower intracellular ATP amounts by inhibiting complex I of the mitochondrial electron transport chain (2). Metformin is derived from a compound found first in a medicinal plant, the lilac *Galega officinalis*. By the same proposed manner, other plant products, including resveratrol from grapes and red wine, epigallocatechin gallate from green tea, and capsaicin from peppers, activate AMPK (2). In an interesting parallel with those natural products derived from herbal medicines, Hawley *et al*. show that AMPK is also activated by the plant product salicylate.

The connection between diabetes drugs and AMPK activation, and AMPK's control of hepatic and muscle glucose and lipid homeostasis through phosphorylation of metabolic enzymes and transcriptional regulators (6), has prompted interest in designing AMPK activators (7). However, identifying direct kinase activators is far more difficult than identifying ATP-competitive inhibitors of kinases. To date, A769662 is the only well-established direct small-molecule activator of AMPK (8). It was discovered through a screen of over 700,000 compounds for agents that activate purified AMPK in vitro. A769662 does not act by mimicking AMP or ADP binding, but by a mechanism involving the β1 subunit of AMPK (9–11). The related β2 subunit can restore AMPK responsiveness to energy stress in β1-deficient cells, yet this heterotrimer cannot be activated by A769662. Haw-

ley *et al*. show that salicylate activation of AMPK also requires the β1 subunit.

Hawley *et al*. deciphered which effects of salicylate require AMPK activation by using mice that were genetically engineered to lack the β1 subunit. Although AMPK β2 can compensate for all the critical functions of AMPK β1 in mouse development and physiology, AMPK β2 cannot be activated by salicylate, though all the other targets of salicylate such as IκB kinase and c-Jun N-terminal kinase remain normal and responsive in these mice. Both salicylate and A769662 induced a switch from carbohydrate to fat utilization in mice; this effect was abolished in the AMPK β1-deficient mice. The authors further demonstrate that fatty acid oxidation by salicylate requires AMPK activation, but thatother effects on blood glucose and insulin concentrations after prolonged salicylate treatment do not require AMPK.

Future studies should carefully delineate, in different physiological and pathological settings, which effects of therapeutic doses of aspirin and related compounds rely on AMPK activation as compared to other pathways affected by salicylate that are independent of the kinase. This is especially relevant given that aspirin and other AMPK activators such as metformin have been linked to lowered cancer risk (12, 13), and the ongoing examination of whether salicylate derivatives show clinical potential as antidiabetics (14).

A full mechanistic understanding of how salicylate activates AMPK through the β1 subunit awaits the isolation of the three-dimensional x-ray crystal structure of salicylate bound to AMPK heterotrimers, as is also needed for the A769662 compound. That information should make it possible to design activators of this kinase that have higher affinity, greater selectivity, and improved pharmacological properties. Most of the current AMPK activators target the nucleotide-binding pockets of the γ subunit. An interesting possibility is that agents that activate AMPK through its β subunits may synergize with γ-targeted agents. No doubt, this ancient sensor has more mysteries yet to reveal.

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CHEMISTRY

Pinning Down the Water Hexamer

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An experimental study resolves a long-standing controversy about isomeric forms of the water hexamer.

Water comes in numerous forms, from isolated clusters to at least one liquid form and 17 known forms of ice. The detailed characterization of water clusters is essential for developing more accurate and detailed models for describing all these different forms. The comparatively simple hydrogen-bond structures in small water clusters help to eluci-

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date the much more complicated cooperative hydrogen bonding in liquid water (1), which is difficult to reproduce quantitatively from simplified models. The water hexamer has received particular attention as the smallest representative of multicyclic three-dimensional hydrogen-bonded structures. On page xxxx of this issue, Perez *et al*. (2) report a landmark experimental study that resolves some of the controversies regarding the relative energies of water hexamer isomers.

Calculations of water properties and dynamics typically use a potential energy surface (PES), which gives the energy of a mole-

cule as a function of its geometry. The PES can either be calculated from first principles (ab initio) or with empirically derived functions. Calculations of important water properties, such as tunneling splittings and hydrogen-bond vibrational frequencies, require a PES that can accurately reproduce large amplitude motions far away from local minima. This is a severe test for even the most extensive ab initio calculations. Popular empirical potentials for water can give qualitatively incorrect results even for the cluster structures. For example, the simple point charge extended (SPC-E) model incorrectly predicts the lowest-energy water pentamer and hexamer to be tetrahedral (*3*), and a three-site transferable intermolecular potential (TIP3P) erroneously gives all-atom planar global minima for the water trimer and tetramer.

Precise measurement of the hydrogen-bond vibrational frequencies and the associated tunneling has been accomplished for the water dimer through hexamer by far-infrared (terahertz) vibration-rotation-tunneling (VRT) spectroscopy and interpreted from the calculated rearrangement pathways (*4*, *5*). These small clusters sample the canonical hydrogen-bond interactions (electrostatics, induction, dispersion, and exchange repulsion) over a wide range of structures, including the linear dimer, the cyclic trimer, tetramer, and pentamer, and the cage geometry of the hexamer. Experiments and ab initio calculations agree very well up to the pentamer. However, there has been controversy regarding the energetic ordering of the low-lying isomers of the hexamer (*2*). It is important to resolve this ambiguity, because the hexamer represents the transition from monocyclic "two-dimensional" to multicyclic "three-dimensional" structures, and global optimization studies indicate that clusters larger than the hexamer all favor such concatenated ring geometries, usually with a number of competing low-energy minima.

According to the tunneling splittings, rotational constants, and torsional vibrations from terahertz VRT spectroscopy, the lowest-energy form of the water hexamer is a "cage" isomer, whereas ab initio calculations and empirical potentials have identified three or four low-energy forms, with most studies identifying a "prism" as the global minimum on the potential surface (*6*). In fact, the highest-level ab initio calculations predict four relatively low-lying cage isomers, but none of them appears to have any low-barrier, single-step rearrangements between permutational isomers (*7*, *8*), which is the usual condition for tunneling splittings to be observed. However, quantum dynamics calculations incorporating only the most important degrees of freedom predict that the lowest vibrational wave functions can be more or less delocalized over the four cage isomers, depending on the intermolecular potential (*7*, *8*). Assignment of the VRT spectrum to a single isomer for the ground vibrational state is thus not inconsistent with the observed tunneling, if the excited states involve different cage isomers. Clearly this system represents a delicate case for theory, in terms of both energetics and dynamics, and both enthalpic and entropic factors must be carefully considered, even at the low temperatures achieved in supersonic expansions.
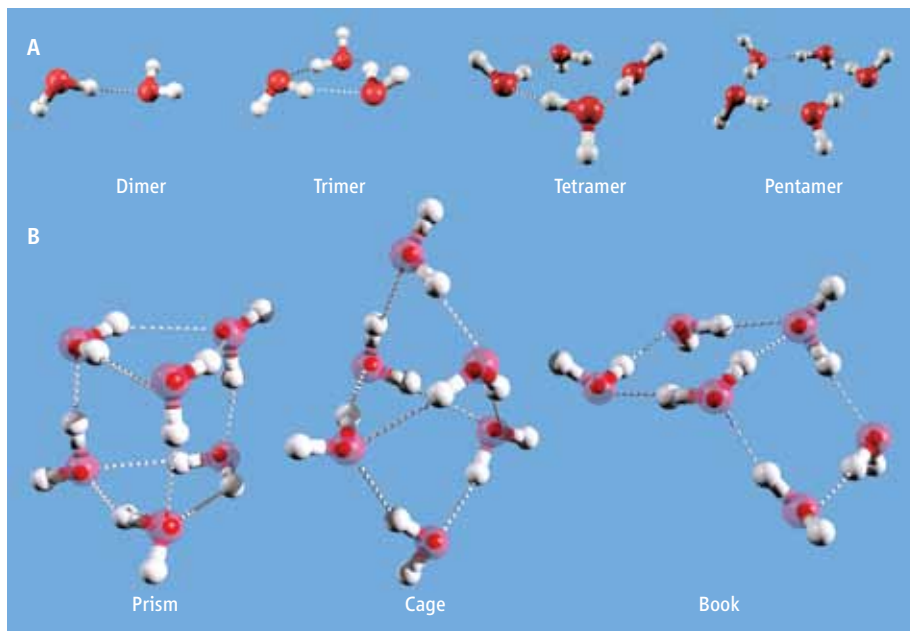
Perez *et al.* have now measured the microwave spectrum of the water hexamer. They unambiguously establish the structures and energy ordering of the lowest three isomers (see the figure) by isotopic substitution and temperature variation. The authors also observe rotational spectra of the heptamer and nonamer, but not of the octamer; this is consistent with predictions of a cuboidal octamer structure, which is nonpolar and therefore not detectable in these experiments.

These results were obtained with the powerful broadband Fourier transform method pioneered by Pate and co-workers (*9*) and comprise the first microwave detection of a water cluster larger than dimer, for which spectra were first reported in 1980 (*10*).

The impressive signal-to-noise ratios obtained by Perez *et al.* promise even more exciting studies of other benchmark water clusters. Along with other new developments, such as the accurate measurement of cluster dissociation energies (*11*), this work will facilitate the long-sought-after "universal" model for water, from which a calculation could accurately predict properties of water in all of its forms (*12*). If such a representation could be found, it would provide a straightforward route to resolving some of the many controversies (*13*) that continue to arise regarding the nature of water.

Hydrogen bonding in small water clusters. Theory and experiment generally agree regarding the global minimum structures for the water dimer through pentamer (**A**), but not for the hexamer, which represents the transition from monocyclic single donor–single acceptor geometries to multicyclic three-dimensional structures. Perez *et al.* report the experimental determination of the structures and relative energies of the lowest three hexamer isomers (**B**). Precise characterization of these archetypal examples of aqueous hydrogen bonding advances the quest for an accurate "universal" model of water that applies under all conditions of interest.

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