

## WATER AND BIOLOGICAL TIME KEEPING

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Our laboratory has investigated extensively the role of water molecules in the control of the activity of a homodimeric, growth-related and time-keeping hydroquinone oxidase (ENOX1) of the eukaryotic cell surface capable of oxidizing externally supplied NAD(P)H. The protein has a binuclear copper center and exhibits characteristics of an ultradian driver of the biological 24 h circadian clock including a defining recurrent 2 +3 set of oscillations with a period length of 24 min. The period length is temperature independent and entrained by light, melatonin and low frequency EMF. Mutant proteins with period lengths of 22, 36 or 42 min transfected into mammalian COS cells yield circadian periods of 22, 26 or 42 h. The oscillations not only require bound copper but are recapitulated in solution by copper salts. Period lengths of both the ENOX1 proteins and of the copper II oscillations are increased from 24 min to 30 min by solvation in D<sub>2</sub>O in keeping with the characteristic 30 h circadian day of organisms grown in D<sub>2</sub>O. Our hypothesis supported by experiment is that the oscillations are inherent in the protein-associated copper II hexahydrate and derive from periodic variations in the ratios of ortho and para nuclear spins of the paired hydrogen or deuterium atoms of the elongated octahedral structure of the protein-bound copper hexahydrate. This is the only microscopic periodic process that occurs on a similar time scale. Interconversion of the ortho and para isomers in liquid water requires from several min to several h to reach a 3:1 ortho to para equilibrium ratio in ortho or para-enriched samples depending on the ionic composition of the medium. Tikhonov and Volkov enriched a sample to a 10:1 ortho-para ratio, and a 18.6 min period was present in the decay kinetics of ortho water. A central question is why the system does not simply reach equilibrium and remain there. What might prevent that from occurring? Many materials preferentially absorb para water due to its non-rotation ground state. The apparent difference in energy between the two states is a significant 1-2 kJ mol<sup>-1</sup> far greater than expected from spin-spin interactions. One possibility is that populations of water molecules spontaneously form some type of collective order whose structure subtly favored formation of ortho water, i.e., exclusion zone water. If so, that water exists as a collectively coherent mode like a laser beam could become an important consideration. Experiments with coherent vs. incoherent water have given no indications that water coherence effected the oscillations. Our current model is that of a limit cycle oscillator where the amplitude tends to be more or less constant but the frequency can vary greatly. Control of heart beat is a classical limit cycle oscillator that exhibits characteristics that parallel the ortho-para oscillatory cycle of liquid water.

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## HEART BEAT RATE MODEL FOR PERIODIC SHIFTS BETWEEN ORTHO AND PARA WATER THAT MAY REGULATE THE CELL'S BIOLOGICAL CLOCK

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A natural oscillatory cycle inherent to liquid water is proposed as the basis for the cell's biological clock. Evidence has evolved experimentally from a clock-related protein-associated copper hexahydrate. The likely candidate resides in instabilities in the water molecule itself, whose two hydrogen nuclei undergo ordered periodic shifts between ortho and para states. However, there is no clear mechanism as to how the ratio of ortho to para water is maintained in a steady oscillation. As ortho water forms, it might locally add to the field generated by the collective order which would further favor the accumulation of ortho water for some period of time. But then it seems that the initial condition favoring ortho water formation as a result of this process produces an increased potential which can accrue but cannot be released or discharged until it achieves some minimum threshold of activation energy. Once the minimum threshold is achieved, there is a discharge of potential and the conditions favorable to para water result and the process now runs for a time in the opposite direction until some threshold level of para water is achieved and the cycle reverses. A defining characteristic of the clock-related oscillations is a characteristic 2 + 3 pattern of oscillations. Within each repeat of 5 oscillations, two are more widely spaced than the remaining 3. Heartbeat rate is an example of a limit-cycle oscillation in that the frequency of beats varies widely, while each individual beat continues to pump about the same amount of blood. Control of heart beat has its origins in a group of specialized cardiomyocytes (heart muscle cells) called the sinoatrial node or primary pacemaker. The action potentials that propagate along the plasma membranes of these excitable cells are generated by a sequence of changes in electrical potential between the interior of the cell and the surrounding extracellular space. The key to the rhythmic firing of pacemaker cells that makes it an attractive model of ortho-para water oscillations is that, unlike other muscles cells and neurons, the pacemaker cells slowly depolarize by themselves. Depolarization, a reduction in the degree of electronegativity in a resting cells (the cells become more positive) occurs when positively charged sodium and calcium ions enter the cytosol to generate inward ionic currents. Depolarization continues until the threshold potential is reached between -40 mV and -50 mV. When threshold is reached, the cells enter phase 0 or upstroke. Following upstroke, the cardiomyocytes repolarize. Repolarization, the return to resting potential, occurs when outward currents restore the membrane potential to its resting level of electronegativity. In the heart, repolarizing currents are generated largely by potassium efflux, possibly supplemented by the inward movement of chloride ions. In order to create the asymmetric 2 + 3 pattern, there must be a regular delay or hold every fifth oscillation. Injection of a brief stimulus pulse will reset the spontaneous periodic activity. A stimulus delivered early in the cycle generally delays the time of occurrence of the next action potential. How this is achieved in the heart is unclear but remains as an important precondition to explain the observed 2 + 3 pattern of ortho/para fluctuations.

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