

Oscillations of the Ortho/Para Ratio of Liquid Water Drive the Ultradian Regulator of the Cell's 24 h Biological Clock

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Our laboratory has investigated extensively 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 recurring complex 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 require bound copper and 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 D₂O in keeping with the characteristic 30 h circadian day of organisms grown in D₂O. 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 18.6 min period was present in the decay of ortho water. This time scale corresponds with the independently measured period length in water's absorption of x-rays at a copper k-edge of 8.9. Data analysis indicated that the bond angles between the equatorial and axial oxygen atoms of the copper (II) hexaaqua ion changed with the same characteristic five-peak pattern as redox potential and NADH oxidation. These results suggest that a ca. 18 min period to ortho-para conversions is inherent in water itself. In the presence of copper salts, the period length is increased to 24 min. Extremely low frequency (ELF) magnetic fields result in phasing the ultradian time keeping mechanism. The target of ELF magnetic fields again appears to be water. Why the ortho-para isomer compositions should exhibit a regular pattern of oscillations from their equilibrium ratio of 3:1 remains unexplained. However, such variation would provide a physical rather than chemical basis for the oscillatory behavior of the redox potential and catalytic activity of aqueous solutions to explain our time keeping model and, at the same time, account for the temperature independence of the circadian period length.

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