Stable Isotope Probing of microbial metabolic water using $\delta^{18}O_P$ of DNA

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It is generally accepted that intracellular water is isotopically identical to extracellular water, due to the near-instantaneous and unimpeded transport of water across cell membranes. However, isotopic measurements of intracellular water extracted from microbial cells indicate that metabolism produces isotopically distinct water, which can account for as much as 70% of intracellular water [1, 2]. Our recent studies of the O isotopic composition of PO_4 in microbial biomolecules (e.g. DNA) also indicate a significant contribution of non-extracellular water (e.g. metabolic water) to the intracellular water pool. Our observations are based on PO_4 in DNA, which has been shown to be a powerful probe of intracelluar water and like bioapatites (e.g., bones, shells, teeth), also records temperature and O-isotope exchange between PO_4 and water [3]. We hypothesize that this is due to the effect of pyrophosphatase--an intracellular enzyme that catalyzes complete exchange of all 4 O's in PO_4 with ambient water [4] --on the intracellular dissolved PO_4 pool from which both phosphatic biomolecules and bioapatites are synthesized. When *Marinobacter aquaeolei* was grown in media made using 3 different ^{18}O-labeled waters at 3 different temperatures, the $\delta^{18}O_p$ value of PO_4 in DNA plotted against $\delta^{18}O$ values of extracellular water gave a slope of 0.65, indicating that ~35% of O in DNA-PO_4 is not derived from the extracellular water in the growth medium. Results from these studies suggest the need to determine the percentage and isotopic composition of any possible metabolic water component of intracellular water for isotope probing studies of cellular processes and cellular biomass. These findings further suggest that previous studies based on O isotopic compositions of biogenic minerals (e.g., carbonates, apatites) that assume isotopic equilibrium between these biominerals and intracellular/body water, which has also been assumed to have the same isotopic composition as extracellular water, may need re-evaluation.

[1] Kreuzer-Martin *et al* (2005) *PNAS* **102**, 17337-17341. [2] Kreuzer-Martin *et al* (2006) *Biochemistry* **45**, 13622-13630. [3] Blake *et al* (2014) *PNAS*, submitted. [4] Blake *et al* (2005) *Am. J. Sci.* **305**, 596-620.