# Synthesis of isotopically labelled substrates for KIE studies of human GDP-L-fucose synthase

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Human GDP-l-fucose synthase (hGFS)[1] converts GDP-4-keto-6-deoxy-d-mannose into GDP-l-fucose in three distinctive steps: two epimerisations at C3" and C5" positions of the l-fucose sugar ring followed by NADPH-dependent reduction at C4". Currently, the identity, and the precise roles in catalysis, of active-site residues of hGFS are not fully understood. In this research, therefore, we decided to probe the hGFS reaction by kinetic isotope effects (KIEs), in order to dissect the multistep catalytic mechanism of the enzyme[2, 3].

For kinetic isotope effect measurements, we first show enzymatic synthesis and isolation of site-selectively deuterium labeled substrates: (4S)-[$^2$H]-NADPH, [3"-$^2$H]- and [5"-$^2$H]-GDP-4-keto-6-deoxy-d-mannose. Then, we used a combination of steady state and stopped flow kinetic measurements to obtain KIEs on the turnover number ($k_{cat}$) and the first-order rate constant of the kinetc transient. Using the wildtype hGFS, KIEs due to [3"-$^2$H] and [5"-$^2$H] are not different from unity, indicating that the proton abstractions for epimerisation at the C3" and the C5" are not rate-determining at steady state. The reduction appears to be only partially rate-determining due to (4S)-[$^2$H]-NADPH (KIE $\simeq$ 1.4). Stopped flow experiments reveal a rapid burst of NADPH consumption, suggesting that the $k_{cat}$ is limited by release of the product. Measured KIEs for several active site mutants support the notion that Y143 is involved in hydride transfer for reduction and the pair of general-base and general-acid catalytic residue for epimerisations is represented by C116 and H186.

Bibliographic references:

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