Elucidation of the exquisite reaction selectivity of human GDP-fucose synthase

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L-Fucose is found in a variety of important glycoconjugates in mammalian cells[1]. GDP-fucose (GDP-Fuc) is biosynthesized in two enzymatic steps catalyzed by GDP-mannose-4,6-dehydratase[2] and GDP-Fuc synthase (GFS)[3]. GFS catalyzes the conversion of GDP-4''-keto-6''-deoxy-mannose (GDP-4k6d-Man) into GDP-Fuc using three reactions within a single active site: epimerizations at C-3'' and C-5'', and a NADPH-dependent reduction at C-4''[3]. The mechanism that controls conformational changes is currently not understood. We demonstrate evidence for stereochemical control of GFS by employing a multidisciplinary approach of structural, biochemical and computational analysis.

Solvent derived deuterium incorporation into GDP-4k6d-Man and GDP-Fuc assessed the timing of the epimerization steps, revealing that the first epimerization occurs at C-3" and second one at C-5". C116 acts as a base and H186 as an acid responsible for epimerization. Y143 is the proton donor involved in the final reduction. Crystal structures of hGFS in complex with GDP-Fuc or GDP-4k6d-Man show the deoxy-hexose moiety well positioned for epimerization. However, the conformation is incompatible with C-4" reduction indicating a need for substrate repositioning. QM/MM simulations showed that the sugar conformation switch during catalysis is coupled to a change in protonation states of C116, H186. For each catalytic step a distinct substrate conformation is adopted which is strictly controlled by GFS. Together these results fully resolve reaction mechanism and elucidate the underlying mechanism of hGFS reaction selectivity.

Bibliographic references:

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