THE CONFORMATIONAL FREE ENERGY LANDSCAPE OF *β*-XYLOSE REVEALS A TWO-FOLD CATALYTIC ITINERARY FOR β-XYLANASES

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Unraveling the conformational catalytic itineraries[1] of glycoside hydrolases (GHs) is a growing topic of interest in glycobiology, with major impact in the design of GH inhibitors. β-xylanases are responsible for the hydrolysis of glycosidic bonds in β-xylans, a group of hemicelluloses of high biotechnological interest that are found in plant cell walls. The precise conformations followed by the substrate during catalysis in β-xylanases have not been unambiguously resolved, with three different pathways being predicted from structural analyses. In this work, we compute the conformational free energy landscape (FEL) of β-xylose to predict the most likely catalytic itineraries followed by β-xylanases. The calculations are performed by means of *ab initio* metadynamics, using the Cremer-Pople puckering coordinates as collective variables.[2] The computed FEL supports only two of the previously proposed itineraries, $^{2}S_{O} \rightarrow [^{2,5}B]^{\ddagger} \rightarrow {}^{5}S_{1}$ and $^{1}S_{3} \rightarrow [^{4}H_{3}]^{\ddagger} \rightarrow {}^{4}C_{1}$, which clearly appear in low energy regions of the FEL. Consistently, $^{2}S_{O}$ and $^{1}S_{3}$ are conformations preactivated for catalysis in terms of free energy/anomeric charge and bond distances. The results[3] however exclude the $^{O}E \rightarrow [^{O}S_{2}]^{\ddagger} \rightarrow B_{2,5}$ itinerary that has been recently proposed for a family 11 xylanase.[4] Classical and *ab initio* QM/MM molecular dynamics simulations reveal that, in this case, the observed ^{O}E conformation has been enforced by enzyme mutation. These results add a word of caution on using modified enzymes to inform on catalytic conformational itineraries of glycoside hydrolases.

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