MECHANISM OF ORLISTAT HYDROLYSIS BY FATTY ACID SYNTHASE THIOESTERASE

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Fatty acid synthase (FASN) is the sole protein capable of *de novo* synthesis of free fatty acids. The fatty acid synthesis cycle begins with the condensation of acetyl-CoA and malonyl-CoA, and continues with the elongation of the fatty acid chain, which is tethered to an acyl carrier protein domain (ACP), via a repeating cycle. At the end of elongation, the thioesterase (TE) domain of FASN cleaves the bond between the fatty acid and ACP, releasing the fatty acid. FASN has been found to be over-expressed in a wide variety of human cancers, and this over-expression is correlated to a higher metastatic potential and poorer prognosis in cancer patients. Orlistat, an FDA approved drug for obesity treatment, is a compound found to reversibly inhibit FASN TE by covalently binding to the active site serine within the TE domain. In crystal structure studies, a hydrolyzed form of orlistat can also be observed in the active site of TE, demonstrating that orlistat is not a stable inhibitor of FASN. In this study, we examined the mechanism of orlistat hydrolysis within the TE domain of FASN using molecular dynamics simulations. We found that the hexanoyl tail of orlistat is capable of shifting while covalently bound to the active site serine, and that this shift is accompanied by the destabilization of a hydrogen bond that exists between a hydroxyl moiety of orlistat and the active site histidine, allowing a catalytic water molecule to enter the active site with the proper orientation for catalysis of the covalent bond between orlistat and serine. These findings suggest that the hexanoyl tail of orlistat plays an important role in its hydrolysis and may guide the future design of new inhibitors that target the TE domain of FASN with greater endurance for potential use in the treatment of cancer.

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