Rationalizing the classical Koshland retention mechanism of linamarin hydrolysis with transition state modelling using quantum mechanics

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Epidemiological studies have shown a link between low incident of cancer in communities where cassava is the main staple. The prophylactic action of cassava intake and its deleterious effect on humans are attributed mainly to the toxicity of the aglycon moiety of the cyanogenic glucoside "linamarin", when the latter breaks down. Particularly this study was focused on validating the proposed mechanism by Koshland *et. al.* for glycoside hydrolysis reactions towards hydrolysis of "linamarin". Quantum mechanics calculations in gas phase at B3LYP/6-311+G** level of theory for a simplified model of β-glucosidase active site with linamarin as the substrate, clearly indcated a stepwise mechanism for glycosylation. The rate determining step is nucleophilic substition by Glu373 to form the covalently bonded enzyme substrate intermediate with protonation of the leaving group by Glu165. The geometrical configuration of the transition state for the enzymatic reaction was essentially the same as that found for a gas-phase model involving only the substrate and propionate/propionic acid pair to represent the catalytic glutamate/glutamic acid groups. The model estimated a reaction barrier of 29.65 kcal/mol while the reaction energy being 15.25 kcal/mol for glycosylation step of linamarin. Furthermore it was found that the rate determining step is the first step owing to a higher barrier than the second thus rationalizing the proposed Koshland retention mechanism. The mechanistic insights gained are valuable for not only understanding similar reaction mechanisms but also for rational design of novel linamarin analogs as potent anti-cancer drugs.

Keywords: density functional theory, cyanogenic glycoside, oxocarbenium ion