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DFT study on classical Koshland retention mechanism of Linamarin hydrolysis

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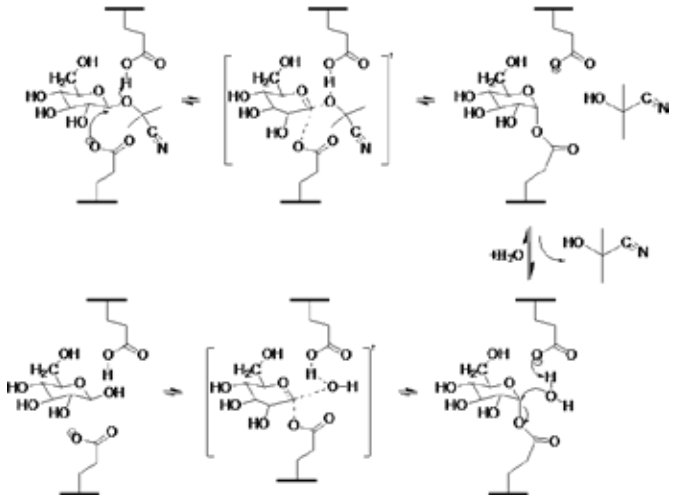

Figure 1. Geometries of transition states (TSg and TSdg) for the glycosylation and deglycosylation of double displacement retaining mechanism for hydrolysis of β-glycosides.[1]

The hydrolysis of glucosidic linkage catalyzed by every carbohydrate-hydrolase is a reaction in which the product retains (α→α or β→β) or inverts (α→β or β→α) the anomeric configuration of the substrate. By releasing α-glucose and β-glucose, respectively, from the common substrates having α or β -glucosidic linkages, α-glucosidase and β-glucosidase are essentially distinguished. The distinction in the substrate specificities of the two enzymes was explained by the subsite affinities in their active sites.2,3 Amino acid sequences of the regions containing the catalytic sites in β-glucosidases from various sources were compared. Particularly this study was focused on validating the proposed mechanism by Koshland et al1 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 indicated a stepwise mechanism for glycosylation. The rate determining step is nucleophilic substitution 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. A reaction barrier of 29.65 kcal/mol was estimated by the model with the reaction energy being 15.25 kcal/mol for the 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.

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Keywords

Density functional theory, Cyanogenic glycoside, Oxocarbenium ion, retaining glycoside-hydrolase

References

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