

## ENZYMES RELATED WITH FORMATION AND DEGRADATION OF CYCLOALTERNAN

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Cycloalterenan (CA) is a cyclic tetrasaccharide having the structure of *cyclo*{→6)-α-D-Glcp-(1→3)-α-D-Glcp-(1→6)-α-D-Glcp-(→3)-α-D-Glcp-(1→)}. It was found as an alternan degrading product by an enzyme produced by *Bacillus* sp. NRRL-21195 [1].

The cycloalternan-forming enzyme (CAFE), a 117 kDa protein, was partially purified by affinity chromatography using isomaltose-gel accompanied by a 140 kDa protein [2]. The partially purified preparation had ability to form CA from maltooligosaccharides [3]. The 140 kDa protein was found to be a disproportionating enzyme (DE) that catalyzes transfer of a D-glucopyranosyl residue from the non-reducing end of one maltooligosaccharide to the non-reducing end of another, forming an isomaltosyl residue at the non-reducing end. CAFE then transfers the isomaltosyl residue to the non-reducing end of another isomaltosyl maltooligosaccharide, to form α-isomaltosyl-(1→3)-α-isomaltosyl-(1→4)-maltooligosaccharide, and subsequently catalyzes a cyclization to produce cycloalternan. Thus, DE and CAFE act synergistically to produce cycloalternan directly from maltodextrin or starch.

We found and purified an intracellular enzyme hydrolyzing CA, cycloalternan-degrading enzyme (CADE), from the cell pellet of *Bacillus* sp. NRRL B-21195 [4]. The enzyme hydrolyzed CA into isomaltose *via* an intermediate, α-isomaltosyl-(1→3)-isomaltose. This enzyme also hydrolyzed panose. Neither maltooligosaccharides nor isomaltosaccharides were hydrolyzed.

The biochemical role of CA is supposed to be involved in a starch utilizing system described in the figure. The synergistic reaction of CAFE and DE first produces cycloalternan from starch outside the cells. Then, the resultant cycloalternan is transported into the cells by a cycloalternan transport system. The transported cycloalternan is finally degraded into isomaltose intracellularly by the action of CADE.

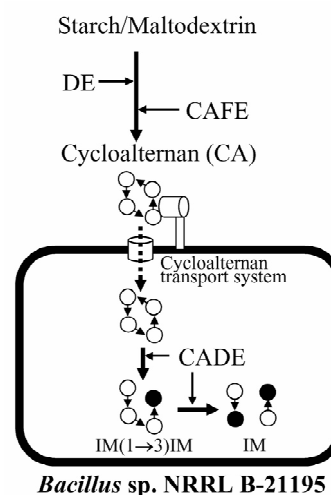


Fig. Starch utilization via CA

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[2] Ahlgren, J. A.; Côté, G. L. *J. Ind. Microbiol. Biotechnol.* **2003**, *30*, 114-117.

[3] Kim, Y.-K.; Kitaoka, M.; Hayashi, K.; Kim, C.-H.; Côté, G. L. *Carbohydr. Res.* **2003**, *338*, 2213-2220.

[4] Kim, Y.-K.; Kitaoka, M.; Hayashi, K.; Kim, C.-H.; Côté, G. L. *Carbohydr. Res.* **2004**, *339*, 1179-1184.