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Determinants of substrate specificity in chitin oligosaccharide deacetylases

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Chitin processing, mainly in the form of depolymerization and de-*N*-acetylation reactions, generates a series of derivatives including chitosan and chitooligosaccharides (COSs), which play remarkable roles in nature. COSs are particularly apparent in molecular recognition events, including the modulation of cell signaling and morphogenesis, the immune response, and host-pathogen interactions. Most of the biological activities associated with COSs seem to be largely dependent on the degree of polymerization and the specific de-*N*-acetylation pattern, which define the charge density and the distribution of GlcNAc and GlcNH₂ moieties in chitosan and COS.

Chitin de-*N*-acetylases (CDAs) catalyze the hydrolysis of the acetamido group in GlcNAc residues of chitin, chitosan, and COS. The deacetylation pattern exhibited by CDAs and related CE4 enzymes active on COSs is diverse, some being specific for a single position, others showing multiple attack. A major challenge is to understand how CDAs specifically define the distribution of GlcNAc and GlcNH₂ moieties in the oligomeric chain.

To address this question, we here report the crystal structure of the *Vibrio cholerae* CDA in four relevant states of its catalytic cycle [1]. The two enzyme complexes with chitobiose and chitotriose represent the first 3D structures of a CDA with its natural substrates in a productive mode for catalysis, thereby unraveling an induced-fit mechanism with a significant conformational change of a loop closing the active site. Based on these novel structures and structural analyses of CE4 enzymes active on COS, we propose a "Subsite Capping Model" in which the deacetylation pattern exhibited by different CDAs is governed by critical dynamic loops that shape and differentially block accessible subsites in the binding cleft of CE4 enzymes. The model might provide the bases for the rational design of CDA enzymes aimed at the biotechnological production of COSs with novel de-*N*-acetylation patterns for industrial and medical applications.

[1] E.Andrés, D.Albesa-Jové, X.Biamés, B.M.Moerschbacher, M.E.Guerin, A.Planas (2014) *Angewandte Chem. Int. Ed.* DOI: 10.1002/anie.201400220

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The Calvin-Benson cycle is not directly linked to transitory starch biosynthesis by means of phosphoglucose isomerase in leaves of plants exposed to microbial volatiles

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It is widely considered that starch is the end-product of a pathway exclusively taking place in the chloroplast that is linked to the Calvin-Benson cycle by means of the plastidial phosphoglucose isomerase (pPGI). Genetic evidence showing that starch biosynthesis occurs solely by this pathway has been obtained with mutants impaired in pPGI. However, mounting evidence has been compiled previewing the occurrence of starch biosynthetic pathways bypassing pPGI and involving the cytosolic compartment. We have recently shown that volatile compounds emitted by microorganisms promote both growth and accumulation of exceptionally high levels of transitory starch in leaves. This phenomenon (designated as MIVOISAP for **M**icrobial **V**olatiles **I**nduced **S**tarch **A**ccumulation **P**rocess) is accompanied by enhanced expression of the plastidial glucose-6-phosphate/Pi translocator GPT2 (Ezquer et al. 2010, Li et al. 2011). To investigate the role of pPGI and GPT2 in this phenomenon, in this work we characterized pPGI null *pgi1-2*, GPT2 null *gpt2*, and *pgi1-2/gpt2* Arabidopsis mutants cultured in the presence or absence of volatiles emitted by the plant pathogen *Alternaria alternata*. These studies revealed that, in the absence of fungal volatiles (FVs), *pgi1-2* and *pgi1-2/gpt2* leaves accumulate 10% of the wild type starch, whereas *gpt2* leaves accumulate nearly WT starch. Most importantly, FVs-treated *pgi1-2*, *gpt2* and *pgi1-2/gpt2* leaves accumulated exceptionally high starch levels in the chloroplasts of mesophyll cells. The overall data presented in this work show that, when plants are exposed to microbial volatiles (i) the Calvin-Benson cycle is not directly linked to leaf starch biosynthesis by means of pPGI, (ii) GPT2 does not provide cytosolic carbon to the chloroplast for its subsequent conversion into starch, and (iii) either cytosolic glucose-1-phosphate and/or ADP-glucose enter the chloroplast to be subsequently converted into starch.

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