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植物遗传学

胡萝卜抗冻蛋白失去PGIP家族抑制多聚半乳糖醛酸酶的活性

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摘要

含有LRR基序的胡萝卜抗冻蛋白虽然具有抗冻活性，但却属于植物PGIP家族。胡萝卜抗冻蛋白虽然在氨基酸序列上属于PGIP家族，但却失去了抑制外源真菌的PGase活性，并且获得了一个重要的活性——抑制冰晶的生长和重结晶。胡萝卜抗冻蛋白的这种活性的变化一直被认为是由于植物自身长期进化的结果，并认为最初的DcAFP也应当具有抑制PGase的活性。采用酵母双杂交来分析DcAFP是否还拥有PGIP的活性。通过RT-PCR克隆了真菌互格链格孢（Alternaria alternata）的PGase的cDNA，然后分别将PGase与DcAFP的完整编码框构建成酵母双杂交的捕获质粒和诱饵质粒，经过预实验表明两者都不能产生自激活作用，酵母双杂交实验表明两者不能产生相互作用，说明DcAFP完全失去了抑制PGase的活性，这种活性的丢失是由于位于β-螺旋上凹面的LRR基序中非保守的氨基酸残基发生了大量的碱性氨基酸的取代，导致结合的凹面从负电荷富集区变成了正电荷表面，从而不能通过静电作用与PGase的正电荷表面相结合。

关键词 植物抗冻蛋白；多聚半乳糖醛酸酶；多聚半乳糖醛酸酶抑制蛋白；LRR；酵母双杂交

分类号

Carrot Antifreeze Protein Does Not Exhibit the Polygalactu- ronase-inhibiting Activity of PGIP Family

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Abstract

<P>The carrot (Daucus carota) antifreeze protein (DcAFP) has a strong antifreeze activity and identified as belonging to the

plant polygalacturonase-inhibiting protein (PGIP) family based on its sequence similarities, including the presence of a leucine-rich repeat (LRR) motif. In this study, yeast two-hybrid technology was used to analyze whether the carrot AFP could act as a PGIP. The complete DcAFP and polygalacturonase (PGase; obtained from fungus Alternaria alternata by RT-PCR) coding sequences were cloned into the bait and capture vectors, respectively, and yeast two-hybrid assays were performed. The results revealed that there was no evidence of an interaction between DcAFP and PGase, which suggests that DcAFP probably lacks PGIP activity. An analysis of the electrostatic potential of DcAFP and other PGIPs revealed that a large number of nonconservative residues within the β-helix of the DcAFP LRR motif had been substituted to basic amino acids, thus changing the surface from negative to positive. This will electrostatically prevent DcAFP from binding with the positively charged surface of PGase. This is the first report that showed the correlation between nonconservative amino acids within the LRR motif of the DcAFP and its loss of polygalacturonase inhibiting activity.</P>

Key words plant antifreeze protein；polygalacturonase；polygalacturonase-inhibiting protein；leucine-rich repeat；yeast two-hybrid

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