Protein engineering, cofactor engineering and surface display engineering to achieve whole-cell catalytic production of chondroitin sulfate A

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Abstract

Chondroitin sulfate A (CSA) is a valuable glycosaminoglycan that has great market demand. However, current synthetic methods are limited by requiring the expensive sulfate group donor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) and inefficient enzyme carbohydrate sulfotransferase 11 (CHST11). Herein, we report the design and integration of the PAPS synthesis and sulfotransferase pathways to realize whole-cell catalytic production of CSA. Using mechanism-based protein engineering, we improved the thermostability and catalytic efficiency of CHST11; its T_m and half-life increased by 6.9°C and 3.5 h, respectively, and its specific activity increased 2.1-fold. Via cofactor engineering, we designed a dual cycle strategy of regenerating ATP and PAPS to increase the supply of PAPS. Through surface display engineering, we realized the outer membrane expression of CHST11 and constructed a whole-cell catalytic system of CSA production with a 89.5% conversion rate. This whole-cell catalytic process provides a promising method for the industrial production of CSA.

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