ENZYMATIC BIOTRANSFORMATION OF ADIPIC ACID TO 6-AMINOCAPROIC ACID AND 1,6-HEXAMETHYLENEDIAMINE USING ENGINEERED CARBOXYLIC ACID REDUCTASES AND AMINOTRANSFERASES

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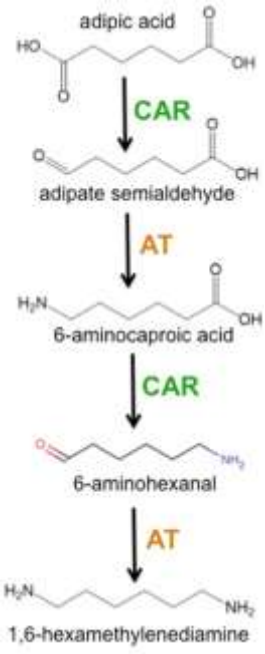

Biocatalytic reduction of carboxylic acids is gaining importance for the production of polymer precursors and different chemicals. Carboxylic acid reductases (CARs) reduce carboxylic acids to aldehydes using ATP and NADPH as cofactors under mild conditions. Recently, we demonstrated that several bacterial CARs can reduce a broad range of bifunctional carboxylic acids containing amino group or second carboxylic group including adipic acid, which is a precursor for nylon-6-6 (Khusnutdinova et al., 2017). In this project, we demonstrate application of CARs and aminotransferases for further bioconversion of adipic acid to 6-aminocaproic acid and hexamethylenediamine, two other important precursors for nylon synthesis. Based on the crystal structure of the adenylating domain of the CAR enzyme MCH22995 from *Mycobacterium chelonae*, we generated a structural model of the CAR enzyme MAB4714 from *M. abscessus*, which is active toward adipic acid. Aiming at improving MAB4714 activity toward 6-aminocaproic acid, we used structure-based protein engineering and generated 16 MAB4714 mutant proteins. Screening of 16 purified MAB4714 variants against 6-aminocaproic acid,identified one protein, which was 10 times more active than the wild-type protein. We also identified several bacterial aminotransferases producing 6-aminocaproic acid from adipic acid in combination with CARs. Further optimization of reaction conditions and application of cofactor regeneration systems resulted in efficient biotransformation of adipic acid to 6-aminocaproic acid (88% conversion) and further to 1,6-hexamethylenediamine (78% conversion).

Figure 1 – Biotransformation of adipic acid to 6-aminocaproic acid and 1,6-hexamethylenediamine using carboxylic acid reductases (CAR) and aminotransferases (AT).

Khusnutdinova, AN; Flick, R; Popovic, A; Brown, G; Tchigvintsev, A; Nocek, B; Correia, K; Joo, JC; Mahadevan, R; and Yakunin, AF (2017) Exploring bacterial carboxylate reductases for the reduction of bifunctional carboxylic acids. *Biotechnology J*, 12, 1600751.