

Biodegradation of 7-Ketocholesterol and Studies into Cholesterol Oxidase

The oxidation of cholesterol in food results in the formation of oxysterols such as 7-ketocholesterol (7KC), which are implicated in a number of age-related disorders. According to preliminary studies, 7KC levels were detected to be higher in milk powder samples than raw milk, thus implying the role of heating in 7KC formation. The concept of 'medical bioremediation' was proposed to mine therapeutic bacterial enzymes to degrade 7KC *in-vitro* and *in-vivo*. *Pseudomonas aeruginosa* PseA and *Rhodococcus erythropolis* MTCC 3951 were identified as potential bacterial strains with high capability of degrading 7KC. Cholesterol oxidase was reported as the major enzyme involved in the degradation pathway. Since the level of cholesterol oxidase was low in *P. aeruginosa* PseA, cloning and overexpression of its gene were attempted. The gene was cloned through pGEM-T Easy vector, into *E. coli* XL1 Blue-MRF' and was found to be highly conserved in nature with a size of 1794 bp. Several structures were predicted using Phyre² and I-Tasser, showing high similarity with *B. sterolicum* cholesterol oxidase and another solvent tolerant cholesterol oxidase. Restriction cloning was done through pET22b (+) vector into *E. coli* Rosetta (DE3) strain. However, activity was only obtained on plates and not in solution. In case of cholesterol oxidase from *R. erythropolis* MTCC 3951, process optimization was done for high yield. The protein was then purified to homogeneity by Q-sepharose followed by Phenyl Sepharose column chromatography. A novel monomeric 35 kDa protein was obtained in SDS-PAGE, which is uncommon in *Rhodococcus* strains. The enzyme was further structurally and biochemically characterized. In order to increase the applicability, the enzyme was immobilized on magnetic Iron (II, III) oxide and Silica nanoparticles. The kinetic properties of the enzyme were found to improve after immobilization, along with increase in stability over a wide range of temperature and pH. The nanobioconjugates were reusable up to 10th cycle of operation. Anti-obesity drugs 4-cholesten-3-one and 4-cholesten-3, 7-dione were produced through biotransformation of cholesterol and 7KC respectively using the nanobioconjugates. Further, magnetic nanoparticles were explored for their capability to act as artificial chaperones to refold thermally denatured cholesterol oxidase. The activity of denatured cholesterol oxidase was regained by a large extent. The current investigation thus encompasses a connection between the multiple perspectives of food cytotoxicity, biodegradation, molecular biology, enzymology, nanotechnology and structural biology.