

Expression, purification and characterization of extradiol dioxygenase carbb involved in carbazole degradation pathway

ABSTRACT

Extradiol dioxygenase is a family of enzymes essential for ring cleavage reactions in aromatic compounds degradation pathways. Extradiol dioxygenases belonging to the majority of aromatic compound degradation pathways are single peptide proteins, while a small subset of in this family was reported to be two subunits complex proteins. The extradiol dioxygenase CarB protein is a protein complex consisting of catalytic subunit CarBb with a smaller subunit CarBa. This enzyme was reported to show no ring cleavage activity without the expression of both peptides. However, to date, there was no specific study to confirm CarBb protein dependency on CarBa protein for its ring cleavage activity. In this study, we cloned, heterologously expressed and purified CarBb in *E. coli*. CarBb protein showed appreciable ring cleavage activity without expression of CarBa protein. The K_m and V_{max} values calculated were 163.68 μM and 1.19 $\mu M/min$. The effects of pH and temperature suggested that the CarBb protein was significantly unstable, suggesting that the CarBa protein may be responsible for the structural stability of the CarBb protein to function as an effective ring cleavage enzyme.

Keyword: Aromatic compounds degradation; Carbazole; Extradiol dioxygenase; Ring cleavage enzyme