

## ELUCIDATION OF CATALYTIC PROPERTY AND STRUCTURAL FEATURE OF HUMAN CARBOXYLESTERASE2

**Sou Tanoue, Kouske Tanaka, Seiya Fujihara, Kayoko Ohura and Teruko Imai**

School of Pharmacy, Kumamoto University, 5-1 Oe-honmachi, Kumamoto,  
862-0973, Japan

E-mail: 085p2023st.kumamoto-u.ac.jp

### ABSTRACT

Mammalian carboxylesterases (CESs) comprise a multigene family (CES1-CES5), and CES1 and CES2 family play important roles in the metabolism of xenobiotics. Although amino acid sequence of human CES1 (hCE1) and CES2 (hCE2) is 48% homology, they conserve a catalytic triad consisting of Ser-Glu-His, Gly-Gly as an oxanion hole, four Cys residues for disulfide bonds, and endoplasmic reticulum retention signal (KDEL-COOH). The major differences between hCE1 and hCE2 are the number of N-linked *glycosylation* site (one for hCE1, two for hCE2), and the lack of loop consisted from 15 amino acid residues in the vicinity of the active site. hCE1 and hCE2 show significantly different substrate specificity and predominantly contribute human hepatic and intestinal esterase activity, respectively. Therefore, the analysis of substrate recognition based on the protein structure is important for drug discovery. In this study, we examine the involvement of the deficient loop structure in catalytic function of hCE2 using mutant hCE2 inserted lacking 15 amino acids. We selected baculovirus/insect cell (Sf9) expression system to produce a recombinant enzyme. The site-directed mutagenesis of hCE2 cDNA was performed by inverse-PCR. In order to simplify the purification of recombinant enzyme, C-terminal cDNA coded KDEL sequence was removed, and 6×His-tag sequence was inserted. We are expressing mutant hCE2 in Sf9 cell and evaluating its enzyme activity.

Reproduced with permission of copyright  
owner. Further reproduction prohibited  
without permission.