

Engineering of a hybrid enzyme to study thromboxane A₂ mediating vascular diseases

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Abstract:

Thromboxane A₂ (TXA₂) is synthesized by a coupling reaction between cyclooxygenase (COX) and TXA₂ synthase (TXAS). However, the mechanism of this coordination is poorly understood. To uncover the details of this coupling, it is crucial to have a model showing their coordination. This study focuses on creating a hybrid enzyme to mimic the coupling between COX-1 and TXAS in the ER membrane. The two enzymes, COX-1 and TXAS, were joined by a 10 amino acid (aa) linker to create a hybrid enzyme, COX-1-10aa-TXAS. The cDNA of the engineered COX-1-10aa-TXAS was successfully cloned into the pcDNA 3.1 vector containing a cytomegalovirus early promoter and used for gene expression in HEK293 cells. The expression of the hybrid enzyme in the ER membrane, which is similar to that of the co-expressed COX-1 and TXAS, was demonstrated by immunostaining. The hybrid enzyme could mimic the triple catalytic functions of the wild type COX-1 and TXAS, as confirmed by the coupling assays from HPLC and LC/MS as well as Western blot analyses. The results indicated that the biosynthesis of TXA₂ is likely through the configuration of the two enzymes in which the orientation of the COX-1 C-terminal domain is toward the TXAS N-terminal domain, and the distance between the two enzymes is likely very short. This study provides a model to understand the molecular mechanism of the coupling between COX-1 and TXAS in biosynthesis of TXA₂, which is involved in strokes.