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Molecular cloning of sea cucumber oxidosqualene cyclases

Key words: oxidosqualene cyclase genes, sterols, sea cucumbers

Sea cucumbers contain rare Δ^7- or $\Delta^{9(11)}$-sterols including methyl sterols in their cell membranes instead of main zoosterol – cholesterol. Also, sea cucumbers produce a huge number of diverse triterpene glycosides with 7(8)- or 9(11)-double bond in their aglycones [1, 2, 5]. It has been suggested that the holothuroid sterols play an important role in resistance of the cell membrane to a lytic action of saponins due to their ineffective interaction with each other compared to cholesterol-saponin one [3].

Biosynthesis of sea cucumber sterols and saponins is still a mystery taking into account the unusual cyclization of oxidosqualene into parkeol, the presence of unusual 4,14-dimethyl- and 14-methyl-$\Delta^{9(11)}$-sterols, and the inability of polycyclic precursors (parkeol, lanosterol and cycloartenol) to been transformed into glycosides. In addition, it is still difficult to explain how glycosides with a 7(8)-double bond acquire 9β-H configuration [4]. Previously, Makarieva et al. (1993) proposed the existence of two pathways of sterol biosynthesis in sea cucumber *Eupentacta fraudatrix*. The first pathway involves *de novo* biosynthesis of 14-methylcholest-9(11)-en-3β-ol from parkeol and the second one involves biosynthesis of triterpene saponins from lanosterol. Later, it was suggested that some glycosides with 7(8)-double bond can be biosynthesized via cyclization of oxidosqualene into 9β-H-lanosta-7,24-dien-3β-ol [4]. Therefore, it can be assumed that an oxidosqualene cyclase from sea cucumbers must have structural features in an active center compared with those of other animals. Herein we report the results of molecular identification of OSC genes from the holothurians *E. fraudatrix* as well as structure and evolutionary analysis of these enzymes.

Two partial-length cDNAs, encoding OSC1 and OSC2, were obtained by reverse transcription, RACE and molecular cloning from the total RNA isolated from body wall and intestine samples of the sea cucumbers *E. fraudatrix*. Sequence comparison showed that OSC1 and OSC2 are 89% identical to each other and share 51-62% identity to the other animal OSCs. Structure comparison analysis identified the conserved SQCY_1 and ISOPREN_C2 domains specific for class II terpene synthases. Both sequences showed the presence conservative motifs, which are important for carbocation formation and protein structure maintenance. However, OSC1 and OSC2 have some substitutions of functionally important residues in the active center that could be responsible for the formation 7(8)- or 9(11)-double bond in a triterpene nucleus. Phylogenetic

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tree of OSCs demonstrates that OSCs of *E. fraudatrix* do not cluster with other echinoderm OSCs but take a position basal to the clade of other animal OSCs (100% bootstrap support).

Thus, the coevolution of sea cucumber saponin-sterol pairs is triggered by the appearance of the second and extensive diversification of both OSC genes.

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