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PRODUCTION OF DIFFERENT FORMS OF HUMAN RECOMBINANT VALYL-TRNA SYNTHETASE USING THE HEK293 CELL LINE

Background. Valyl-tRNA synthetase (VRS) catalyzes valine attachment to its cognate tRNA that is required for protein biosynthesis. Importantly, biallelic mutations in VRS gene were found to be responsible for the neurodevelopmental disorder (neurodegeneration with microcephaly, seizures, and cortical atrophy) with reduction of VRS catalytic activity, signifying partial loss of enzymatic function [1]. Thus, **the aim** of our work was to obtain full-length valyl-tRNA synthetase and its truncated forms required for further functional and structural studies. **Methods.** The genetic constructs pcDNA3.1(+)/VRS(FL) (full-length), pcDNA3.1(+)/VRSΔ684 (lacking 684 bp from the N-end), pcDNA3.1(+)/VRS(cat.) (catalytic domain only) were used to transfect HEK293 cells that enable mammalian-specific protein glycosylation and proper folding. The recombinant protein synthesis in transfected cells was tested by Western-blot analysis using anti-His-tag and anti-VRS specific antibodies. The recombinant proteins were purified from soluble cell extracts using affinity chromatography on Cobalt Superflow and gel filtration on Superose 6 as a polishing step. Protein purity was evaluated by SDS-PAGE. **Results.** Western-blot analysis with anti-His-tag and specific anti-VRS antibodies confirmed the ectopic overexpression of all used genetic constructs at the protein level. Small-scale purification of the proteins of interest including affinity chromatography and gel filtration yielded homogenous protein preparations as judged by SDS-PAGE. In contrast to its truncated forms, VRS(FL) showed a lower yield of the recombinant protein and a tendency to aggregation. Molecular weight of the purified recombinant proteins corresponded to the theoretical value of their monomers as judged by analytical gel filtration. Surprisingly, anti-His-tag antibodies failed to detect full-length VRS, while successfully detected its truncated forms. This failure is most probably due to His-tag epitope masking by the N-terminal domain of VRS(FL). **Conclusions.** Full-length VRS and its truncated forms were successfully expressed in HEK293 cells and purified to homogeneity. These cultured human cells may be also used for expression of VRS mutant forms in order to understand the functional and structural significance of the different point mutations in this enzyme that may be involved in the developmental of neurological disorders.

Keywords: human valyl-tRNA synthetase, recombinant protein production, protein chromatography.

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