English Abstract

Glutamate carboxypeptidase II (GCPII) is a membrane metallopeptidase expressed in many human tissues, predominantly in prostate, brain and small intestine. In brain it cleaves the most abundant peptide neurotransmitter N-acetyl-L-aspartyl-α-L-glutamate into N-acetyl-L-aspartate and free L-glutamate. Thus, GCPII participates in glutamate excitotoxicity through the release of free glutamate into the synaptic cleft. Inhibition of this activity has been shown to be neuroprotective in rats. In the human jejunal brush border, GCPII cleaves off terminal glutamate moieties from poly-γ-glutamylated folates, which can be then transported across the intestinal mucosa. The function of GCPII in human prostate is unknown but it is overexpressed in prostate cancer. Therefore, GCPII is an important marker of prostate cancer and its progression.Moreover, it could become a perspective target for treatment of prostate cancer as well as neuronal disorders associated with glutamate excitotoxicity.

For the development and testing of novel drugs and therapeutics it is necessary to have an appropriate animal model. Mouse (*Mus musculus*) is such a model and it is widely used by many experimentators. However, no detailed comparison of mouse and human GCPII orthologs regarding their enzymatic activity, inhibition profile and expression has been provided yet. Possible variations in expression and activity profiles among the human and mouse orthologs are very relevant for the development of novel GCPII-based anticancer and neuroprotective drugs and therapeutical methods.

We cloned, expressed, purified and subsequently characterized recombinant mouse GCPII and compared it to its human ortholog. Activity, substrate specificity and inhibition of mouse GCPII are similar to human GCPII. We show that monoclonal antibodies raised against denatured human GCPII recognize mouse GCPII. On the contrary, antibodies raised against native human GCPII do not interact with mouse GCPII. Significant differences in GCPII expression in several mouse and human tissues were observed. We also established stable mammalian cell line for inducible expression of mouse GCPII. Finally, microcrystals of mouse GCPII were prepared during crystallization trials; crystallization conditions will be further optimalized for obtaining sufficiently large monocrystals that could be used for mouse GCPII structure determination.

(In Czech)