Krupp, A. (2006); "Cloning, Expression, Purification and Characterisation of the Tropomyosin-related Kinase B (TrkB)", Diploma Thesis, Institute of Biochemistry, University of Cologne

Abstract

The tropomyosin-related kinase B (TrkB) is a neurotrophin receptor from the family of receptor tyrosin kinases. It regulates differentiation, survival, cell cycle, axon outgrowth and protein expression of neurones. TrkB also plays an important role in anoikis (apoptosis of cells that lost contact to the adjacent tissue).

The catalytic activity and activation mechanisms of TrkB have only been poorly characterised, to date. Thus, the aim of my project was to elucidate the influence of dimerisation on its activation and the interaction of TrkB with its preferred substrate "Src homology domain containing protein" (Shc).

Therefore, fusion proteins of the intracellular domain of TrkB with both a GST-tag and a hexahistidin-tag were cloned, co-transfected into *Sf*9 cells, expressed and purified. Both fusion proteins were also generated where tyrosine$^{490\ (a)}$ was changed to phenylalanine by site-directed mutagenesis to remove the PTB binding site of TrkB. p52^{Shc} is a physiological substrate of the Trk receptors. A full-length protein and two fragments of p52^{Shc} were cloned as both GST- and hexahistidin fusion proteins. Shc fusion proteins were expressed in *E.coli* and purified.

In vitro reaction conditions for the phosphorylation of p52^{Shc} by TrkB were established, which enabled not only stoichiometric but also catalytic phosphorylation. In addition to autophosphorylation, TrkB was also shown to phosphorylate the substrate p52^{Shc} on serine, yielding a rate of 30% of serine phosphorylation based on the total phosphate content of Shc.

GST fusion proteins form artificial dimers due to dimerisation of the GST-tags. Artificially dimeric GST-TrkB was chosen as a model of the dimeric receptor. Its catalytic activity was compared with the catalytic activity of the monomeric $(His)_6$-TrkB

[a] The amino acids of TrkB are numbered analogous to TrkA/NGFR because TrkB numbering is very inconsistent in the literature.

to assess the influence of dimerisation on the activation of the kinase. Despite the fact that TrkB is classified as a tyrosine kinase, significant autophosphorylation on serine could be shown.

Kinetic data were obtained using a novel method that was developed in this project. The method enables a fast, precise and quantifiable determination of the transfer of the phosphoryl group by tyrosine kinases. It is based on the phosphorylation of the substrate *tyrTide* which is recognized specifically by tyrosine kinases. *tyrTide* was developed in our group and can easily be separated into its phosphorylated an unphosphorylated form by PAGE. The amount of phosphorylated *tyrTide* can subsequently be quantified densitometrically to analyse the activity of the respective kinase.

This method was employed to compare the activities of monomeric $(His)_6$- and dimeric GST-TrkB. In doing so it could be shown that TrkB is activated at least 6-fold by dimerisation.