Abstract

Transcription factors play a key role in the management of cell growth and differentiation and their deregulation is associated with many cancers. TEAD proteins utilise highly conserved DNA binding domain to recognise specific DNA sequences. This domain could facilitate new drug design and development.

The goal of this master thesis includes recombinant preparation of DNA binding domain of transcriptional factor TEAD4 extended by a part of an unstructured variable sequence, which connects this domain with transactivation domain. Purification steps include affinity chromatography followed by size exclusion chromatography. The characterization of produced protein was performed by mass spectrometry and finally, native gel electrophoresis was used to prove the ability of the produced protein to bind DNA.

During purification steps, a fragmentation from C-terminus was observed. Based on analysis of the mass spectra, three most represented forms of produced protein were described all of which were fragmented. The most abundant form (55%) consisted of amino acids 30–131 from TEAD4 protein. Second most abundant form (18%) consisted of amino acids 30–144 and the third form consisted of amino acids 30–81. Native gel electrophoresis verified the ability to bind DNA, the efficiency was however lower than expected. Future improvements could include changes in the length of the construct to reduce the effect of fragmentation on the relevance of DNA interaction studies.

Key words: recombinant expression, protein purification, DNA binding protein, transcription factor, cell signaling, TEAD4, native electrophoresis, mass spectrometry

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