Abstract

Transcription factors are proteins that regulate gene expression in different cell types. They play an important role in many cellular processes including regulation of cell cycle and cell differentiation. They possess DNA binding domains to recognize and bind specific DNA sequences. One type of DNA binding domain is the forkhead domain, which contains a region of 100–110 amino acid residues. This sequence is referred to DBD FOX and its spatial arrangement resembles a "winged helix". Proteins of the FOX family interact with double-stranded DNA via the α-helix H3, which represents highly conserved region within the proteins of this family. Other regions of the DBD further contribute in DNA binding, but as not significantly conserved, and their different properties are responsible for variable affinities of individual FOX proteins against binding motifs. Differences in three-dimensional structure may also alter biological functions of FOX proteins in the organism. FOX proteins are divided into 19 subfamilies, including the FOXK subfamily, consisting of two members, FOXK1 and FOXK2. FOXK proteins regulate aerobic glycolysis, cell proliferation and carcinogenesis. Their increased expression has been reported in cancer cells of skeletal tissue, stomach, colon, breast, lung, ovary, etc. However, the biological role of FOXK proteins in different types of cancer is still not well understood.

The aim of this work was to prepare a native DBD FOXK1 protein that recognizes and binds a specific DNA motif. Five oligonucleotides (CRTC 2_14, CRTC 2_16, INSR_13, S16 and S1FLC) with the length of 13, 14 and 16 base pairs were used to examine the binding of DBD FOXK1. Intermolecular interaction was monitored by native electrophoresis and native mass spectrometry. Established purification procedure provided a highly pure functional DBD FOXK1 protein that binds to all tested oligonucleotides containing the specific binding sequence. Using native electrophoresis, an additional form of the complex with lower mobility was observed at higher excess of the protein, which indicates binding of the second DBD FOXK1 molecule outside the highly conserved binding motif.

Key words

transcription factors, DNA binding domain, oligonucleotide, three-dimensional structure, carcinogenesis

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