

Abstract

Galectins are animal lectins with affinity to terminal β -galactosides. These lectins are characterized by an evolutionarily conserved carbohydrate recognition domain (CRD). Galectins are involved in many processes *in vivo*, such as cell adhesion; signaling; cell proliferation and carcinogenesis. They can be used as markers in certain types of cancer and also for the targeted binding of therapeutics in the diagnosis and therapy of cancer. Galectins have been defined as a protein family composed of 15 members (11 of which are known in humans). They are classified into three subgroups, according to their structure: prototypical; chimeric; tandem-repeat type. Galectin-8 belongs to tandem-repeat galectins, which consist of two different subunits connected with a peptide linker; they contain two different CRD domains, and are therefore bivalent.

Galectin-glycan interactions are based on the affinity of galectin CRDs to glycans containing a terminal β -galactoside, and this affinity is formed by the set of conserved amino acids within each CRD. These interactions can be investigated by many methods, such as X-ray crystallography; competitive ELISA; biolayer interferometry. Due to the extensive involvement of galectins in biological processes, there are many methods of labeling galectins for their detection. The most used methods are fluorescence methods and biotinylation of galectins, for which it is advantageous to use the so-called AVI-tag (GLNDIFEAQKIEWHEGGSGGS).

In this work, a gene construct of one galectin (N-terminal domain of galectin-8) carrying a peptide tag for selective biotinylation of proteins *in vivo* (AVI-tag) was prepared. This construct, along with other galectin constructs (available in the supervisor's laboratory), was recombinantly expressed in *Escherichia coli*. The galectin binding affinity to selected standard carbohydrate ligands was determined for the construct of the whole galectin-8 together with its N-terminal domain and its respective form carrying a biotinylated AVI-tag. The presence of biotinylation was confirmed in galectin forms with a biotinylated AVI-tag.

Key words

biotin ligase; labeling; ELISA; galectin; carbohydrate; Western blot