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High level expression of monomeric and dimeric human α1,3-fucosyltransferase V

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Abstract

α3/4-Fucosyltransferases play a crucial role in inflammatory processes and tumor metastasis. While several human fucosyltransferases (FucTs) with different acceptor substrate specificities have been identified, the design of specific inhibitors for therapeutic approaches is hampered by the lack of structural information. In this study, we evaluated the expression of different constructs of human fucosyltransferase V to generate the large amounts required for structural studies. The truncated constructs lacking the transmembrane region and the cytosolic N-terminus, were expressed in baculovirus-infected *Trichoplusia ni* (Tn) insect cells and in two non-lytic expression systems, stably transfected human HEK 293 and *T. ni* cells. Since secretion of some glycosyltransferases is controlled by formation of dimeric molecules via disulfide bonds, one of the fucosyltransferase V constructs contained the N-terminal cysteine residue 64 for dimerization, whereas this residue was replaced in the other construct by serine. In both human and insect cells dimerization did not prove to be essential for efficient expression and secretion. On the basis of enzymatic activity, the yield of secreted fucosyltransferase V was approximately 10-fold higher in stably transfected insect cells than in HEK 293 cells. In particular the monomeric form of the enzyme provides a valuable tool for structural analyses to elucidate the fine specifity of fucosyltransferase V-mediated fucosylation of Lewis type glycans.

Keywords: Fucosyltransferase V; Insect cells; Enzyme dimerization; Protein engineering

1. Introduction

Fucosylated glycans of the Lewis type mediate recognition and adhesion processes occurring in inflamation and metastasis, and play an important role in foetal development and haematopoietic cell differentiation (Lowe, 1993). As a result, there is considerable

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interest in the design of specific fucosyltransferase (FucT) inhibitors and in the synthesis of glycomimetics that inhibit these processes. For example, the sialyl-Le^x determinant or structural variants of it which mediate as ligands for E-, P-, and L-selectins normal leukocyte trafficking and leukocyte extravasation in inflammatory reactions (McEver et al., 1995), have been demonstrated to act effectively as anti-inflammatory drugs in animals (Mulligan et al., 1993a, 1993b; Buerke et al., 1994).

The transfer of fucose onto type 1 and type 2 carbohydrate core structures is catalyzed by fucosyltransferases which are found throughout a variety of species (for review see Becker and Lowe, 2003). Several human FucTs have been distinguished in various tissues on the basis of acceptor substrate specificity and other biochemical properties (Macher et al., 1991; de Vries and van den Eijnden, 1992; Mollicone et al., 1990; Stroup et al., 1990). Interestingly, the acceptor substrate specificity of FucTs differs significantly although some of these enzymes exhibit high sequence homology (de Vries et al., 1995; Kukowska-Latallo et al., 1990; Weston et al., 1992a, 1992b; Koszdin and Bowen, 1992). For example, within the catalytic domain of FucT III, V, and VI approximately 280 of 300 amino acid residues are identical (Breton et al., 1998; Oriol et al., 1999; Oulmouden et al., 1997; Leiter et al., 1999; Kageyama et al., 1999). However, FucT III exhibits high activity with type 1 disaccharide acceptor Galβ1,3GlcNAc, whereas type 2 disaccharide acceptor Galβ1,4GlcNAc is utilized exclusively by FucT VI (de Vries et al., 1995, 1997; Kukowska-Latallo et al., 1990; Weston et al., 1992a, 1992b; Koszdin and Bowen, 1992; Chandrasekaran et al., 1996) and almost exclusively by FucT V (Nguyen et al., 1998). Mutagenesis approaches point to a crucial role of single residues for transfer specificity of FucT III (Dupuy et al., 1999, 2004). Furthermore, FucT V and VI can bind the same acceptor substrates but facilitate the synthesis of different products. Mammalian cells transfected with FucT V have been shown to express cell surface antigens recognized by anti-difucosyl-sialyl-Le^x and anti-VIM 2, whereas cells transfected with FucT VI proved to be reactive only with anti-difucosyl-sialyl-Le^x (Weston et al., 1992a, 1992b). Thus, despite the high degree of sequence homology, FucT III, V, and VI display different functional properties, the molecular basis of which remains to be determined.

While three-dimensional structural analyses are most informative, they require substantial amounts of protein which is present in native tissues in only low amounts (de Vries and van den Eijnden, 1992). Therefore, several human FucTs have been produced recombinantly as soluble secretory proteins (de Vries et al., 1995; Gallet et al., 1998) to obtain the large amount required for structural analyses. In particular human FucT III and VII have been expressed in high quantities in baculovirus-infected cells (Morais et al., 2001; Shinkai et al., 1997).

In this study we compared the expression level of a soluble form of human FucT V in baculovirus-infected *Trichoplusia ni* (Tn) insect cells and in two non-lytic expression systems, stably transfected human HEK 293 and Tn cells, to generate large amounts of human FucT V for structural analyses. Stably transfected insect cells proved to be most efficient. Since the FucT V construct was found to be secreted as a mixture of monomeric and dimeric molecules, the cysteine residue responsible for dimer formation was replaced by serine. The resulting FucT V mutant (FucT V_{C64S}) is secreted exclusively as monomer and, therefore, provides a valuable tool for structural studies.

2. Materials and methods

2.1. Antibody production and purification

A DNA fragment encoding human FucT V (A^{58}–T^{374}) was amplified by PCR using genomic DNA isolated from HEK 293 and the oligonucleotides FUT5BamHIfw: (5′-CGGGGATTCCTCCCAATGGGTCCCGCTGCCAG-3′) and FUT5PstIrev (5′-CCAAGCTCTCAGGTGAACCAAGTGGCTATGCT - 3′). This fragment was introduced into the vector pRSETB (Invitrogen, Karlsruhe, Germany) via *Bam*H I and *Pst* I restriction sites and used to express the FucT V construct in *Escherichia coli* BL21(DE3) cells according to standard procedures. The cells were resuspended in phosphate-buffered saline (PBS), pH 7.2, containing 8 M urea and incubated overnight at room temperature. Debris was removed by centrifugation at 10,000 × *g* and the supernatant applied to Ni-NTA-agarose (Qiagen, Hilden, Germany). Protein containing fractions were pooled and separated by preparative SDS–PAGE. Gel slices containing FucT V