Journal of Biotechnology 145 (2010) 130–138

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Production and *N*-glycosylation of recombinant human cell adhesion molecule L1 from insect cells using the stable expression system. Effect of dimethyl sulfoxide

Ricardo Gouveia[a], Sebastian Kandzia[b], Harald S. Conradt[b], Júlia Costa[a,c,*]

[a] *Instituto de Tecnologia Química e Biológica, Avenida da República, 2780-157 Oeiras, Portugal*
[b] *GlycoThera GmbH, Feodor-Lynen Strasse 35, 30625 Hannover, Germany*
[c] *Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal*

ARTICLE INFO

Article history:
Received 14 July 2009
Received in revised form 23 October 2009
Accepted 28 October 2009

Keywords:
N-glycosylation
Insect cells
Stable expression system
Dimethyl sulfoxide
Cell adhesion molecule L1

ABSTRACT

L1 is a cell adhesion molecule that is heavily glycosylated and is essential for normal development of the central nervous system. In this work, we compare the *N*-glycosylation of the L1 mutant that consists of immunoglobulin domains 5 and 6 (L1/Ig5–6), expressed in insect *Spodoptera frugiperda* Sf9 and *Trichoplusia ni* Tn cells, using the stable expression system. L1/Ig5–6 levels of 30 and 8 mg l^{-1} were obtained from the two cell lines, respectively. The *N*-glycans were characterized by high-performance anion-exchange-chromatography with pulsed-amperometric-detection and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The *N*-glycans from Sf9 cells were more homogeneous and consisted predominantly of the paucimannose-type structure Manα6(Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAc. On the other hand, the *N*-glycans from Tn cells were more heterogeneous and consisted of paucimannose-type structures with or without terminal *N*-acetylglucosamine. Allergenic proximal α3-linked fucose was only found in Tn cells.

Dimethyl sulfoxide at 1.5% concentration has been found to increase the levels of L1/Ig5–6 and the L1 ectodomain in the Sf9 and Tn cells, without affecting cell viability nor protein integrity. Furthermore, the *N*-glycan composition of L1/Ig5–6 was not affected by dimethyl sulfoxide, with only a slight increase in the percentage of the minor high-mannose-type structures.

1. Introduction

Insect cell lines have been widely used for the expression of recombinant proteins, and are particularly suitable for the production of eukaryotic glycosylated proteins, since they are able to perform *N*-glycosylation required for folding and, consequently, secretion while still achieving production yields in the milligram range. The *N*-glycans of proteins produced by insect cells consist primarily of paucimannose-type structures (±GlcNAcMan$_3$GlcNAc(±Fucα6)(±Fucα3)GlcNAc) and high-mannose-type structures to a lower extent (reviewed in Shi and Jarvis, 2007).

Most of the insect cell glycoproteins whose *N*-glycans were structurally characterized were obtained using the baculovirus–insect cell system (reviewed in Grabenhorst et al., 1999; Kost et al., 2005; Shi and Jarvis, 2007). However, there are several drawbacks to this system: since it is a lytic system cellular contents such as hydrolases, including proteases and glycosidases are released to the medium and might compromise protein quality; a large proportion of the protein is accumulated intracellularly since the folding and secretion machinery may be compromised by the infection. As an alternative, the stable expression system in insect cells has been used for the production of recombinant membrane (Kempf et al., 2002) and soluble glycoproteins (Barinka et al., 2004; Chang et al., 2002, 2004; Gouveia et al., 2007; Swiech et al., 2008). For the secretory form of the enzyme fucosyltransferase III, the levels of protein obtained using the stable expression system were higher when compared with the baculovirus expression system (Morais and Costa, 2003). The *Trichoplusia ni*, particularly, the BTI-Tn-5B1-4 (High-Five®) Tn cell line has been shown to produce higher amounts of protein than the *Spodoptera frugiperda* Sf9 cell line, using the baculovirus expression system (Morais et

Abbreviations: dHex, deoxyhexose; DMSO, dimethyl sulfoxide; EDTA, etylenediaminetetraacetic acid; Endo H, Endo-β-*N*-acetylglucosaminidase H; Fuc, fucose; Gal, galactose; GlcNAc, *N*-acetylglucosamine; Hex, hexose; HexNAc, *N*-acetylhexosamine; HPAEC-PAD, high-performance-anion-exchange-chromatography with pulsed-amperometric-detection; Ig, immunoglobulin; MALDI/TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; Man, mannose; PNGase F, peptide-*N*-glycosidase F; PNGase A, peptide-*N*-glycosidase A.

* Corresponding author at: Instituto de Tecnologia Química e Biológica, Avenida da República, 2780-157 Oeiras, Portugal. Tel.: +351 214469437; fax: +351 214411277.
E-mail address: jcosta@itqb.unl.pt (J. Costa).

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doi:10.1016/j.jbiotec.2009.10.018

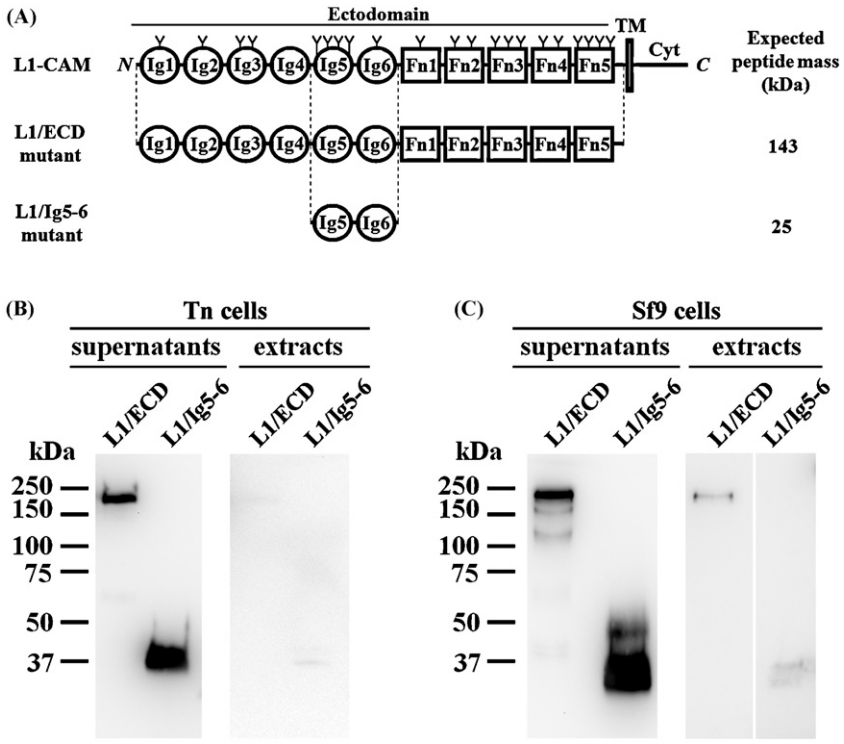

Fig. 1. Recombinant L1 mutants produced in insect Sf9 and Tn cells. (A) A schematic representation of the L1 protein and the two L1 truncated mutants L1/ECD and L1/Ig5–6. L1 has six Ig domains (*circles*), five Fn domains (*boxes*), a transmembrane region (*TM*), and a cytoplasmic domain (*Cyt*). The 21 potential *N*-glycosylation sites (*arrowheads*) are represented. Expected molecular mass of L1 truncated mutants is shown. The two mutants contained V5 followed by hexa-histidine tags at their *C*-terminus. Analysis of L1/ECD and L1/Ig5–6 produced by (B) Tn and (C) Sf9 cells. Supernatant samples of 50 (0.8×10^5 cells) and 20 μl (1.6×10^5 cells) of Tn and Sf9 stably transfected cell cultures, respectively, and corresponding cell extracts were analyzed by Western blot. As primary antibody an anti-V5 antibody at 1:5000 dilution was used. Detection was performed by the ECL method.

al., 2001), but it has been less used with the stable expression system (Chang et al., 2004; Dojima et al., 2009; Farrell et al., 1998).

Detailed *N*-glycosylation analysis of recombinant proteins produced in insect cells using the stable expression system has been reported for Schneider S2 cells (reviewed in Barinka et al., 2004; Gårdsvoll et al., 2004; Kim et al., 2009; Rendić et al., 2008) and, recently, for Tn cells (Dojima et al., 2009), but to our knowledge not for Sf9 cells.

The cell adhesion molecule L1 is a heavily *N*-glycosylated member of the immunoglobulin (Ig) superfamily, comprising an ectodomain region with six Ig-like domains (Ig1–Ig6) and five fibronectin-type III domains (Fn1–Fn5), a single transmembrane region and a cytoplasmic domain (Fig. 1A, reviewed in Bateman et al., 1996). Recent evidence indicates a regulatory role for carbohydrates in L1-mediated homophilic adhesion (He et al., 2009). L1 is critical for proper development of the human central nervous system, as it is implicated in many cellular mechanisms, including neurite outgrowth, cell-cell adhesion, migration, myelination, synaptic plasticity and axon guidance and fasciculation. Neurite outgrowth is due to signalling events triggered by L1–L1 homophilic interaction and by binding of the Arg-Gly-Asp (RGD) sequence from the Ig6 domain to various classes of integrins (reviewed by Maness and Schachner, 2007). The various cellular activities of the L1 protein indicate that it can be used as a potent neuronal growth factor or differentiation promoter; however, endogenous L1 is only available in low amounts.

In addition, L1 plays an important role in different types of cancer. It is associated with increased cell growth rate, motility, transformation and tumorigenic capacity of colon cancer cells (reviewed in Raveh et al., 2009). L1 was also found to be overexpressed in ovarian and uterine carcinomas and it was associated with bad prognosis (Fogel et al., 2003). The RGD sequence from the Ig6 domain was found to be important for cell-cell binding, cell motility, cell invasiveness and tumor growth (Gast et al., 2008).

Previously, in our laboratory, we have expressed the full-length human L1 and truncated soluble forms including L1 ectodomain (L1/ECD) and Ig domains 5 and 6 (L1/Ig5–6) in *S. frugiperda* Sf9 cells using the stable expression vector pMIB/V5-His under the control of the OpIE2 promoter for high-level constitutive expression and the signal sequence of honeybee melittin for efficient protein secretion in insect cells, and recombinant L1/ECD and L1/Ig5–6 from Sf9 cells were found to induce neurite outgrowth of human NT2N neurons (Gouveia et al., 2007; Gouveia et al., 2008).

In an attempt to increase the yield of recombinant L1, the use of small molecules to attain higher levels of protein expression was considered; one such compound is dimethyl sulfoxide (DMSO). DMSO is a small amphipathic molecule soluble in both aqueous and organic media that exerts a wide variety of biological effects, by altering lipid membrane structure and metabolism, DNA synthesis and transcription, cell proliferation, differentiation and apoptosis (reviewed in Yu and Quinn, 1994). More specifically, DMSO was shown to increase production of recombinant proteins in stably transfected *Drosophila melanogaster* S2 (Chang et al., 2002; Swiech et al., 2008) and Tn insect cells (Chang et al., 2004).

In the present study, the *N*-glycosylation of recombinant L1/Ig5–6 produced from Sf9 and Tn insect cells using the stable expression system has been characterized in detail. The *N*-glycans from Sf9-L1/Ig5–6 were more homogeneous and consisted predominantly of the paucimannose-type structure Manα6(Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAc. On the other hand, the *N*-glycans from Tn-L1/Ig5–6 consisted mostly of paucimannose-type structures with or without terminal GlcNAc. Furthermore, addition of DMSO caused 1.7 and 1.3-fold-increased