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Human TPST1 Transmembrane Domain Triggers Enzyme Dimerisation and Localisation to the Golgi Compartment

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TPST1 is a human tyrosylprotein sulfotransferase that uses 3′phosphoadenosine-5′phosphosulfate (PAPS) to transfer the sulfate moiety to proteins predominantly designated for secretion. To achieve a general understanding of the cellular role of human tyrosine-directed sulfotransferases, we investigated targeting, structure and posttranslational modification of TPST1. Golgi localisation of the enzyme in COS-7 and HeLa cells was visualised by fluorescence imaging techniques. PNGase treatment and mutational studies determined that TPST1 bears N-linked glycosyl residues exclusively at position Asn60 and Asn262. By alanine mutation of these asparagine residues, we could determine that the N-linked oligosaccharides do not have an influence on Golgi retention of TPST1. In concert with N and C-terminal flanking residues, the transmembrane domain of TPST1 was determined to act in targeting and retention of the enzyme to the trans-Golgi compartment. This domain exhibits a pronounced secondary structure in a lipid environment. Further *in vivo* FRET studies using the transmembrane domain suggest that the human tyrosylprotein sulfotransferase may be functional as homodimer/oligomer in the trans-Golgi compartment.

Keywords: dimerisation; FRET; glycosylation; sulfotransferase

Introduction

Tyrosine-*O*-sulfation is a widespread posttranslational modification found in many secreted proteins and a variety of cellular surface receptors and lysosomal enzymes.[1] It triggers inflammatory leukocyte adhesion to blood vessel walls,[2,3] controls the interaction between proteins of the blood clotting cascade[4–8] and plays a significant role in viral cell attack and antigen recognition.[9,10] In all those processes, the sulfotyrosine moiety acts as modifier of enzymatic activity or as "molecular glue" by strengthening biomolecule interaction.

The process of tyrosine-*O*-sulfation is catalysed by tyrosylprotein sulfotransferases (TPSTs) and takes place in the lumen of the trans-Golgi network,[11] which in interplay with the endoplasmic reticulum is primarily responsible for proper sorting of lipids and proteins in eukaryotic cells. TPSTs receive the sulfate moiety from the ubiquitous sulfate carrier 3′phosphoadenosine-5′phosphosulfate (PAPS) and attach it onto a tyrosine side-chain of a target protein in a transesterification reaction.[12] To date, two human isoforms, TPST1 and TPST2, have been described and cloned.[13,14] They consist of about 370 residues and share 64% sequence identity. Both transferases

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Abbreviations used: TPST, tyrosyl protein sulfotransferase; TMD, transmembrane domain; (E)GFP, (enhanced) green fluorescence protein; (E)CFP, (enhanced) cyan fluorescent protein; DsRed, Discosoma sp. red fluorescent protein; CD, circular dichroism; TFE, trifluoroethanol; nOG, *n*-octylglucoside; FRET, fluorescence resonance energy transfer; wt, wild-type.

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are predicted to be type II transmembrane proteins anchored in the lipid bilayer by a single N-terminal hydrophobic, membrane-spanning domain. A so-called stem region of about 40 amino acid residues follows immediately beneath the membrane.[15] The catalytic domain of hTPSTs is localised inside the lumen of the Golgi apparatus where protein substrates are posttranslationally modified. The luminal part of TPST2 can be expressed as soluble active recombinant fusion protein in higher eukaryotic cells.[14] As a posttranslational modification, it was shown that TPST1 purified from bovine adrenal medulla[16,17] carries N-linked glycosyl moieties. TPST1 and 2 are expressed in various human tissues[13,14,18] and are co-expressed in primary umbilical vein endothelial cells (HUVECs) in a shear stress-dependent manner.[19]

Despite the fact that mammalian TPST variants have already been studied for several years, their function is barely understood and many questions concerning their biochemical and structural characterisation remain open. Although it is generally accepted that TPSTs are localised in the trans-Golgi apparatus, the determinant responsible for Golgi targeting has not been described so far. Moreover, the specific residues hosting posttranslational glycosylation modifications have not been identified. Here we report detailed biochemical and cellular studies on the localisation of TPST1 and its targeting determinant, the predicted transmembrane domain (TMD). N-Glycosylation of TPST1 could be determined to take place exclusively at amino acid residues Asn60 and Asn262 and it seems to have no influence on the targeting of the sulfotransferase to the Golgi apparatus. In addition, we investigated the secondary structure of the TMD in a membrane-like environment by CD spectroscopy and provided evidence for its dimerisation using *in vivo* fluorescence resonance energy transfer (FRET) measurements.

Results

TPST1-EGFP colocalises with Golgin97 in COS-7 cells

A first hint for possible Golgi localisation of TPSTs in mammalian cells was provided by Lee and Huttner,[20] who detected protein sulfation activity in crude Golgi membrane fraction obtained upon cell fractionation. To investigate the localisation of TPST1 in intact cells, we transfected COS-7 cells with a plasmid encoding a full-length wild-type (wt) TPST1-EGFP fusion construct. Simultaneously we visualised the Golgi apparatus by antibody staining of the marker protein Golgin97 for verifying the TPST targeting proposed by studies of cell fractionation. TPST1 fusion construct and Golgin97 colocalised in the Golgi network of COS-7 cells (Figure 1). This immunofluorescence experiment performed in intact mammalian cells supports the abovementioned localisation data of TPST obtained indirectly, using cell fractionation techniques. However, the targeting signal responsible for the Golgi localisation and the mechanisms of retention of TPST inside the membranes of this cell compartment are unknown to date.

N-Glycosylation occurs at positions 60 and 262 and has no influence on Golgi retention of TPST1

For a protein species carrying protein sulfation activity purified from bovine adrenal medula,[16] the

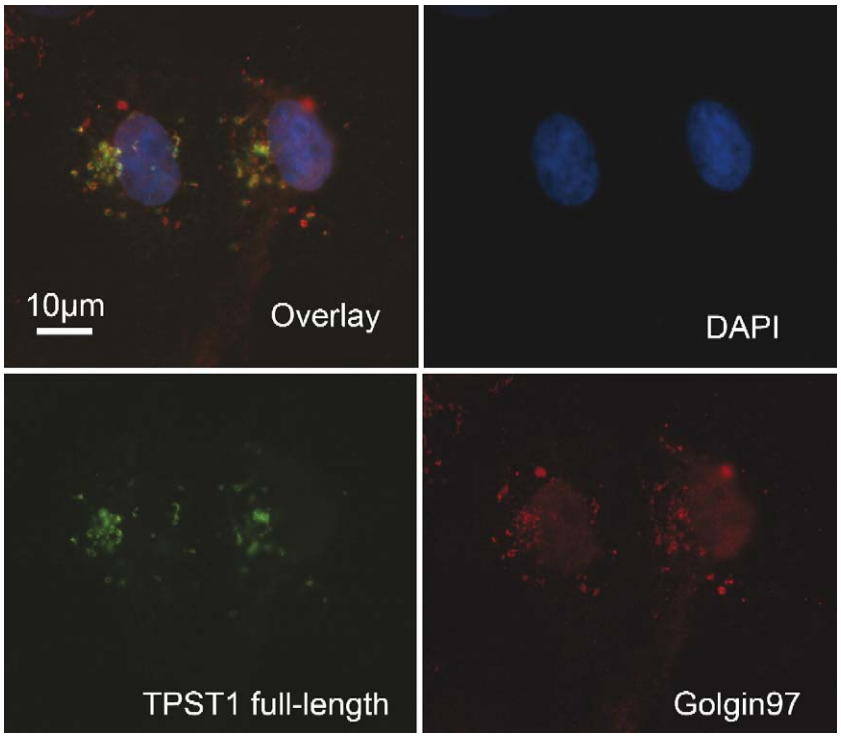

Figure 1. TPST1 colocalises with Golgin97 in mammalian cells. Transfection of COS-7 cells with full-length EGFP-coupled TPST1 shows that the construct colocalises with Golgin97 in vesicles of the Golgi apparatus around the cell nucleus. The channel displaying DAPI staining of nucleus is only shown as separated in Figure 1, in the following Figures being only visible in the overlay panels. The bar represents 10 µm.