Principal Investigator: Kohji Itoh

Grand Title: Development of neoglycobiologics by utilizing transglycosylation technology

Abstract

Object

The enzyme replacement therapy (ERT) has been clinically applied for genetic lysosomal enzyme deficiencies (lysosomal diseases) by utilizing functional N-glycans with terminal mannose 6-phosphate (M6P) residues attached to recombinant lysosomal enzymes produced by mammalian cell lines. However, high costs of the enzyme drugs, the immunogenicity leading to production of the neutralizing antibodies due to continuous administration and decrease in QOL of the patients have been problematic. In recent years we have established transgenic (TG) silkworms overexpressing human lysosomal enzyme genes in the silk glands to produce the recombinant enzyme drugs at low cost. In this study we aimed to develop a novel transglycosylation technology to produce neoglycoenzymes using microbial endo-β-N-acetyl-glucosaminidase (ENGase) mutants, functional N-glycans with M6P residues as donors and recombinant human lysosomal enzymes derived from TG silkworms as N-glycan acceptors for stable production and increase in the effectiveness of neoglycoenzymes for the ERT.

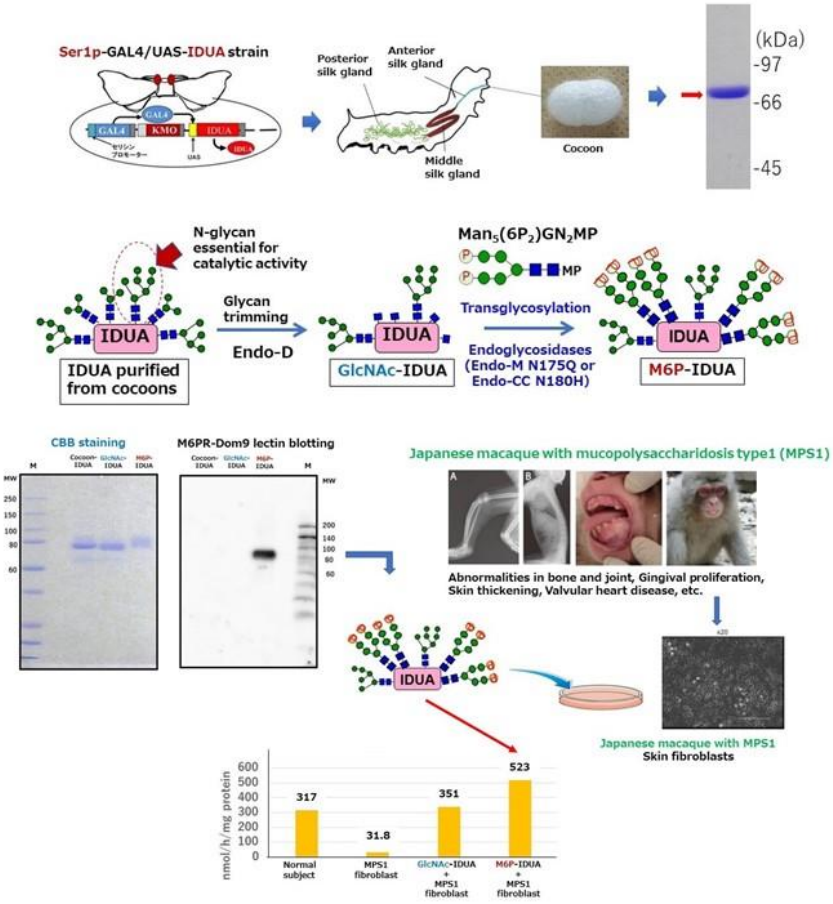
Methods

The recombinant human lysosomal α-iduronidase (IDUA) was easily purified from the cocoons from TG silkworm overexpressing *IDUA* in the middle silk glands. We utilized two ENGase mutants including Endo-M N175Q and Endo-CC N180H to transglycosylate the N-glycans attached to the IDUA to synthetic branched M5(6P2)GN2 glycans with terminal M6P residues or homogenous branched sialyloligosaccharides with terminal α2,6-sialic acid residues to produce chemoenzymatically neoglycoIDUA. We also evaluated the effectiveness of the neoglycoIDUA by assessing the incorporation through binding with cell surface M6P receptor (M6PR) to be transported to lysosomes and restoration of IDUA activity in the fibroblasts derived from mucopolysaccharidosis type I (MPSI) patients and Japanese macaque with a genetic IDUA deficiency. One-pot production of the neoglycoIDUA was performed by utilizing magnetic beads conjugated with Endo-CC N180H.

Results

The enzymatically active neoglycoIDUA (M6P-IDUA) was successfully produced by subsequent treatment of IDUA (acceptor) with Endo-D at pH5.0, the catalytic activity being retained, and then Endo-M N175H at pH6.0 or Endo-CC N180H at pH7.5 in the presence of M5(6P2)GN2 (donor) (acceptor : donor = 1 : 1,000 molar ratio). The M6P-IDUA was incorporated into both of fibroblasts derived from MPSI patients and Japanese macaque with MPSI through binding with cell surface M6PR to be transported to lysosomes, the deficient IDUA activity being restored. We succeeded in transglycosylating the N-glycans attached to IDUA to sialylglycans using sialylglycopeptides as donors and Endo-CC N180H conjugated to magnetic beads in one pot at pH6.0 and 30℃ for 24hrs.

Figure


Ser1p-GAL4/UAS-IDUA strain
GAL4
KMO
IDUA
Posterior silk gland
Anterior silk gland
Middle silk gland
Cocoon
(kDa)
-97
-66
-45
N-glycan essential for catalytic activity
IDUA
IDUA purified from cocoons
Glycan trimming
Endo-D
GlcNAc-IDUA
Man5(6P2)GN2MP
MP
Transglycosylation
Endoglycosidases (Endo-M N175Q or Endo-CC N180H)
M6P-IDUA
CBB staining
M6PR-Dom9 lectin blotting
Japanese macaque with mucopolysaccharidosis type1 (MPS1)
Abnormalities in bone and joint, Gingival proliferation, Skin thickening, Valvular heart disease, etc.
Japanese macaque with MPS1
Skin fibroblasts
nmol/h/mg protein
317
31.8
351
523
Normal subject
MPS1 fibroblast
GlcNAc-IDUA + MPS1 fibroblast
M6P-IDUA + MPS1 fibroblast