Supplementary Material (ESI) for Molecular BioSystems

Targeting lectin activity into inclusion bodies for the characterisation of glycoproteins

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Materials

HotStarTaq Master Mix Kit, QIAquick PCR Purification Kit, QIAprep Spin Miniprep Kit were from QIAGEN (Hilden, Germany). The linearized plasmid vector pET-34b(+) and T4 DNA Polymerase, that is suitable for ligation-independent cloning, were from Novagen (Madison, WI). *Helicobacter pylori* genomic DNA was obtained from American Type Culture Collection (ATCC 700824D). Gene Pool Human Normal Liver cDNA and primers were from Invitrogen Corporation. CelLytic™ B Cell Lysis Reagent (Sigma, B7435-500ML) was used for active inclusion bodies preparation. α(2-3,6,8,9) neuraminidase from *Arthrobacter ureafaciens* (Sigma, N8271) was used to cleave sialic acid. Fetuin (F2379), asialofetuin (A4781), BS albumin (A7030) and other reagents of analytical grade were from Sigma-Aldrich (St. Louis, MO).

Cloning, expression and isolation of active inclusion bodies

Both truncated versions of HP0662 gene were amplified from genomic DNA and truncated version of siglec8 (Entrez GeneID: 27181) was amplified from human normal liver gene pool cDNA; in 50 μL PCR reaction using forward
5'gacgacgacaagttgggctttttgtgagcgcg3'
5'gacgacgacaagttggaagacaacggctttttt3'
5'gacgacgacaagttgggggatggttacttgctg3'
and reverse
5'gaggagaagcccggttaaggtatcatcgccttaat3'
5'gaggagaagcccggttaagggatagaactaaagag3'
5'gaggagaagcccggttatgctgtggcatctccttg3'
primers.

The target genes with LIC extensions was purified, treated with T4 DNA polymerase for preparation of overhangs and annealed with the linearized vector. DNA sequence analysis confirmed the presence of truncated genes.
Freshly transformed *E. coli* BL21(DE3) cells harboring the recombinant plasmid were grown overnight (30 °C, 225 rpm) in 30 ml of LB medium (10 g/l tryptone, 5 g/l yeast extract, 10 g/l NaCl) supplemented with kanamycin (30 μg/ml). Then 10 ml of the culture was transferred into fresh LB (100 ml) medium containing kanamycin and grown at 37 °C. When A_{600} value reached 0.9–1.0, temperature was decreased to 25 °C, the agitation rate was reduced to 100 rpm and after addition of 400 μM isopropyl-1-thio-β-D-galactopyranoside (IPTG) allowed to proceed for 20 h.
The cells were harvested by centrifugation (4500×g, 10 min, 4 °C) and lysed with 10 volumes of the non-ionic lytic detergent. After centrifugation of the lysate (20,000×g, 10 min, 4 °C), the debris were three times washed with 25 volumes of buffer (50mM Tris–HCl, pH 7.5) and resuspended in same buffer or in PBS (150 mM NaCl, 100mM KH_2PO_4, pH=7.2).

IBs-hemagglutination

25 μl IBs (0.5 mg/mL) were 2-fold diluted from column 1 to column 10 in PBS or in PBS-fetuin solution (fetuin 5 mg/mL), titrated with 50 μl fresh human RBCs (washed in PBS), and incubated 1 hour at room temperature and then 4 hours at 4 °C.

IBs-agglutination

Asialofetuin, fetuin, BSA (25 μl, 67 mg/mL in 50 mM Tris–HCl, pH 7.5) were 1.5-fold diluted from column 1 to column 11 in the same buffer, titrated with 25 μl IBs (5 mg/mL), and incubated 4 hours at 30 °C (two time mixed with 100 μl pipette) and then overnight at 4 °C.

Neuraminidase reaction / IBs-agglutination

25 μl Neuramnidase (N8271) was diluted in 200 μl provided buffer and then 25 μl of the solution was pipetted into 25 μl 50 mM Tris–HCl, pH 7.5, A1. Then it was 2-fold diluted from A1 to F1 in the Tris. Fetuin (24 mg/mL) was added from A1 to G1, and 1.25-fold diluted from column 1 to column 11 in the same buffer. It was then titrated with 25 μl IBs (5 mg/mL), incubated 4 hours at 30 °C (two time mixed with 100 μl pipette) and then overnight at 4 °C.

Detection of interaction SAB-A with fetuin by Western Blot

Soluble cell fractions and IBs fractions were separated by SDS-PAGE on polyacrylamide gel (10%) according to Laemmli [1]. Proteins were transferred by electroblotting to nitrocellulose membrane with transfer buffer without SDS (1 h at 80 mA) (Amersham, U.K.) according to the method of Towbinet et al., [2]. Proteins interacting with fetuin were detected by specific staining with (4, 2, 1 μg/mL) solution of fetuin conjugated with biotin (prepared according to Diamandis and Christopoulos [3]; contained 1 mol biotin

per 1 mol fetuin) and avidin linked to horseradish peroxidase (Sigma) using the ECL detection system (Amersham U.K.) and Kodak scanning system CF 440.

References

[1] Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* **1970**, *227* (5259), 680–685.

[2] Towbin, H.; Staehelin, T.; Gordon, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. U. S. A.,* **1979** *76* (9), 4350–4354.

[3] Diamandis, E.P, Christopoulos, T.K. The biotin (strept)avidin system: principles in biotechnology. *Clin. Chem.*, **1991,** 37, 625-636.