Glycosilation of recombinant protein in hamster ovary cells

GLYCOSILATION OF RECOMBINANT PROTEIN IN CHINESE HAMSTER OVARY CELLS

- MANISHA YADAV

INTRODUCTION :

- GLYCOSYLATION OF PROTEINS

The phenomenon of addition & subsequent processing of carbohydrates in most of the plasma- membrane and secretory proteins is called glycosylation. This is the principle chemical modification or post-translational modification to most of such proteins. Glycosylation reactions occur in the lumen of Endoplasmic Reticulum (ER) and can also occur in the Golgi cisternae [1]. There are two different forms of oligosaccharide chains: O-linked oligosaccharides and N-linked oligosaccharides. The O-linked oligosaccharides are short structures and contain 1-4 sugar residues whereas; the N-linked oligosaccharides always contain mannose along with N-acetyl glucosamine. The structures of N-linked oligosaccharides have various branches terminating with sialic acid residues which are negatively charged groups [1].

Approximately 50% of the proteins manufactured in the eukaryotic cells are glycosylated. Many glycoproteins are produced from different glycoforms with different glycan structure which are attached to a single peptide backbone [2]. These glycan structures affect the biological properties of various glycoproteins which include pharmacokinetics, secretion, solubility, bioactivity, *in vivo* clearance, antigenicity and receptor recognition [2].

- CHINESE HAMSTER OVARY CELLS (CHO CELLS)

For the production of biopharmaceuticals, animal cell cultures are used due to their abilities of post-translational modification of proteins involving glycosylation [2]. Chinese Hamster Ovary (CHO) cells were first used in the year 1919 as a laboratory specimen for typing pneumococci [3]. CHO cells are the most commonly used cell lines for studying gene expression (particularly to express recombinant proteins), toxicity screening, genetics and nutrition. They are frequently used mammalian hosts for production of recombinant protein therapeutics at an industrial level [3]. Due to their adaptability to several culture conditions, relatively fast generation time, easy to maintain and also due to their plasticity in the surroundings of genetic alterations, CHO cells are technologically more prominent and highly useful [2].

- DIFFERENT EXPERIMENTS AND STUDIES ON GLYCOSYLATION OF RECOMBINANT PROTEIN IN CHO CELLS

Alterations in the glycosylation of recombinant proteins depend on a range of parameters which include: metabolic flux, cellular metabolism and the efficiency of the glycosylation process [4]. CHO cell lines are used in identifying the biological roles of mammalian glycans and the pathways to synthesise them [5]. An analysis of N- glycans and major O- glycans in CHO mutants was performed using glycomic analysis by MALDI-TOF (Matrix Assisted Laser Desorption Ionization- Time of Flight) and time of flight- Mass Spectroscopy (TOF-MS). To enhance the applications of CHO mutants and to obtain its insights, alterations in the glycosyltransferase activity was done. It was found that lectin resistant CHO cells make very large and various complex N-glycans. Also it was observed that terminal addition of sialic acid

enhances polylactosamine extension whereas fucose addition reduces polyLacNAc extension [5].

To determine the structural characteristics of recombinant envelope glycoprotein (rgp120) present in human immunodeficiency virus type-1 (HIV-1), the virus was produced by expression in CHO cells. To confirm the primary structure of rgp120 protein, enzymatic cleavage was done to assign intrachain disulphide bonds and the potential sites for N-glycosylation were determined. To carry out such technique reverse-phase high performance liquid chromatography method was employed [6]. For tryptic peptide mapping studies, N-glycosylation of 24 potential sites were characterized by determining the susceptible carbohydrate structures attached to the peptides which include: endo-β-N-acetyl glucosaminidase H and N-glycosidase F [6]. It was found that in all the 24 sites of gp120 protein, 13 consisted of complex-type oligosaccharide structures and the remaining 11 consisted of mannose-type oligosaccharide structures. It also contained certain hybrid-type oligosaccharide structures [6]. Analysis of the carboxyl terminal using carboxypeptidase digestions demonstrated that the glutamic acid residue 479 is the carboxylterminus molecule secreted by CHO cells [6]. The gp120 protein has a polypeptide core of approximately 60, 000 Daltons, an extensive modification in this polypeptide by N-linked glycosylation results in an increase in its molecular weight to 120, 000 Daltons [6].

Glycosylation is a cellular process in which achieving a precise glycan distribution is challenging, because it is a non-template driven process. Hence, it results in significantly uncontrolled variability in the glycan distributions [7]. Different cell types differ in their ability to carry out specific

post-translational modifications; for example: glycosylation, which affects the different processes such as receptor binding, bioactivity, susceptibility to proteolysis, clearance rate of therapeutic recombinant protein in vivo and immunogenicity [8]. As a result of this difference, various forms of glycans are formed and their attachment to proteins increases variability and non-uniformity [7]. Hence, an experiment was conducted to study the heterogeneity of a recombinant protein in different animal systems including Chinese hamster ovary cells [8]. CHO cells have proven produce proteins with glycoforms which are both bioactive and compatible in humans [3]. CHO cells produce recombinant IFN-γ which has 3 sources of variation: site-specific N-glycan microheterogeneity, variable N-glycosylation site occupancy and C-terminal polypeptide truncations [8]. It has examined that for efficient secretion and dimerization of IFN-γ, glycosylation of Asn [25] is necessary [8].

For optimal enzymatic activity, glycosylation is required. Neutrophils release granules such as Myeloperoxidase (MPO) into the phagosome during the process of phagocytosis of exogenous structures. Respiratory burst is initiated which reduces oxygen and releases superoxide radical anions. These superoxides upon dismutation produce Hâ,, Oâ,, which mediates MPO-driven oxidation of chloride to antimicrobial HOCl [9]. To increase the production of MPO protein, recombinant human MPO (r-MPO) are produced by expression in CHO cell lines. However, the r-MPO in CHO cell lines consists of some missing proteolytic steps as compared to the dimeric leukocyte enzyme (h-MPO). As a result, the r-MPO protein differs in the glycan structure and is a monomeric protein [9]. Tryptic digestion of reduced carboxamido-

methylated proteins was carried out to determine the glycan structures. A method like con A-immobilized affinity chromatography was used to enrich the glycopeptides. These glycopeptides were fractionated and analyzed by HPLC. The N-deglycosylated forms along with their glycopeptides were then identified by MALDI-MS. The result indicated that both r-MPO and h-MPO consists of high-mannose structures. The r-MPO has high amount of complex-type glycans like di- & trisialylated triantennary structures [9].

A glycoprotein hormone called human erythropoietin (EPO) regulates the level of circulating erythrocytes in humans. They play an important role in maturation of erythrocytes. In normal human, EPO is produced in the kidney. Various recombinant human erythropoietins are being produced in non-mammalian & mammalian cells. There are two forms of EPO (erythropoietin), EPO-bi and EPO- tetra. Each one of them have different biological activities. These were isolated from culture medium of CHO cell line (B8-300) encoding human EPO [10]. The culture was purified by reverse-phase chromatography, anion-exchange chromatography and gel filtration. The highly purified recombinant human erythropoietin from mast cells was used as a control and it is the standard EPO [10]. The results obtained showed that EPO-bi has only 1/7 in vivo activity and the invitro activity was 3 times higher than in vivo activity. Whereas EPO-tetra have similar in vivo and in vitro activities as the standard EPO. EPO-tetra, the standard EPO and EPO-bi had same immunoreactivity and amino acid composition. The structural analysis of N-linked sugar chains showed that EPO-tetra and the standard EPO contain tetraantennary complex-type as their major sugar chain, whereas EPO-bi contains biantennary complex-type as their major sugar chain [10].

Heparin is most widely used in modern medicine to control blood coagulation and is in great demands. Therefore, CHO cells are used for producing heparin. CHO cells are capable of producing HS (Heparan Sulfate) naturally, which is a related polysaccharide as heparin [11]. Human N-deacetylase/N-sulfotransferase (NDST2) and mouse heparan sulfate 3-O-sulfotranferase 1 were sequentially transfected into CHO host cells. Screening of these transfectant cells was carried out using quantitative RT-PCR and western blotting technique. The heparan sulphate produced by CHO cells is a less sulfated glycosaminoglycan (GAG). This is due to the glycosylation process resulting in heterogeneity in glycan structures. This HS has similar disaccharide units as heparin, but HS has lower anticoagulant activity than heparin [11].

- TREATMENT OF DISEASES:

Glycosylation process has been proved efficient in treating various diseases. Few examples are as follows:

1. Fabry disease : it is an X-linked genetic disease. It arises due to a deficiency in the lysosomal enzyme called α-galactosidase A (GLA). This enzyme hydrolyzes the terminal α-galactosyl moieties from glycoproteins and glycolipids. An enzyme replacement therapy is carried out to treat the disease. CHO cell lines are used to produce recombinant GLA with very high productivity. When the recombinant GLA was compared to an approved GLA (agalsidase beta), the charge and size of the recombinant GLA was found to be more neutral and smaller respectively. This difference was due the absence of terminal

sialic acid. To increase the sialic acid content, combined reaction of sialyltransferase, galactosyltransferase and their sugar substrates was developed. This product generated had same isoelectric points, size and sialic acid content as in approved GLA (agalsidase beta) [12].

2. <u>Gaucher disease</u> : it is an inherited metabolic disorder and is caused due to impaired activity of glucocerebrosidase (GCR) enzyme. This is a lysosomal enzyme which is responsible for hydrolysis of glucocerebroside to ceramide and glucose. The treatment of this disease consists of enzyme replacement therapy in which exogenous GCR is administered in the patients. This protein was expressed in CHO-DXB11 (dhfr $^-$) cells. The glycosylated GCR produced due to transfection and gene amplification with methotrexate is then detected by immunoblotting assay. CHO cells are the highest producer of glucocerebrosidase enzyme [13].

- <u>CULTURE PARAMETERS THAT CAN AFFECT GLYCOSYLATION:</u>

Culture conditions such as pH, nutrient content, temperature, ammonia and oxygen have an important effect on distribution of glycan structures in recombinant protein [2, 4, 10].

1. <u>pH</u> : any adverse change in the external pH conditions can change the internal pH of Golgi apparatus, resulting in reduction in the activity of glycosylating enzymes [2].

2. <u>Oxygen</u> : to maintain optimum growth and metabolism of producer cells in bioprocesses, the dissolved oxygen level should be under control. At high oxygen level there is an increase in sialyltransferase activity. By controlling dissolve oxygen (DO) set points, the

galactosylation of IgG immunoglobulin was changed efficiently and a decrease in digalactosylated glycans was observed [2, 4].

3. Effect of producer cell line on protein glycosylation : in the Golgi of the cell, the protein glycosylation pattern depends on the expression of several glycosyltransferase enzymes. Differences in the activities of these enzymes result in significant changes in the protein structure [2].

4. Fucosylation : the Fucosylation pathway synthesizes the fucose donor called GDP-fucose. α-1, 6-fucosyltransferase enzyme is a glycosyltransferase enzyme which catalyzes the transfer of fucose to Asn-linked GlcNAc residue from GDP-fucose [2, 6]. This enzyme is found in the N-glycans of several glycoproteins. Few studies suggest that fucose residues play a vital role in defining the oligossacharide conformations which are required for specific protein- carbohydrate interactions [2].

- CONCLUSION :

The properties of a mature protein depend on the carbohydrate moiety of glycoproteins. These properties include protein stability, solubility, molecular recognition or immunogenicity [9, 4]. CHO cells are being widely used in biopharmaceutical industry. By optimizing glycosylation, the therapeutic efficacy can be improved. The bioprocesses to produce monoclonal antibodies and fusion proteins have shown complex relationship between these variables and its protein quality [14]. Glycosylation can be controlled by understanding the cellular and metabolic alterations which results in the modification of protein structures [4]. It has been observed that by decreasing the concentration of glutamine to 0mM, there is reduction in

fucosylation, sialylation and antennarity, but an increase in neutral N-linked glycans has been observed [4, 7]. Stable CHO cells wih functional recombinant proteins which are properly glycosylated and secreted can be easily generated. The screening strategies can be used to produce biopharmaceuticals and other biological products which are highly cost-effective and is used for public health value [12, 13].

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