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Rational design of hyper-glycosylated interferon beta analogs: A computational strategy for glycoengineering

Mojtaba Samoudi[a], Fatemeh Tabandeh[a], Zarrin Minuchehr[a,*], Reza Ahangari Cohan[b], Davoud Nouri Inanlou[b], Mahvash Khodabandeh[a], Mohammad Sabery Anvar[a]

[a] Institute of Industrial and Environmental Biotechnology (IIEB), National Institute of Genetic Engineering and Biotechnology (NIGEB), P.O. Box 14965/161, Tehran, Iran

[b] Department of Research and Development, Pasteur Institute of Iran, P.O. Box 1316942551, Tehran, Iran

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ABSTRACT

Glycoengineering has been successfully used to improve the physicochemical and pharmaceutical properties of therapeutics. One aspect of glycoengineering is to introduce new N-linked glycosylation consensus sequences (Asn, X, Thr/Ser) into desirable positions in the peptide backbone by mutational insertion to generate proteins with increased sialic acid content. In the current work, human interferon beta (huIFN-β) was used as a model to identify the potential positions for the addition of new N-glycosylation sites. A computational strategy was employed to predict the structural distortions and functional alterations that might be caused by the change in amino acid sequence. Accordingly, three-dimensional (3D) structures of the designed huIFN-β analogs were generated by comparative modeling. Molecular dynamics (MD) simulation was carried out to assess the molecular stability and flexibility profile of the structures. Subsequently, for the purpose of glycoengineering huIFN-β, analogs with 3D structures more similar to the wild-type huIFN-β and exposed Asn residue in the new N-glycosylation site were identified. These modeling procedures indicated that the addition of the new N-glycosylation site in the loop regions had lower constraining effects on the tertiary structure of the protein. This computational strategy can be applied to avoid alterations in the 3D structure of proteins caused by changes in the amino acid sequences, when designing novel hyper-glycosylated therapeutics. This in turn reduces labor-intensive experimental analyses of each analog.

1. Introduction

Interferons (IFNs) are considered important cytokines which have a multiplicity of biological actions including anti-viral, anti-proliferative, and immunomodulatory activities. These biological properties have been leveraged into clinical usage to treat various diseases such as hepatitis, multiple sclerosis (MS) and several cancers [1]. In the case of MS, the recombinant huIFN-β is considered as the first line of treatment [2]. Although fibroblasts were originally identified as a source of IFN-β when responding to a number of stimuli *in vitro*, but other cell types have also been found to produce IFN-β, which include endothelial and epithelial cells, and various leukocytes [3]. IFN-β belongs to type I interferon family along with IFNαs, IFNω and IFNτ. Type I interferons exert their biological activities by binding to the type I interferon receptor, which is composed of two subunits, IFNAR1 and IFNAR2 [1]. IFNγ is the only known member of the type II interferon family. HuIFN-β adopts the standard fold of type I interferons, which consists of five α helices labeled A–E. A long overhand loop, known as the AB loop, connects the A and B helices and can be conceptually subdivided into three segments: the AB1 (residues 23–35), the AB2 (residues 36–40), and the AB3 (residues 41–50) loops. Three shorter loops (named BC, CD, and DE) connect the rest of the helices [4]. Fig. 1 shows the 3D structure of huIFN-β with respect to regions that are involved in receptor binding interactions. It is created from the PDB file of a crystal structure using the Discovery Studio visualization tool [4–6]. The blue residues (B, BC, C2, D and DE1) correspond to the regions interacting with the IFNAR1 subunit, and the green residues (A2, AB1, AB2 and E) correspond to the regions

* Corresponding author at: Department of Systems Biotechnology, Institute of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), P.O. Box 14965/161, Tehran, Iran. Tel.: +98 21 44787363.

E-mail addresses: m.samoudi@nigeb.ac.ir (M. Samoudi), taban_f@nigeb.ac.ir (F. Tabandeh), minuchehr@nigeb.ac.ir (Z. Minuchehr), cohan_r@yahoo.com (R. Ahangari Cohan), d_nouriinanlou@yahoo.com (D. Nouri Inanlou), saba@nigeb.ac.ir (M. Khodabandeh), mohammad_sa@nigeb.ac.ir (M. Sabery Anvar).

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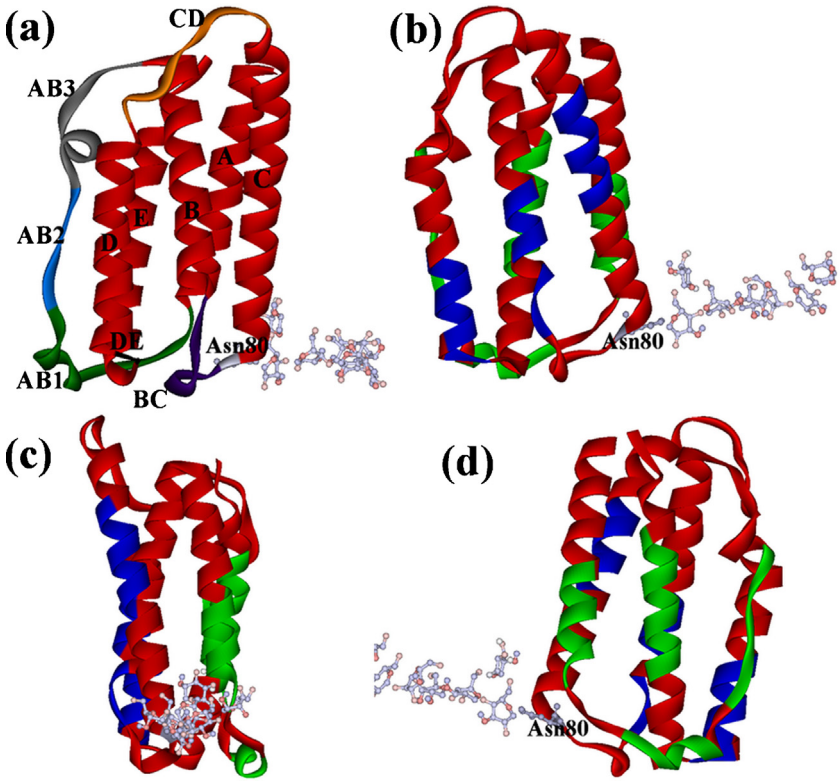

Fig. 1. (a) Crystal structure of huIFN-β visualized by the discovery studio program (Accelrys Software Inc., San Diego) from the PDB file of huIFN-β (PDB code: 1AU1, chain A). HuIFN-β adopts the standard fold of type I interferons and consists of five α helices (A–E) and connecting loops. AB loop connects helices A and B and is conceptually subdivided into three segments: the AB1 (green), the AB2 (blue), and the AB3 (gray) loops. Three shorter loops named BC (purple), CD (orange) and DE (black) loops connect the rest of the helices. A single N-glycosylation site exists at residue Asn-80 that involves a biantennary complex-type carbohydrate chain. (b) Front, (c) lateral and (d) back views of the crystal structure of huIFN-β with respect to the receptor binding regions. The blue regions correspond to the domain interacting with the IFNAR1 subunit and the green regions correspond to the domain interacting with the IFNAR2 subunit of the receptor.

interacting with the IFNAR2 subunit of the receptor. As illustrated in Fig. 1, the regions interacting with the IFNAR1 and IFNAR2 subunits are located on the opposite sides of the molecule.

HuIFN-β is a 166 amino acid glycoprotein with a non-glycosylated molecular weight of 18 kDa, which can increase to 23–25 kDa due to the presence of N-linked glycans at the single glycosylation site, Asn-80. The glycosylated IFN-β1a and non-glycosylated IFN-β1b produced in *Escherichia coli* and CHO cells, respectively, are both commercially available for clinical usage [7]. IFN-β1a is more potent and has been reported to have higher specific activity due to the stabilizing effect of the carbohydrate [7,8]. Additionally, glycosylation of huIFN-β reduces *in vitro* aggregation [7]. In spite of benefits resulting from glycan addition, the therapeutic usage of huIFN-β is limited. A major problem in MS patients treated with recombinant huIFN-β is the development of neutralizing antibodies (NAbs) against both IFN-β forms. This is primarily due to the low clinical effectiveness which increases the frequency of IFN-β administration [9]. Protein-based drugs routinely display suboptimum therapeutic efficacies due to their poor physicochemical and pharmacological properties [10]. Different strategies have been employed to improve therapeutic behavior of protein drugs. Glycoengineering is one of the most promising procedures as it has been shown to simultaneously improve most of the parameters necessary for optimization the *in vivo* efficacy of protein drugs. This approach has been successfully used to improve the biological activity of a number of therapeutics by increasing molecular stability and serum half-life [11,12]. The addition of glycans to a protein can also decrease aggregation by increasing solubility and/or masking the hydrophobic patches on the protein surface [7]. Specifically, protein aggregation is a challenging problem for recombinant huIFN-β due to the high degree of hydrophobic interactions among exposed residues located on the molecular surface [13].

One aspect of glycoengineering is to introduce new N-glycosylation consensus sequences (Asn, X, Thr/Ser) into desirable positions in the peptide backbone, so as to generate proteins with increased carbohydrate content. The glycan moiety can attach to the protein through the Asn residue in the consensus tripeptide sequence. However, the addition of a new tripeptide sequence will change the amino acid sequence of the protein. Such a modification must not alter the 3D structure, stability and receptor–ligand interaction of the protein. Inappropriate positions can affect folding and destroy the 3D structure of the active protein [11]. Additionally, factors such as surface accessibility of the N-linked Asn residue and probability of the enzymatic glycosylation of the new N-glycosylation site must be considered. Experimental screening of all possible analogs would be time-consuming and costly. Therefore, rational selection of suitable positions for introducing new N-glycosylation sites is very valuable. The main goal of this research is to develop a computational procedure for designing, evaluating and screening novel hyper-glycosylated huIFN-β analogs in order to find molecules in which changes in amino acid sequences have the least effect on the 3D structure, stability and receptor binding, whilst ensuring the glycosylation process is carried out efficiently. Due to the high degree of aggregation encountered during the production of huIFN-β and associated poor clinical outcomes, huIFN-β was selected as the model protein. Analogs of huIFN-β carrying one additional N-glycosylation site were examined with regard to molecular stability, flexibility regions, similarity to the wild-type huIFN-β and surface area accessibility of the new Asn residue. This computational procedure will determine if the designated analogs have increased numbers of carbohydrate chains