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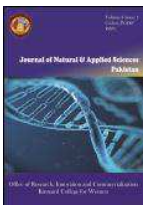

ENHANCEMENT OF INSULIN STABILITY THOUGH PROTEIN ENGINEERING TECHNIQUES: A MINI REVIEW

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Abstract
Insulin is a significant peptide hormone that helps in the regulation of blood glucose, and mainly utilized for treating diabetes. It is renowned hormone that suffers from several chemical modifications easily, that reducing the storage time of this extremely useful hormone. As the size of insulin is larger and intricate as compared to traditional drugs, the successful delivery of insulin undergoes various hindrances. Nevertheless, along with various unwanted modifications there are few beneficial modifications that enhances the stability and function of insulin. In this review, various strategies for enhancing the stability, biological performance and properties of insulin were discussed.

1. Introduction

Insulin is the first recombinant protein developed for therapeutic use. It is vital for controlling hormone level in human metabolic processes (Glidden et al., 2018). It is a globular protein consists of two peptide chains and 51 amino acids, having two disulfide bridges between chains and one intrachain. As the size of insulin is larger and intricate as compared to traditional drugs, the successful delivery of insulin undergoes various hindrances. The 3-D structure of insulin that is essential for binding of receptors and biological activity, extremely prone to chemical modifications and stress posed by environment. Thus, the stability of insulin remains a significant approach for conserving its biological activity. There are various extracellular and intracellular compounds that chemically modify insulin. Simultaneously, these compounds attach with insulin are targeted by various chemicals during modification. Consequently, the function of insulin may be disrupted by chemical modification (Drake & Smith, 2016).

2. Structure Of Insulin

The molecular weight of human insulin is 5,808 Da, having 51 amino acids in both the chains (A and B) (Brange, 2012a). The primary structure of insulin contains two chains A and B having 21 and 30 amino acids respectively. The structure of insulin contains three disulfide linkages, which includes two amongst A and B chain and one inside the A chain. When crystallization occur, the B chain might be in two different conformations, T and R state. Disulfide bonds between two cysteine residues play a role in providing sustainability to insulin. The entire 3-dimensional structure of insulin is well-organized and sustained by the association of specific side chains of amino acids. The N-terminal present in the A chain and C terminal in B chain are essential for binding of insulin

receptor with the insulin, probably the variation in configuration of both secondary and tertiary structure of insulin direct protein stability. The N-terminal present in the B chain have essential part in stabilization. As there are both non-polar and polar residues present on the surface, making it susceptible for homo-oligomerization. Insulin dimer can be easily damaged, which make sure splitting into monomers in solution and in the presence of zinc ions it become stable by forming hexamer while accumulating in β-cell of pancreas. Owing to the changeable feature of insulin dimer, Due to the transient nature of insulin dimer, immediate examination is intrinsically problematic. To study the association between insulin function and stability and insulin oligomerization, two monomers are covalently linked through a disulfide bond has been engineered. UV absorption, near-UV circular dichroism spectroscopy and equilibrium sedimentation are the techniques to study the association of insulin molecule (Fu, R Gilbert, & Liu, 2013).

3. Physiochemical Properties Of Insulin

Insulin is a therapeutic hormone utilized from long time. Meanwhile, while formulation and preservation proteins can effortlessly be degenerated. Few techniques are proposed to enhance the stability of proteins which includes protein modification, adding stabilizers and nanoparticle techniques. Drying procedure that desiccate proteins to make the protein stable than before in compact form are normally utilized for therapeutics. Consequently, the tendency of insulin for aggregation and precipitation can be altered by chemical modification and substitution by amino acids. For making insulin beneficial for pharmaceutical purposes, it is important to consider the basis of insulin aggregation and creating appropriate conditions for long-lasting storage of this hormone. For decreasing aggregation of insulin while storage and injecting, the recent techniques involved using chemically modified analogs of insulin and two-zinc hexamer (Brange, 2012b; Brange & Langkjœr, 1993; Emami, Vatanara, Park, & Na, 2018; Jang et al., 2012).

4. Strategies Used For Enhancement Of Insulin Stability

4.1 Modification of Insulin

Insulin is renowned hormone that suffer from several chemical modifications effortlessly, that reducing the storage time of this extremely useful hormone. Nevertheless, along with various unwanted modifications there are few beneficial modifications that enhances the stability and function of insulin. It has been reported that the shelf-life of few intracellular hormones can be increased by attaching with particular serum proteins. Insulin analogs being formed that can both be short-acting and long-acting analogs. Short-acting analogs were formed since insulin is in hexamer form in solution, which needs to be split so that insulin from the injection area is assimilated. Conversely, C-terminal of the B chain of insulin which together with two arginine molecules and replacement of asparagine with glycine enhances the isoelectric point. This led to the crystallization of analog at the intravenous injection spot, results in delayed absorption. (Jalili, Yousefi, Papari, & Moosavi-Movahedi, 2011; Radermecker & Scheen, 2004; Szewczak, Bierczyńska-Krzysik, Piejko, Mak, & Stadnik, 2015; Yousefi et al., 2016).

4.2 Insulin Conjugates

Conjugates of insulin have been formulated by binding of various compounds with amino acids of insulin for enhancing its permeability, solubility, stability, enhance the shelf-life in body, and reduce the chance of denaturation when administered orally. For this purpose, numerous approaches for conjugation have been utilized (Figure.1). PEG conjugated with insulin have been reported to have greater shelf-life, producing less allergic and immune reactions and also reduce the glucose level in blood efficiently (Hinds & Kim, 2002; Shechter et al., 2008). Insulin conjugated with human serum albumin (HAS) also showed to have longer half-life (Thibaudeau et al., 2005). Human transferrin (Tf) loaded with iron that binds to insulin by covalent linkage, that is cleaved when insulin absorb in the circulatory system. Insulin conjugated with Tf showed increased stability in the gut, function slowly and also efficiently reduce the blood glucose level (Kalra, Kalra, Agrawal, & Unnikrishnan, 2011; Shechter et al., 2008; Xia, Wang, & Shen, 2000).

IN-105 is a conjugate, updated form is HIM2, has been reported to resistant to denaturation in GI and efficacious than insulin in this way. Yet,

it pharmacologically more active than insulin. It is a recombinant insulin linked to an oligomer and has better solubility, stability and also higher absorption (Shao, Zaro, & Shen, 2016). Insulin-deoxycholic acid (In-DOCA) formed by conjugating DOCA with insulin, does not change its tertiary structure showed increase binding with insulin and have longer half-life and more stable (Baghban Taraghdari, Imani, & Mohabatpour, 2019a).

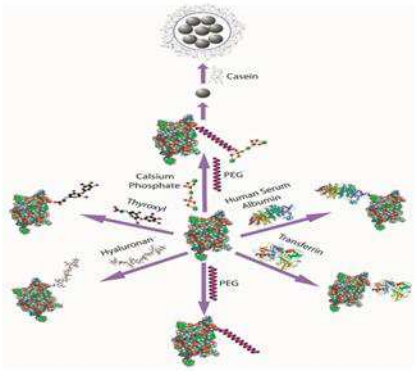

Figure: 1. A schematic representation of common insulin conjugates utilized for enhancing the stability of insulin (Baghban Taraghdari, Imani, & Mohabatpour, 2019b).

4.3 Simulation Method

The increasing progress in molecular simulation method has enabled us to comprehend the molecular procedures fundamental biochemical methods and molecular functions. A study was conducted to investigate the association between Polyethylene glycol (PEG) and a protein that is conjugated. A model consisting on PEG-Insulin conjugate was developed with varying length of PEG chain and a simulated method was constructed enable the system to attain equilibrium at increased rate devoid of altering the precision of the simulation. The Molecular dynamics (MD) simulation exhibited that directly conjugated with PEG results in a huge amount of conjugate and the surface of insulin become less exposed. The stability of conjugate model has been evaluated at increasing degeneration conditions. At elevated temperatures, Conjugates exhibited remarkable thermal stability and sustained their secondary structure, whereas unbound insulin become denatured completely. The distinct feature of PEG sustain a liquid layer that holds water molecules essential for the protein to function and also improves the stability of conjugated protein (Jevševar, Kunstelj, & Porekar, 2010; C. Yang, Lu, & Liu, 2011).

Recently, alterations by genetic modification in insulin are tested to delay and stabilize the formulations of long acting insulin. Another approach to enhance the stability of insulin is to raise the isoelectric point of insulin from pH 5.4 to neutral by forming analog that have positively charged amino acids in large number. This results in making the analog insoluble at the site of injection, allowing the sustained release of molecule in blood. Insulin is not chemically stable. Breakdown of insulin occur by two groups of chemical reactions include: intermolecular transformation and hydrolysis results in the formation of insulin dimers. By analyzing chemical modification of residues while preservation, insulin with enhanced stability can be formed (Jevševar et al., 2010). Single chain insulin (SCI) shows increase thermodynamic stability as compared to wild type (WT) insulin. CI-a exhibits enhanced thermodynamic stability relative to WT insulin. This has been resulted by NMR studies. Single chain insulin proved to have extraordinary resistance to denaturation physically at high temperature during fibrillation assays. These results showed that SCIs having promising biological properties offer promising therapeutic source without the need of refrigeration (Phillips, Whittaker, Ismail-Beigi, & Weiss, 2012;Weiss, 2013;Y. Yang et al., 2010).

4.4 Nanoparticles-Mediated Stability of Insulin

Dendrimers are used for modifying the aggregation tendency of insulin in less amount. Polyamidoamine (PAMAM) dendrimers in reduce amount trigger some alterations in the secondary structure of insulin. In addition to stabilize insulin, nanoparticles play essential role in delivering insulin. There are various NPs include Poly (Lactic-co-Glycolic Acid) (PLGA)-insulin nanoparticles, chitosan-insulin nanoparticles, dextran-insulin nanoparticles, solid lipid-insulin nanoparticles and polyalkylcyanoacrylated-insulin nanoparticles recognized as next generation of nanocarrier-based insulin delivery systems nanoparticles. Foe oral delivery of insulin, encapsulation of insulin in dextran sulfate-chitosan nanoparticles

showed promising results (Nowacka, Shcharbin, Klajnert-Maculewicz, & Bryszewska, 2014; Sharma et al., 2015; Tomalia, Christensen, & Boas, 2012).

5. Conclusion

The enormously liable structure of insulin is extremely prone to aggregation and unfolding by a wide range of chemical agents present intracellular. Nevertheless, in research it is still challenging to resolve the complicated structure of insulin and its receptors. The collected information of the structure of insulin has enabled us to precisely plot a unique, highly-stable and single-chain insulin analog and offered an innovative method to form rapid-acting, less expensive and highly stable formulations of insulin for diabetic patients. By exploiting engineering techniques in fabricating insulin delivery system have revealed extraordinary consequences in insulin treatment. Taking into account the smart biomaterials, which might react to variations in environment (like temperature, pH, ionic concentration), insulin-delivery system could assist the patient's rate of insulin delivery nearer to the physical condition. Though, these biomaterials have not still came into clinical trials, aiming these advanced approaches providing a motivation for rapid transformation of bio responsive insulin delivery systems to marketed product. It is favorably predicted that with the help of developments in drug delivery systems and biomaterials, the existing trials of insulin treatment will be overwhelmed.

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