BPR MCS | Bulletin of Pioneering Researches of Medical and Clinical Science

Bulletin of Pioneering Researches of Medical and Clinical Science

Available online: https://bprmcs.com
2023 | Volume 2 | Issue 2 | Page: 8-14

Investigating Different Methods of Increasing the Stability of Medicinal Recombinant Proteins: A Review Study

Xinyi Chen[1], Chun Li[1-3], Hu Liu[1*]

[1]Key Laboratory of Medical Molecule Science and Pharmaceutics Engineering, Ministry of Industry and Information Technology, Institute of Biochemical Engineering, School of Chemistry and Chemical Engineering, Beijing Institute of Technology, Beijing, China.
[2]Key Laboratory for Industrial Biocatalysis, Ministry of Education, Department of Chemical Engineering, Tsinghua University, Beijing, China.
[3]Center for Synthetic & Systems Biology, Tsinghua University, Beijing, China.

Abstract

One of the significant aspects of the production of proteins is the discussion of their stability and solubility. Studying the stability of proteins and identifying factors that increase their stability, is one of the important and interesting topics in scientific societies. The production of biotech medicinal proteins plays an important role in the fight against diseases. Proteins are stable only in a limited range of temperature and acidity conditions and are very susceptible to physical and chemical degradation. Today, various solutions have been proposed to increase the solubility and stability of proteins, which include guided changes in the protein molecule and optimization in the instructions for expression, purification, and the solubility process of proteins, which most of the time, the first approach is not possible and achievable. This review study provides solutions to increase the stability of proteins at different levels, including investigating the impact of the design of the expression structure, sequence, and protein sequences on stability, the optimal selection of the host cell, and the improvement of the expression conditions be also discussed. In addition, the effect of optimization of buffers used in pharmaceutical formulations, such as the selection of suitable amino acids and osmolytes to increase protein stability, and the effect of sugars and polyols on the stability of recombinant pharmaceutical proteins are also investigated.

Keywords: Stability, Solubility, Proteins, Pharmaceutical proteins

Corresponding author: Hu Liu
E-mail ✉ liuubit@bit.edu.cn

How to Cite This Article: Chen X, Li Ch, Liu H. Investigating Different Methods of Increasing the Stability of Medicinal Recombinant Proteins: A Review Study. Bull Pioneer Res Med Clin Sci. 2023;2(2):8-14. https://doi.org/10.51847/bxQo3dIbnf

Introduction

One of the significant aspects of the production of proteins is the discussion of their stability and solubility. The stability of proteins during the process of expression and purification is one of the important and challenging issues, because many recombinant proteins are unstable under the conditions they are expressed and lose their correct folding or undergo proteolytic failure, and finally, their stability decreases at the spatial structure level. Therefore, studying the stability of proteins and identifying factors that increase their stability, is one of the important and interesting topics in scientific societies [1-4]. In the production of recombinant proteins, it is very important to produce a stable protein with correct folding and proper function for medicinal use [5-7]. Today, various strategies have been proposed to increase the solubility and stability of proteins, which include directed modifications. It is in

the protein molecule or the optimization is done in the expression protocols, purification, and solubility process of proteins, which is often not possible and achievable in the first approach [1, 8, 9]. One of the problems faced by the production of recombinant proteins is the production of a protein with correct and active folding in the host cell. Unfortunately, many recombinant proteins are not expressed in a stable and soluble folded form, and this issue is a big obstacle in the pharmaceutical and biotechnology industry. The production of biotechnology medicinal proteins plays an important role in the fight against diseases. In recent decades, the growth of research on these products has significantly outpaced that of small-molecule drugs, and this trend is expected to continue in the coming decades [10-13].

The production of a biological drug is not a direct path, because on the one hand, this process requires a lot of work, and on the other hand, there is the issue of product instability during the process and storage; In addition, there are other challenges such as the need for strong laws, the scrutiny of Good Manufacturing Practice (GMP) regulations and fierce competition in the consumer market. Therefore, one of the significant aspects of the production of proteins is the discussion of their stability and solubility during the process of expression and purification. Because proteins are only stable in a limited range of temperature and acidity conditions (marginal stability) and are very susceptible to physical and chemical degradation. Many recombinant proteins are unstable under the conditions they are expressed and lose their correct folding or undergo proteolytic failure which eventually decreases their stability at the spatial structure level. In addition, medicinal proteins have a great tendency for aggregation, especially when they are kept at high concentrations required for doses; therefore, the main problem in the production of medicinal proteins, which are a large class of drugs, is the issue of stability and maintaining their activity [14-18].

Factors affecting the stability of proteins

In addition to temperature and the presence of stabilizers, other variable factors that affect the stability of proteins can be the effect of acidity, pH, salts in different concentrations, and the help of solvents, osmolytes, preservatives and surfactants [10, 11]. A change in each of these factors can in some cases cause a change in the folding and condensation of proteins and, as a result, cause a decrease in their stability and loss of activity [19]. In general, with some exceptions, electrostatic interactions play a minor role in protein stability in the native state. The binding of ligands, such as inhibitors to enzymes, also increases the stability of proteins. The number of disulfide bonds in extracellular proteins is high. This is even though intracellular proteins with disulfide bonds are less common in them. The presence of a large number of disulfide bonds in proteins increases the stability in the natural state of proteins, and as a result, reduces the amount of structural entropy of proteins. Considering that each of the amino acid roots in the protein sequence has different properties, hence they do not have the same contribution to the stability of proteins. Studies have shown that the lack of access of the amino acid roots located on the inner side of the protein to the solvent molecules has caused these amino acid roots in the primary structure to contribute more to the stability of proteins than the amino acid roots located on the outer side. They are located outside the proteins.

Solutions to increase the stability of proteins

The effect of structure design, T sequence expression, and protein sequences on the stability of proteins

The solutions that are offered to increase the stability of proteins are sequential and serial methods. Genetic engineering of protein production is a general approach to increase the stability of proteins, through which expression, purification, and protein activity can be increased. This topic is a wide and widely used field that has received a lot of attention today. Among the methods used in this field are targeted mutations (site-directed mutagenesis, addition or removal of segments (segment domain deletion and ligation), optimization of amino acid codons (using novel non-native amino acids), chemical modifications and additives and containment methods with the help of nanoparticles (confinement in nanoparticles) [20].

Designing an expressive structure

Designing the expression structure is the first step that structural biologists must be sure of to increase stability in the process of protein expression and purification. For example, the structural stability of proteins may be affected due to the presence of proteolytic cleavage sites. The presence of these sites has caused the proteins to be more affected by the proteases that exist inside the host cell. The process of proteolysis can be reduced with the help of genetic engineering techniques or the removal of breakpoints from the expression structure during the production of recombinant protein. Because of removing these breakpoints, proteins are produced with real size and on a large scale [21, 22]. First Structure Some proteins are inherently unstable and have a short half-life. For example, proteins whose sequences are rich in the amino acids proline, glutamate, serine, and threonine (PESTS) often have half-lives of less than two hours [23, 24]. In addition, the N-terminal sequence is an important factor for measuring the half-life of a protein in vitro. For example, if this N sequence is rich in amino acids methionine, serine, alanine, glycine, treolin, valine, or proline, the half-life of the protein is more than 20 hours and the protein

becomes stable. On the other hand, if this sequence consists of phenylalanine, aspartate, lysine, or arginine, the protein becomes unstable and its half-life is less than 3 minutes, and the instability in this method is through the ubiquitin pathway. Therefore, the effect of the N terminus on protein instability is not significant in bacterial expression systems. Therefore, to increase the stability of this type of protein, using genetic engineering techniques and optimizing the codons of amino acids in these proteins, their half-life can be increased [25, 26].

The effect of protein sequences on the stability and increasing the solubility of proteins

Many recombinant proteins are produced in bacterial systems such as Escherichia coli, and the main challenge in the production of these proteins is the formation of inclusion bodies and the transformation of proteins into insoluble structures. With the help of genetic engineering techniques, sequences can be used in the design of gene structures expressed in bacteria, which not only help to increase the solubility of proteins but also make it easier to purify proteins. Another point to be noted here is that the N sequence, whose sequence affects the stability of proteins, is covered in systems that use (TAG) sequences; therefore, these sequences cannot affect the stability of proteins. In addition to the effect that sequences have on increasing the stability of proteins, they can also be considered as a factor for increasing the solubility of the target protein. Like MBP TAG (Maltose Binding Protein), which increases the solubility of the target protein and, like a chaperone, induces the correct folding of the target protein in Ecoli bacteria. Protein sequences are also effective in the process of separation and increasing the purification efficiency of recombinant proteins. After the completion of purification, the general method to remove desired sequences is to use proteases such as thrombin and factor a, because of which a non-specific fragmentation is performed and a decrease in stability is created in the target protein. Therefore, using sequences in purification can increase structural stability if these sequences themselves can be separated from proteins without the need to use proteases. Sequences such as intein-based tags, which themselves can be separated from proteins, prevent this type of instabilities created under the influence of proteases in proteins, which can also be used to separate from nickel without the need for internal proteases [27]. In bacterial expression systems, to prevent the formation of inclusion bodies and considering that some proteins are not stable in the cytoplasmic space, the pMal vector system (NEB)) pal in connection with the malk marker to transfer the protein from us between the membrane Cytoplasmic to periplasmic space are used. As a result, proteins are transferred to other parts of the cell; therefore, their proteolysis is prevented and increases the compositional stability. In addition, the use of chaperones such as Fab antibody fragments, which increase the stability of membrane proteins, also helps to increase the stability of proteins. Other chaperones that increase the stability of proteins during expression include DnaK and GroEL. Today, the use of advanced genetic engineering methods to create changes in the expression plasmid and the host cell is considered; therefore, there is a need for accurate and fast methods that can record the folding events in the early moments and directly in the living cell. These selective methods have the necessary efficiency to check the folding and solubility in the environmental conditions of living cells. They can also be used to investigate compounds that inhibit aggregation and factors that increase solubility naturally or with the help of mutation [21]. All these methods, despite being valuable, are very time-consuming and require complex and precise designs. In addition, if there is a change in any of the amino acids of the protein, detailed simulations should be done regarding the effect of this change on the entire complex, which makes the process of stabilization by this method difficult. On the other hand, in pharmaceutical proteins, it is preferred that there is not much change compared to the biological system. Because any change requires heavy and expensive studies and checking the effects of these changes on the body. To replace this method, it is possible to use permitted additives, suitable buffers, surfactants, ions, and osmolytes to increase protein stability.

Host cell selection and protein stability

Recombinant proteins may be affected by intracellular proteases and thus cause structural instability in them, or the protein itself may be toxic to the host cell; therefore, the choice of host cell type has a great impact on the stability of recombinant proteins. The toxicity caused by the expression of proteins in the host cell is controlled by the precise regulation of the level of expression with the help of regulatory promoters, and the expression level of internal proteases and in general, proteins within the host can be controlled. For example, with the help of pLys S plasmid, which expresses T7 lysozyme, it acts as a natural inhibitor of the T7RNA polymerase enzyme and stops its activity. In addition, this promoter is used in the pET (Vector) system and reduces the expression of internal proteins [28, 29]. Using mammalian expression systems to express human proteins is better and more practical. Because the codons in these expression systems are optimized and post-translational modifications are also applied to the proteins; as a result, the proteins expressed in these systems have the correct folding, are stable, and have maintained their activity [30].

Improving expression conditions and stability of proteins

To ensure the production of a protein with correct and stable folding that has activity, it is necessary to provide suitable conditions by the host cell. Many of these factors are provided by the culture medium and host cells during the expression process. However, many others are not synthesized by the host cells or in sufficient quantities; it is not available and must be provided to the cell. The solubility of proteins increases during the expression process with the help of additives to the culture medium, such as trehalose glycine, proline, mannitol, L-arginine, potassium citrate, xylitol, and potassium phosphate [31]. In addition, temperature is considered a factor to increase protein solubility and stability. Among the other benefits of this system, it can be pointed out that at low temperatures, cell proliferation is stopped and the expression of internal proteins such as proteases is reduced; Therefore, the target protein is more protected and its purity increases [32].

The effect of buffer optimization on the stability of proteins

Choosing the type of additive and their concentration to increase protein stability is a difficult and complex task. Because the number of additives that can be added to buffers to increase protein stability is many and varied. Optimizing the buffer conditions to achieve the best solubility and stability in the protein is obtained with the help of screening. The need to optimize the buffer for proteins with low initial solubility and stability is felt more and more screening should be done. Since the buffer compounds, ligands, and reducing agents in the buffer in which the protein is dissolved and stored have a great impact on the stability of proteins, screening the type of buffer used is a very efficient method in protein stability and crystallography and it is their biological formulation. One of the primary methods used in the screening of buffers is Thermofluor. In this method, various buffers with different compositions and different pH are examined and finally, their effects on the stability of proteins are determined by measuring the protein melting point (Tm). The screening of ligands and additives is also effective in the stability of proteins. The binding of ligands specifically increases the stability of proteins, especially increases the structural stability. In addition to the effects of small molecules on the stability of proteins, ions or other organic additives also cause the stability of proteins [33].

Selection of suitable amino acids and osmolytes to increase protein stability

The solubility and stability of proteins can be significantly increased with the help of additives such as ionic compounds, salts, and osmolytes in the buffer [20, 34]. Today, amino acids and osmolytes are used to increase the stability of proteins. The amino acid arginine is used as an active molecule involved in pharmaceutical formulation because it increases the solubility of proteins from inside the Incologne and prevents protein condensation during purification. Unlike common denaturants, such as urea and guanidinium hydrochloride, this amino acid does not affect the stability of proteins by increasing their solubility. Adding charged amino acids such as arginine and glutamate to the buffer increases the solubility of proteins, but does not affect the protein structure and does not prevent protein-protein and protein-RNA interactions. In addition, the addition of apple charged amino acids increases the life span and stability of proteins and prevents their breakdown and sedimentation over time. As a result, in their presence, proteins with high concentration and solubility can be obtained. The mechanism through which arginine increases solubility without affecting stability is still unknown and debatable. Therefore, if the effect of arginine on protein and its solubility were fully determined, this amino acid could be used as a reliable additive to increase protein stability [35]. In order to check the effect of these additives, they can be added to the environment with different concentrations and finally the stability of the proteins can be measured.

The effect of sugars and polyols on protein stability

The reason for using the term osmolyte for such compounds is that these substances were first known as osmotic pressure regulators in the biological system and later their effect on the structure of cell proteins was proven. Using temperature-sensitive polymers, degradable polymers, and nanoparticles also helps to increase the stability of proteins [36]. Sugars have been introduced as an efficient additive to protect proteins against loss of activity and chemical and thermal denaturation. Among sugars, trehalose is a suitable stabilizer to protect biological materials against water loss and desiccation. This sugar is a compatible osmolyte in organisms whose synthesis rate increases under stress conditions. Due to these unique characteristics, there is a great desire to understand the mechanisms that occur in organisms [37]. Trehalose, due to its unique physical and chemical properties such as low chemical reactivity, high non-reduction property of glass transition (Tg), high tendency to bond with water molecules, and its existence as a polymer structure, as a stabilizer is suitable to protect the protein structure. Trehalose is very effective in the stability of proteins during lyophilization. In addition, when the proteins are exposed to high temperatures in the solution, it prevents their aggregation and folding. Sugars generally protect proteins from water loss. They can make hydrogen bonds with proteins instead of water molecules. Sugars replace the water molecules lost in dried protein [38]. Many studies conducted by Timasheff and his colleagues have shown that sugars and polyols stabilize the folded structure of proteins in solution. Therefore, in

the folded state where water coverage is less, sugar molecules enter into hydrogen bonds with protein molecules As a result, it makes them stable. Proteins are expected to interact with solvents in different ways, which depends on their physicochemical properties. In general, it has been observed that apple trehalose protects proteins in different ways, and the efficiency of this protection depends on the structure of proteins. Despite the existence of such information, the exact role of proteins and their physicochemical properties in the environment in the presence of trehalose to increase stability is not yet clear [39].

Results and Discussion

One of the main problems in the production of medicinal proteins, which are a large class of drugs, is their stability. Medicinal proteins have a very high tendency to condense, especially when they are kept at the high concentration required for doses [40]. Considering that the activity of proteins depends on their structure and flexibility, any connection between proteins and other molecules may cause changes in their conformation. Potentially, perturbations that alter protein flexibility may also interfere with their function, and how protein function, flexibility, and stability are related is still hotly debated, and a deep understanding of The relationship between the properties of proteins is not only a scientific issue, but also has a great impact on the design of the function and application of proteins [41]. Although protein stability has a different meaning for many scientists, in general, protein stability can be defined as the ability of proteins to maintain their structure and function in different environments. In addition, if the proteins are in normal physiological conditions and the sum of all contributing forces is negative towards G, then the folded protein is stable. Although not all proteins are located in physiologically stable environments, they tend to be unstable. Since the introduction of the first therapeutic recombinant protein to the market in the 1980s, many products have been successfully commercialized and other new drugs are in clinical trials. Since protein, products have shown beneficial therapeutic effects in human diseases and abnormalities, there is a lot of luck for them in the consumer market. Meanwhile, protein drugs must go through the process of production, transportation, maintenance, and storage without being destroyed. Providing these conditions is very difficult because proteins are vulnerable to chemical and physical degradation and are exposed to many damaging factors. Therefore, studying the stability of proteins and identifying the factors that increase their stability is one of the important and interesting topics in scientific communities and is the main challenge in the production of medicinal proteins, which has attracted a lot of attention today. Because many recombinant proteins become unstable under the conditions, they are expressed, lose their folding, or undergo proteolytic failure, which ultimately causes a decrease in the stability of the spatial structure of the protein. Therefore, in the production of recombinant proteins, it is very important to produce a stable protein with proper folding and function that can be used medicinally. Unfortunately, many recombinant proteins are not expressed in a stable and soluble folded form, and this issue is a big obstacle in the fields of pharmaceuticals and biotechnology [35]. In this era, with the advent of bioinformatics and the creation of genomic changes, along with the efforts and research that has been done on proteomics, many advances have been made in the use of protein sequences, which can be used to produce proteins at a high level and purify them to the condition of maintaining their structure and function is effective. It also has a very influential role in structural biological studies. The use of these protein sequences has made the process of purification and isolation of these proteins easier and economical. They also have positive effects on the pharmaceutical industry and drug design. It is also predicted that in the field of protein sequences, many developments will be made in the future. By using this technique, which has a high efficiency, it is possible to investigate the relationship between the communication networks of proteins and offer new protein drugs. In addition, the ability to understand the relationship between the structure and function of proteins increases in places that are not available due to limitations in techniques and information. The study of host cells has also developed greatly in recent years. All high-volume production lines appear to be cell lines that have been genetically engineered to increase viability or carry growth-inducing transgenes. Reports from academic laboratories show many examples of how it is possible to increase the growth, viability, and production of recombinant cell lines with genetic engineering methods. Proto-oncogenes, cell cycle control genes (cyclins), growth factor genes (such as insulin and growth factor), and antiapoptotic genes are inserted into cell lines to produce high-production hosts [42].

Conclusion

In this review study, various strategies have been presented to increase the solubility and stability of proteins, which include guided changes in the protein molecule or optimization in the expression protocols, purification and solubility process of proteins, which are carried out in most of the time, the first approach is not possible and achievable [1]. Therefore, it can be said that a complete investigation of the environment around proteins to increase their stability can be efficient enough and a uniquely stable protein is provided to us, without the

need for complex methods. Genetic engineering. Therefore, the different influencing factors that are examined during the expression and purification of proteins to increase their stability are nowadays mostly used as a combination design approach. In this approach, methods with high efficiency and capability are combined and used to obtain a suitable strategy to increase the stability of proteins in different optimal conditions [5, 17].

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

References

1. Savchenko A, Yee A, Khachatryan A, Skarina T, Evdokimova E, Pavlova M, et al. Strategies for structural proteomics of prokaryotes: Quantifying the advantages of studying orthologous proteins and of using both NMR and X-ray crystallography approaches. Proteins. 2003;50(3):392-9.
2. Das N, Kalita PP, Sarma MP, Bhattacharjee M. Molecular modeling of HEV core protein and active compounds from northeast folk medicine. J Biochem Technol. 2021;12(2):12-8.
3. Bazhenova AA, Guryanova NI, Guryanov GS, Alieva HA, Kachmazova DT, Khripunova AA, et al. In-vitro study of the properties of components for the synthesis of sorbent for low-density lipoprotein apheresis. Pharmacophore. 2021;12(3):37-41.
4. Suragan M, Durmuşkaya S. The long-run relationship between ict indicators and stock market indexes for g7 and e7 countries. J Organ Behav Res. 2022;7(1):42-57.
5. Deller MC, Kong L, Rupp B. Protein stability: A crystallographer's perspective. Acta Crystallogr F Struct Biol Commun. 2016;72(2):72-95.
6. Kosilov VI, Kubatbekov TS, Semak AE, Salikhov AA, Baranovich ES, Makeeva VM, et al. The impact of steer genotype on protein and mineral metabolism. J Biochem Technol. 2021;12(3):22-6.
7. Alrahimi J, Yousuf M, Pushparaj P, Basingab F, Zaher K, Hassan M, et al. Investigating the changing levels of immune checkpoint proteins in the serum of breast cancer patients. Pharmacophore. 2022;13(6):125-36.
8. An HT, Linh TK, Ha TN, Huy PQ, Luc PT. Value co-creation activities of students on the covid-19 pandemic: Empirical evidence from economics students in Vietnam. J Organ Behav Res. 2022;7(2):214-28.
9. BaSalamah M, AlMghamsi R, AlTowairqi A, Fouda K, Mahrous A, Mujahid M, et al. The effect of coffee consumption on blood glucose levels. J Biochem Technol. 2022;13(2):64-9.
10. Craik DJ, Fairlie DP, Liras S, Price D. The future of peptide-based drugs. Chem Biol Drug Des. 2013;81(1):136-47.
11. Leader B, Baca QJ, Golan DE. Protein therapeutics: A summary and pharmacological classification. Nat Rev Drug Discov. 2008;7(1):21-39.
12. Tang L, Persky AM, Hochhaus G, Meibohm B. Pharmacokinetic aspects of biotechnology products. J Pharm Sci. 2004;93(9):2184-204.
13. Sonbol HS, AlRashidi AA. Cloning and expression of receptor of egg jelly protein of polycystic kidney disease 1 gene in human receptor of egg jelly protein. Pharmacophore. 2022;13(6):97-105.
14. Nayak AK. Advances in therapeutic protein production and delivery. Int J Pharm Pharm Sci. 2010;2(2):1-5.
15. Sun J, Rao S, Su Y, Xu R, Yang Y. Magnetic carboxymethyl chitosan nanoparticles with immobilized metal ions for lysozyme adsorption. Colloids Surf A Physicochem Eng Asp. 2011;389(1-3):97-103.
16. Sun YL, Patel A, Kumar P, Chen ZS. Role of ABC transporters in cancer chemotherapy. Chin J Cancer. 2012;31(2):51-7.
17. Papaneophytou CP, Kontopidis G. Statistical approaches to maximize recombinant protein expression in Escherichia coli: A general review. Protein Expr Purif. 2014;94:22-32.
18. Hoang TT, Nguyen TH, Nguyen TT, Hoang LP, Ho TT, Nguyen TH. Research factors affecting students' academic results in learning project subjects oriented CDIO in vinh university. J Organ Behav Res. 2022;7(1):14-28.
19. Robertson AD, Murphy KP. Protein structure and the energetics of protein stability. Chem Rev. 1997;97(5):1251-68.
20. Bagby S, Tong KI, Ikura M. Optimization of protein solubility and stability for protein nuclear magnetic resonance. Methods Enzymol. 2001;339:20-41.
21. Sorensen HP, Mortensen KK. Advanced genetic strategies for recombinant protein expression in Escherichia coli. J Biotechnol. 2005;115(2):113-28.
22. Spiegel H, Schinkel H, Kastilan R, Dahm P, Boes A, Scheuermayer M, et al. Optimization of a multi-stage, multi- subunit malaria vaccine candidate for the production in Pichia pastoris by the identification and removal of protease cleavage sites. Biotechnol Bioeng. 2015;112(4):659-67.
23. Spencer ML, Theodosiou M, Noonan DJ. NPDC-1, a novel regulator of neuronal proliferation, is degraded by the ubiquitin/proteasome system

through a PEST degradation motif. J Biol Chem. 2004;279(35):37069-78.
24. Rogers S, Wells R, Rechsteiner M. Amino acid sequences common to rapidly degraded proteins: The PEST hypothesis. Science. 1986;234(4774):364-8.
25. Hara K, Schmidt FI, Crow M, Brownlee GG. Amino acid residues in the N-terminal region of the PA subunit of influenza A virus RNA polymerase play a critical role in protein stability, endonuclease activity, cap binding, and virion RNA promoter binding. J Virol. 2006;80(16):7789-98.
26. Sung D, Kang H. The N-terminal amino acid sequences of the firefly luciferase are important for the stability of the enzyme. Photochem Photobiol. 1998;68(5):749-53.
27. Kopera E, Bal W, Lenarcic Zivkovic M, Dvornyk A, Kludkiewicz B, Grzelak K, et al. Atomic resolution structure of a protein prepared by non-enzymatic His-tag removal. Crystallographic and NMR study of GmSPI-2 inhibitor. PLoS One. 2014;9:e106936.
28. Guzman LM, Belin D, Carson MJ, Beckwith J. Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J Bacteriol. 1995;177(14):4121-30.
29. Studier FW. Use of bacteriophage T7 lysozyme to improve an inducible T7 expression system. J Mol Biol. 1991;219(1):37-44.
30. Fischer S, Handrick R, Otte K. The art of CHO cell engineering: A comprehensive retrospect and future perspectives. Biotechnol Adv. 2015;33(8):1878-96.
31. Leibly DJ, Nguyen TN, Kao LT, Hewitt SN, Barrett LK, Van Voorhis WC. Stabilizing additives added during cell lysis aid in the solubilization of recombinant proteins. PLoS One. 2012;7(12):e52482.
32. Quistgaard EM. A disulfide polymerized protein crystal. Chem Commun (Camb). 2014;50(95):14995-7.
33. Reinhard L, Mayerhofer H, Geerlof A, Mueller-Dieckmann J, Weiss MS. Optimization of protein buffer cocktails using Thermofluor. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2013;69(2):209-14.
34. Bondos SE, Bicknell A. Detection and prevention of protein aggregation before, during, and after purification. Anal Biochem. 2003;316(2):223-31.
35. Platts L, Falconer RJ. Controlling protein stability: Mechanisms revealed using formulations of arginine, glycine and guanidinium HCl with three globular proteins. Int J Pharm. 2015;486(1-2):131-5.
36. Akash MS, Rehman K, Chen S. Polymeric-based particulate systems for delivery of therapeutic proteins. Pharm Dev Technol. 2016;21(3):367-78.
37. Mensink MA, Frijlink HW, van der Voort Maarschalk K, Hinrichs WL. How sugars protect proteins in the solid state and during drying (review): Mechanisms of stabilization in relation to stress conditions. Eur J Pharm Biopharm. 2017;114:288-95.
38. Kaushik JK, Bhat R. Why is trehalose an exceptional protein stabilizer? An analysis of the thermal stability of proteins in the presence of the compatible osmolyte trehalose. J Biol Chem. 2003;278(29):26458-65.
39. Xie G, Timasheff SN. Xie G, Timasheff SN. Mechanism of the stabilization of ribonuclease A by sorbitol: Preferential hydration is greater for the denatured then for the native protein. Protein Sci. 1997;6(1):211-21.
40. Akash MS, Rehman K, Chen S. IL-1Ra and its delivery strategies: Inserting the association in perspective. Pharm Res. 2013;30:2951-66.
41. Teilum K, Olsen JG, Kragelund BB. Protein stability, flexibility and function. Biochim Biophys Acta. 2011;1814(8):969-76.
42. Betenbaugh MJ, Arden N, Nivitchanyong T. Cell engineering blocks cell stress and improves biotherapeutic production. BioProcess J. 2004;3:23-8.