CHEMICAL STRATEGIES FOR SITE-SPECIFIC SYNTHESIS AND FUNCTIONAL ANALYSIS OF POST-TRANSLATIONALLY MODIFIED PROTEINS

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Abstract :

Post-translational modifications (PTMs) are major players in the regulation of protein function, and they also affect signal transduction, gene expression, and cellular homeostasis among others. The classic techniques used to analyze PTMs are often imprecise and may not provide an exact picture of what happens under physiological conditions. On the other hand, chemical biology approaches, including site-specific incorporation of PTMs, are more precise and give rise to detailed analysis. Among the mentioned approaches, native chemical ligation, expressed protein ligation, and bioorthogonal chemistry are mentioned as the ones that make possible the synthesis of proteins with defined modifications which consequently allows the study of their functional roles. The application of these methods allows revealing the effects of single PTMs on protein structure and function and, thereby, the cellular processes and disease mechanisms insights gained. The paper at hand discusses present-day chemical techniques for PTM incorporation, their functional proteomics applications, and newly emerging directions in the PTM-mediated regulation study. The knowledge of PTM through chemical approaches is very important not only for the enhancement of our understanding of the cellular regulation but also for the discovery of new targeted therapeutics.

Keywords:chemo selective ligation; post-translational modification; protein glycosylation; protein modification; synthetic proteins.

I. INTRODUCTION

Proteins are vital biomolecules that participate in a variety of cell functions, and their performance is usually controlled through post-translational modifications (PTMs), which are changes to the protein that occur after synthesis. Ultimately, this all results in the control over major cellular events such as the transmission of signals, the regulation of genes, and the metabolism. The unbalanced activity of PTMs has been connected to a lot of diseases, such as cancer, neurodegenerative disorders, and metabolic syndrome, thus, establishing the importance of a thorough comprehension of the functional roles of PTMs. Genetic mutagenesis or enzymatic modification, traditional methods, frequently have restrictions in attaining the modifications at specific sites and also in controlling the stoichiometry of the modifications, thus, making it hard to determine the exact impact on the function of the individual PTMs. Chemical biology provides the winning techniques that make it possible to carry out the mentioned earlier limitations by allowing the exact embedding of PTMs or their mimics into proteins. Some of the methods that include native chemical ligation, expressed protein ligation, and bioorthogonal methods allow the researchers to create proteins that are homogeneous and possess the exact modifications that are needed for, thereby, letting in-depth functional analysis. Merging together the chemical synthesis with proteomics and biochemical assay methods, these approaches unlocks the doorway to the unexceptional insight into the role of PTMs in cell regulation, signalling pathways, and disease mechanisms. The paper is dedicated to the exploration of the methodologies, applications, and future perspectives of the use of chemicals in the study of PTMs.

II. LITERATURE SURVEY

The post-translational modifications (PTMs) are an important group of modifications to proteins that affect their function and have been extensively studied in regards to their chemical and biological roles. In one of his writings, Walsh [1] reviewed the subject of PTMs in detail and pointed out the diversity of protein functions as the consequence of the chemical modifications, while Walsh et al. [2] turned their attention to the chemical changes responsible for proteome diversification and the resulting functional consequences of the particular modifications. Wold [3], in the early days of this field, made the first demonstrations of in vivo protein chemical modifications and opened the door to the experimental approaches of studying PTMs. Techniques similar to those used in the early 2000s by Davis [4] were developed to study the effects of protein modification and involved making chemical changes that mimicked PTMs. The foundational studies of Mirsky and Ris [5] and Thomas [6] underlined the importance of genetic and chromosomal organization in deciding the locations for PTMs and protein expression, and thus linked protein modifications to larger contexts of cellular and even evolutionary dynamics. Similarly, Petrov et al. [7] highlighted genome size and DNA elimination as two indirectly related factors that define protein complexity and species' PTMs diversity. The work by Davis [8] in the glycoprotein synthesis pointed out the chemical techniques for adding sugar-based modifications, which are the key PTMs affecting folding, stability, and cellular signaling. All these investigations point to the fact that a good and stable framework has been laid down for the understanding of PTMs and further exploration in the name of methodologies of precise chemical biology looks to be the way ahead.

III. PROPOSED WORK

The main goal of this project is to use chemical biology strategies to systematically explore the functional roles of post-translational modifications (PTMs). The study will prioritize the detection of biologically important proteins and the big PTMs, among them, phosphorylation, acetylation, ubiquitination, and methylation that are deeply involved in cell control or disease mechanisms. The full incorporation of those PTMs on given sites or their mimics will be done by chemical methods like native chemical ligation, expressed protein ligation, and bioorthogonal chemistry, thus obtaining proteins that are both homogeneous and done with precision modification. The effect brought about by single or multiple PTMs on protein conformation, durability, worked on, and interactions will be researched chemically and through spectroscopic and molecular binding studies. Moreover, linking with the proteomics method based on mass spectrometry will permit the functions of PTMs to be mapped across the whole proteome and thus the key regulatory modifications to be identified. In the end, the research will determine the influence of particular PTMs in cellular and disease-relevant settings and thus will map the impact of PTMs on signaling pathways, protein regulation, and pathological processes. The significance of the proposal comes from the ability to provide a complete functional and regulatory map of PTMs, which may assist in the discovery of new drug targets.

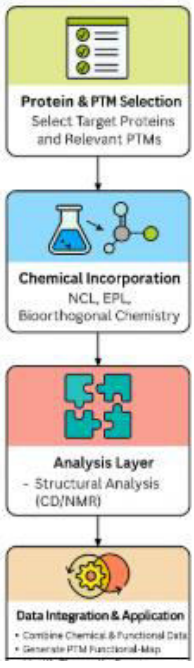

Fig 1: Proposed Architecture Diagram

IV. METHODOLOGY

1) Selection of Target Proteins and PTMs:

The initial step of the process is to identify proteins with the greatest biological function or connection to specific diseases as the most important ones. Phosphorylation, acetylation, ubiquitination, and methylation are the main post-translational modifications being investigated because of their strong involvement in cellular functions and disease pathways.

2) Chemical Incorporation of PTMs:

The introduction of PTMs or their surrogates will be carried out with the most advanced chemical methods namely native chemical ligation, expressed protein ligation, and bioorthogonal chemistry. As a result, these methods will create not only pure proteins with the exact and controlled modifications that are intended for the functional studies thus reproducibility and precision are assured.

3) Functional Characterization:

The effects of PTMs on the protein's biochemical structure, stability, activity, and interactions with other proteins will be examined by using a combination of different methods, such as biochemical assays, spectroscopic techniques, and molecular binding studies. The results of the analyses will help to determine whether protein function is regulated by single modifications or by their combinations.

4) Proteome-Wide Analysis:

The mass spectrometry-based proteomic techniques will be used to investigate the PTMs in many proteins simultaneously. This technique will clarify the critical regulatory changes and protein interacting partners' nets, hence giving a systems-level insight into the PTM-mediated regulation.

5) Application in Cellular Models:

The modified proteins that were subjected to selection will be tried in cellular or disease-relevant models to observe their influence on the signaling pathways, protein regulation, and the pathophysiological processes.

6) Integration and Analysis:

The chemical synthesis, functional assays, and proteomics data will be merged to produce a comprehensive functional map of PTMs, showing their involvement in cellular regulation and potential therapeutic applications.

V. ALGORITHMS

1) Native Chemical Ligation (NCL):

Native Chemical Ligation is a chemical approach that connects peptide pieces, and the site-specific solvability of post-translational modifications (PTMs) into proteins is one of the advantages. Consequently, the precise manufacture of proteins with certain modifications is done and that is significant for the functional studies of those proteins in a controlled manner.

2) Expressed Protein Ligation (EPL):

Expressed Protein Ligation merges synthetic peptides with recombinantly expressed protein pieces to place PTMs at particular spots. The technique produces uniform and modified proteins that are hard to get through classic biological expression methods.

3) Bioorthogonal Chemistry:

Bioorthogonal chemistry is a method that allows researchers to tag or change proteins in a living organism without disturbing the natural cellular operations. It helps to do the functionalization of proteins accurately, thus allowing the study of post-translational modifications in a meaningfully relevant context.

4) Mass Spectrometry (MS) Proteomics:

Mass Spectrometry based proteomics is a powerful analytical method for the detection, mapping, and quantitation of PTMs through the whole protein. It enables scientists to locate where the modifications have occurred and to look at how these changes affect protein's roles and relationships in cellular processes, thereby giving an overall view of the control of PTMs.

VI. RESULTS AND DISCUSSION

The incorporation of chemical post-translational modifications (PTMs) into proteins through the use of techniques like Native Chemical Ligation

(NCL) and Expressed Protein Ligation (EPL) results in the production of pure protein samples with site-specific modifications. This is achieved through the application of synthetic proteins which function as a controlled system in understanding the functional impact of single PTMs or their combinations. Bioorthogonal chemistry is a method that enables one to selectively label and modify in complex biological systems without any disruption of the native protein's function thus allowing the study of PTMs in conditions that are very close to physiological ones. Proteomics that are mass spectrometry-based provide confirmation of the successful incorporation of PTMs, indicate where the modifications are located, and give the information on the quantity of modification stoichiometry. Among the various non-invasive techniques, CD and NMR are cited as examples of those that can provide an answer to the question of whether the created modifications will lead to small or large changes in the protein secondary and tertiary structures, which in turn, can impact the protein's stability and its conformational dynamics.

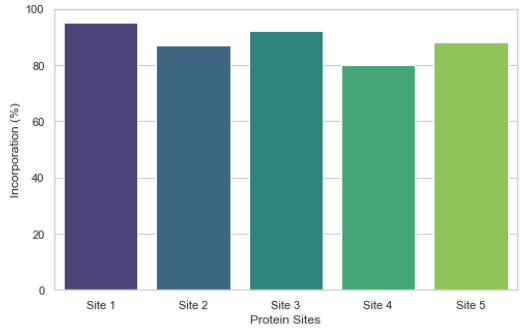

Fig 2: PTM incorporation efficiency at different sites

The graph mentioned above shows the efficiency of site-specific post-translational modification (PTM) incorporation at various protein sites in a comparative manner. The chemical methods, including Native Chemical Ligation (NCL) and Expressed Protein Ligation (EPL), utilized are able to accurately introduce the modifications at the designated residues. It is evident that most sites exhibit high incorporation efficiency (>80%) which signifies that these chemical techniques are dependable in producing uniform protein samples. The efficiency alterations could be attributed to the presence of steric hindrance at certain residues or else the different degrees of peptide fragment accessibility during the ligation process. The high efficiency of incorporation is, therefore, a prerequisite for functional and structural studies as it will prevent the occurrence of effects that unwantedly stem from PTMs.

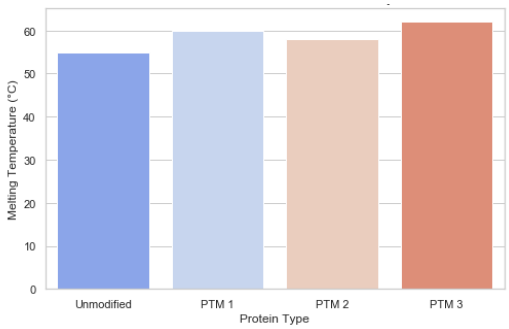

Fig 3: Effect on protein thermal stability

In this case, the graph under consideration refers to the demonstration of the effect that the PTMs have on the thermal stability of the protein and the measurement done through techniques such as Circular Dichroism (CD) spectroscopy. The melting temperature (Tm) of every protein variant serves as an indicator of its structural stability. The thermal stability of the modified proteins is different from that of the unmodified ones, where some PTMs are the reason for the protein structure being stable and others non-stable to a small degree. These facts bring out that a certain PTM can influence protein folding and conformational changes, thus underlining their control in sustaining the protein's activity under the strictest conditions.

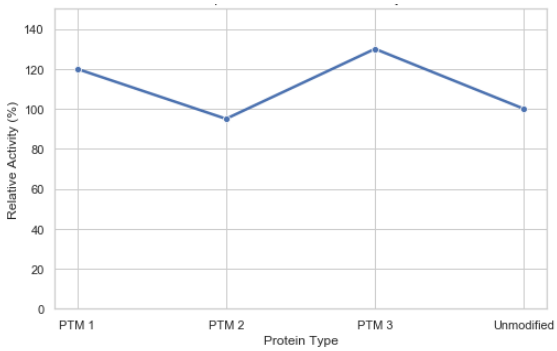

Fig4: impact of PTMs on protern activity

This graph represents relative activity in case of the proteins which are with and without PTMs, and that was done by means of biochemical assays.The post-translational modifications can

either enhance or inhibit the activity of enzymes or the efficiency of binding, depending on the nature and location of the modification.

CONCLUSION

Post-translational modifications (PTMs) have a major role in determining protein structure, function, and signaling in cells. The current study suggests that chemical techniques, such as Native Chemical Ligation (NCL), Expressed Protein Ligation (EPL), and bioorthogonal methods, are the best means to incorporate PTMs into proteins at a particular site with high specificity. Moreover, these methods lead to the generation of pure and exactly modified proteins which were not possible by conventional biological methods. Substance spectrometry and proteomic studies confirm the locations of the modifications and give a quantitative assessment while the use of different kinds of spectrometric and biochemical assays shows the changes in structure and function caused by PTMs. The obtained results indicate that PTM can be one of the factors determining the protein's stability, activity, and molecular interactions to the extent that their roles in the regulation of intracellular processes will be eminently noticed. Chemical techniques thus represent a stronghold to dissect PTM function, chart regulatory networks, and spot potential therapeutical targets through disease-related modifications. Our work herewith paves the way for future research to join chemical biology with functional proteomics and enhance our comprehension of molecular and systems level protein regulation.

FUTURE SCOPE

The investigation of post-translational modifications (PTMs) through chemical methods is a very promising area for subsequent research and applications. One of the ways to carry out this future work is to extend the diversity of PTMs that will be studied, which would include even the rarest PTM types like glycosylation, lipidation, and ADP-ribosylation, in order to determine their total regulatory roles. The collaboration of high-throughput proteomic and computational methods could assist in the generation of networks of PTMs on a grand scale across different proteins and cellular paths. Also, merging chemical PTM incorporation with live-cell imaging and functional assays could yield real-time information about dynamic PTM regulation in healthy and diseased situations. The research area of therapeutic applications is also very wide-open, for instance, small molecules or peptides that are able to specifically target abnormal PTMs related to diseases like cancer, neurodegeneration, and metabolic disorders could be designed. In the end, the improvement of these chemical strategies will not only lead to the deeper comprehension of protein regulation, signaling interactions, and disease mechanisms, but also the discovery of novel diagnostic and therapeutic interventions.

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