Some Methods about Protein Cyclisation

Lezhi Wang

West China School of Pharmacy, Sichuan University, Chendu Sichuan 610065,P.R. China

ABSTRACT

Proteins are crucial to exerting biological functions, while they are stable under some specific circumstances limiting their widely use in various fields. One approach called protein cyclisation, especially cyclised polypeptides which are proved to be beneficial to the stability of proteins both in laboratory and nature world, they are expected to have a widely use in many fields including food, health care and pharmaceutical industries. So it is of great importance to develop approaches to achieve the cyclisation of protein and peptides, fortunately, a lot of related researches have been reported over the past few decades. This review illustrates some approaches about protein cyclisation, including chemical methods and enzymatic methods, and some features of each approach and under what conditions it would be the best choice are discussed, advantages and limitations are also discussed in this article.

KEYWORDS

Protein cyclisation; Chemical methods; Enzymatic methods; Advantages and limitations

DOI: 10.47297/taposatWSP2633-456903.20230402

1 Introduction

As it is known to us all, proteins are one of the most important component in tissues and cells. Proteins are widely used in human bodies including applying energy to physiological activities, constructing and restoring damaged cells, keeping cellular structure and function and so on. Most proteins are structurally linear instead of cyclical which are made up from twenty essential amino acids. However, most of proteins and peptides are not stable to heat and other factors which seriously affect the use of proteins and related products in various areas. In the past few decades, it is reported that some effective were proved to be efficient for the cyclisation of proteins. Moreover, cyclised peptides are stable to many factors which enhances their biophysical properties and broadens their scope of application as therapeutics and medications[1]. For instance, they are more stable to high temperature, extreme PH and low energy supporting condition. So it is promising to develop more methods for protein cyclisation. There are many approaches which can be used for protein cyclisation, including chemical methods, enzymatic methods, using protein tags for cyclisation[2].In fact, chemical methods for cyclisation are more difficult to deal with long-chain substances, but they are easier to put into production. Enzymatic methods for cyclisation are more targeted, but usually face difficulties in extraction and preparation.

The purpose of this work is to give some brief introductions of these methods, then will try to simply analyse their superiority and potential applications, limitations, working conditions. Each method has its suitable fields, and the choice of the appropriate method depends on whether it is convenient for production, whether it has application prospects, and the difficulty of material

preparation, research and development.

2 Chemical Methods for Protein Cyclisation

A lot of chemical methods have been proposed in the aspect of protein cyclisation nowadays. In this section, I will briefly talk about the process and limitation of some chemical methods. Though there

are a lot of method regarding protein cyclisation, this article will only talk about some of them, direct amide bond formation builds amide bond formation between various biomolecules which can be performed by condensation reactions including using extreme temperature or radiation methods. Native chemical ligation is an efficient method to connect two exposed peptide segments. Bioorthogonal reactions relates to two nested bioorthogonal functions that are nonreactive with commonly existing biomolecules but optionally interact with each other during bodily environment.

(1) Building amide bond formation

Typical approaches to build amide bond formation between different biomolecules always use equivalent quantities of activator reagents including EDC, HATU, thionyl chloride and T3P [4]. However, these methods may cause another problem which means the raw materials are heavily wasted. One recent research which is developed by Nuria Martín and Francisco G. Cirujano shows that the use of heterogeneous catalysts is better than using homogeneous catalysts, this is because of their advantages regarding the difficulty of isolation and recycling[5]. And some scientists consider that using high temperatures or microwave irradiation are of great importance to direct amide bond formation[6]. Some perspective makes sense for green chemistry [7]. However, these conditions are not suitable for most proteins and peptides. Therefore, we need to find mild conditions for these reactions, fortunately, there are some effective solutions to address problems related to harsh conditions. Such as converting the hydroxyl in C-terminal into a more easily leaving group including an acyl azide, an acyl halide, an anhydride and an activated ester, via utilizing coupling agentias[8]. So the study of building direct amide bond formation has good prospects for production application in different fields such as biotechnology and pharmaceuticals, and is worthy of further study.

(2) The native chemical ligation

The native chemical ligation reaction is to construct a native chemical bond between two peptide segments, connecting one's C-terminal and the other's N-terminal[9]. This reaction is promising because it considerably improves the size of proteins and peptides which are able to be synthesized by total synthesis. Moreover, it has a huge number of applications in various fields, such as biopharmaceutical, healthcare and molecular research industries.The typical mechanism of NCL involves several steps. These steps are very sophisticated and precise. The first step named thiol–thioester exchanges is fundamental to the whole reaction, it provides dynamic acyl with opportunities to transfer between Cys thiols and possibly thiol additives, and lots of computer programme are made to develop the more detailed mechanism regarding the S,S-acyl transfer reactions[10]. The second step called nucleophilic catalysis. In the process of this reaction, nucleophiles to the thioester are addicted so that the yield of dynamic and energetic acylates is improved. The third step is the formation of the intermediates that are linked by thioester, during that step, peptide aryl thioesters are captured which is followed by the folding of template-assisted ligations, then intramolecular ligations are followed. And the last step is the rearrangement of the thioester-linked intermediate, actually, it is much faster than the exchanges

of thiol–thioester. Therefore, the intramolecular S,N-acyl migration step limits the rate and applications of the native chemical ligation[11]. Though the future of the use of native chemical ligation is promising, we should also develop new experimental conditions so that we can obtain more proteins which are challenging in synthesis.

(3) Bioorthogonal reactions

One bioorthogonal reaction includes two nested bioorthogonal compounds, which are nonreactive with common biological molecules, but optionally interact with each other in human bodies. The cyclisation of protein and peptide using bioorthogonal reactions improve the decreased proportion of side reactions and targetability of some compounds. For instance, in 2010, it is reported that salicylaldehyde ester-induced chemoselective peptide lgations can lead to the production of peptide bonds at the serine or threonine locus[12]. The team found the method to connect Ser and Thr at the N-terminal to complete an optional chemical ligation leading to the natural Xaa-Ser or Xaa-Thr bonds on the ligation site. Another team researched some bioorthogonal reactions which happened in mouse bodies, they used track probes during cellular internalization to monitor the changes of health of laboratory animals that they implemented bioorthogonal reactions[13]. Bioorthogonal reactions can also be used to label and monitor proteins[14]. Actually, we can even build any peptide and protein cyclisation by using bioorthogonal reactions in theory, however, there are some difficulties involved in practical applications. For example, the mostly used approach named Staudinger ligation can merely be relied on reduction reaction producing amine, which results in the limitation of its practical application. Another disadvantage of bioorthogonal reactions is that most of these reactions which are reported so far need non-native functionalities, though they may be addressed by the use of solid-phase peptide synthesis[15].

3 Enzymatic Methods for Protein Cyclisation

Scientists have made efforts in the research of enzymes for decades, and enzymes are famous for their high efficiency, specialized selectivity and mild working environment. Herein I will briefly talk about some enzymatic methods and their limitations. Actually, almost any enzyme that catalyse the formation of protein and peptide bonds can be prospectively applied for protein and peptide cyclisation. In this review will discuss some of them, non-ribosomal peptide synthetases can generate the preparation of lots of natural cyclic proteins and peptides. Transglutaminase facilitates the formation of bonds among side chains of different amino acids. Aspartyl endopeptidases are predominantly found in plants, many of which mediate transpeptidation to produce naturally occurring cyclic peptides.

(1) Synthetases of non-ribosomal peptides

Synthetases of non-ribosomal peptides belong to microbial metabolites, they are wildly used and research especially in the fields of medicine and pharmacy because of their special biological structures.The structure of these enzymes that are used for biosynthetic are composed of various parts which take the responsibility for the fabrication of some single and specialized parts into a natural and entire product[16]. Actually, the structure of non-ribosomal peptide synthetases are really sophisticated, because of their structural diversity, they are able to generate chemical and biological diversity in order to combine specific biomolecules and their targets. Though the structures of non-ribosomal peptide synthetases are various, their modes of synthesis are not very complex but

conservative, including three main kinds, occurring in a linear, in an iterative , or in a nonlinear way[17]. The structure of non-ribosomal peptide synthetases can be divided into different domains and herein I am only going to briefly talking about the core domains. The core non-ribosomal peptide synthetases domains can also be divided into three different parts. The domain of adenylation is the key domain to select substrate, it works in a two-step process to recognize different amino acids[18]. It selectively combines with the cognate amino acid and then it spends ATP to transfer the related amino acid into an aminoacyl adenylate intermediate. After that, the aminoacyl adenylate intermediate are attacked by PCP which result in the forming of a thioester which is bound with aminoacyl-S-PCP. The peptidyl carrier protein domain is the central to direct the synthesis of nonribosomal peptides and the structure of peptidyl carrier protein domain can be transferred with the development of the reaction it catalyzed. The condensation domain connects different molecules which are generated during the process of non-ribosomal peptide synthetases synthesis. It catalyses the generation of the peptide bond between the activated acyl group and the free amino group locating at nearly parts on an amino acid. This method has some advantages, such as easily to be used in commercial fields, being able to connect artificial amino acids. However, it also has some drawbacks, for example, it is hard to generate heterologous expression because of its large size.

(2) Transglutaminase

Transglutaminase catalyzes an acyl transferred reaction between the γ-carboxyamide group of a peptidebound glutaminyl residue and one variety of essential amines. The application of this bond can be widely used in the development of the process of food and the conservation of food[19]. For example, it is reported that using beef, pork, chicken or fish as the matrix, after gelation with transglutaminase. And the results of related experiments indicated that the improved solubility, moisture retention ability and thermal stability of food proteins make the enzymatic method potentially useful in the food industry. So the first advantage of this enzymatic method is its promising commercial use, especially in the field of food. Another noticeable advantage of this method is that its endurance towards different PH and various temperature is considerably good. Although the crystal structure of transglutaminase has been studied, its large-scale production is still difficult, because it is difficult to achieve industrial mass production using the inclusion body method. In fact, some of them witness limitations including the requirement of precursors of esters, low cyclisation productivity, restriction regarding a small range of peptide types and the requirement of some particular amino acid fragments locating at the peptide[20].

(3) Asparaginyl endopeptidases

The proteases found from the vacuole of plant cells are called asparaginyl endopeptidases[21]. Several of these isoforms from different plants are reported to generate the transpeptidase activity that is essential to the head-to-tail cyclisation reaction happening at the cyclotides synthesis. Asparagine endopeptidase has a wide range of application prospects. It can be used to label and track proteins that have been transcribed and translated. It can also be used in the production of biopharmaceuticals, such as protein-drug conjugate products. And in the future, with significant progress which might be made in this field, they can be potentially used as bio-catalyst. The main reaction mechanisms of asparaginyl endopeptidases are that the core domain of them attracts the catalytic cysteine residue as a nucleophile[22]. And it is proved that these enzymes has been witnessed a wide range of PH endurance and a high rate of catalytic efficiency[23]. And both intramolecular ligation by asparaginyl endopeptidases and intermolecular ligation by asparaginyl endopeptidases can lead to peptide and protein cyclisation. Though the usage of this method is promising, the access

of active enzyme of asparaginyl endopeptidases is difficult which limits the application of this method. So we still need to improve the methods to obtain more asparaginyl endopeptidases.

4 Conclusion and Perspectives

This review mainly discusses about the overview of some methods which are available for the cyclisation of proteins. We all know that it is very promising to apply these methods in various fields, especially in medical, food and pharmaceutical industries. Though we want to find one method for protein cyclisation that is specific, targetable, easily to prepare, it is very hard to achieve this goal as all methods that have been reported so far have their advantages and limitations. Generally speaking, the widely use of chemical and enzymatic methods to produce cyclized proteins and peptides still needs a long process to achieve. In order to make these methods more commercially available, we should pay more attention to their value of application, the cost to produce related products, the sequence of the whole synthesis and some other factors. In spite of some difficulties, chemical and enzymatic approaches for protein and peptide cyclisation are still really prospective, and making researches of them are profitable and are potential in curing some disease and designing chemical, biological targets. All in all, we need to make more researches of various methods for protein and peptide cyclisation, then we can make progress in the fields of food, health care industries and pharmaceutical industries.

About the Author

Lezhi Wang is an undergraduate from Sichuan University, and his research field is pharmacy.

References

[1]Aboye, T. L., & Camarero, J. A. (2012). Biological synthesis of circular polypeptides. Journal of Biological Chemistry, 287(32), 27026-27032.

[2]Hayes, H. C., Luk, L. Y., & Tsai, Y. H. (2021). Approaches for peptide and protein cyclisation. Organic & biomolecular chemistry, 19(18), 3983-4001.

[3]de Araujo, A. D., Nguyen, H. T., & Fairlie, D. P. (2021). Late-Stage Hydrocarbon Conjugation and Cyclisation in Synthetic Peptides and Proteins. ChemBioChem, 22(10), 1784-89.

[4]Martin, N., & Cirujano, F. G. (2022). Heterogeneous catalytic direct amide bond formation. Catalysis Communications, 164, 106420.

[5]Perreux, L., Loupy, A., & Volatron, F. (2002). Solvent-free preparation of amides from acids and primary amines under microwave irradiation. Tetrahedron, 58(11), 2155-62.

[6]Sabatini, M. T., Boulton, L. T., Sneddon, H. F., & Sheppard, T. D. (2019). A green chemistry perspective on catalytic amide bond formation. Nature Catalysis, 2(1), 10-17.

[7]Valeur, E., & Bradley, M. (2009). Amide bond formation: beyond the myth of coupling reagents. Chemical Society Reviews, 38(2), 606-31.

[8]Montalbetti, C. A., & Falque, V. (2005). Amide bond formation and peptide coupling. Tetrahedron, 61(46), 10827-10852.

[9]Agouridas, V., El Mahdi, O., Diemer, V., Cargoët, M., Monbaliu, J. C. M., & Melnyk, O. (2019). Native chemical ligation and extended methods: mechanisms, catalysis, scope, and limitations. Chemical reviews, 119(12), 7328-7443.

[10]Castro, E. A. (2007). Kinetics and mechanisms of reactions of thiol, thiono and dithio analogues of carboxylic esters with nucleophiles. An update. Journal of Sulfur Chemistry, 28(4), 401-29.

[11]Adams, A. L., Cowper, B., Morgan, R. E., Premdjee, B., Caddick, S., & Macmillan, D. (2013). Cysteine promoted C-terminal hydrazinolysis of native peptides and proteins. Angewandte Chemie, 125(49), 13300-13304.

[12]Li, X., Lam, H. Y., Zhang, Y., & Chan, C. K. (2010). Salicylaldehyde ester-induced chemoselective peptide ligations: enabling generation of natural peptidic linkages at the serine/threonine sites. Organic letters, 12(8), 1724-27.

[13]Sletten, E. M., & Bertozzi, C. R. (2011). From mechanism to mouse: a tale of two bioorthogonal reactions. Accounts of chemical research, 44(9), 666-76.
[14]Lang, K., & Chin, J. W. (2014). Bioorthogonal reactions for labeling proteins. ACS chemical biology, 9(1), 16-20.
[15]Row, R. D., & Prescher, J. A. (2018). Constructing new bioorthogonal reagents and reactions. Accounts of chemical research, 51(5), 1073-1081.
[16]Hur, G. H., Vickery, C. R., & Burkart, M. D. (2012). Explorations of catalytic domains in non-ribosomal peptide synthetase enzymology. Natural product reports, 29(10), 1074-98.
[17]Mootz, H. D., Schwarzer, D., & Marahiel, M. A. (2002). Ways of assembling complex natural products on modular nonribosomal peptide synthetases. ChemBioChem, 3(6), 490-504.
[18]Lee, S. G., & Lipmann, F. (1977). Isolation of amino acid activating subunit-pantetheine protein complexes: Their role in chain elongation in tyrocidine synthesis. Proceedings of the National Academy of Sciences, 74(6), 2343-47.
[19]Yokoyama, K., Nio, N., & Kikuchi, Y. (2004). Properties and applications of microbial transglutaminase. Applied microbiology and biotechnology, 64, 447-54.
[20]Touati, J., Angelini, A., Hinner, M. J., & Heinis, C. (2011). Enzymatic cyclisation of peptides with a transglutaminase. ChemBioChem, 12(1), 38-42.
[21]Hara-Nishimura, I., Takeuchi, Y., & Nishimura, M. (1993). Molecular characterization of a vacuolar processing enzyme related to a putative cysteine proteinase of Schistosoma mansoni. The Plant Cell, 5(11), 1651-59.
[22]Dall, E., & Brandstetter, H. (2013). Mechanistic and structural studies on legumain explain its zymogenicity, distinct activation pathways, and regulation. Proceedings of the National Academy of Sciences, 110(27), 10940-10945.
[23]Tang, T. S., & Luk, L. Y. (2021). Asparaginyl endopeptidases: enzymology, applications and limitations. Organic & Biomolecular Chemistry, 19(23), 5048-62.