Application of Protein Chemistry in Biomedical Research

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Abstract: Application of protein chemistry in biomedical researchAt present, with the completion of human genome sequence sketch, human beings have entered the post-genome era from genome project, and the genome sequencing of many model organisms has been basically completed. Chemical proteomics is the latest development of chemical biology in the post-genome era. Chemical proteomics is a new generation of functional proteomics, which uses small chemical molecules as tools and means to detect in vivo proteomics with a new strategy based on the function of target proteins. The strategy of protein analysis, the principle of protein chip analysis based on array, the related preparation methods and detection technologies, and their applications in biological research, medicine and experimental diagnosis were described.

1. Introduction

With the successful completion of human genome sequencing, it is possible to study functional genomes, and proteomics with proteins as the research object is becoming more and more important. Because of the influence of diseases and drug treatment, the proteome expressed by cells or tissues in humans and animals will change obviously. Its research object is no longer a single or few proteins, but focuses on comprehensiveness and integrity. It is necessary to study the physical, chemical and biological properties and functions of all protein components in the system, and finally obtain large-scale information on the properties, expression changes and post-translation processing of each protein [1]. In order to obtain the functional integration information of physiological pathology and signal transduction process of organisms, the proteins expressed in genomes and their post-translational modifications were studied from a systematic, holistic and interrelated new perspective.

Because the ultimate goal of genetic engineering is protein, the real product as a commodity is protein. After people get all the sequence information of genes, they only solve the problem of genetic information base, which is far from the end of genome research. The application of proteomics in biomedicine mainly includes several aspects: By looking for differentially expressed proteins, we can find disease-related proteins. It is necessary to have a comprehensive, multidimensional and dynamic understanding of this biological system, and to link these understandings with biological phenotype, including all life activities including its birth, death and illness, so as to finally clarify the mystery of life.

2. Study on Protein Chemical Biotechnology

Labeling is to introduce the substances that can distinguish the element differences into the difference samples. Using the information obtained by mass spectrometry analysis, the differences of relative quantities can be distinguished and displayed. The modification of protein side chain groups is realized by chemical reaction between selective reagents or affinity labeling reagents and specific functional groups on the side chain of protein molecules. Generally speaking, every time a multi-segment peptide fragment is connected, it needs to be purified and freeze-dried, which is to remove the reaction by-products and update the connecting reaction solvent. The labeling of amino acids is to replace the carbon atoms and hydrogen atoms of amino acids with stable elements, and

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then add such stable amino acids into a certain culture medium. In this way, cells will add amino acids to the markers introduced in proteins [2]. In fact, gene protein dynamics is not a definite linear relationship, and most protein structure modifications, abundance changes and active forms are not controlled by a single gene, but are the result of multiple genes and multiple environmental factors [3].

After gene chip, protein chip technology has gradually developed. Gene chip, also called DNA chip, is made of DNA fragments, while protein chip is made of protein or polypeptide. If proteins are expressed differently in different environments, it is important to find different egg autoplasm species. Using ICAT technology, the same amount of mixture was followed by enzymolysis. Finally, these labeled peptides were enriched by streptavidin column, and the differences were analyzed by mass spectrometry. The display of differential proteomics can borrow the current conventional proteomics technology. Under the condition of a large number of thiol additives and cysteine residues in peptide fragments, it is precisely because of the rapidity and reversibility of thioester exchange reaction that the thioester intermediate of N- terminal cysteine always exists in NCL system.

3. Protein and Antibody Microarray Chip

3.1 Principle Overview

Protein chip is a high-throughput, miniaturized and automated protein analysis technology. As we all know, proteins are not as stable as nucleic acids, and there will be many difficulties in processing protein samples. The operation of protein chip will be more strict than that of gene chip. By analyzing whether a protein can interact with a protein with known function, we can get clues to reveal its function. After labeling, all samples were mixed in advance and then loaded and run together, so that the display differences of proteome can be compared in the same gel, which greatly overcomes the systematic error between two-dimensional electrophoresis gel and gel. This is due to the moderate activation of C- terminal polypeptide thioester, which makes thioester relatively stable to nucleophiles such as hydroxyl and amino in neutral aqueous solution. This technology has beautiful concept and low technical content. It works hard but can get results quickly, and does not depend on expensive equipment.

3.2 Preparation of Protein and Antibody Array Chip

At present, protein chip and surface enhanced laser desorption ionization mass spectrometry are mainly combined to find differentially expressed proteins. According to different research purposes, some specific bioactive proteins such as antigens, antibodies, enzymes and receptors can be selected to make low-density chips. In addition, it is not easy to amplify and purify proteins in vitro through expression vectors, so it is difficult to prepare a large number of proteins. At present, with the maturity of combinatorial chemistry and natural product chemistry, the provision of a large number of new compounds is no longer a problem in the research and development of new drugs. The natural chemical connection method has great practical value. The separation and purification process of polypeptide thioester fragment is the same as that of other unprotected polypeptide fragments, without special treatment. Therefore, the study of drug-resistant and sensitive strains of pathogenic microorganisms by two-dimensional electrophoresis is of great value for clarifying the mechanism of drug resistance, identifying new drug targets and diagnostic markers of drug resistance.

Nowadays, protein chemistry and molecular biology have their own mature technologies. Protein separation and purification technology, sequencing technology, synthesis technology, connection technology and computer simulation technology of three-dimensional structure have been quite developed; On the other hand, how to improve the natural chemical linkage to get rid of the restriction of cysteine is of great value for the total chemical synthesis of proteins (especially those without cysteine residues). However, the pharmacological activities of most new lead compounds are unpredictable. Even if there is foreseeable activity information, there is often no sensitive and convenient method for tracing and screening. Proteome research is essentially a large-scale parallel

separation and analysis of proteins at the cellular level, which often involves processing thousands of proteins simultaneously. Among them, two-dimensional electrophoresis, two-dimensional liquid chromatography and their combination with mass spectrometry have become powerful tools for proteomics research.

The speed of the joining reaction is the same as that of the conventional cysteine peptide fragment joining reaction (Figure 1), which is a thioester exchange reaction. These modified groups are stable to HF, TFA and other acidic conditions when they are attached to amino groups due to the regulation of the electron-donating substituent methoxy on benzene ring. When the reaction is finished, when they are connected with the newly generated amide bond, they can be removed under the action of strong acid without damaging the polypeptide chain [4].

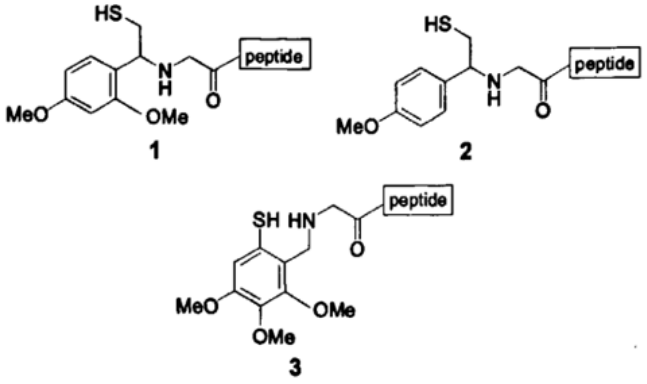

Fig.1 Modifying Groups Dissociated under Acidic Conditions

Because protein is more difficult to synthesize than DNA, protein and antibody array chip is much more complicated than DNA chip. Therefore, it is necessary to find a new way of immobilization, select a suitable carrier and modify it. It is impossible to achieve complete labeling, which mainly depends on the metabolism of protein.Sequential ligation of peptide fragments will lead to the accumulation of by-products, and the ultimate ligation efficiency depends on the ligation efficiency of each step. When there are many ligation times, the ultimate efficiency decreases exponentially. Alkylation of amino groups and aminoacylation by cyanate are important lysine modification methods. In the era of functional genomics, two-dimensional electrophoresis technology is used to separate complex protein samples and establish two-dimensional electrophoresis database, and it is mainly used to find differentially expressed proteins in different tissues or cells for the study of disease-related proteins or genes.

3.3 Detection of Protein and Antibody Array Chip

At present, the most commonly used standard method is to modify the carboxyl groups of protein molecules with water-soluble carbodiimides, and the products are generally esters or amides, which can be carried out under mild conditions. For example, the two-color fluorescence display system adopted by Zhang Weiwei et al. [5] uses Cy5 to mark the protein in the sample, and uses Cy3 marker with a fixed concentration as the built-in reference. By testing its fluorescence gray scale ratio, the protein or antibody level in the sample can be reflected. The disease tissues and normal tissues were separated by two-dimensional electrophoresis, and then the generated 2-DE map was analyzed by computer software to find out the differentially expressed proteins. The main methods of sample pre-grading include grading according to protein solubility and protein localization in different organelles, such as separating the protein components of organelles such as nucleus, mitochondria or Golgi apparatus. This method will be more efficient and simple for subsequent affinity chromatography separation and detection.

The microarray protein chip was used to study the interaction between small chemical molecules and proteins. The differentially expressed protein spots were cut off from the glue, digested by enzyme (commonly used trypsin), and identified by mass spectrometry or tandem mass spectrometry and database search. Flying under the action of electric field force, the mass-to-charge ratio of ions is

calculated by detecting their flight time, which can be used to analyze the molecular mass and relative content of proteins. Using protein chips to detect the interaction between proteins and small chemical molecules at the proteome level has been well developed, and future technological breakthroughs may gradually solve the above problems.

4. Application of Protein Chemistry in Biomedical Research

4.1 Protein Expression Profile

Two-dimensional electrophoresis and image analysis can be used to quantitatively study the overall changes of protein expression in tissues, cells or body fluids, and obtain the total protein expression at the detection level. Protein arrays are most likely to be used in laboratory diagnosis of autoimmune diseases in clinical medicine to determine the existence of specific autoantibody spectrum. Two-dimensional electrophoresis requires operators to master electrophoresis, staining and software analysis techniques, wear experimental clothes and gloves during the whole experiment, and try to avoid pollution such as keratin, so as to obtain better repeatability. For example, as a diagnostic tool in medical clinic. Look for bladder or kidney cancer markers in urine. Compare normal tissues with β-cells, and look for the changes of insulin dependence in diabetes.

According to a large number of experimental results, Liu Wenfeng et al. [6] summed up the thermodynamic rules for judging the binding force between biomacromolecules and micromolecules and biomacromolecules themselves, that is, hydrophobic force may increase the ΔS of the system, hydrogen bond or Van der Waals force may decrease the ΔH and ΔS of the system, and electrostatic force may make ΔH very small and close to zero. According to the data listed in Table 1, the interaction force between PF and BSA is mainly hydrogen bond and Van der Waals force.

Table 1 Thermodynamic Parameters Of Bsa-Pf System

T(℃)	ΔH (kJ/mol)	ΔS (kJ/mol)	ΔG (kJ/mol)
17	-114.3	-258.2	-36.5
36	-114.3	-255.1	-34.2
44	-114.3	-271.6	-28.6

With the development of bio-imaging mass spectrometry technology, chemical proteomics in the future may be able to directly regulate the life activities of proteome in vivo at the level of living cells, enabling scientists to study the overall biological functions of proteins in vivo under physiological conditions. The separation and purification process of polypeptide thioester fragment is the same as that of other unprotected polypeptide fragments, without special treatment. Therefore, the requirements for biological samples are low, which can simplify the pretreatment of samples, and even directly use biological materials (blood samples, urine samples, cells and tissues, etc.) for detection. At present, the study of differentially expressed proteins by multidimensional liquid chromatography-mass spectrometry usually relies on isotope labeling technology.

4.2 Application of Protein Chemistry in Clinical Medical Research

Because the protein array chip technology is operated in vitro, it breaks through the limitation of yeast two-phase hybridization system technology and can directly detect the target protein. Because proteomics represents the functional state of biological system, clinical research based on proteomics has developed rapidly, and many human diseases such as cancer, cardiovascular and cerebrovascular diseases and infectious diseases have been studied by proteomics. Comparing the changes of protein expression abundance between normal cells and cells treated with drugs can suggest the toxic mechanism of drugs. With the maturity of genetic engineering technology, people have shifted from designing functional proteins to designing genes, and tried gene therapy and DNA immunization. Human Genome Project found that the most common structural unit is cysteine-rich zinc finger protein domain, and all these cysteine proteins and protein domains can be obtained by natural chemical linking method in principle [7].

Two-dimensional electrophoresis can be used to study the pathogenesis of malignant tumors at the molecular level. Because if there is a sulfhydryl group in the unprotected peptide fragment, the sulfhydryl group will be removed nonselectively in the final desulfurization step. In addition, the further organic connection and integration of combinatorial chemistry and proteomics will give birth to new chemical proteomics technology for high-throughput screening and development of new drugs. Proteomics plays an important role in the study of various types of cancers, such as revealing the pathological process of tumors, finding tumor marker proteins, and treating and diagnosing them. By comparing with the reverse DNA chip and two-dimensional gel electrophoresis results, protein expression can be organically linked with DNA sequence information. Comparing the protein profiles of healthy and diseased cells, it will be possible to recognize the intracellular signal transduction and metabolic pathways.

4.3 Identification of Protein Noncovalent Complexes

The second important application of protein chemistry research is the identification of proteins with different expression in cells or systems and their noncovalent complexes, which helps us to know more about their biological functions. Protein array chip is helpful to understand the interaction between drugs and their effect-related proteins. The basic idea of this method is to obtain sulfhydrylation structural units of various amino acid side chains by chemical synthesis, to realize the connection of these sulfhydrylation sites by NCL reaction, and then to selectively remove sulfur. This change pattern is similar to many kinds of malignant tumors they studied before, and it may be helpful for studying the pathogenesis of malignant tumors. To provide experimental basis for guiding clinical medication, and to further establish and develop the database of exogenous chemical drugs and protein expression profiles, so as to promote the research of pharmacology and toxicology [8].

The ICAT-labeled peptide was eluted and analyzed by liquid chromatography-mass spectrometry. On the mass spectrogram, the samples with different expression levels and the control showed characteristic double peaks with a difference of 8 mass numbers (Figure 2).

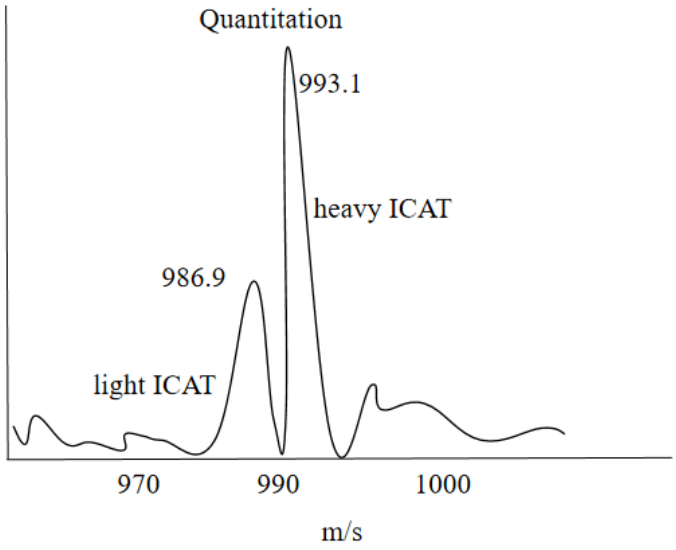

Fig.2 Mass Spectra of Samples Labeled with Icat Reagent after Enzymatic Hydrolysis and Separation by Reversed-Phase Chromatography

The principle of using ICAT reagent to label protein and find differentially expressed protein is: after the sample and control are labeled with heavy reagent and light reagent respectively, the two samples are mixed, hydrolyzed and passed through streptavidin column, so that ICAT labeled peptide fragments are adsorbed on streptavidin column. Another important factor to improve the production of protein array is to improve the protein expression system, so that it can produce a large number of soluble and nascent state-expressed recombinant proteins. In the process of signal transduction research, the purification of proteins and protein complexes and the corresponding mass spectrometry identification play a key role [9]. Because the auxiliary group is connected with the newly generated

amide bond, the electronic effect changes, and strong acids such as TFA can be used to remove the auxiliary group without damaging the polypeptide chain.

5. Summary

Protein is the most important functional component encoded in genome, and the most important thing for protein analysis in post-genome era is to be able to analyze complex protein mixtures on a large scale. Compared with traditional analysis equipment, the main advantage of current protein microarray technology is that it is possible to analyze thousands of proteins in parallel with a single sample. With the development of proteomics technology, such as liquid phase 2-DE, protein chip combined with SELDI-MS technology, proteomics will be further developed and play an important role in the pathogenesis, diagnosis and treatment of diseases. New technologies, such as nano-biology and biosensors, will be applied to chemical proteomics in the future, which will further develop in the direction of micro-sensitivity, automation and high efficiency, so as to meet the needs of practical application.

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