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Research Progress in Antigen Synthesis

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Abstract: *In recent years, immunoassay using small molecular compound as hapten has been applied in many fields, such as food and drug, environmental protection, etc., which has achieved ideal detection results. Only when the small molecule compound can only be coupled with the carrier to form an artificial antigen, can it indirectly induce B cells to proliferate and differentiate by T cell epitope, and then specific antibodies can be produced. The synthesis of efficient artificial antigen is the premise and key to immunoassay. This essay is a literature review at home and abroad about the design and synthesis methods of small molecular haptens, the selection of carriers, the coupling methods of haptens and carriers, the purification and identification methods, which are involved in the synthesis of artificial antigens.*

Keywords: *Antigen, Carrier, Identification*

1. Research on the Current Status of Synthesis Methods

Antigens enable the body to have immune reactions and produce antibodies that specifically bind to them. It is called artificial antigens to prepare antigens containing a known cluster of chemical determinants by chemical synthesis or gene recombination.

It includes artificial binding antigens, synthetic antigens and genetic engineering antigens.

1.1. Artificial Binding Antigen

The principle overview: The operation of binding no immunogenic chemical groups or organic molecules with the carriers to form the conjugates of carriers and haptens is called Artificial binding antigen method. In the past few years, various studies derived from this principle mainly manifest in antigen preparation of small molecules. The main process is the design of hapten, coupling reaction of half antigen and carrier, identification and purification of synthetic antigen and so on.

1.1.1. The Design of Hapten

Hapten refers to a substance that can only have antigen-antibody reactions with antibodies but cannot cause the body to produce the corresponding antibodies. That means that hapten only possess immunogenicity rather than immune reactivity.

Many small molecules to be synthesized are unstable, so structural analogues of small molecules should be used, and attention should be paid to retaining the characteristic groups of small molecules. If the determinand itself has no active groups that can directly coupling with carriers such as COOH, -OH, -NH2, etc., or although the active groups are important for the group to maintain the immune properties to be tested and cannot be used to couple with the carrier, it must be based on immune theory and combined with the structure of the object under test for a redesign of hapten. It should be noted that: first of all, the design of haptens should try to keep the characteristic groups of the tested object, so as to ensure that the obtained antibodies can have specific immune reaction with the tested object; Secondly, the more complex the spatial structure of the hapten is, the stronger the immunogenicity of the produced antigen is, so higher valence antibodies can be produced. Therefore, the structure of the hapten should also have a certain complexity, such as including groups with high immunoactivity such as benzene ring, heterocyclic ring and branched-chain [1].

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1.1.2. Coupled Reaction of the Hapten to the Vector

Carriers play a crucial role in the synthesis of small molecular antigens. Since the relative molecular weight of hapten is too small to induce immune reaction, it is necessary to combine with macromolecular carrier to obtain immunogenicity. The carrier needs to meet the following requirements :(1) The carrier needs to have enough active groups as binding sites to conduct coupling reaction with haptens; (2) The carrier has no toxic effect on the body; (3) The carrier is relatively cheap and easy to obtain.

Currently commonly used carriers include protein carriers, polypeptides and macromolecular compounds. Protein carriers are the most commonly used, such as bovine serum protein (BSA) and chicken egg albumin (OVA), among which bovine serum protein is the most commonly used. This is because bovine serum protein is stable in physical and chemical properties, has more free amino groups in the molecule. It can maintain good solubility in different pH, ionic strength and organic solutions. Moreover, it is cheap and easy to obtain.

Although most haptens have certain active groups, they still cannot be directly coupled with the carrier. Moreover, the characteristic structure of some haptens directly linked with the macromolecular carrier is easily interfered by the local microchemical environment or steric hindrance of the carrier, which will affect the recognition of the immune system. This requires the use of cross-linking agent to activate the hapten groups, and then couple with the carboxyl, amino, sulfhydryl groups and other groups on the carrier to form the artificial antigen. At the same time, some introduced cross-linking groups are not removed, so that the hapten is separated from the carrier, which becomes the spacer arm. It has shown that the introduction of spacer arms of a certain length can make hapten protruded on the surface of the carrier, which can be easily recognized by the immune system of the body and increase the possibility of producing antibodies against haptens [2].

According to the different hapten groups, the coupling methods are mainly divided into the following kinds: The hapten containing carboxylic group (-COOH) generally adopts carbodiimide method, mixed anhydride, N-hydroxysuccinimide active ester method, etc. The hapten containing amino (-NH2) is generally used glutaraldehyde method, halogenated nitrobenzene method, diisocyanate method, diazotization method, etc. The hapten containing hydroxyl (-OH) is generally available to succinic anhydride method, phosgene method, halogenated carboxylic acid method, etc. The haptens containing sulfhydryl (-SH), aldehyde (-CHO) and ketone (-CO-) structures can also be conjugated to proteins using the corresponding bifunctional group reagents [3].

(1) Carbodiimide method to synthesize complete antigen

The method is mild and can be performed in neutral pH. The carboxylic acid (-COOH) or the amino group (-NH2) in the compound forms an amide bond with the amino group or the carboxylic group on the protein with the carbodiimide (commonly used DCC, etc.) as the dehydrating agent. However, since the condensation reaction of carbodiimide is not selective, it is easy to form the self-polymerization of carrier protein and produce non-homogeneous products. To reduce the coupling between carrier proteins, the carboxyl-containing hapten molecule may be reacted with carbodiimides to be activated by the carboxylic group before adding to the carrier protein [4]. Wu et al. [5] derived 17-β-estradiol (E2) with succinic anhydride, and then conjugated E2 with BSA by carbodiimide method to obtain E2-BSA with coupling ratio of 18.6:1.

(2) Glutaraldehyde method to synthesize complete antigen

The glutaraldehyde method is one of the mildest coupling reactions. It can be performed in a buffer solution of pH 6.0~8.0, with the temperature range generally controlled in 4~40 ℃. This method is similar to carbodiimide method, because it also causes coupling between carriers with poor product uniformity. This method is mostly used in the preparation of enzyme marker antibodies. Peng H J et al. [6] used glutaraldehyde as a coupling agent to conjugate sulfamethoxydiazine (SMD) with bovine serum albumin or ovalbumin to form complete antigen, and established an ELISA method for the determination of SMD, with the detection limit less than 5ng mL-1. Other scholars have also used this method to synthesize artificial antigens [7].

(3) Diazotization method to synthesize complete antigen

The compound containing aromatic amines can react with nitrite to form a diazonium salt, which then binds directly to the ortho position of the phenolic hydroxyl group on the tyrosine residue of the carrier protein. As this method has more side reactions, it is generally limited to the synthesis of small molecule artificial antigen.

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(4) Mixed acid anhydride method to synthesize complete antigen

The reaction process of mixed anhydride method is simple. There is no need to prepare and separate intermediate products, but its operation is complicated and the control conditions are strict. In the mixed anhydride method, isobutyl chloroformate is needed to be added, which is highly toxic.

Zhu et al. successfully prepared Caoz-KLH2 immune antigen using the mixed anhydride method. Compared with the active ester method, the coupling of this method ratio is lower. A large number of domestic scholars have used this method for antigen synthesis [8].

(5) Activated ester method to synthesize complete antigen

The active ester method is an improved method for the carbodiimide method, which avoids the direct action of the carbon diamine on the protein, and thus it avoids the coupling between the protein molecules. Small molecular haptens containing carboxyl groups can react with N-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide (DCC) to form active ester derivatives, which can react with the amino group on the carrier protein to form an amide bonded conjugate.

This method is widely used, and many domestic scholars have adopted this method as their method of synthesizing artificial antigen [9].

(6) Physical method to synthesize complete antigen

Physical method is a kind of method which is suitable for the drug containing amino, carboxyl or lactam structure. At pH7 ~ 8, it reacts with the amino or carboxyl group and binds to a protein. Liu Chengmei [10] and Zhu Xuan et al. [11] all reported the method for preparation of artificial antigen by constant temperature shaker reaction at 37℃ in barbiturate buffer solution. They indicate that the coupling rate of artificial antigen prepared by physical method is much higher than that of glutaraldehyde method, with better immunogenicity and simpler reaction conditions, which is a good method for preparing artificial antigen.

1.1.3. Identification of Complete Antigen Synthesis

After the coupling reaction, it is necessary to determine the success of the synthesis by identifying the relative molecular weight and spectral distribution of the synthesis.

1) Electrophoresis method

Electrophoresis methods mainly include polyacrylamide gel electrophoresis, high performance capillary zone electrophoresis, agarose gel electrophoresis and so on.

Polyacrylamide gel electrophoresis (SDS-PAGE) separates protein molecules according to the size of the relative molecular mass of protein, the protein shape and the amount of charge. High efficiency capillary zone electrophoresis is driven by high voltage electric field and capillary as the separation channel according to the difference between flow or distribution behavior in the sample. The difference between agarose gel electrophoresis from other supportive electrophoresis is that it plays the dual role of both a "molecular sieve" and "electrophoresis".

2) UV absorption method

The UV absorption spectrum belongs to the molecular spectrum, which is generated due to the valence electron transition of the molecule. The composition, content and structure of a substance can be analyzed, determined and inferred by the ultraviolet visible spectrum and the degree of absorption generated by the absorption of ultraviolet light by the molecule or ion of a substance. Compared with uncoupled haptens and proteins, the coupling products may introduce new ultraviolet absorbent groups or change the ultraviolet absorption due to the action of the coupling arm. Whether the coupling is successful can be judged by this theory. UV spectroscopy can calculate coupling ratio at the same time. It is simple and quick to operate and widely used.

3) Nuclear magnetic resonance method

In the NMR hydrogen spectrum diagram, the number of characteristic peaks reflects the kind of chemical environment of the hydrogen atoms in the molecule. The intensity ratio of different characteristic peaks, namely the height ratio of characteristic peaks, reflects the number ratio of hydrogen atoms in different chemical environments. This method can infer the structure of the identification substance by analyzing the attribution of hydrogen. It has a strong ability to identify the structure, but its disadvantage is that the instrument is expensive.

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4) Infrared spectroscopy

Infrared spectroscopy is one of the important characteristics of organic compounds, with high specificity and specificity. This method is one of the important methods for the identification of synthetic products. Its advantage lies in its strong characteristic, which can intuitively reflect the structural characteristics of substances.

1.2. Synthetic antigen

Activated amino acids are chemically polymerized into synthetic peptides. Polymers that consist of only one amino acid are called homopolypeptides, such as common Poly peptides pll (PLL) formed from L-lysine. Polymeric peptides formed by two or more kinds of amino acids are called copolymeric peptides, such as tyrosine, glutamate and polyalanine and lysine polymerization of polypeptide (T, G) -AL.

This synthetic polypeptide can be used to study the relationship between the types, sequences of amino acids, the antigenicity and immunogenicity of proteins, as well as the relationship between the heredity and immunity of the body.

Research on natural protein antigens proves that Any amino acid fragment with the right configuration has antigenicity. Moreover, even a small synthetic peptide linked to the right carrier can induce the production of antibodies and bind to its natural molecular configuration. It is suggested that the synthetic peptide vaccine can be constructed according to the amino acid sequence analysis of the immunogenicity fragments of the natural protein antigen or the amino acid sequence derived from its coding DNA.

1.3. Gene Engineering Antigen

Due to advances in molecular biology technology, it has been possible to clone genes encoding immunogenic amino acid sequence with appropriate carriers, such as bacterial grains or viruses, and then introduce expression in receptor cells (such as E. coli or eukaryotic yeast and mammalian cells), which enable immunogenicity fusion proteins to be purified as vaccines, genetically engineered vaccines[12].

2. Application

Compared with the traditional detection methods, it has the advantages of simplicity, rapidness, high sensitivity, low cost, convenience for field detection and high specificity. In addition, immunoassay technology also plays an important role in drug identification, drug metabolism research, pesticide and heavy metal residue detection, detoxification and environmental monitoring in medicinal materials [13]. Immunoassay is almost suitable for the detection of almost all bioactive substances.

3. Summary and Prospects

At present, there have been many reports on synthetic antigens at home and abroad. Its synthesis route and its synthesis technology are getting more and more mature and developing continuously. There is much experience that can be summarized and used for reference. It is believed that polymer-supported reagents will have a wide application prospect in this field. Although the synthesis methods have been greatly developed, the immunogenicity of the artificial antigens obtained is greatly different, and some artificial antigens cannot meet the requirements of immunoassay [14]. Due to the complexity of the immune response, as for a specific object to be measured, the synthesis of the artificial antigen should be analyzed and treated according to its own structural characteristics to design and combine multiple schemes, such as introducing different property groups from different positions, spacing arms of different lengths, combining half antigen with multiple carriers, and obtaining different binding ratio, etc. The screening and preparation scheme was optimized through the immune activity test. Therefore, the research of artificial antigen synthesis is not only a chemical synthesis problem, but also involving the principle of immunology. It is an important research direction in this field how to synthesize the artificial antigen with high immunogenicity according to the principle of immunology.

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