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Research Status of Antigen Synthesis Methods

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Abstract: *Most haptens are small molecules compounds. Because of its small molecular mass itself alone can not induce an immune response, it is necessary to form combined with the carrier hapten-carrier protein conjugate can stimulate the body's immune response. In recent years, in-depth research has been conducted at home and abroad on the synthesis of artificial antigens and the analysis of the results. Artificial antigen synthesis technology has been applied to many fields such as food and environment, which have more or less impact on our lives. This article reviews how to select carrier protein and the coupling method of hapten and carrier in the process of artificial antigen synthesis.*

Keywords: *Hapten, Carrier protein, Artificial antigen, Coupling*

1. Introduction

Antigen refers to a substance that can induce an immune response. Antigens are divided into complete antigens and incomplete antigens. Incomplete antigens are also called haptens. The hapten is only immunoreactive but not immunogenic. After the hapten is coupled with the carrier protein, an artificial antigen is synthesized to obtain immunogenicity. At present, synthetic artificial antigens have been widely used in immunoassays, and artificial antigen synthesis technologies are present in many fields.How to choose the carrier protein and how to couple the hapten to the carrier protein affects the quality of the synthesized artificial antigen.This article reviews the selection of carrier proteins and coupling methods involved in the synthesis of artificial antigens at home and abroad.

2. The Choice of Carrier Protein

Carrier is a vital presence in the process of artificial antigen synthesis.Since the hapten itself is small molecular mass, only relying on the carrier protein can induce the matrix to produce an immune response. When choosing a carrier protein, it is necessary to comprehensively consider the cost, experimental results and the structure of the carrier protein itself. Currently carrier proteins mainly include Bovine Serum Albumin (BSA), Ovalbumin (OVA), Keyhole Limpet Hemocyanin (KLH), in addition to Rabbit Serum Albumin (RSA)[1], Porcine Serum Albumin (PSA), Thyroglobulin[2], etc. Wherein the most widely used is Bovine Serum Albumin (BSA), mainly in its lower cost, stable properties and has better immunological activity.

3. Method for Linking Hapten with Carrier Protein

The coupling method of hapten and carrier is mainly chemical coupling method. Most small molecule compounds have functional groups, such as -OH, -COOH, -NH2 and so on. According to different functional group haptens, different methods can be selected for coupling with the carrier.

3.1. Synthetic Method of Functional Group Containing -OH Structure

3.1.1. Synthesis of Complete antigen by N, N-Carbonyldiimidazole Reaction Method

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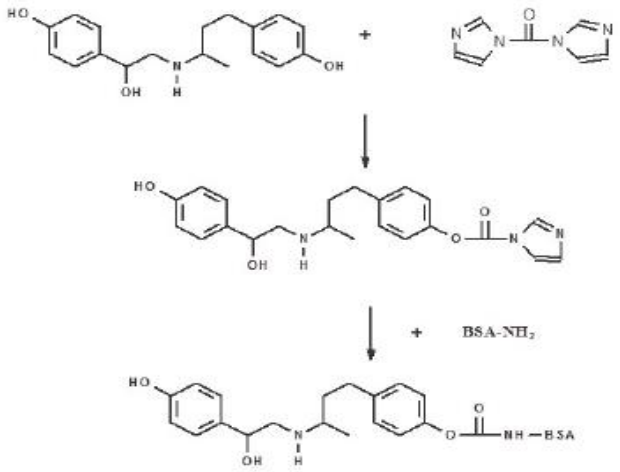

Figure 1: N, N-carbonyldiimidazole Reaction Schematic.

3.1.2. Synthesize Complete Antigen Using Linker Method(1,4-Butanediether Method)

The hapten selects a suitable carrier protein and connects it with 2,2-(ethylenedioxy) bis (ethylamine) to form an ultra-long chain complete antigen.

3.1.3. Synthesis of Complete Antigen by Mixed acid Anhydride Reaction

The hapten reacts with glutaric anhydride to activate the hydroxyl group into a carboxyl group to form a hemiacetal compound and then couples with the carrier protein to synthesize a complete antigen.

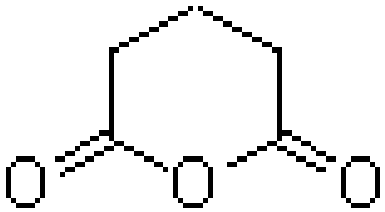

Figure 2: Glutaric anhydride structure.

Use N,N-carbonyldiimidazole reaction method, Linker method and mixed acid anhydride method to prepare Ractopamine(RAC) artificial antigen. Comparing the three methods comprehensively, it can be found that the Linker method has simple steps and few by-products during the preparation process. The mixed acid anhydride method has complicated steps and many by-products. In the process of preparing artificial antigens by N'N-carbonyldiimidazole method, since the coupling formed ester bonds, it is not as stable as peptide bonds. The specific effect needs to be further studied.For comparison of immune effects, the artificial antigens prepared by the Linker method and the mixed acid anhydride method have good immunogenicity. The titer of artificial antigen antiserum prepared by N'N-carbonyldiimidazole method is lower than that of Linker method and mixed acid anhydride method [3].

3.1.4. Synthesis of Complete Antigen Using Sodium Periodate Oxidation Method

Periodic acid is used to oxidize the hydroxyl group to an aldehyde group, and then it is coupled with the protein to form a linking arm.For example, aloin is an anthraquinone compound with o-dihydroxyl groups. By using sodium periodate oxidation method to oxidize the o-dihydroxyl group into aldehyde group, and then couple it with the amino group on the protein to synthesize artificial antigen. After testing, the artificial antigen of aloin synthesized by sodium periodate oxidation method has good immunogenicity and can induce BALB/c mice to produce specific antibodies against aloin[4].

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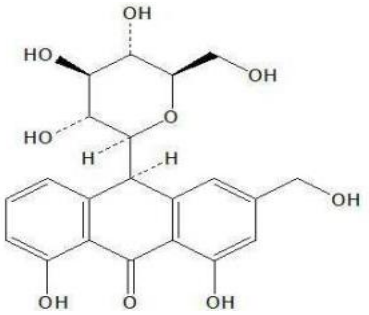

Figure 3: Aloin structure diagram.

3.1.5. Synthesis of Complete Antigen using N, N-Disuccinimidyl Carbonate Reaction Method

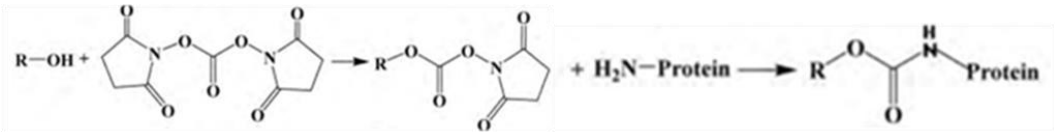

Figure 4: Schematic diagram of N, N-disuccinimidyl carbonate reaction method.

The hapten PMp6[6-(4-(4,6-dimethylpyrimidin-2-ylamino)phenyl)hexanoic acid (3)] is reacted with N,N-disuccinimidyl carbonate and then coupled with bovine serum albumin to prepare a BSA-PMp6 conjugate[5].

3.2. Synthetic Method of Functional Group Containing -COOH Structure

3.2.1. Synthesize Complete Antigen using DDC Method[6]

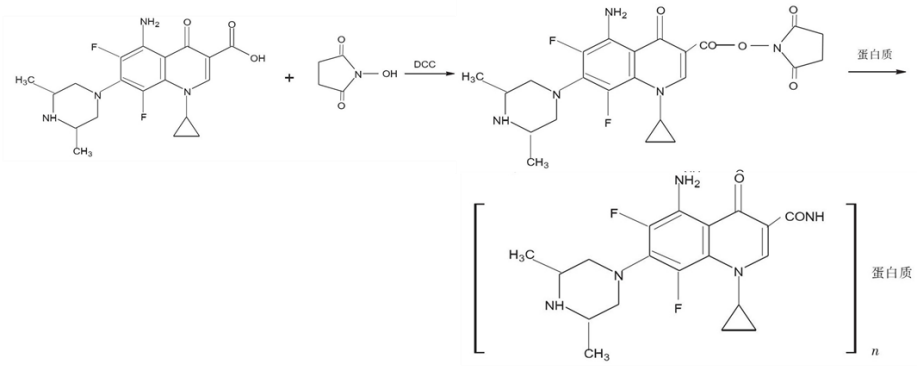

Figure 5: DDC method principle diagram.

3.2.2. Synthesis of Complete Antigen by Mixed Acid Anhydride Reaction

The carboxyl group in the hapten molecule reacts with isobutyl chloroformate to generate a mixed anhydride intermediate and then reacts with the amino group on the carrier protein[7].

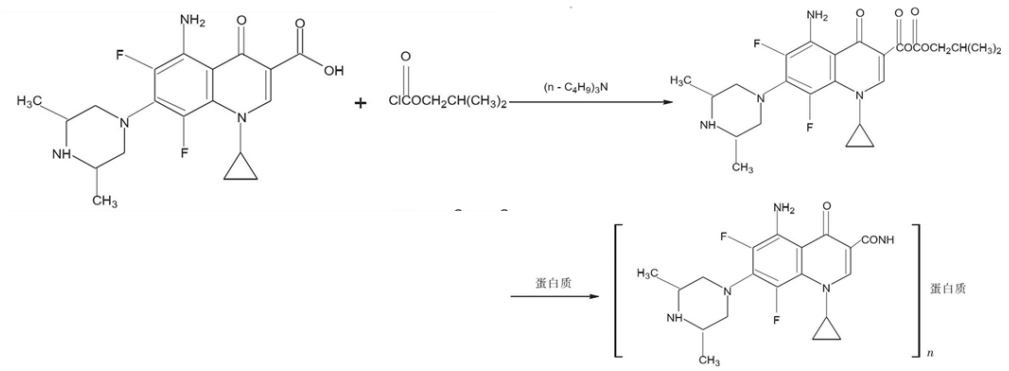

Figure 6: Schematic diagram of the mixed acid anhydride method.

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Synthesize sparfloxacin artificial antigen by DDC method and mixed acid anhydride reaction method respectively. Both methods can synthesize artificial antigens, but the DDC method is complicated to operate and has many processes that require attention. At the same time, it is easy to produce by-products and difficult to remove. The mixed acid anhydride method is relatively simple to operate, but it also has shortcomings such as serious reaction loss. Experiments have found that the artificial antigen synthesized by the mixed acid anhydride method is relatively stable and can better stimulate the production of antibodies in animals.

3.2.3. Synthesis of Complete Antigen using Carbodiimide Reaction Method[8]

Using the carbodiimide reaction method, the carboxyl group on the hapten and the amino group on the carrier protein are dehydrated and condensed to form an amide bond to synthesize the hapten. For example, in the synthesis of the leucomalachite green artificial antigen, the leucomalachite green hapten contains not only the active group carboxyl group but also the amino group. The method of synthesizing artificial antigen is not limited to the carbodiimide method, but also the diazotization method can be used to make the amino group and the carboxyl group on the carrier protein be coupled to synthesize[9].

3.2.4. Synthesis of Complete Antigen using Azobenzoic Acid Method

The hapten is modified with azobenzoic acid so that the hapten contains a carboxyl group in its structure, and then it is coupled to the protein. For example, there is no amino or carboxyl group in the structure of doxycycline (DC), which cannot be directly coupled with protein. Therefore, the azobenzoic acid method was used to convert it to doxycycline containing carboxyl group and then coupled with protein [10].

3.2.5. Synthesize Complete Antigen by Active Ester Reaction Method

The active ester method uses N-hydroxysuccinimide (NHS) as the activator and cyclodihexylcarbimide (DCC) as the dehydrating agent to directly connect small molecules to the carrier protein without a coupling bridge. This method has also been shown to produce fewer by-products and strong antibody specificity. For example, Furazolidone [N-(5-nitro-2-furfurylidene-3-amino)-2-oxazolidinone] belongs to nitrofuran antibiotic drugs. By using the active ester method to synthesize complete antigens, the antibodies obtained by this method are superior to other coupling methods. This is due to the higher conjugation ratio of the active ester method[11].The active ester method improves the carbodiimide method and avoids the direct action of the carbodiimide method with the carrier protein to reduce the intermolecular cross-linking reaction.

3.3. Synthetic Method of Functional Group Containing -NH2 Structure

3.3.1. Synthesis of Complete Antigen using Glutaraldehyde Reaction Method

The hapten is reacted with glutaraldehyde and then coupled with the carrier protein to synthesize an artificial antigen. For example, Huperzine A is a new type of natural alkaloid of Lycopodium. The glutaraldehyde method is used to couple Huperzine A to the carrier protein. The two aldehyde groups on the glutaraldehyde are connected to the hapten and the amino group on the carrier protein by a five-carbon chain bridge to synthesize artificial antigens[12].

3.3.2. Synthesis of Complete Antigen using Carbodiimide Method

Introducing -COOH into the hapten structure and condensing it with $-NH_2$ on the protein to synthesize an artificial antigen.

3.3.3. Synthesis of Complete Antigen by Diazotization Reaction Method

The $-NH_2$ in the hapten structure undergoes an electrophilic reaction to form a diazonium salt, and then forms a diazonium bond with the phenolic hydroxyl group to form an azo compound.

3.3.4. Synthesis of Complete Antigen using BS3 Method

The -NH2 in the hapten structure and the -NH2 in the protein are respectively coupled to the -CO at both ends of the BS3 through a condensation reaction.

The cimaterol (CIM) was synthesized by the above three methods respectively. It can be seen from the comparison that the hapten-carrier complex synthesized by the diazotization method is formed by direct coupling, while the carbodiimide method and the BS3 method are both randomly coupled. The products produced are not uniform and the structure is more complicated. By detecting the coupling

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ratio, it is not difficult to find that the complete antigen coupling ratio synthesized by the diazotization method is more suitable and has strong immunogenicity[13].

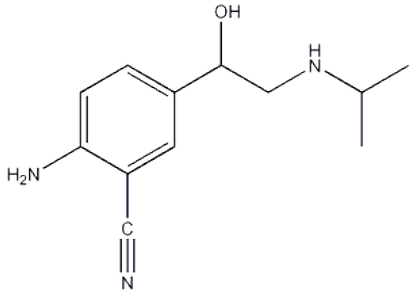

Figure 7: Cimarot structure.

3.4. Synthetic Method of Functional Group Containing C=O Structure

3.4.1. Synthesis of Complete Antigen using P-Hydrazinobenzoic Acid Reaction Method

The hapten and carrier protein use p-hydrazinobenzoic acid as the cross-linking arm to synthesize a complete antigen. For example, tylosin tartrate is used as a raw material and tylosin dystamycin is used as a hapten to prepare a complete antigen. After testing, the complete antigen was successfully synthesized[14].

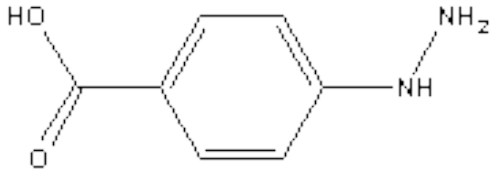

Figure 8: Structural formula of p-hydrazinobenzoic acid.

3.4.2. Synthesis of Complete Antigen using Amine Methylation Reaction Method

Under acid catalysis, the amine group in the carrier protein is dehydrated with formaldehyde to produce methyleneamine carbocation.The α hydrogen atom on the carbonyl group of aflatoxin B1 reacts with methyleneamine carbenium ion to synthesize an artificial antigen[15].This method is simple and easy to implement and has low cost. It is suitable for ordinary laboratories.

3.4.3. Synthesis of Complete Antigen using Epoxide Method

In dichloromethane, the double bond of the aflatoxin difuran ring is oxidized by ethylene oxide or peroxide meta-chloroperoxybenzoic acid to form aflatoxin yellow oxide and then couple with the carrier protein. This method is simple to operate but easy to produce by-products[16].

4. Summary and Outlook

The key to artificial antigen synthesis is to have good immunogenicity. The successful synthesis of artificial antigens with haptens requires constant attempts. Countless people at home and abroad have provided more ideas for the synthesis of artificial antigens.It is necessary to seek experience from past failures and extract the essence from success. Look for a simpler and easier way. How to integrate modern advanced technology into daily research requires continuous efforts to explore.

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