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Review of the Preparation and Applications of Chemically Synthesized Peptide Antibodies

Chemically synthesized peptide antibodies refer to a strategy where specific short peptide sequences are artificially prepared as antigens or directly as antibody mimics through chemical methods such as solid-phase peptide synthesis. These are then applied in immunological research, disease diagnosis, and treatment. This approach differs from traditional methods involving immunization of animals with intact proteins or antibodies obtained through cellular recombinant technology. The core advantage of this strategy lies in its ability to precisely control antigenic epitope sequences, introduce non-natural modifications, and rapidly generate specific recognition tools for challenging targets. Chemically synthesized peptide antibodies have become a key technology in fundamental research, in vitro diagnostics, and drug development.

I. Core Concepts and Synthesis Fundamentals

1. Basic Concepts

Chemically synthesized peptide antibodies primarily exist in two forms:

Antibodies generated against peptides as immunogens: Short chemically synthesized peptides (typically 10-20 amino acids) are conjugated to carrier proteins and administered to animals to induce the production of specific polyclonal or monoclonal antibodies against that particular peptide segment.

Peptides as antibody mimetics: Directly synthesizing peptide segments with antigen-binding functionality. These peptides themselves mimic antibody complementarity determining regions (CDRs), enabling specific binding to targets. They are termed “peptide ligands” or “peptide antibodies.”

2. Chemical Synthesis Cornerstone: Solid-Phase Peptide Synthesis

Advancements in this field rely entirely on the maturity of solid-phase peptide synthesis technology.

Basic Principle: The carboxyl terminus of the first amino acid is covalently anchored to an insoluble resin. Subsequently, following the target sequence, a cyclic process of amino acid deprotection, activation, coupling, and washing is performed to sequentially attach amino acids to the growing peptide chain. Upon completion, the intact peptide is cleaved from the resin using a cleavage reagent, and side-chain protecting groups are removed.

Key Advantages:

Fully Controllable Sequence: Capable of synthesizing any specified

sequence, including those not found in nature.

Special Modifications: Facilitates the introduction of non-natural amino acids, fluorescent groups, biotin, phosphorylation, methylation, and other post-translational modifications at specific sites for studying modification-specific antibodies.

Rapid and Efficient: Automated synthesizers can synthesize peptides up to several dozen amino acids within days, accelerating the R&D process.

II. Primary Preparation Strategies and Applications

Antibody strategies based on chemically synthesized peptides are primarily categorized into two approaches: “peptide-based antibody production” and “peptide-based antibody mimicry.”

1. Strategy One: Preparing Specific Antibodies Using Synthetic Peptide Antigens

This represents the most classical and widely adopted technical approach.

Process:

1. Epitope Analysis and Peptide Design: Analyze the target protein sequence using bioinformatics software to predict linear B-cell epitopes (hydrophilic, accessible, flexible regions) or directly select known functional domains to design synthetic peptides.

2. Peptide Synthesis and Conjugation: Chemically synthesize the peptide, typically adding an extra cysteine residue at its N- or C-terminus. This

enables conjugation with carrier proteins like keyhole lecithin or bovine serum albumin via crosslinking agents to enhance immunogenicity.

3. Animal Immunization and Antibody Purification: Immunize animals with the conjugate to obtain antiserum. Subsequently, immobilize the synthetic peptide on a chromatography medium to affinity-purify the specific antibody.

Core Applications:

Research Tool Development: For difficult-to-purify membrane proteins, low-abundance proteins, or specifically modified proteins, synthesizing partial peptides offers a viable approach to obtain research antibodies.

Disease Diagnostic Marker Detection: In infectious disease, autoimmune disorder, and tumor diagnostics, synthesizing pathogen-specific antigen peptides or autoantigen peptides enables antibody production for detection or direct use as diagnostic antigens. Examples include synthetic peptide-based HIV and HCV antibody test kits.

Vaccine Development:

Synthetic peptides representing key antigenic epitopes of pathogens serve as vaccine candidates to induce neutralizing antibodies.

2. Strategy Two: Screening and Synthesis of Peptide Ligands (Mimetic Antibodies)

This strategy aims to obtain peptide molecules that directly bind to

targets, serving as alternatives or complements to traditional antibodies.

Process:

1. Library Screening: Typically not designed directly, but rather screened from libraries containing billions of random sequences—such as phage display peptide libraries or mRNA display peptide libraries—to identify peptide sequences with high-affinity binding to the target protein.

2. Chemical Synthesis Optimization: Selected lead peptides undergo chemical synthesis followed by structure-activity relationship studies and optimization through alanine scanning, sequence truncation, and incorporation of non-natural amino acids to enhance affinity, stability, and protease resistance.

Core Applications:

Molecular Imaging Probes: Optimized targeting peptides are conjugated with radionuclides or fluorescent groups for PET/CT or fluorescence imaging of tumors and other diseases. Their small molecular weight, rapid penetration, and efficient clearance result in low imaging background.

Targeted Therapeutic Carriers:

Cytotoxic drugs are conjugated to tumor-targeting peptides to create peptide-drug conjugates (PDCs) as alternatives to antibody-drug conjugates (ADCs). Alternatively, cell membrane-penetrating sequences are integrated into the peptide sequence for intracellular delivery of drugs

or nucleic acids.

Diagnostic Detection:

Directly employed as capture or detection probes immobilized on biosensors or detection platforms.

III. Strengths, Challenges, and Future Outlook

1. Unique Advantages

For “undruggable” targets: When natural proteins are unavailable or targets exhibit complex structures, synthesizing key epitope peptides offers a streamlined approach to developing recognition tools.

Powerful Tool for Post-Translational Modification Studies: Precisely synthesize peptides bearing specific modifications like phosphorylation, acetylation, or ubiquitination to prepare modification-state-specific antibodies—critical for signal transduction and epigenetics research.

High Safety: Synthetic peptide antigens avoid pathogen or tumor cell culture, ensuring greater safety. Peptide ligands typically exhibit lower immunogenicity than antibodies.

Cost and Timeline: For short peptides, chemical synthesis is faster and more economical than recombinant protein expression.

2. Key Challenges

Conformation Restriction: Chemically synthesized linear short peptides may fail to mimic conformation epitopes found in native proteins, resulting in antibodies that cannot recognize the natural protein.

Affinity Bottleneck: Peptide ligands obtained through screening typically exhibit affinity levels several orders of magnitude lower than monoclonal antibodies.

In Vivo Stability: Linear peptides undergo rapid proteolytic degradation in vivo, exhibiting extremely short half-lives that limit direct therapeutic applications.

Structural Optimization Complexity: Enhancing peptide affinity and stability often requires intricate chemical modifications and cyclization designs, increasing R&D complexity and costs.

3. Future Outlook

Structural Stabilization Technologies: Develop more efficient cyclization strategies and stable secondary structure modeling techniques to constrain peptide conformation with rigid structures, enhancing recognition of conformational epitopes and in vivo stability.

Multifunctional Integrated Design: Design “multivalent” or “bispecific” peptides targeting two distinct epitopes or cells simultaneously, boosting binding affinity and functionality.

Computational and AI-Assisted Design: Utilize artificial intelligence to predict antigenic epitopes, simulate peptide-target interactions, and design high-affinity, antibody-like peptide molecules, reducing reliance on large-scale screening.

Advanced Delivery Systems: Develop nanotechnology-based

delivery systems to protect therapeutic peptide ligands during circulation and enable targeted controlled release.

IV.Conclusion

Chemically synthesized peptide antibody technology combines the precision of chemical synthesis with the specificity of immunology, pioneering a unique pathway for producing molecular recognition tools. Whether serving as immunogens for producing specific antibodies or functioning as antibody mimetics directly applied in diagnostics and therapeutics, it demonstrates flexibility and precision unmatched by traditional antibody technologies. Despite ongoing challenges in conformational mimicry, affinity, and stability, the convergence of peptide chemistry, structural biology, and computational science is propelling this field toward designing smarter, more potent “synthetic antibodies.” It not only provides indispensable tools for life science research but also lays a solid technical foundation for developing next-generation in vitro diagnostic reagents, molecular imaging probes, and targeted therapeutic drugs, becoming a crucial link in the biomedical innovation chain.