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Solubilization Tags: Core Solution for Difficult Hydrophobic Protein Chemical Synthesis

1.Research Background

Chemical protein synthesis enables precise customization of modified and artificial proteins that are hard to obtain via microbial expression, serving as core raw material preparation technology for target protein drugs, ion channel proteins and biomedical research samples. However numerous membrane-derived and hydrophobic polypeptide fragments easily form α -helix, β -sheet via hydrogen bonding and hydrophobic aggregation, leading to poor solubility in common organic/aqueous solvents. Poor solubility severely blocks HPLC purification, mass spectrometry characterization and native chemical ligation assembly, becoming a major bottleneck restricting full chemical synthesis of IL-2, Kir5.1, FCER1G and other key therapeutic proteins. Solubilizing tag modification is one of the most mature and practical technical routes to solve aggregation troubles of hydrophobic peptide fragments.

2.Research Purpose and Significance

This paper systematically sorts three mainstream tag modification

routes classified by modification positions: terminal attachment, amino acid side-chain grafting and peptide backbone reversible modification, summarizes corresponding detachable tag structures, deprotection conditions and practical application cases including histone, virus protein and ion channel peptide. Systematic induction of existing technical pros and cons provides clear process reference for R&D personnel to select proper solubilization scheme during difficult peptide customization, lowers trial-and-error cost for industrial peptide synthesis and accelerates the R&D progress of novel protein-based pharmaceuticals.

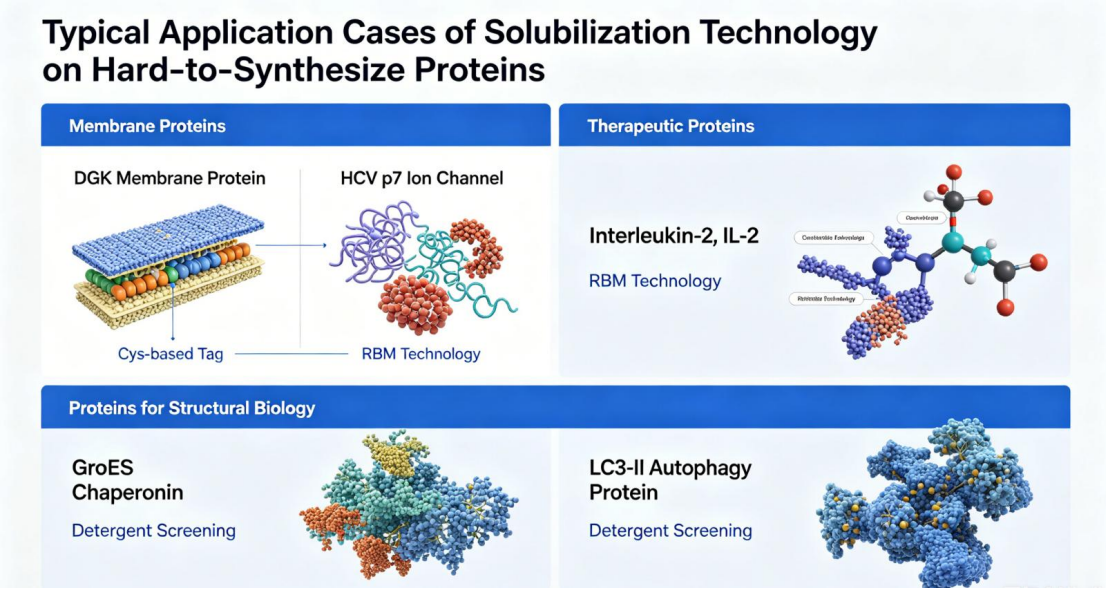
3.Research Content

Terminal solubilizing tags include N-terminal and C-terminal hydrophilic labels: C-terminal thioester-type arginine tags fall off automatically during NCL reaction and have been applied for DGK membrane protein synthesis; alkali/protease cleavable N-terminal tags improve solubility of transmembrane peptides yet risk side-chain deamidation under strong alkaline cleavage.

Side-chain modification is the most widely used technical system, with three common modification sites on Cys, Lys, Asn/Gln residues. Cys-based tags adopt $PdCl_2$, TFA or reducing agent triggered removal; Lys-derived tags enjoy high amino acid abundance in natural sequences and multiple removable structures such as Ddae/Ddap, which have successfully realized GroES, IL-2 full synthesis; Asn/Gln sites introduce photosensitive

solubilizing groups cleaved under UV irradiation for amyloid peptide regulation and LC3-II preparation, though UV treatment may oxidize sensitive amino acids.

Reversible backbone modification (RBM) is designed to break intramolecular hydrogen bonds via temporary Hmb/Dmb group on peptide amide nitrogen to suppress secondary structure formation, removable by neat TFA after ligation; this technology has accomplished HCV p7 ion channel and Kir5.1 transmembrane domain synthesis, while irreversible backbone methylation is limited to research use due to permanent structural change.



4.Conclusion & Outlook

Various removable solubilizing tags effectively overcome hydrophobic peptide aggregation and solubility defects, covering terminal, side chain and backbone multi-dimensional modification routes and have realized industrial-level preparation of many difficult target proteins; at

present each modification technology has inherent limitations including harsh cleavage conditions, amino sequence restriction or side oxidation risk, future development will focus on developing mild, enzyme-triggered recyclable solubilizing tags with simple grafting operation and wide sequence compatibility, meanwhile combining continuous flow peptide synthesis technology to further optimize solubilization and ligation integrated process, expand the application scope of chemical synthesis toward longer-chain complex therapeutic proteins and novel biomaterial peptides.