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TFA-Assisted Ligation: A Novel Route for Challenging Protein Chemical Synthesis

1.The Research Background

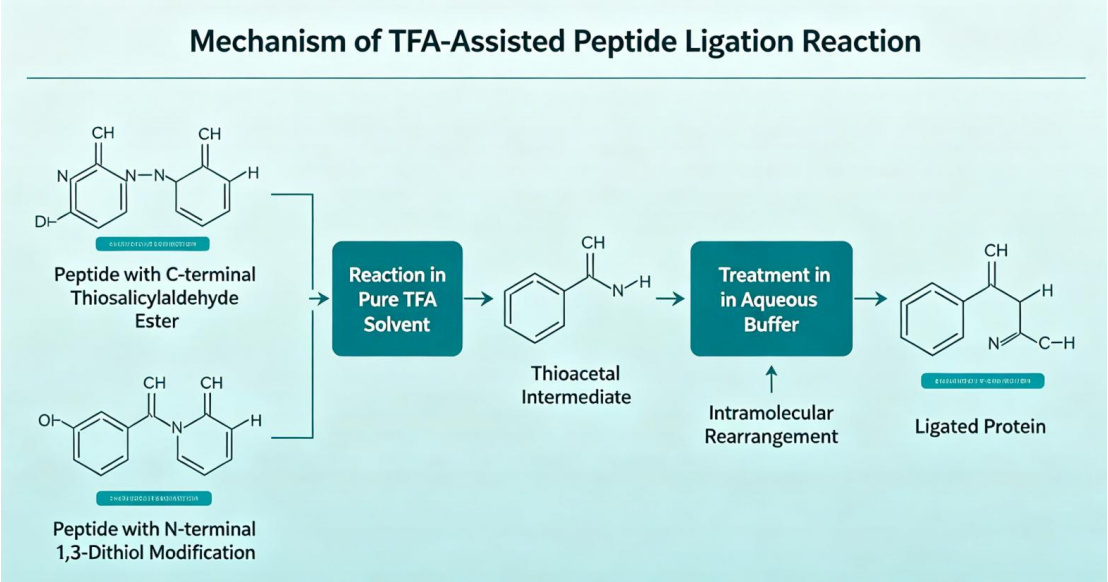
Natural chemical ligation, serine/threonine ligation and KAHA ligation are mainstream protein assembly technologies, widely adopted for hydrophilic peptide splicing. However, most hydrophobic and membrane-prone peptide fragments easily aggregate and form colloids in conventional aqueous denaturing solvents such as guanidine hydrochloride, leading to poor solubility and extremely low ligation efficiency, which severely restricts the full chemical synthesis of hard-to-make proteins like viral envelope protein and nanobody. To break through this bottleneck, researchers developed an innovative TFA-assisted peptide ligation (TAL) method using trifluoroacetic acid as the core reaction solvent.

2.Research Purpose and Significance

This study establishes a brand-new peptide linking system based on C-terminal thiosalicylaldehyde ester peptide and 1,3-dithiol modified peptide. The research verifies the reaction rules, applicable amino acid range and solvent compatibility of TAL, and completes the total synthesis

of multiple difficult proteins including SARS-CoV-2 E protein and GFP-targeted nanobody. The new method provides a reliable industrializable technical path for chemically synthesizing hydrophobic/membrane proteins, fills the technical gap of traditional ligation methods, and lays a foundation for the R&D of peptide drugs and biological raw materials.

3. Research Content



The TAL reaction proceeds in pure TFA medium: two types of peptide raw materials dissolve thoroughly without aggregate generation, rapidly forming thioacetal intermediates at room temperature; subsequent mild aqueous buffer treatment triggers intramolecular rearrangement to generate natural amide bonds, and auxiliary groups can be removed via simple acidolysis to obtain full-length target protein. Systematic screening proves the reaction features outstanding chemoselectivity, tolerating most common amino acids including sterically hindered proline with over 85% yield. The method adapts to wide pH and concentration ranges from 0.1

mM to 100 mM. In practical verification, conventional NCL failed to assemble Haemophilus influenza DNA ligase fragment, SARS-CoV-2 E protein and β-rich nanobody due to severe peptide aggregation, while TFA solvent eliminated colloid formation and realized high-efficiency splicing; after in vitro folding and characterization, synthesized proteins matched the structure and antigen-binding activity of recombinantly expressed counterparts.

4.Conclusion & Outlook

TFA-assisted ligation effectively solves the aggregation and poor solubility issues of hard-to-splice hydrophobic peptides that puzzle traditional linking technologies, featuring fast reaction speed, wide substrate compatibility and high product purity, and has been validated on multiple typical challenging protein samples. Moving forward, the team will further optimize auxiliary group design and expand compatible reagent systems, continuously broaden the application boundary of TAL technology, accelerate process simplification and cost reduction, and promote the large-scale industrial application of this new method in custom peptide and innovative protein drug production.