Lecture 3
Discipline: Bioorganic Chemistry
Lecturer: Associate Professor, Dr. Gulnaz Seitimova

Title: Methodology for creating a peptide bond for the design of peptides similar to natural ones. Solid-phase method for peptide synthesis.

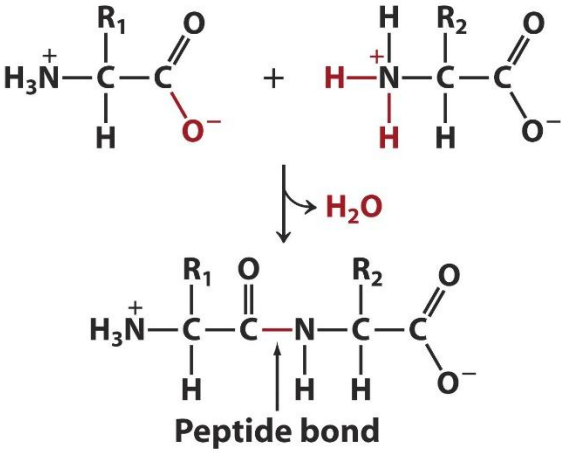
Objective: The aim of this lecture is to explain the chemical strategies for forming peptide bonds, describe the principles of designing peptides analogous to natural peptides, and outline the solid-phase peptide synthesis (SPPS) methodology, which allows efficient and controlled production of peptides for research and therapeutic applications.

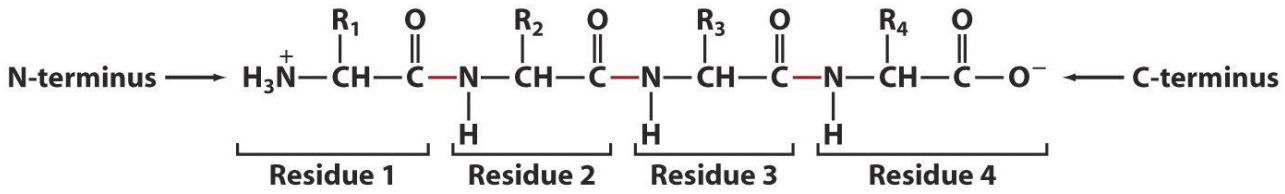
Main Questions: Definition and significance of peptide bonds in biology and bioorganic chemistry. Strategies for peptide bond formation in solution-phase and solid-phase synthesis. Protecting groups used in peptide synthesis (amino, carboxyl, side-chain functional groups). Activation methods for carboxyl groups to promote peptide bond formation. Solid-phase peptide synthesis (SPPS) methodology: principles and advantages. Stepwise elongation, deprotection, and cleavage in SPPS. Comparison between Fmoc and Boc strategies for SPPS. Purification and characterization of synthetic peptides. Applications of synthetic peptides in drug development, research, and diagnostics.

Key Notes and Theses

Peptide Bonds and Their Importance

- Peptide bonds (–CO–NH–) connect amino acids in proteins and peptides.
- Responsible for the primary structure of proteins, influencing folding, stability, and biological function.
- Peptide bond formation is a condensation reaction releasing water.





Methods for Peptide Bond Formation

Solution-phase peptide synthesis:

- Amino acids are sequentially coupled in solution using activating agents (DCC, EDC, HATU) and protecting groups.
- Advantages: well-established chemistry, suitable for small peptides.
- Disadvantages: labor-intensive, low yield for longer peptides.

Solid-phase peptide synthesis (SPPS):

- Developed by R. B. Merrifield in 1963, now standard for peptide synthesis.
- Amino acids are attached to an insoluble resin, and peptide elongation occurs while anchored to the solid support.
- Advantages: simplified purification (wash away excess reagents), automation possible, high efficiency for long sequences.

Protecting Groups

- Amino group protection: Boc (tert-butyloxycarbonyl), Fmoc (9-fluorenylmethoxycarbonyl).
- Carboxyl group protection: typically esterified or attached to resin.
- Side-chain protection: specific groups for reactive side chains (Ser, Lys, Asp, Cys).
- Protecting groups prevent undesired reactions and allow selective bond formation.

Activation of Carboxyl Groups

- Carbodiimides (DCC, EDC) form active esters to react with amino groups.
- HATU, HBTU, PyBOP are commonly used coupling reagents.
- Activators increase reaction rates and yields.

Solid-Phase Peptide Synthesis Steps

1. Attachment of the first amino acid to the resin through a linker.
2. Deprotection of the amino-terminal protecting group.
3. Coupling of the next protected amino acid using activation chemistry.
4. Repeat elongation cycle until desired sequence is complete.
5. Cleavage of the peptide from the resin and removal of side-chain protecting groups.

Solid phase peptide synthesis

Why are chemists needed?

Gene expression is very popular, relatively easy and cheap method: it is good for long linear peptides or proteins containing L-amino acids.

However:

- no *D*-amino acids
- no unnatural amino acids
- no post translation (Hyp, Pyr, glyco- and phosphopeptides)
- no branches
- no cyclic peptides
- no fluorescent or isotop labeling

Peptides as drugs: there are not too many, because of the price and their fast biodegradation.

"Peptides have and will continue to be important sources of lead compounds in many drug discovery programs. However, due to their generally poor pharmacokinetic

properties and hydrolytic instability, natural peptide structures are usually substituted with mimics of the actual peptide constuction."

Fmoc vs Boc Strategies

- Boc strategy: acid-labile protecting group, removed with TFA; requires strong acid treatment.
- Fmoc strategy: base-labile protecting group, removed with mild base (piperidine); compatible with many modern automated synthesizers.

PEPTIDE SYNTHESIS

Coupling of amino acids:

NH_2-CH(R)-COOH + NH_2-CH(R')-COOH

↓ - H_2O

NH_2-CH(R)-CO-NH-CH(R')-COOH; NH_2-CH(R')-CO-NH-CH(R)-COOH;

NH_2-CH(R)-CO-NH-CH(R)-COOH; NH_2-CH(R')-CO-NH-CH(R')-COOH;

+ oligomers and polymers with different composition

Protecting groups: *amino-; carboxyl-; side chain protecting groups*

X-NH-CH(R)-COOH + NH_2-CH(R')-COOY

↓ - H_2O

X-NH-CH(R)-CO-NH-CH(R')-COOY;

Removal of the protecting groups together or selectively

Purification and Characterization

- Crude peptides are purified by reverse-phase HPLC or preparative chromatography.
- Characterization techniques: mass spectrometry, amino acid analysis, NMR spectroscopy, and optical rotation.

Applications of Synthetic Peptides

- Drug development: insulin analogs, peptide hormones, antimicrobial peptides.
- Vaccines: synthetic epitopes for immunization.
- Diagnostics and research: receptor binding studies, enzyme inhibition, structural studies.

Questions for Knowledge Assessment

1. What is a peptide bond and why is it important in bioorganic chemistry?
2. What are the main differences between solution-phase and solid-phase peptide synthesis?
3. Name the commonly used amino-protecting groups in peptide synthesis.
4. How do carbodiimide activators facilitate peptide bond formation?
5. What is the principle of solid-phase peptide synthesis (SPPS)?
6. Compare the Fmoc and Boc strategies in terms of stability and deprotection.
7. Describe the steps of SPPS from the first amino acid attachment to peptide cleavage.
8. Which analytical methods are used to characterize synthetic peptides?
9. Give examples of therapeutic applications of synthetic peptides.

Recommended Literature

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