Chem 109 C
Bioorganic Compounds

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http://labs.chem.ucsb.edu/~zakariangroup/courses.html
Primary structure is the sequence of amino acids in a protein and the location of disulfide bridges

strategy for determining the primary structure:

1. break down peptide into individual amino acids to determine composition

2. use selective reagents to determine sequence
first steps - overall composition:

cleaving disulfide bridges: \( \text{HSCH}_2\text{CH}_2\text{OH} \)
first steps - overall composition:

cleaving disulfide bridges: \( \text{HSCH}_2\text{CH}_2\text{OH} \)

complete hydrolysis: \( 6 \text{ M HCl, H}_2\text{O, 100 °C, 24 h} \)
first steps - overall composition:
cleaving disulfide bridges: $\text{HSCH}_2\text{CH}_2\text{OH}$

complete hydrolysis: 6 M HCl, H$_2$O, 100 °C, 24 h

protein $\xrightarrow{100 \, ^\circ \text{C}}$ amino acids

then use amino acid analyzer...
selective reagents to break amide bonds:

**for N-terminal amino acid**

Edman’s reagent:

\[
\text{phenyl isothiocyanate} \quad \xrightarrow{\text{react}} \quad \text{PTH-amino acid}
\]

from N-terminal amino acid

can be repeated up to 50 times in sequencer
selective reagents to break amide bonds:

**partial hydrolysis with dilute acid into smaller pieces**

Ala-Lys-Phe-Gly-Asp-Trp-Ser-Arg-Met-Val-Arg-Tyr-Leu-His
### Sequencing an oligopeptide...

#### PRACTICE PROBLEM
A decapeptide undergoes partial hydrolysis to give peptides whose amino acid compositions are shown. Reaction of the intact decapeptide with Edman’s reagent releases PTH-Gly. What is the sequence of the decapeptide?

<table>
<thead>
<tr>
<th>Choice</th>
<th>Amino Acid Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ala, Trp</td>
</tr>
<tr>
<td>2.</td>
<td>Val, Pro, Asp</td>
</tr>
<tr>
<td>3.</td>
<td>Pro, Val</td>
</tr>
<tr>
<td>4.</td>
<td>Ala, Glu</td>
</tr>
<tr>
<td>5.</td>
<td>Trp, Ala, Arg</td>
</tr>
<tr>
<td>6.</td>
<td>Arg, Gly</td>
</tr>
<tr>
<td>7.</td>
<td>Glu, Ala, Leu</td>
</tr>
<tr>
<td>8.</td>
<td>Met, Pro, Leu, Glu</td>
</tr>
</tbody>
</table>
Sequencing an oligopeptide...

**PRACTICE PROBLEM**
A decapeptide undergoes partial hydrolysis to give peptides whose amino acid compositions are shown. Reaction of the intact decapeptide with Edman’s reagent releases PTH-Gly. What is the sequence of the decapeptide?

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
</table>
Wednesday, October 16, HFH 1104, 8 – 8:50 am

• Chapter 20. Carbohydrates.

  All Sections except 20.13, 20.17, 20.19

• Chapter 21. Amino acids, Proteins.

  All sections Up to 21.11, including 21.11

structures of carbohydrates (except glucose, mannose, and galactose) and amino acids will be provided
PRACTICE PROBLEM

Name the following monosaccharide and draw a Fischer projection for the open form.
selective reagents to break amide bonds:

for C-terminal amino acid:

exopeptidases:

carboxypeptidase A: all but Arg and Lys

carboxypeptidase B: only Arg and Lys
selective reagents to break amide bonds:
partial hydrolysis with endopeptidases:

**Trypsin**: C-side of Arg and Lys

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Ala-Lys-Phe-Gly-Asp-Trp-Ser-Arg-Met-Val-Arg-Tyr-Leu-His
```

**cleavage by trypsin**
selective reagents to break amide bonds:
partial hydrolysis with endopeptidases:
**Trypsin**: C-side of Arg and Lys
selective reagents to break amide bonds:

partial hydrolysis with endopeptidases:

**Trypsin**: C-side of Arg and Lys

![Trypsin cleavage example]

**Chymotrypsin**: C-side of Phe, Tyr and Trp

![Chymotrypsin cleavage example]
selective reagents to break amide bonds:
partial hydrolysis with endopeptidases:

**Trypsin**: C-side of Arg and Lys

**Chymotrypsin**: C-side of Phe, Tyr and Trp

**Elastase**: C-side of Gly and Ala
selective reagents to break amide bonds:

for endo- and exopeptidases,
no reaction at Pro:

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala-Lys-Pro</td>
<td>trypsin will not cleave</td>
</tr>
<tr>
<td>Leu-Phe-Pro</td>
<td>chymotrypsin will not cleave</td>
</tr>
<tr>
<td>Pro-Phe-Val</td>
<td>chymotrypsin will cleave</td>
</tr>
</tbody>
</table>
Proteins: Structure

selective reagents to break amide bonds:

cyanogen bromide: BrCN,

C-side of Met

Ala-Lys-Phe-Gly-Asp-Trp-Ser-Arg-Met-Val-Arg-Tyr-Leu-His

BrCN will cleave next to proline (Pro)
### Table 21.4 Specificity of Peptide or Protein Cleavage

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical reagents</td>
<td></td>
</tr>
<tr>
<td>Edman’s reagent</td>
<td>removes the N-terminal amino acid</td>
</tr>
<tr>
<td>Cyanogen bromide</td>
<td>hydrolyzes on the C-side of Met</td>
</tr>
<tr>
<td>Exopeptidases*</td>
<td></td>
</tr>
<tr>
<td>Carboxypeptidase A</td>
<td>removes the C-terminal amino acid (not Arg or Lys)</td>
</tr>
<tr>
<td>Carboxypeptidase B</td>
<td>removes the C-terminal amino acid (only Arg or Lys)</td>
</tr>
<tr>
<td>Endopeptidases*</td>
<td></td>
</tr>
<tr>
<td>Trypsin</td>
<td>hydrolyzes on the C-side of Arg and Lys</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>hydrolyzes on the C-side of amino acids that contain aromatic six-membered rings (Phe, Tyr, Trp)</td>
</tr>
<tr>
<td>Elastase</td>
<td>hydrolyzes on the C-side of small amino acids (Gly and Ala)</td>
</tr>
</tbody>
</table>

* Cleavage will not occur if Pro is on either side of the bond to be hydrolyzed.
Primary structure is the sequence of amino acids in a protein and the location of disulfide bridges

- obtained from sequencing a protein

Secondary structure describes the common conformations of segments of a protein

three types:
**Proteins: Secondary Structure**

α-Helix

one turn: 3.6 aa, 5.4 Å repeat distance
Proteins: Secondary Structure

**β-Pleated Sheet**

Average two residue repeat distance is 7.0 Å
Proteins: Secondary Structure

Coils or Loops
**Proteins: Tertiary Structure**

**Tertiary structure** describes the 3D arrangement of all the atoms in the protein.
Tertiary structure describes the 3D arrangement of all the atoms in the protein.
Quaternary structure aggregates of proteins: each is called a subunit
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hemoglobin

mutation at position 6: glutamate $\rightarrow$ valine: sickle cell anemia
Proteins: Quaternary Structure

nicotinic acetylcholine receptor (nAChR)
Protein denaturation: is destruction of the highly organized tertiary structure, results in [irreversible] loss of function

Factors that can cause denaturation:

- changing acidity (pH)
- temperature
- some reagents (urea, guanidine)
- detergents
- organic solvents
Protein Structure and Analysis

structure: primary, secondary, tertiary, quaternary
parameters of alpha-helix, beta sheet, loops/coils
and tertiary structures

structure determination, reagents:
HSCH₂CH₂OH
6M HCl

Edman’s reagent
cyanogen bromide BrCN

exopeptidases: carboxypeptidase A
carboxypeptidase B
endopeptidases:
trypsin
chymotripsin
elastase
PROBLEM 48
Determine the primary structure of an octapeptide from the following data:

- Acid-catalyzed hydrolysis gives 2 Arg, Leu, Lys, Met, Phe, Ser, and Tyr
- Carboxypeptidase A releases Ser
- Edman’s reagent releases Leu
- BrCN forms two peptides with the following composition:
  1. Arg, Phe, Ser
  2. Arg, Leu, Lys, Met, Tyr

- Trypsin-catalyzed hydrolysis forms the following two amino acids and two peptides:
  1. Arg
  2. Ser
  3. Arg, Met, Phe
  4. Leu, Lys, Tyr