Chem 109 C
Bioorganic Compounds

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HFH1104

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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: $\text{HSCH}_2\text{CH}_2\text{OH}$
Proteins: Structure

first steps - overall composition:

cleaving disulfide bridges: HSCH$_2$CH$_2$OH

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

protein $\xrightarrow{6 \text{ M HCl}}$ amino acids

2-mercaptoethanol

2-mercaptoethanol

iodoacetic acid

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Proteins: Structure

**first steps - overall composition:**

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

2-mercaptoethanol

**complete hydrolysis:** 6 M HCl, H\(_2\)O, 100 °C, 24 h

protein \( \xrightarrow{6 \text{ M HCl}} \) 100 °C \( \xrightarrow{24 \text{ h}} \) amino acids

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

for *N*-terminal amino acid

Edman’s reagent:

\[
\begin{array}{c}
\text{phenyl isothiocyanate} \\
N=C=S \\
\end{array}
\quad \rightarrow \quad \begin{array}{c}
\text{PTH-amino acid} \\
\end{array}
\]

from *N*-terminal amino acid

can be repeated up to 50 times in sequencator
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?

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?

1. Ala, Trp  
2. Val, Pro, Asp  
3. Pro, Val  
4. Ala, Glu  
5. Trp, Ala, Arg  
6. Arg, Gly  
7. Glu, Ala, Leu  
8. Met, Pro, Leu, Glu
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

![Amino acid sequence with cleavage by trypsin](image)
selective reagents to break amide bonds:
partial hydrolysis with endopeptidases:

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

```
Ala-Lys-Phe-Gly-Asp-Trp-Ser-Arg-Met-Val-Arg-Tyr-Leu-His
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**Chymotrypsin:** C-side of Phe, Tyr and Trp

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

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

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

**Elastase**: C-side of Gly and Ala

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

selective reagents to break amide bonds:

partial hydrolysis with endopeptidases:

Trypsin: C-side of Arg and Lys
selective reagents to break amide bonds:

for endo- and exopeptidases,
no reaction at Pro:

- Ala-Lys-Pro: trypsin will not cleave
- Leu-Phe-Pro: chymotrypsin will not cleave
- Pro-Phe-Val: chymotrypsin will cleave
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)
## Proteins: Structure

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

**Quaternary structure** aggregates of proteins: each is called a subunit

*mutation at position 6: glutamate → valine: sickle cell anemia*
Proteins: Quaternary Structure

**Quaternary structure** aggregates of proteins: each is called a subunit

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