Review
Protein Synthesis / General
** Important Reacs **
** Structure / 1°, 2°, 3°, 4° **
1° / content
Hydrolysis / chromatography (Ninhydrin)
1° / sequence

** Sequence problem: find the order the acid is in **
(file the C & N terminal)

** Edman Degradation **

\[ \text{Ph} - \text{N} = \text{C} = \text{S} \xrightarrow{\text{N}, \text{H} - \text{AA} - \text{AA} - \text{AA} - \text{CO}_2 \text{H} + \text{enoughtime} \rightarrow \text{Phenylisocyanate reacts with N terminal a.a.}} \]

[ i ]

\[ \xrightarrow{\text{H}^+} \]

[ i ]

\[ \xrightarrow{\text{H}_3\text{O}^+} \]

[ i ]

\[ \xrightarrow{\text{H}_2\text{N} - \text{AA} - \text{AA} - \text{CO}_2 \text{H}} \]

A * phenylthiohydantoin * (automated up to 50 before stop) + clean up
Sequence c-terminal end

\[ \text{H}_2\text{N}-(\text{AA}_1-\text{AA}_2)\underbrace{\text{AA}_2-\text{CO}_2\text{H}} \]

\[ \text{NH}_2-(\text{AA}_1-\text{AA}_2-\text{CO}_2\text{H} + \text{AA}_3) \]

"Carboxypeptidase A" (determining the C-terminal end) removes fragments of peptide bonds at the end of a peptide chain but not arginine or lysine.

Partial hydrolysis:


Enzymes cleave this mixture of spots not the same spot in every molecule but hydrolyzes some of the peptide bonds.

\[ \text{H}_2\text{N}-\text{AA}-\text{AA}-\text{CO}_2\text{H} \quad \text{H}_2\text{N}-\text{AA}-\text{AA}-\text{AA}-\text{AA}-\text{AA}-\text{CO}_2\text{H} \quad \text{H}_2\text{N}-\text{AA}-\text{AA}-\text{CO}_2\text{H} \]

Analyze each in detail:

The sequence of the original molecule protein can be determined by lining up the peptides and looking for points of overlap.
2° protein structure

C-N partial double bonds

α-helix

β-pleated sheets

PARTIAL C-N DOUBLE BONDS

α-helix

R̂₂

R̂₁

H₂N-C-C=C-N-CH₂-C=CH₂...Rₜ

P-sheet

α-helix

ÅAA₁-ÅAA₂-ÅAA₃-ÅAA₄-ÅA₅
B-pleated sheets (antiparallel)

Also parallel B-pleated sheets
see in both

50% of local structure is α-helix & β-pleated sheet

Other forms: loop conformation
Coil (random coil)
Edman's reagent determines the N-terminal amino acid of a peptide or protein.

Edman's reagent:

\[
\text{Ph-N=C=S} \quad \text{Ph-N=C=S}
\]

Phenyl isothiocyanate

\[
\text{Ph-N=C=S} + \text{HNC} + \text{NHCH}_{2} \text{C} = \text{NHCH}_{2} \text{C} = \text{NHCH}_{2} \text{C} \\
\text{Edman's reagent}
\]

\[
\begin{align*}
\text{Ph-N-C} & \text{H} \\
\text{S} & \text{R}_{1} \\
\text{R}_{2} & \text{R}_{3}
\end{align*}
\]

\[
\text{Ph-N-C} \quad \text{NHCH}_{2} \text{C} = \text{NHCH}_{2} \text{C} = \text{NHCH}_{2} \text{C} \\
\text{Edman's reagent}
\]

\[
\begin{align*}
\text{Ph-N-C} & \text{H} \\
\text{H} & \text{C} \\
\text{S} & \text{R}_{1} \\
\text{R}_{2} & \text{R}_{3}
\end{align*}
\]

Cleaved peptide

\[
\text{HC} \quad \text{HN} \\
\text{N} \quad \text{C} \\
\text{S} \quad \text{HN}
\]

Thiazoline derivative

(5 cleaved with mild acid)

Peptide without original N-terminal AA

Reagents:

IN the presence of acetic acid

\[
\text{NH}_{2} \text{C} \text{H}_{2} \text{C} = \text{NHCH}_{2} \text{C} = \text{NHCH}_{2} \text{C} \\
\text{R}_{2} \\
\text{R}_{3}
\]

PTh – aca