

### Protein Sequencing Exercise

You are in a South American rain forest looking for naturally occurring peptides with potential as drugs. You have a mobile biochemistry lab with common reagents and enzymes, an amino-acid analyzer, gel-filtration and ion-exchange chromatography, and electrophoresis. You also have an Edman Sequenator, but one or more of your reagents has become contaminated, and as a result, you cannot sequence peptides longer than about 12 residues before contaminants obscure the results. While screening extracts from the ovaries of a tropical orchid, you find a peptide with potential as an antiviral. Deduce its amino-acid sequence using the available tools.

- 1) MW by electrophoresis can tell you how big a sequencing problem you are up against.

**Result: about 4000**

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- 2) Amino-acid analysis can help you decide how to fragment the peptide for sequencing:

**Result: A<sub>2</sub>C<sub>2</sub>D<sub>2</sub>E<sub>4</sub>FG<sub>3</sub>HKLMN<sub>2</sub>P<sub>2</sub>Q<sub>2</sub>R<sub>4</sub>S<sub>4</sub>T<sub>3</sub>W**

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- 3) How many peptides expected from each of these possible cleavage reagents?

- Cyanogen bromide (C-side of M). \_\_\_\_\_
  - *Staph. aureus* V8 protease (C-side of D and E). \_\_\_\_\_
  - Trypsin (C-side of K and R). \_\_\_\_\_
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- 4) Cleavage by trypsin followed by gel-filtration chromatography gives the expected 6 products, which you sequence (shown in order of emergence from column):

T-1 ETMESSAGEFGR

T-2 SQTWALDHSECR

T-3 GPQDNK

T-4 TCR

T-5 NP

T-6 R

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- 5) Cleavage by *Staph. aureus* V8 protease followed by gel-filtration chromatography gives the expected 7 products, which you sequence (shown in order of emergence from column):

S-1 RSQTWALD

S-2 FGRGPQD

S-3 NKTCRNP

S-4 SSAGE

S-5 TME

S-6 CRE

S-7 HSE

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**Deduce the primary structure of this polypeptide.**

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**Additional Questions to Think About:**

- Why would cyanogen bromide not be a good choice as a cleavage reagent?
- Can you account for the order of elution of peptides from the two chromatographies?
- Predict the order of elution of the tryptic peptides from a cation-exchange column eluted with pH-8.5 buffer and a salt gradient.
- Predict the order of elution of the V8 protease peptides from an anion exchange chromatography column eluted with a pH-6.5 buffer and a salt gradient.
- For both sets of peptides, predict the order of elution from a hydrophobic interaction chromatography column.
- When the first proteins were sequenced, it was somewhat disappointing that the sequences exhibited no simple patterns that provided clues to what other sequences might be like. Unlike the example on this page, which contains a hidden message (did you notice it?), real protein sequences contain no easily understandable messages that might help us in predicting the sequence of other proteins.