Table for use with problems:

| ENZYME | SITE OF CLEAVAGE | AMINO ACID RESIDUE |
|---|---|---|
| Trypsin | Carboxy side of basic residues | Arg, Lys |
| Chymotrypsin | Carboxy side of hydrophobic aromatic residues | Phe, Trp, Tyr |
| Thermolysin | Amino side of hydrophobic non-aromatic residues | Ile, Leu, Val |

Problems discussed in the videos:

Video (25)
Provide the structure of an octapeptide, Pep8, that would lead to the following results after three different enzyme digestion reactions. You would use three separate samples of this octapeptide to obtain these results.

Reaction of Pep8 with Chymotrypsin gave 2 dipeptides and 1 tetrapeptide.

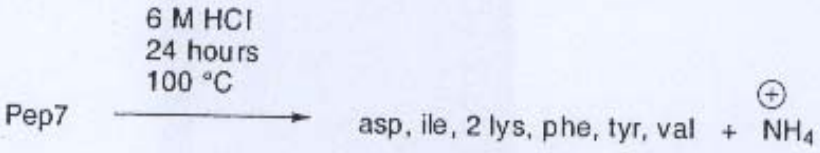
Reaction of Pep8 with Trypsin gave 2 tripeptides and 1 dipeptide.

Reaction of Pep8 with Thermolysin gave one heptapeptide and the amino acid isoleucine (Ile).

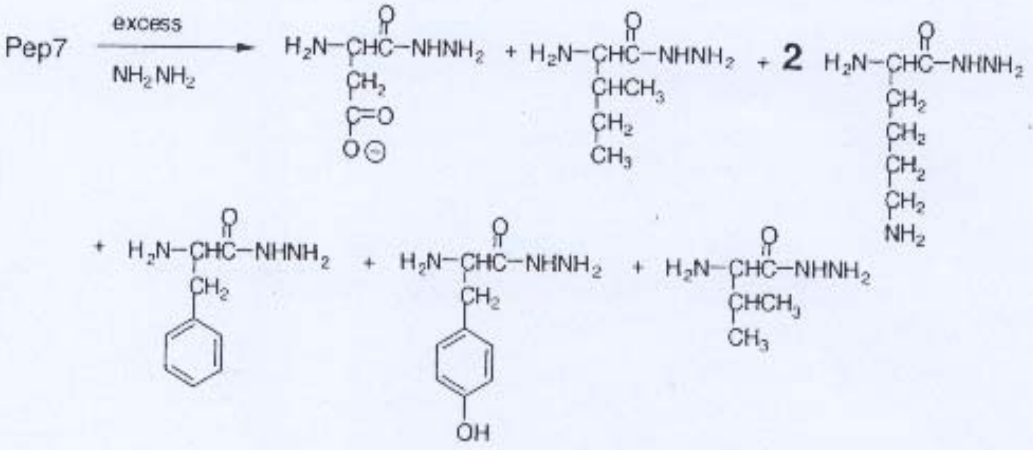
Videos (27)-(29)
Problem begins on next page.

6. From the information below deduce the primary sequence of the heptapeptide, Pep7. Credit will be provided for answers to each section! Information on the proteases is provided on page 2. (40 pts)

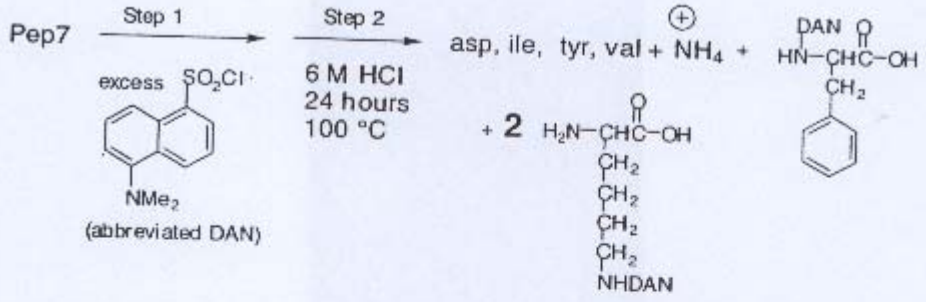
**A**. Total acid hydrolysis of Pep7 provided the results shown below. Based on these results, what is the possible source(s) of the ammonium ion?



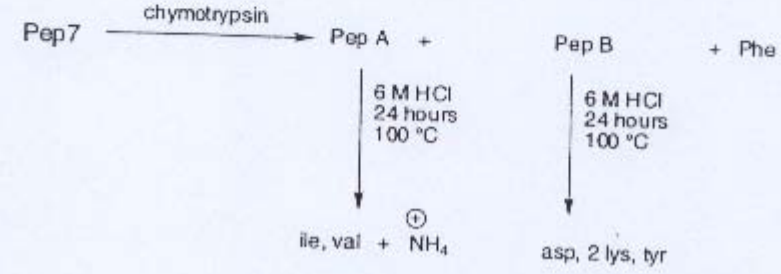

**B.** Reaction of Pep7with excess hydrazine gave the following results. What do you now know about Pep7 that you did not know from Part A? Be specific.



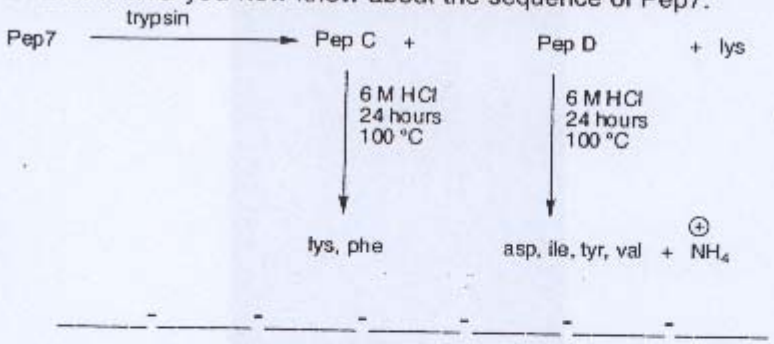
**C.** Reaction of Pep7 with excess Dansyl chloride followed by total acid hydrolysis gave the results shown. On the template, indicate everything you know about the sequence of Pep7 at this point.



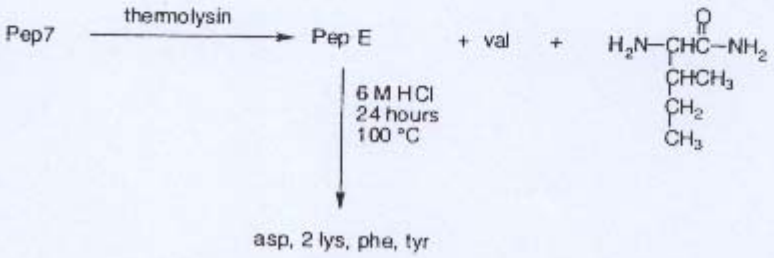

**D.** Complete enzymatic digestion of Pep7 with chymotrypsin (see page 2) gave two peptides and one free amino acid. Each peptide was subjected to total acid hydrolysis and the results are shown below. On the template, indicate EVERYTHING you now know about the sequence of Pep7.



**E.** Complete enzymatic digestion of Pep7 with trypsin (see page 2) gave two peptides and one free amino acid. Each peptide was subjected to total acid hydrolysis and the results are shown below. On the template, indicate EVERYTHING you now know about the sequence of Pep7.




**F.** Complete enzymatic digestion of Pep7 with thermolysin (see page 2) gave one peptide, one free amino acid and one derivatized amino acid. The peptide (PepE) was subjected to total acid hydrolysis and the results are shown below. On the template, indicate EVERYTHING you now know about the sequence of Pep7.



Video (29)
A hexapeptide, $Peptide_6$, of unknown sequence was isolated from a starfish.
Total acid hydrolysis of $Peptide_6$ led to five amino acids and one equivalent of ammonium ion. Clearly one amino acid is missing after total acid hydrolysis. Which one is it?