Amino acids, Peptides and Proteins: Exam Question 06534/06594, 2007

1. Answer **all** of the following parts.

(a) Describe briefly the technique of SDS-PAGE, explaining why it is particularly useful for the analysis of peptides and proteins.

(*6 marks*)

(b) The following peptides are subjected to normal electrophoretic analysis at pH 6.0. State whether the peptides will migrate towards the cathode or anode and predict the relative rate of migration of each peptide.

(i) Gly.Arg
(ii) Phe.Gly.Arg
(iii) Phe.Glu.Glu
(iv) Phe.Gly.Glu

Arg is $HO_2CCH(NH_2)CH_2CH_2CH_2CH_2NHC(=NH)NH_2$, and
Glu is $HO_2CCH(NH_2)CH_2CH_2CO_2H$, and
Phe is $HO_2CCH(NH_2)CH_2Ph$, and
Gly is $HO_2CCH_2NH_2$.

(*6 marks*)

(c) The peptide neurotoxin alpha-pompilidotoxin **A** was purified from the venom of the solitary wasp *Anoplius samariensis*. Explaining fully your workings, determine the sequence of **A** from the following experimental results.

(i) Total acid hydrolysis of **A** gives:
A = (Arg, Asp, Glu, Gly, 2×Ile, 3×Leu, 2×Lys, Phe, Ser)

(ii) Treatment of **A** with Edman's reagent gives the thiohydantoin of Arg.

(iii) Treatment of **A** with chymotrypsin gives peptides **B** and **C** with composition:
B = (Arg, Gly, 2×Ile, Leu, Lys, Phe)
C = (Asp, Glu, 2×Leu, Lys, Ser)

(iv) Treatment of **C** with Edman's reagent gives the thiohydantoin of Glu.

(v) Treatment of **A** with trypsin gives peptides **D** and **E** with composition:
D = (Asp, Glu, Gly, Ile, 2×Leu, Lys, Phe, Ser)
E = (Ile, Lys)
plus free Arg and free Leu

(vi) Partial acid hydrolysis of **A** releases the following dipeptides:
F = Leu.Phe
G = Glu.Asp
H = Ser.Lys
I = Ile.Gly
J = Arg. Ile

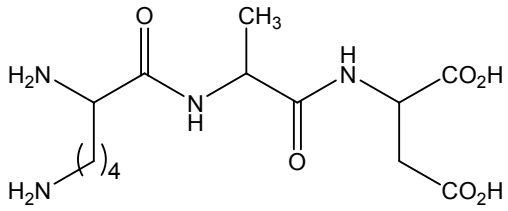
(*NB:* peptides in brackets with amino acids separated by commas indicate composition and not sequence)

2. Answer **all** of the following parts.

(d) Explain briefly the advantages of carbamates, such as *tert*-butyloxycarbonyl (BOC) and benzyloxycarbonyl (Z), over other functional groups for amine protection during peptide synthesis.

(*4 marks*)

(e) Devise a synthesis of the peptide Lys.Ala.Asp (given below) starting from its constituent unprotected amino acids. Give reagents for your synthesis (mechanisms are not required) and explain your strategy.



(*9 marks*)

(f) Explaining fully your workings, determine the sequence of the polypeptide **A** from the following experimental results.

(i) Total acid hydrolysis of **A** gives:
A = (Ala, Arg, Gly, 2×Lys, Met, Phe, Pro, 2×Ser, Tyr, Val)

(ii) Treatment of **A** with Edman's reagent gives the thiohydantoin of Val.

(iii) Treatment of **A** with carboxypeptidase first releases Ala.

(iv) Treatment of **A** with cyanogen bromide gives two peptides **B** and **C** with composition:
B = (Ala, 2×Lys, Phe, Pro, Ser, Tyr)
C = (Arg, Gly, Met, Ser, Val)

(v) Treatment of **A** with chymotrypsin gives three peptides **D**, **E** and **F** with composition
D = (2×Lys, Phe, Pro)
E = (Arg, Gly, Met, Ser, Tyr, Val)
F = (Ala, Ser)

(vi) Treatment of **A** with trypsin gives three peptides **G**, **H** and **I** with composition:
G = (Gly, Lys, Met, Tyr)
H = (Ala, Lys, Phe, Pro, Ser)
I = (Arg, Ser, Val)

(*12 marks*)

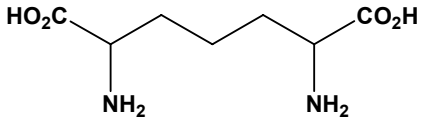
Amino acids, Peptides and Proteins: Exam Question 06534/06594, 2005

1. Answer **all** of the following parts.

(a) Define the term *isoelectric point*.

(*1 mark*)

(b) Draw the structure of diaminopimelic acid when in solution at i) pH 1, and ii) pH 12. Sketch the acid-base titration curve for this amino acid and mark on this curve where you would expect to find the isoelectric point.



diaminopimelic acid

(*7 marks*)

(c) Describe briefly the technique of isoelectric focusing electrophoresis, explaining why it is particularly useful for the separation of peptides and proteins.

(*7 marks*)

(d) Mycobacillin, a fungal metabolite, is a cyclic peptide. Total acid hydrolysis gave the following amino acid analysis: (Ala_1, Asp_5, Glu_2, Leu_1, Pro_1, Ser_1, Tyr_2). Partial acid hydrolysis gave 15 fragments **A** to **O** which were isolated and their *N* termini determined by the end group analysis. The remaining residues were determined by amino acid analysis, but without sequences being determined for the tri- and tetrapeptides. Explaining your reasoning, work out the structure of mycobacillin.

A Asp.Glu
B Ser.Asp
C Glu.Asp
D Asp.Ala
E Asp.Tyr
F Ala.Asp
G Glu.Tyr
H Glu.(Ala, Asp)
I Asp.(Glu, Tyr)
J Leu.(Asp, Glu)
K Asp.(Ser, Tyr)
L Ser.(Asp, Leu)
M Ala(Asp, Pro)
N Leu.(Ala, Asp, Glu)
O Ser.(Asp, Glu, Leu)

(*8 marks*)

(e) Considering the answer you have given for (c), how might you modify the structure you propose for mycobacillin given that this cyclic peptide was ring-opened and partially degraded by the action of carboxypeptidase?

(*2 marks*)