7.013 Solutions to Enzymes/Protein Structure

a) i) leu-thr-phe-ala-ser (unchanged)   ii) trp-tyr-lys + ala-phe   iii) 2 lys + 2 arg

GENERIC ENZYME:

ACTIVE SITE -
peptide bond cleavage
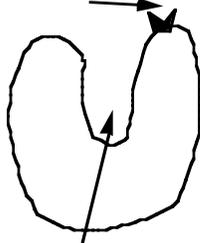
RECOGNITION POCKET -
specific substrate regognition

Schematics of enzymes: (side chains shown in black)
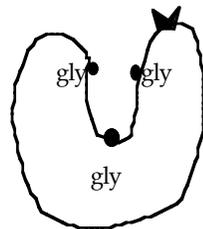
protease A
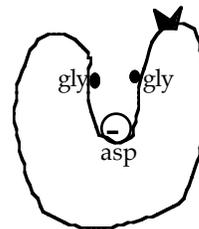
protease B
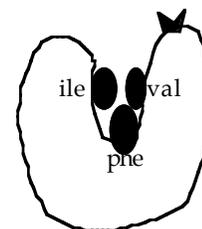
protease C

Schematics of substrates:

lysine, arginine

peptide backbone

(+)

long & + charged
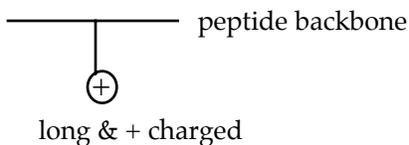
phenylalanine,
tryptophan, tyrosine

large & hydrophobic
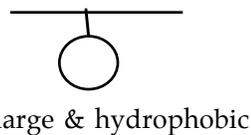
glycine, alanine
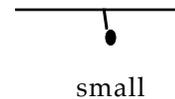
small

b) Matching them up:

protease A - large open pocket. Could be Lys/Arg or Phe/Trp/Tyr.

protease B - large open pocket with (-) charge at bottom. Therefore, Lys/Arg, which means that protease A must cut after Phe/Trp/Tyr

protease C - small pocket. Gly, Ala.

c) Change the asp in the bottom of the pocket in protease B to a lys or arg.

d) It might still bind lys or arg, but if the space in the pocket were constrained, there might not be enough room because glu is longer than asp.

e) The others are required to hold the essential ones in place. The also provide the correct folding to bring these amino acids in close proximity and orientation.

GLY	ALA	VAL	LEU	ILE	SER	THR	ASP	ASN	GLU	GLN	LYS	ARG	HIS	TRP	PHE	TYR	PRO	CYS	MET
G	A	V	L	I	S	T	D	N	E	Q	K	R	H	W	F	Y	P	C	M

Figure by MIT OCW.

AA sidechains. 7.013 CG 2004. 1
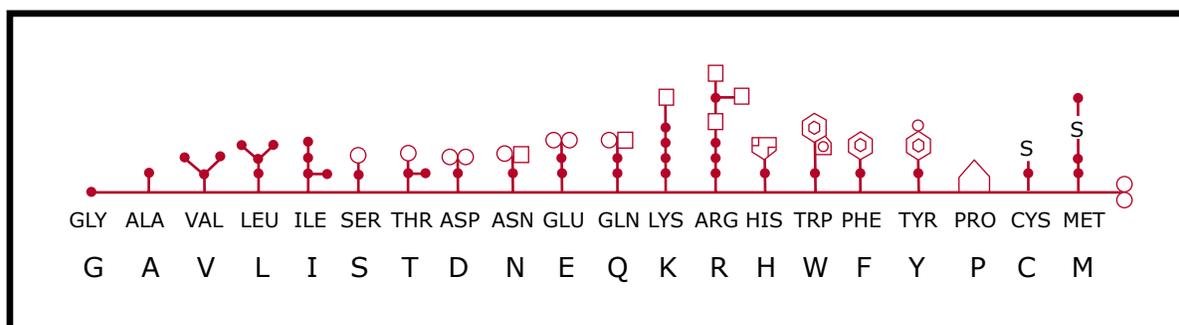
f) Protease A is a protein, therefore other protease A molecules can cleave it and thereby inactivate it. Having casein around decreases the chance that a protease A molecule will cleave a protease A molecule, because it will be more likely to cleave the more numerous casein molecules.

g) The protease enzyme must be able to bind to the target amino acids. If they are buried inside the target protein, the protease can't "see" them and therefore can't cut at them. Eventually, the structure of the target protein gets so broken down that the inside amino acids are exposed to the protease.

h) To protect protease A from degradation by other protease A molecules, bury all the phe/trp/tyr inside the protein so that they cannot be recognized and cleaved.

i) The graph should show an exponential rise – the more enzyme, the faster enzyme will be produced. Eventually, the enzyme will be degraded as in part (e). This is sketched below.