

Biochemistry Midterm

Take Home portion

You are free to use your textbook for help, however you may not ask any other student or instructor except Dr. Z for help. You are also allowed to use a computer, and, if you like, you can submit your answer electronically by E-mailing your answer to me at michaelzehfus @ bhsu.edu

Problem 1

I have just purified a new protein. When I treat the protein with mercaptoethanol and cyanogen bromide I get the following peptides:

WG

TGQAPGYCYTAANKNKGIM

IAYLKKATNEGDVEKGKKIFIM

KCSQCHTVEKGGKHKGTGPNLHGLFGRKM

When I forget to treat the peptide with mercaptoethanol first I get 3 peptides instead of 4 peptides.

When I treat the peptide with mercaptoethanol and then with trypsin I get the following peptides:

K (3 times)

GK

HK

NK

GGK

IAYLK

IFIMK

GIMWG

ATNEGDVEK

CSQCHTVEK

TGPNLHGLFGR

MTGQAPGYCYTAANK

What is the sequence of the protein? Are there any disulfide bonds in this protein, if so, where are they located

Peptide

IAYLKKATNEGDVEKGKKIFIMKCSQCHTVEKGGKHKGTGPNLHGLFGRKMTGQAPGYCYTAANKNKGIMWG

1 disulfide between residue 58 and either 24 or 27. The disulfide cannot be between 24 and 27 because they are on the same CNBr fragment and that would not have made the change between 3 and 4 fragments.

Problem 2

I have conducted a series of kinetic experiments on this protein when the protein concentration was $5 \mu\text{M}$. The results of these initial rate experiments are as follows:

S (mM)	V_o (mMoles/minute)
1	6.15
2	10.1
5	16.6
10	21.1
50	26.9

What are the K_M , V_{MAX} , K_{cat} and specificity constant for this enzyme. (Note: you will get more accurate results and more points if you do a linear transformation of this data and determine your parameters using a line of best fit.)

I wanted a Lineweaver-Burke plot. From this plot you would find that:

$$V_{max} = 28.9 \text{ mmol/min}$$

$$K_m = 3.7 \text{ mM}$$

$$K_{cat} = V_{max}/5\mu\text{M} = 28.9 \times 10^{-3} \text{ M/min} / 5 \times 10^{-6} \text{ M} = 5.78 \times 10^3 \text{ min}^{-1} \text{ (or } 96 \text{ s}^{-1}\text{)}$$

$$\text{Specificity} = K_{cat}/K_M = 5.78 \times 10^3 \text{ min}^{-1} / 3.7 \text{ mM} = 1.56 \times 10^3 \text{ mM}^{-1} \text{ s}^{-1}$$