CHEM 191
Module 4
Structures and reactions of biological molecules

Lecture 8
Peptides – hydrolysis, including enzymes

Lecturers: Dr Andrea Vernall andrea.vernall@otago.ac.nz
Dr Eng Wui Tan ewtan@chemistry.otago.ac.nz

1

Module 4 Lecture 8
Learning objectives

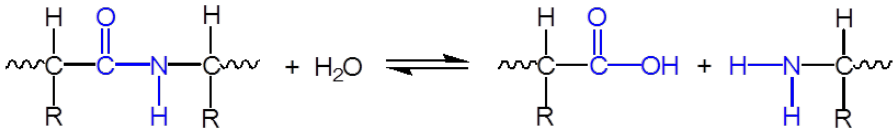
Learning Objectives:

- Recognise that peptides are quite stable to 'chemical' conditions but are easily hydrolysed by enzymes.
- Understand the mechanism of amide bond hydrolysis for zinc ion proteases such as carboxypeptidase A.
- Rationalise the binding of a substrate (peptide or drug) into an enzyme active site of a zinc ion protease using hydrogen bonding, ionic bonding and metal coordination concepts.

2

Chemical peptide hydrolysis

- Proceeds with difficulty because
  - H_2O is a poor nucleophile
  - the C=O of an amide is unreactive towards nucleophiles (L5 recap)



- Can be achieved by heating with strong aqueous acid eg 6 M HCl, but even then, the reaction proceeds slowly

3

Peptide hydrolysis in the body

- Protein is a necessary component of our diet. Our metabolism must efficiently hydrolyse the peptide bonds to liberate the amino acids (and in particular, the essential amino acids, i.e. the ones we don't make).

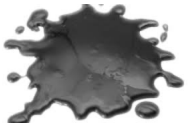
- This is achieved by
  - the low pH of our stomach (chemical hydrolysis of peptides is possible)
  - Mostly by **enzymes** called **proteases**, that effect hydrolysis of the peptide bond.

4

Enzymes

- There are many types of large proteins that are enzymes, that catalyse the cleavage of different bonds in different compounds
- Washing powders can contain different types of enzymes to break down the different compounds in stains…..

- **Lipases** cleave **triglycerides** into glycerol and fatty acids



- **Proteases** cleave **amide bonds**

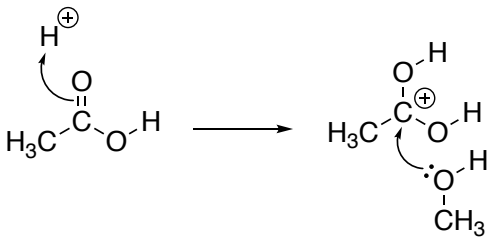
- **Amylases** break down starches

- Washing powders usually only contain one type of enzyme, though some have two or all three.
- Some people have an immune response/a reaction to enzymes in washing powder

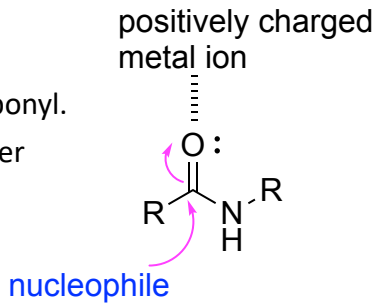
5

Proteases – hydrolysis mechanism

- Many proteases hydrolyse peptides in a manner analogous to the hydrolysis of esters (Lecture 5) -



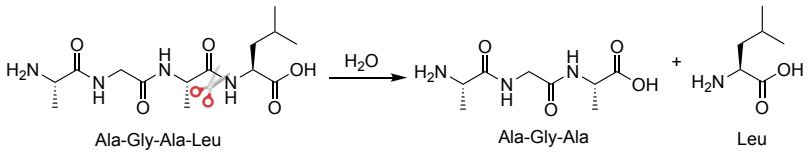
- For proteases hydrolysing peptide bonds
  - instead of the acid (H^+) a metal ion often polarizes the carbonyl.
  - the **nucleophile** is often an amino acid side chain, or a water that has reacted with an amino acid side chain.

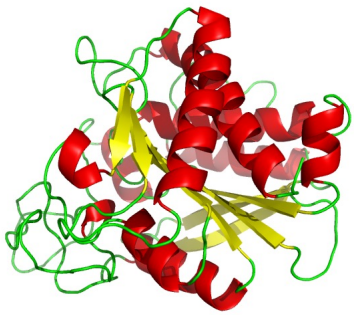


6

Carboxypeptidase A

- Different proteases are very specific in the peptide sequence and site they cleave.
- Carboxypeptidase A recognises the carboxylate of a C-terminal amino acid next to an amino acid with a non-polar side chain and efficiently hydrolyses the adjacent peptide bond.
- Thus, it effectively clips off the C-terminal amino acid





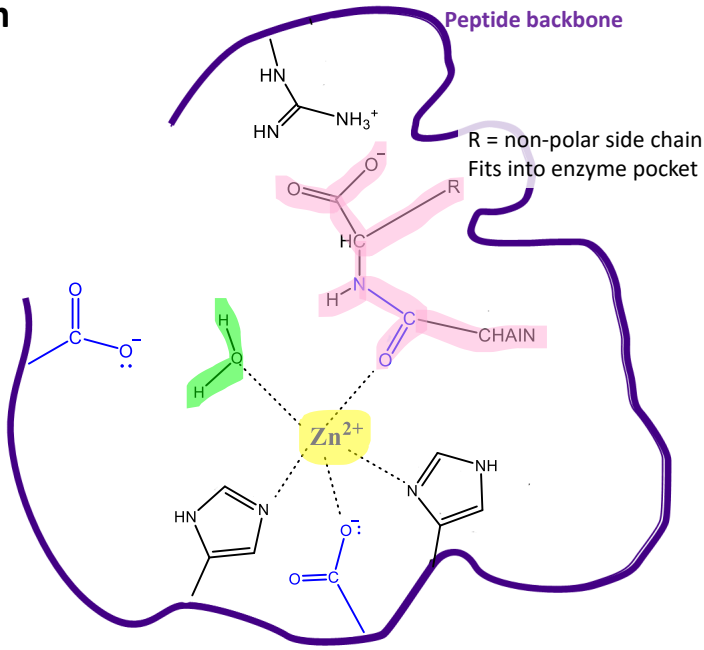
- It is an example of a metalloenzyme, with Zn^{2+} playing an integral role at the active site.

7

Carboxypeptidase A, mechanism

Key factors:

1. The reactants are held close together, the peptide to be cleaved, the soon to be nucleophile and the zinc ion are all in close proximity
2. Zn^{2+} makes the carbon of the C=O more susceptible to nucleophilic attack

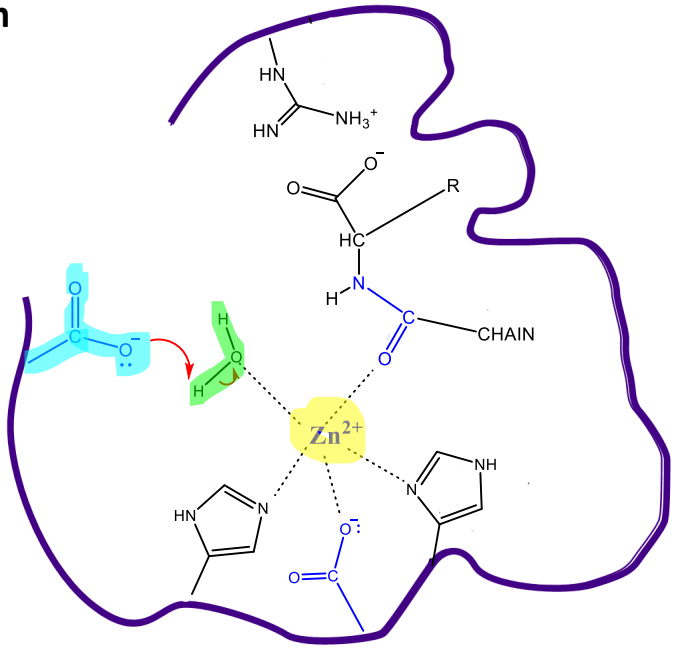


8

Carboxypeptidase A, mechanism

A glutamic acid side chain (i.e. a carboxylate) reacts with the water that is co-ordinated to the zinc ion

This is not something 'chemically possible' in a solution but is possible in an enzyme active site because of the co-ordination of the water to the zinc ion. The zinc ion lowers the pK_a of the water (refer back to module 1, lecture 6)



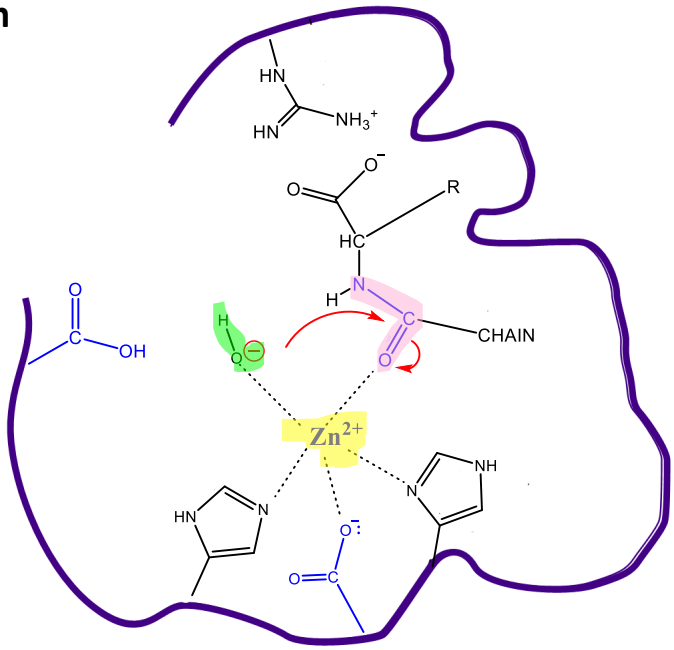
9

Carboxypeptidase A, mechanism

The hydroxide nucleophile can now attack the carbonyl carbon of the amide bond to be cleaved

This is possible because of the zinc ion polarising the carbonyl bond (drawing electron density away)

Like with chemical hydrolysis, this is the SLOW step, the attack of the nucleophile



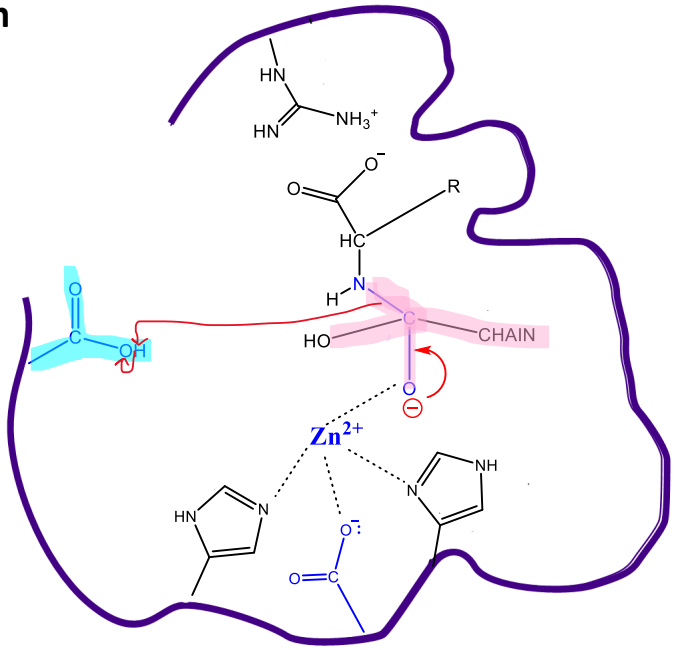
10

Carboxypeptidase A, mechanism

This forms a tetrahedral intermediate, analogous to the chemical hydrolysis reactions we learnt about previously.

The carbonyl is then reformed and the C-N breaks, the 'extra' NH_2 is 'picked up' from the same glutamic acid, this regenerating the carboxylate

(catalytic! The enzyme 'active site' is regenerated and ready to go again)



11

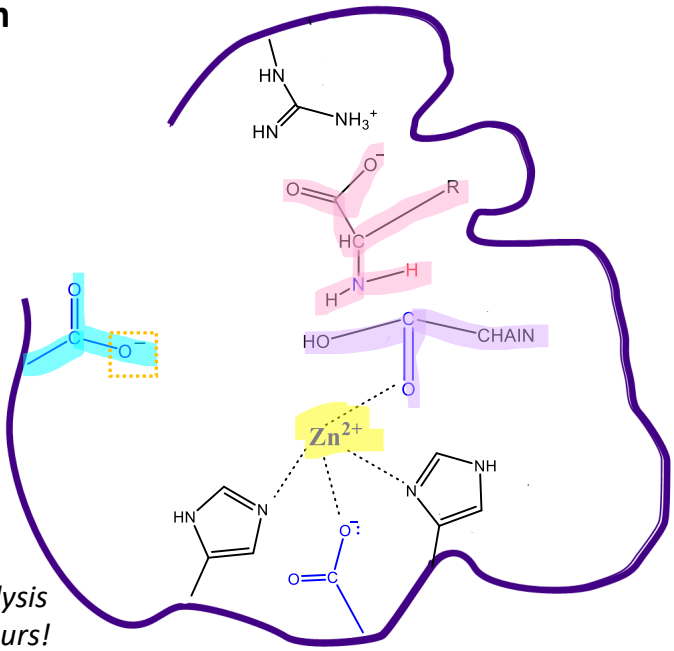
Carboxypeptidase A, mechanism

The C-terminal amino acid is now cleaved from the rest of the peptide

Both diffuse out of the enzyme active site

The zinc ion, the glutamic acid carboxylate, and another water are ready to do the reaction again on another peptide

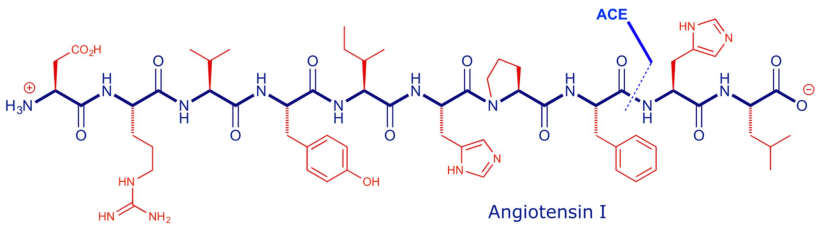
The enzyme takes minutes to effect the hydrolysis, whereas acid (or base,) hydrolysis in a flask/as a chemical reaction takes hours!

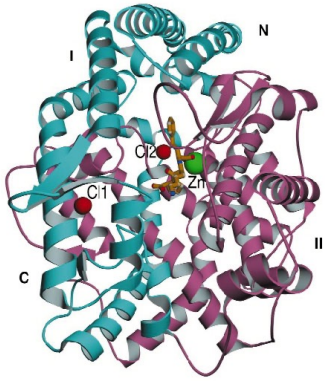


12

ACE – another zinc ion-based protease

- Angiotensin converting enzyme (ACE) is part of the renin-angiotensin system
- ACE breaks down angiotensin I (a **decapeptide**) into angiotensin II (**octapeptide**) by hydrolysing the second amide bond in from the C-terminus





13

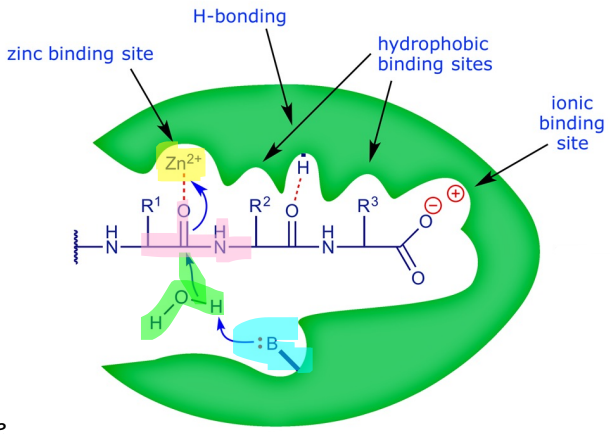
ACE – mechanism of cleavage

- ACE catalysed peptide hydrolysis is a very similar mechanism to carboxypeptidase A.

An amino acid side chain reacts with the water that acts as a nucleophile and attacks the carbon of the C=O amide

Attack of the amide C=O is possible because the zinc ion is coordinated to the C=O oxygen

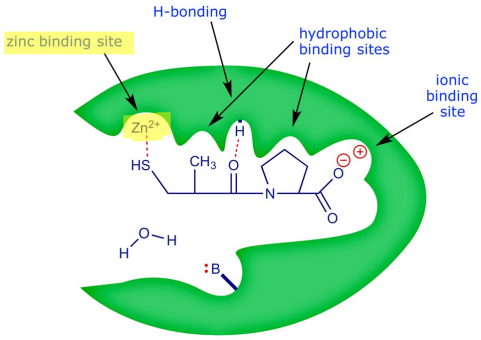
… the next steps are also the same as before



14

Drugs that are ACE inhibitors

- We can rationally design enzyme inhibitors by understanding how enzymes bond to and cleave their peptide substrates.
- Various ACE inhibitor drugs have been designed, with the key feature of having a functional group that **bonds well to the important zinc ion** inside the enzyme active site.
    - This ACE inhibitor binds tightly in the active site of ACE (bonding of the **sulfur** to **zinc ion**, the drug also mimics natural hydrogen bonding and ionic bonding) but does not undergo a reaction.
    - The drug stays there and the natural peptide substrate can not bind to the enzyme thus can not be hydrolysed – enzyme inhibitor



ACE inhibitors are primarily prescribed to treat high blood pressure.

15

* Homework *

No homework

16