BCH 4054 Chapter 26 Lecture Notes

Slide 1

Chapter 26

Nitrogen and Amino Acid Metabolism

Slide 2

Outline

- No time to cover entire chapter, therefore concentrate on a few **focal points**
  - Assimilation of inorganic nitrogen
  - Transamination (aminotransferases)
  - Nitrogen removal from amino acids
  - Urea synthesis
  - A few metabolic defects in catabolism

Slide 3

Assimilation of Inorganic Nitrogen

- Primary sources are NO_3^-, NO_2^-, N_2 and NH_3
- Most reduction of first three species occurs in microorganisms and plants
- N_2 to NH_3 is called **nitrogen fixation**
  - It occurs in bacteria, some in symbiotic relation with plants
- Nitrifying bacteria convert NH_3 to NO_3^-
- Denitrifying bacteria reduce NO_3^- to NH_3
  - See Fig 26.1

Slide 4

Nitrogen Fixation

- Enzyme is **nitrogenase**, catalyzing

$N_2 + 8\ H^+ + 8\ e^- \rightarrow 2\ NH_3 + H_2$

- Two proteins in complex
  - Nitrogenase reductase (Fe-protein)
  - Nitrogenase (an MoFe-protein)
  - See Fig 26.5 for metal cluster structure
- ATP required in the reaction
  - 16 ATP for each N_2 reduced
- Electrons come from ferredoxin originally

ATP requirement is explained as energy needed to overcome a high activation energy for breaking the N_2 triple bond. Other texts suggest the ATP lowers the reduction potential of the reductase complex. *Rhizobia* grow in symbiotic association with leguminous plants and fix nitrogen for them. Chemical fixation of nitrogen is by the **Haber process**, and is a major industrial chemical process used to produce fertilizer.

Slide 5

Regulation of Nitrogen Fixation

- ADP inhibits activity of nitrogenase
- NH_4^+ represses synthesis of many of the enzymes involved in nitrogen fixation
  - Known as the *nif* genes

Slide 6

Nitrate Assimilation

- Nitrate reductase
  - Two electron reduction of nitrate to nitrite
  - Involves a cytochrome and a molybdenum cofactor (**MoCo**) (Fig 26.2a)
- Nitrite reductase
  - Six electron reduction of nitrite to ammonia
  - **Siroheme** is prosthetic group (Fig 26.2b)
  - Ferredoxin produced in light reaction is electron donor
  - Plant enzyme is in chloroplasts

Nitrate assimilation accounts for 99% of the inorganic nitrogen assimilation into organisms.

Slide 7

Ammonia Assimilation

- Several reactions
  - Carbamoyl phosphate synthetase
    - For urea cycle
  - Glutamate dehydrogenase
    - Reversible, also a catabolic enzyme
  - Glutamine Synthetase
    - Primary assimilation mechanism
  - Glutamate Synthase
    - Equilibrium favors glutamate formation

Slide 8

Carbamoyl Phosphate Synthetase

- Two ATP'S required
  - One for activation of HCO_3^-
  - One for phosphorylation of carbamate

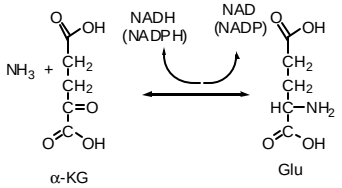
$$NH_4^+ + HCO_3^- + 2\ ATP \longrightarrow H_2NCOPO_3^{2-} + 2\ ADP + 2\ P_i$$

N-acetylglutamate activates the enzyme.

This enzyme is found in mitochondria and begins the urea cycle.

Slide 9

Glutamate Dehydrogenase



- Primarily catabolic enzyme
- Neurospora crassa has two enzymes
  - Mitochondrial NAD—catabolic
  - Cytosolic NADP—anabolic

Not clear to what extent this enzyme plays a role in nitrogen assimilation in addition to catabolic role of nitrogen release. Regulation is that of a catabolic enzyme: activation by ADP, inhibition by GTP.

Slide 10

Glutamine Synthetase

- ATP coupled to synthesis of amide bond
- Phosphate anhydride intermediate formed
  - See Fig 26.10
- Major pathway of ammonia assimilation
  - Ammonia is toxic, so GS provides a way to lower ammonia concentrations in tissues
- Glutamine is the **N** donor in synthesis of many Nitrogen containing compounds

Fig 26.14 shows the subunit organization of the bacterial glutamine synthetase.

Slide 11

Glutamine Synthetase, con't.

- Bacterial enzyme is highly regulated
  - Allosteric **feedback inhibition** by many nitrogen products (See Fig 26.15)
  - Covalent modification by **adenylation** at Tyr^{297} inhibits
    - (See Fig 26.16 and 26.17)
    - Adenylation inhibited by αKG, stimulated by Gln
    - Deadenylation inhibited by Gln, stimulated by αKG
  - Regulation also at gene transcription level
    - Fig 26.18

Slide 12

Glutamate Synthase

(Glutamate:oxo-glutarate aminotransferase)

- Catalyzes the reductive amination of αKG by the amide N of glutamine
  - See Fig 26.12
- Overall reaction fixes two nitrogens into glutamine at expense of 2 ATP
  - See Fig 26.13

Slide 13

Transamination (aka aminotransferase)

- Transfer of N from an amino acid to a keto acid
- Glutamate/ αKG is usually a partner
  - GOT aka glutamate aspartate aminotransferase
  - GPT aka glutamate alanine aminotransferase
  - See Fig 26.19
- Pyridoxal phosphate is an enzyme bound prosthetic group

GOT is glutamate-oxaloacetate transaminase; GPT is glutamate pyruvate transaminase.

Slide 14

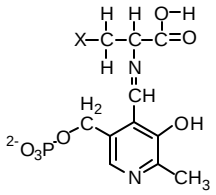
Transamination, con't.

- Pyridoxal phosphate is bound in Schiff base linkage to a lysine residue.
- The amino group of an amino acid replaces the nitrogen of lysine.
- Tautomerization followed by hydrolysis yields a keto acid and **pyridoxamine phosphate**
- Reversal of the process converts another keto acid to an amino acid
  - See Fig page 869

Slide 15

Pyridoxal Phosphate Amino Acid

- An intermediate in many reactions
  - Racemization, decarboxylation, dehydration, alpha-beta C-C bond cleavage (See Fig 18.26)

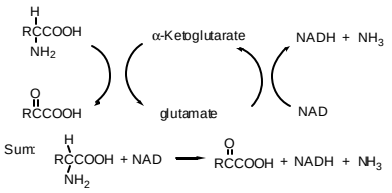


See also Figure page 892 for the serine dehydratase reaction

Slide 16

Nitrogen Removal from Amino Acids

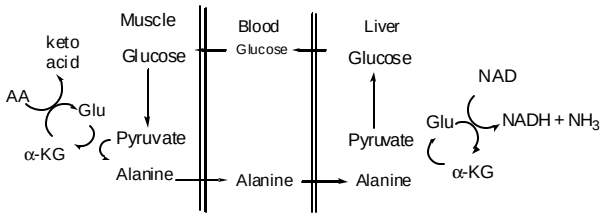
- Glutamate dehydrogenase
  - When coupled with transaminase, can represent removal of N from any AA to form NH_3



Slide 17

Glucose-Alanine Cycle

- General AA transferases and **GPT** involved in moving nitrogen from muscle to liver.



GPT is **glutamate pyruvate transaminase.** Note the similarity to the Cori cycle!

Slide 18

Other Roles for Transaminases

- Remember in the complete oxidation of glucose, cytoplasmic NADH has to be re-oxidized, and two mechanisms were suggested:
  - Glycerol-phosphate shuttle
  - Malate-aspartate shuttle
    - The latter involves Aspartate aminotransferase (GOT) See Figure 21.34, page 703

Slide 19

Other Mechanisms for Nitrogen Removal

- Amino Acid oxidase

AA + E-FMN $\rightarrow$ keto acid + NH_3 + E-$FMNH_2$

E-$FMNH_2$ + O_2 $\rightarrow$ E-FMN + H_2O_2

- L-AA oxidase activity very low
- D-AA oxidase activity high

Slide 20

Other Mechanisms for Nitrogen Removal, con't.

- Aspartate elimination reactions

Aspartate $\rightarrow$ fumarate + R-NH_2

- Serine and threonine deamination
  - See Fig 892 for serine dehydratase reaction
  - (Note that pyridoxal phosphate is a cofactor in this reaction as well)

Slide 21

Excretion of Nitrogen

Three classes of organisms:

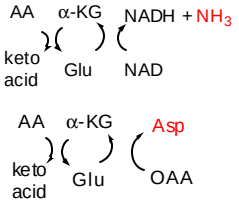
- Ammonotelic
  - Excretes ammonia
    - Microorganisms, aquatic animals
- Ureotelic
  - Excretes urea
    - Terrestrial vertebrates
- Uricotelic
  - Excretes uric acid
    - Birds, reptiles

When tadpoles go through metamorphosis to frogs, their nitrogen metabolism changes from ammonia excretion to urea excretion. The enzymes of the urea cycle are introduced.

Slide 22

Synthesis of Urea (Occurs in Liver)

- One nitrogen comes from ammonia
- The other comes from aspartate



Slide 23

Urea Cycle Enzymes

- Arginase (forms urea from arginine)
- Carbamoyl phosphate synthetase (activates NH_3)
- Ornithine transcarbamoylase (converts ornithine to citrulline)
- Argininosuccinate synthetase (attaches aspartate, requires ATP)
- Argininosuccinate lyase (releases fumarate, regenerates arginine)

Ornithine and citrulline are new amino acids

Slide 24

Urea Cycle, con't.

- Pathway is partitioned between
  - mitochondria (CS-I and OTC) and
  - the cytoplasm (AS synthetase, AS lyase, Arginase)
- See Fig 26.23

Slide 25

Urea Cycle, con't.

- For complete stoichiometry calculations, should show where nitrogens come from ultimately, and regeneration of the aspartate from fumarate.

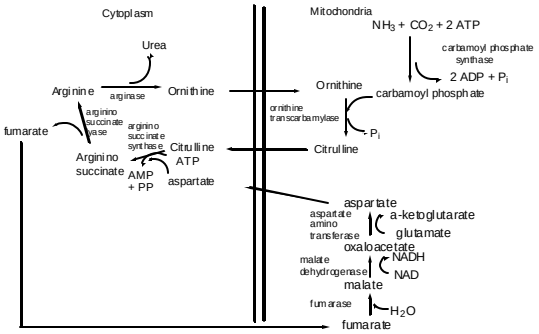
Slide 26

Urea Cycle, con't.

- NH_3 is produced in the mitochondria by two enzymes:
  - Glutamate dehydrogenase
  - Glutaminase (hydrolysis of glutamine)
- NH_3 delivered from other tissues either by
  - glucose-alanine cycle (discussed earlier) or
  - glutamine (sequestering ammonia from tissues, releasing in liver)
  - Free ammonia in blood is toxic

Slide 27

Urea Cycle, con't.



Slide 28

Amino Acid Biosynthesis

- First the keto acid is synthesized, then the amino acid added by a transaminase.
- Many are simple: glu, ala, asp for example
- We have lost the ability to make many of the AA's, and therefore require them in the diet. (See table on page 26.2)

We won't cover the specific biosynthetic pathways, many of which occur only in plants or microorganisms.

Slide 29

Essential Amino Acids

- Early nutritional experiments with rats to determine which amino acids are essential involved measuring **nitrogen balance**.
  - Excrete less nitrogen than consumed—positive nitrogen balance (in growth)
  - Excrete more nitrogen than consumed—negative nitrogen balance (starvation)
    - If an essential amino acid is omitted from diet, get negative nitrogen balance no matter how much is consumed.

Slide 30

Amino Acid Catabolism

- The keto acids are degraded by specific catabolic pathways.
- Amino acids TCA cycle intermediates are **glycogenic** or **glucogenic** (they can be converted to glucose)
- Amino acids leading to acetyl-CoA are **ketogenic**
  - See Fig 26.41

They can be classified experimentally as well. A rat is starved enough to deplete glycogen stores, then fed one of the amino acids. If the glycogen is restored, the amino acid is glycogenic. If instead ketone bodies are produced, the amino acid is ketogenic.

Slide 31

Metabolic Defects in Amino Acid Metabolism

- Defects in urea cycle enzymes lead to **hyperammonemia.** Treatment is to lower protein content in diet.
- Defects of Phe catabolism
  - Alkaptonuria (accumulation of homogentisate)- urine turns black on standing. (Fig 26.47)
  - Phenylketonuria (PKU) (accumulation of phenyl pyruvate (Fig 26.48) and other products)

Defective enzyme in alkaptonuria is **homogentisate dioxygenase**. Condition is relatively harmless. Defective enzyme in phenylketonuria is **phenylalanine hydroxylase**. Condition can lead to mental retardation. Should be identified early, and low Phe diet instituted.

Slide 32

Metabolic Defects in Amino Acid Metabolism, con't.

- Methyl malonate aciduria (MMA)
  - Defect in methyl malonyl CoA mutase
- Maple syrup disease
  - Defect in oxidation of alpha-keto acids from valine, leucine and isoleucine. (Fig 26.45 and 26.46)
  - Urine smells like maple syrup from accumulated keto acids.