II. Amino Acid Oxidation and the Production of Urea

1. INTRODUCTION

A. All organisms need a source of nitrogen.

B. Nitrogen-containing compounds : amino acids and their derivatives, nucleotides, nucleic acids and proteins.

C. Metabolic roles of amino acids : protein constituents, precursors for hormones, vitamins, coenzymes, porphyrins, pigments and neurotransmitters.

D. Amino acids (from proteins in the diet or from degradation of intracellular proteins) : oxidation makes a significant contribution to the generation of metabolic energy.

E. The fraction of metabolic energy : varies with the type of organisms and with the metabolic situation.

E.1 Carnivores : 90 %.

E.2 Herbivores : a small fraction.

E.3 Microorganisms : scavenge amino acids from their environment.

E.4 Photosynthetic plants : amino acids catabolism is concerned with the production of metabolites for other biosynthetic pathways.

F. In animals, amino acids undergo oxidative degradation in three different metabolic circumstances.

F.1 During the normal synthesis and degradation of cellular proteins, some amino acids released during protein breakdown undergo oxidative degradation.

F.2 When a diet is rich in protein and ingested amino acids exceed the body's needs for protein synthesis, the surplus is catabolized : amino acids can not be stored.

F.3 During starvation or in diabetes mellitus, when carbohydrates are either unavailable or not properly utilized, cellular proteins are used a fuel.

G. Amino acids lose amino groups, the α-keto acids so formed may undergo oxidation to CO_2 and H_2O, or provide three- or four- carbon units that can be converted to glucose, the fuel for brain, skeletal muscle and other tissues.

H. Amino acid degradative pathways are quite similar in most organisms.

I. Overview of the catabolism of amino acids in mammals.

2. METABOLIC FATES OF AMINO GROUPS

A. Nitrogen state

A.1 Virtually all biological important N-compounds contain nitrogen in a reduced form.

A.2 The principal inorganic forms of N in the environment are in an oxidized state.

B. Overview of catabolism of amino groups in vertebrate liver.

B.1 Most amino acids are metabolized in the liver.

B.2 Branched-chain amino acids are degraded in extrahepatic tissues.

C. Excretory forms of nitrogen

C.1 From comparative biochemical studies of different animal species (adapted to their lifestyles), amino nitrogen is excreted in one of 3 major forms : ammonia, urea and uric acid.

C.2 Ammonotelic animals (most aquatic vertebrates) : ammonia.

C.3 Ureotelic animals (many terrestrial vertebrates, sharks) : urea.

C.4 Uricotelic animals (birds, terrestrial reptiles, insects) : uric acid.

D. Glutamate and glutamine play especially critical roles in nitrogen metabolism. In most tissues, glutamate and glutamine or both are present in higher concentrations than other amino acids.

D.1 Glutamine as amino group transporter.

D.2 Glucose-alanine cycle : in muscle.

E. Dietary protein is enzymatically degraded to amino acids.

E.1 In humans, the degradation of ingested proteins into their constituent amino acids occurs in the gestrointestinal tract.

E.2 Gastric glands in stomach lining.

a. Gastric mucosa secretes hormone gastrin (17 amino acids, pyroglutamyl…..Phe-$CONH_2$) : stimulated by dietary proteins.

b. Parietal cells secrete HCl. The acidic gastric juice is an antiseptic and a denaturing agent.

c. Chief cells secrete pepsinogen (40 kD, 42 amino acid residues + pepsin : 33 kD). Pepsin begins the process of protein degradation in the stomach (amino-terminal side of the aromatic amino acid residues).

E.3 Exocrine cells of pancreas.

a. Duodenal mucosa cells liberate hormone secretin (27 amino acids; Val-$CONH_2$) by HCl and is carried by the blood stream to the pancreas.

b. Secretin stimulates the pancreas to secrete bicarbonate into the small intestine (pH to about 7).

c. The mucosa of the upper small intestinal tract releases hormone cholecystokinin (pancreozymin, 33 amino acids).

d. Cholecystokinin stimulates secretion of several pancreatic enzymes with activity optima at pH 7 to 8.

e. Trypsinogen (trypsin), chymotrysinogen (chymotrypsin), proelastase (elastase), procarboxypeptidases A & B (carboxypeptidase A and B).

f. Enteropeptidase (enterokinase, glycoprotein, 196 kD) : a highly specific duodenal protease acts only on trypsinogen.

g. Trypsin activates trypsinogen, chymotrypsinogen, proelastase, procarboxypeptidase.

h. Pancreatic trypsin inhibitor.

i. Trypsin and chymotrypsin hydrolyze the peptides produced by pepsin.

j. Carboxypeptidase A and B (Zn-containing enzymes) and aminopeptidase.

k. Acute pancreatitis.

F. Pyridoxal phosphate participates in the transfer of α–amino groups to α–ketoglutarate.

F.1 Transamination.

a. Transamination for amino acid synthesis and degradation.
In degradation: transaminase works in concert with glutamate dehydrogenase.

b. Glutamate and α-ketoglutarate are star player in transamination.

c. Transamination provides a route for redistribution of amino acid nitrogen.
Transamination is the reversible transfer of an amino group from an amino acid to a keto acid, with pyridoxal phosphate as a coenzyme.

d. Transaminases (aminotransferases) : ping-pong mechanism.

e. Enzyme specificity.

F.2 Pyridoxal phosphate.

a. Vitamin B_6 : pyridoxine, pyridoxal.

b. Coenzyme forms : pyridoxal phosphate (PLP), pyridoxamine phosphate (PMP).

c. Pyridoxal phosphate is a remarkable versatile coenzyme. It participates in amino acid transamination, decarboxylation and racemization and numerous modification of amino acid side chains.

d. Enzyme-PLP Schiff base and aldimine intermediate.

e. The mechanism of PLP-dependent enzyme-catalyzed transamination.

f. Pyridoxal phosphate in glycogen phosphorylase and starch phosphorylase.

F.3 The amino groups from most amino acids are consequently funneled into the formation of glutamate or aspartate.

F.4 Assays for tissue damage.

a. Analysis of some enzyme activities in blood serum gives valuable diagnostic information for a number of disease conditions.

b. Alanine aminotransferase (glutamate-pyruvate transaminase, GPT) and aspartate aminotransferase (glutamate-oxaloacetate transaminase, GOT) are important in the diagnosis of heart and liver damage.

c. Creatine kinase (creatine phosphate kinase).

d. Lactate dehydrogenase.

G. Glutamate releases ammonia in the liver.

G.1 Oxidative deamination of glutamate occurs in the mitochondria of hepatocytes.

G.2 Transdeamination.

G.3 L-Glutamate dehydrogenase.

a. Glutamate dehydrogenase reaction is reversible : glutamate formation (bacteria, plants) and catabolic direction (animals).

b. NAD^+ (NADH) or $NADP^+$ (NADPH).

c. Animal enzyme (hexamer of identical subunits) is located in mitochondria (for energy generation), and is inhibited by ATP or GTP and stimulated by ADP or GDP. The enzyme is activated under conditions of low energy charge.

d. Reaction close to equilibrium.

e. Ammonia in high concentration is quite toxic, at lower levels it is a central metabolite and serves as substrate for five enzymes.

H. Glutamine transports ammonia in the blood stream.

H.1 In most animals excess ammonia is converted into a nontoxic compound before export from extrahepatic tissues into the blood and transport to the liver or kidneys.

H.2 Glutamine is a nontoxic transport form of ammonia, and serves as a source of amino groups in a variety of biosynthetic reactions.

H.3 Glutamine synthetase.

H.4 Glutaminase.

a. Ammonia for urea synthesis.

b. Glutamate is processed in the liver by glutamate dehydrogenase, releasing more ammonia and producing carbon skeletons.

H.5 In the kidney, the NH_4^+ forms salts with metabolic acids, facilitating their removal in the urine.

I. Alanine transports ammonia from muscles in the liver.

I.1 Glucose-alanine cycle :

a. Alanine serves as a carrier of ammonia and of the carbon-skeleton of pyruvate from muscle to liver.

b. The ammonia is excreted and the pyruvate is used to produce glucose.

I.2 Alanine aminotransferase.

I.3 Muscle : ATP produced by glycolysis for rapid contraction.

I.4 Liver : ATP used in synthesis of glucose during recovery.

J. Ammonia is toxic to animals.

J.1 Ammonia toxicity on the brains.

J.2 Glutamate dehydrogenase and glutamine synthetase are present at high levels in the brain.

J.3 Toxic concentrations of NH_4^+ may interfere with the very high levels of ATP production

required to maintain brain function.

J.4 Glutamate and γ–aminobutyrate (neurotransmitters) : sensitivity of the brain to ammonia may reflect a depletion of neurotransmitters as well as changes in cellular ATP metabolism.

3. NITROGEN EXCRETION AND THE UREA CYCLE (KREBS-HENSELEIT CYCLE)

A. Introduction.

A.1 In ureotelic organisms, the ammonia deposited in the mitochondria of hepatocytes is converted to urea in the urea cycle.

A.2 Urea production occurs almost exclusively in the liver.

A.3 Urea passes into the bloodstream and to the kidneys and is excreted into the urine.

B. Urea is produced from ammonia in five enzymatic steps.

B.1 The reactions of the urea cycle occur in both mitochondria and cytosol of liver cells. Glutamate dehydrogenase, carbamoyl phosphate synthetase I (CPS I) and ornithine transcarbamoylase are located in the mitochondria, while the rest of the cycle occurs in the cytosol.

a. Carbamoyl phosphate synthetase : acquisition of the first urea nitrogen atom.

- CPS I in mitochondria for urea biosynthesis.
  CPS II in cytosol for pyrimidine biosynthesis.

, CPS I catalyzed reaction is the rate-limiting step of the urea cycle.

b. Ornithine transcarbamoylase.

c. Argininosuccinate synthetase : acquisition of the second urea nitrogen atom.

d. Argininosuccinase.

e. Arginase.

B.2 The overall urea cycle reaction : urea's two nitrogen atoms are contributed by ammonia and aspartate, its carbon atom comes from HCO_3^-.

B.3 The net reaction for one turn of the urea cycle : 3 ATPs and 4 ATPs.

B.4 Blood urea nitrogen (BUN) levels represent a sensitive clinical test of kidney function.

C. The citric acid and urea cycles can be linked.

C.1 Krebs bicycle : fumarate produced in the argininosuccinate lyase reaction is also an intermediate of the citric acid cycle.

C.2 Fumarase and malate dehydrogenase are present both in the mitochondria and cytosol.

C.3 Aspartate-argininosuccinate shunt.

D. The activity of the area cycle is regulated at two levels.

D.1 The diet is primarily protein, urea production increases. All five enzymes are synthesized

at higher rate during prolonged starvation or animals on very high protein diets.

D.2 Animals on protein-free diets produce lower levels of urea cycle enzymes.

D.3 CPS I is allosterically activated by N-acetylglutamate.

D.4 The remaining enzymes are controlled by the concentration of their substrates.

D.5 N-Acetylglutamate is synthesized by N-acetylglutamate synthase.

a. As an activator for CPS I : in liver.

b. As an intermediate for arginine synthesis from glutamate in plants and microorganisms.

E. Pathway interconnections reduce the energetic cost of urea synthesis.

E.1 The overall equation of the urea cycle : 4 ATPs are required.

E.2 Fumarate production : OAA regeneration produces NADH and generates 2.5 ATPs during mitochondrial respiration.

F. Genetic defects in the urea cycle can be life-threatening.

F.1 Nonessential and essential amino acids.

F.2 Treatment for deficiencies in urea cycle enzymes.

a. Free ammonia can not be converted to urea and exported.

b. Aromatic acids benzoate and phenylacetate administered in the diet combine with glycine and glutamine, the products are excreted in the urine.

- Benzoate + glycine → hippurate (benzoylglycine)
- Phenylacetate + glutamine→ phenylacetylglutamine

c. Synthesis of glycine and glutamine to replenish the pool of these intermediates removes ammonia from the blood-stream.

d. Carbamoyl glutamate : N-acetylglutamate analog.

e. Arginine.

G. Natural habitat determines the pathway for nitrogen exertion.

G.1 Ureotelic animals : urea synthesis and exertion.

G.2 Bacterial free-living protozoa and ammonotelic animals : ammonia.

G.3 Brids and reptiles (uricotelic animals) : uric acid.

H. Urea can be reutilized for amino acid synthesis in hibernating animals (black bear) and some ruminant animals (cow and camel).

4. PATHWAYS OF AMINO ACID DEGRADATION

A. Introduction

A.1 Amino acids are degraded to compounds that can be metabolized to CO_2 and H_2O or used

in gluconeogenesis.

A.2 Oxidative breakdown of amino acids accounts for 10 to 15 % of the metabolic energy generated by animals.

A.3 Summary of the points of entry of the standard amino acids into the citric acid cycle.

A.4 Five products from amino acid degradation.

a. Acetyl-CoA.

b. α–Ketoglutarate.

c. Succinyl-CoA.

d. Fumarate.

e. Oxaloacetate.

A.5 Fates of the amino acid carbon skeletons : glucogenic, ketogenic, glucogenic and ketogenic.

B. Several enzyme cofactors play important roles in amino acid catabolism.

B.1 Some enzyme cofactors important in one-carbon transfer reactions.

a. Biotin : CO_2.

b. Tetrahydrofolate.

c. S-Adenosylmethionine.

B.2 Tetrahydrofolates are one-carbon carriers.

a. Discovery and chemistry of folic acid.

a.1 Discovery.

a.2 Folic acid has 3 distinct moieties.

- 6-Methylpterin (a bicyclic, heterocyclic pteridine ring).
- *p*-Aminobenzoic acid (PABA).
- Glutamic acid (1 to 6 or more, a modified peptide bond).

a.3 Most enzymes use folate coenzymes bind more tightly to polyglutamated forms than to monoglutamate. Folate coenzymes contain multiple glutamate residues, which evidently help them be retained within cells.

b. Conversion of folate to tetrahydrofolate.

b.1 NADPH-specific enzyme dihydrofolate reductase : dihydrofolate is the preferred substrate. The target for a number of useful anticancer, antibacterial and antiparasitic drugs.

b.2 Antimetabolite : a synthetic compound, usually a structural analog of a normal metabolite, that interferes with the utilization of the metabolism to which it is related structurally.

Aminopterin (4-aminofolate), amethopterin (methotrexate, 4-amino-10-methyl folate), trimethoprim, pyrimethamine.

b.3 Folate analogs inhibit dihydrofolate reductase and have been used in treating many different cancers and leukemia.

b.4 Sulfonamides (sulfa drugs) such as sulfanilamide are antibiotics that are structural analogs of PABA constituent of THF.

c. Tetrahydrofolate in the metabolism of single-carbon units

c.1 The coenzymatic function of tetrahydrofolate is the mobilization and utilization of single-carbon functional groups. These reactions are involved in the metabolism of Ser, Gly, Met and His, and in the biosynthesis of purine nucleotides and methyl group of thymine.

c.2 Tetrahydrofolate binds single-carbon units at the methyl, methylene and formyl oxidation levels, equivalent in oxidation level to methanol, formaldehyde, and formate, respectively.

c.3 Single-carbon groups : methyl, formyl, formimino, methylene and methenyl.

c.4 Single-carbon groups on tetrahydrofolate can be carried on N^5 or N^{10}, or bridged between N^5 and N^{10}.

c.5 Interconversion of the single-carbon units carried by tetrahydrofolate.

B.3 S-Adenosylmethionine (adoMet, SAM).

a. Structure and synthesis (methionine adenosyltransferase).

b. The sulfonium ion's highly reactive methyl group as an important biological methylating agent.

c. As the donor of propylamino group in the biosynthesis of polyamines (spermidine and spermine).

d. As the precursor to the plant hormone ethylene (promotes plant growth and development and induces the ripening of fruit).

B.4 Tetrahydrobiopterin as redox cofactors.

a. Phenylalanine hydroxylase : Phe $\rightarrow$ Tyr.

b. Tyrosine hydroxylase : Tyr $\rightarrow$ L-DOPA.

c. Tryptophan hydroxylase : Trp $\rightarrow$ hydroxytryptophan.

d. Nitric oxide synthase : Arg $\rightarrow$ NO + L-citrulline.

C. Ten amino acids are degraded to acetyl-CoA.

C.1 Five amino acids (Ala, Trp, Cys, Ser, Gly) $\rightarrow$ pyruvate $\rightarrow$ acetyl-CoA.

C.2 Five amino acids directly to acetyl-CoA and/or acetoacetyl-CoA.

C-3 Alanine, cysteine, glycine, serine and threonine are degraded to pyruvate.

a. Metabolic roles:

a.1 Alanine : glucose-alanine cycle.

a.2 Cysteine : component of glutathione, methionine biosynthesis from cysteine in plants and bacteria.

a.3 Glycine plays multiple roles : one-carbon units, creatine, glutathione, purine nucleotides, porphyrins.

a.4 Serine is quite active metabolically : phospholipids, cysteine, activated one-carbon

units.

a.5 Threonine as a constituent of proteins and a precursor to isoleucine in plants and microorganism.

b. Degradation.

b.1 Alanine ; transamination to pyruvate.

b.2 Serine :

- Serine → glycine : serine hydroxymethyltransferase.
- , Serine → pyruvate : serine-threonine dehydratase.

b.3 Cysteine : cysteine → pyruvate.

b.4 Glycine.

- via the mitochondrial glycine cleavage system (nonketotic hyperglycinemia).
- , Photorespiration.

b.5 Threonine :

- Threonine → α-ketobutyrate → propionyl CoA → succinyl CoA : in animals.
- , Threonine → α-ketobutyrate → isoleucine : in plants and prokaryotes.
- ƒ Threonine → acetaldehyde + glycine.
- , : Serine-threonine dehydratase.
- ƒ : Threonine aldolase.

C.4 Tryptophan :

a. Tryptophan is degraded to alanine and acetoacetyl-CoA.
(Trp, His and Lys do not undergo transamination at the start of its breakdown).

b. Tryptophan-2,3-dioxygenase (iron-heme protein) is induced by certain hormones.

c. Via kynurenine to glutaryl-CoA and synthesis of nicotinamide nucleotides.
(NAD^+ can be synthesized either from Trp or from vitamin nicotinic acid).

d. Trp → indoleacetate (auxin, plant growth factor).

e. Trp → serotonin, melatonin (*O*-methyl-N-acetyl serotonin) : neurotransmitters in vertebrates.

C.5 Asparagine and aspartate are degraded to oxaloacetate.

a. Metabolic roles :

Aspartate plays multiple roles :

- Precursor for Asn, Met, Lys, Thr and Ile.
- , Precursor for purine and pyrimidine nucleotides.
- ƒ Urea synthesis.

b. Degradation.

- Aspartate : transamination to oxaloacetate.
- , Asparagine → Asp → oxaloacetate.

L-asparaginase is an effective chemotherapeutic agent.

C.6 Arginine, glutamate, glutamine, histidine and proline are degraded to α–ketoglutarate.

a. Metabolic roles

a.1 The amino nitrogen of glutamate and amide nitrogen of glutamine are extremely active in biosynthesis.

a.2 Glutamate is the most active of all the amino acids in terms of its number of metabolic roles for other amino acid biosynthesis and degradation.

a.3 Glutamine occupies a central role in nitrogen metabolism. The amide nitrogen is used in biosynthesis of several amino acids (Glu, Trp, His), purine and pyrimidine nucleotides and amino sugars.

a.4 Arginine.

Precursor for nitric oxide, urea, creatine, polyamines and nicotine biosynthesis.

a.5 Histidine undergoes decarboxylation to generate histamine, a substance with multiple biological actions.

b. Degradation

b.1 Arg, Gln, His and Pro are all degraded by conversion to glutamate, which in turn is oxidized to α-KG by glutamate dehydrogenase.

b.2 Glutamine → glutamate : glutaminase.

b.3 Histidine → urocanate → glutamate + N^5-formimino-THF (active one-carbon unit).

b.4 Proline → glutamate.

Procollagen proline hydroxylase : procollagen proline residue → collagen hydroxyproline.

b.5 Arginine.

- Arg → urea + ornithine.
- , Ornithine → putrescine → polyamines, nicotine alkaloids.
- *f* Ornithine → glutamate.
- „ Nitric oxide synthase : Arg + O_2 → citrulline + NO.
  NO: second messenger and neurotransmitter.

C.7 Isoleucine, methionine and valine are degraded to succinyl-CoA.

a. Metabolic functions

a.1 Ile and Val as constituents of proteins.

a.2 Methionine.

- Cysteine biosynthesis in animals.
- , Polyamines biosynthesis.
- *f* Met → α-ketobutyrate → Ile.
- „ Met → SAM → ethylene.
- … Met → SAM : as methyl donor.

b. Degradation of Met

b.1 Ile, Met, Val → propionyl-CoA → succinyl-CoA.

b.2 Methionine breakdown involves synthesis of S-adenosylmethionine (SAM) and cysteine.

b.3 SAM.

- The sulfonium ion's highly reactive methyl group as an important biological methylating agent.
- , As the donor of propylamino group in the biosynthesis of polyamines (spermine and spermidine)
- ƒ As the precursor to the plant hormone ethylene (promotes plant growth and development and induces the ripening of fruit).

b.4 Met is classified as an essential amino acid for mammals, while Cys is nonessential. Actually, Cys sulfur is derived from Met in animals.

c. Branched-chain amino acids are degraded in extrahepatic tissues and involved in acyl-CoA oxidation.

c.1 Much of the catabolism of amino acids in liver. Val, Ile and Leu are oxidized as fuels in muscle, adipose, kidney and brain tissues.

c.2 They share the first three enzymes in their catabolic pathways.

- Transamination to the corresponding α-keto acids : Branched-chain amino acid aminotransferase.
- , Oxidative decarboxylation to the corresponding acyl-CoA : Branched-chain α-keto acid dehydrogenase complex (The complex is analogous to the pyruvate and α-KG dehydrogenase complexes and requires the same five cofactors. The complex is inactivated by phosphorylation and activated by dephosphorylation).
- ƒ Dehydrogenation by FAD to form a double bond.

c.3 The degradation of the branched-chain amino acids.

c.4 Maple syrup urine disease (genetic deficiency of the complex).

C.8 Leucine and lysine are degraded to acetoacetate and/or acetyl-CoA.

a. Val, Ile, Leu and Lys are essential amino acids, which are not synthesized in mammalian tissues. None of them is known to play significant metabolic roles other than as protein constituents and as substrates for their own degradation.

b. Leucine degradation is the same manner as Ile and Val. The products are acetyl-CoA and acetoacetate (ketone body).

c. The pathway of lysine degradation in mammalian liver (saccharopine pathway).

C.9 Phenylalanine and tyrosine are degraded to fumarate and acetoacetate.

a. The first reaction in Phe degradation is its hydroxylation to Tyr.

b. The pathway of Phe degradation.

c. Phe and Tyr serve as precursors to an enormous number of plant substances, ranging from the polymeric lignin to tannins and pigments to many of the flavor components of spices.

d. They are precursors to many of the nearly 3000 alkaloids (nitrogenous substances synthesized by specific plants).

e. Tyrosine utilization and catabolism in animals.

- Thyroid hormones : thyroxine (T4) and triiodothyronine (T3).

, Melanins : pigment-producing cells (melanocytes).

Albinism : a genetic deficiency of tyrosinase causes an individual to lack pigmentation (albinos).

f Catecholamines : as hormones and as neurotransmitters.

f. Phenylketonuria and alcaptonuria (dark urine disease) result from defects in Phe degradation.

• Alkaptonuria : a hereditary deficiency of homogentisate dioxygenase (iron-containing enzyme).

, Phenylketouria (PKU) : a hereditary deficiency of Phe hydroxylase (iron-containing enzyme).

Phe → phenylpyruvate → phenyllactate + phenylacetate (1 to 2 g per day in urine).

A different form of PKU from a hereditary deficiency of dihydropteridine reductase.

C.10 Some human genetic disorders affecting amino acid catabolism.

C.11 Some amino acids can be converted to glucose, others to ketone bodies.

a. Glucogenic amino acids.

b. Ketogenic amino acids : Leu, Lys.

c. Glucogenic-ketogenic amino acids : Trp, Phe, Tyr, Ile.