THE
Glucosinolates & Cyanogenic Glycosides
Assimilatory Sulphate Reduction

- Animals depend on organo-sulphur

- In contrast, plants and other organisms (e.g. fungi, bacteria) can assimilate it

- Sulphate is assimilated from the environment, reduced inside the cell, and fixed to sulphur containing amino acids and other organic compounds
Assimilatory Sulphate Reduction
The Glucosinolates
The Glucosinolates
- Found in the Capparales order and are the main secondary metabolites in cruciferous crops
The Glucosinolates

- The glucosinolates are a class of organic compounds (water soluble anions) that contain sulfur, nitrogen and a group derived from glucose

- Every glucosinolate contains a central carbon atom which is bond via a sulfur atom to the glycone group, and via a nitrogen atom to a sulfonated oxime group. In addition, the central carbon is bond to a side group; different glucosinolates have different side groups
The Glucosinolates

Central carbon atom
The Glucosinolates

- About 120 different glucosinolates are known to occur naturally in plants.

- They are synthesized from certain amino acids: methionine, phenylalanine, tyrosine or tryptophan.

- The plants contain the enzyme myrosinase which, in the presence of water, cleaves off the glucose group from a glucosinolate.
The Glucosinolates

- Post **myrosinase** activity the remaining molecule then quickly converts to a **thiocyanate**, an **isothiocyanate** or a **nitrile**; these are the active substances that serve as defense for the plant.

- To prevent damage to the plant itself, the myrosinase and glucosinolates are stored in separate compartments of the cell and come together only under conditions of stress or injury.

- Glucosinolates might also be beneficial to insects (signals for feeding or oviposition) that could overcome host toxicity.
The "mustard oil bomb"-- A binary Glucosinolate-Myrosinase chemical defense system

Glucosinolates breakdown products
1- isothiocyanates
2- nitriles and elemental sulfur
3- thiocyanates
4- oxazolidine--thiones
5- epithionitriles

Grubb and Abel, TIPS, 2006
The Glucosinolates

- Due to negative effects, food crops have been developed that contain very low amounts of glucosinolates (e.g. canola).

- On the other hand, plants producing large amounts of glucosinolates are also desirable, because substances derived from these can serve as natural pesticides and are potent in the prevention of cancer (e.g. sulforaphane in broccoli)

Glucosinolates are synonymous to 'Mustard oil glycosides' and 'Thioglucosides.'
The Glucosinolates

- Extensive research in Arabidopsis (at least 37 different GSs in all ecotypes, approx. 7 per ecotype)

- Side chains from mainly methionine (so called aliphatic glucosinolates, AGs) and Tryptophan (Indole Glucosinolates; IGs)

- GSs concentration and types vary significantly among organs and GSs are transported via the phloem to sink tissues, most probably intact

- Seeds have very distinctive GSs profile
The Glucosinolates

- **Biosynthesis** starts (Step i) with side chain elongation that could be further elongated or used to form the core structure (glucon)

- Glucon formation (Step ii) is a 5 steps pathway, starting with the formation of aldoxime through oxidation by P450's

- In the next step (iii), sulfation of desulfo glucosinolates occurs and thereafter secondary modification of the side chain takes place (various kinds of oxidation, esterification etc..)
The Glucosinolates

(a) Side chain elongation

1. Transamination

2. Condensation

3. Isomerization

4. Oxidative decarboxylation

5. Transamination
The Glucosinolates

Grubb and Abel, 2006

(b) Glucone formation

1. Oxidation
   - Aldoxime
     - CYP79F1 (Met<sub>1,8</sub>)
     - CYP79B2 (Trp)
     - CYP79A2 (Phe)
   - e<sub>6</sub>-Nitro compound
     - CYP83A1 (Met)
     - CYP83B1 (Trp, Phe)

2. (i) Oxidation
   - S-Alkyl thiohydroximate
     - Spontaneous?
     - GST?

3. C-S cleavage
   - Thiohydroximate
     - C-S Lyase

4. Glucosylation
   - Desulfoglucosinolate
     - UGT74B1

5. Sulfation
   - Glucosinolate
     - ST5a (Trp, Phe)
     - ST5b (Met)
     - ST5c (Met)
The Glucosinolates

(c) Side chain modification

Oxidation of Met-derived glucosinolates

\[
\begin{align*}
\text{Alkenyl-GS} & \quad \text{AOP2} \\
\text{Hydroxy-GS} & \quad \text{AOP3}
\end{align*}
\]
The Indole and Aliphatic Glucosinolates Metabolism

- The pathways share enzymes
- Genes clones marked by capital letters

Kim and Jander, 2007
The Indole Glucosinolates (from Trp)

As in the case of other GSs types, the formation of IGs is inter-linked with metabolism of other key metabolites

- The Trp derived Indole Aldoxime (in the Glucon formation step) is an important branch point

- The aldoxime is a precursor for IGs, Auxin (IAA) and Camalexin biosynthesis
The Indole Aldoxime is a Major Branch Point
The Indole Glucosinolates (from Trp)

- Complex interplay between IGs biosynthesis and linked pathways

- Inhibition of flux between the aldoxime and the IGs results in increased levels of auxin
The Indole Glucosinolates
(from Trp)

- CYP83B1 knockout (rnt1)
Regulation of the Indole Glucosinolates Pathway

Altered Trp Resistance (ATR1)- A dominant mutant

A Myb homologue, ATR1, activates tryptophan gene expression in Arabidopsis

(Myb transcription factor/translational control)

Judith Bender* and Gerald R. Fink

Regulation of the Indole Glucosinolates Pathway (ATR1)
Regulation of Both Pathways by A Set of MYB Regulators

Omics-based identification of Arabidopsis Myb transcription factors regulating aliphatic glucosinolate biosynthesis

Masami Yokota Hirai*, Kenjiro Sugiyama†, Yuji Sawada*, Takayuki Tohge*, Takeshi Obayashi†, Akane Suzuki*, Ryoichi Araki*†, Nozomu Sakurai†, Hideyuki Suzuki†, Koh Aoki†, Hideki Goda*, Osamu Ishizaki Nishizawa*†, Daisuke Shibata†, and Kazuki Saito*†

6478–6483 | PNAS | April 10, 2007 | vol. 104 | no. 15
Regulation of Both Pathways by A Set of MYB Regulators

Identification of the Arabidopsis MYB28 and MYB29 by co-expression analysis (yellow-enzymes; red- transcription factors)
Regulation of Both Pathways by A Set of MYB Regulators

Downregulation of genes encoding enzymes related to AGs metabolism in MYB28
Regulation of Both Pathways by A Set of MYB Regulators

Down regulation of MYB28 results in reduced accumulation of AGs
Regulation of Both Pathways by A Set of MYB Regulators

Overexpression of MYB28 in Arabidopsis cell cultures results in increased accumulation of AGs related transcripts and AGs.
Regulation of Both Pathways by A Set of MYB Regulators

The transcription factor HIG1/​MYB51 regulates indolic glucosinolate biosynthesis in *Arabidopsis thaliana*

Tamara Gigolashvili, Bettina Berger, Hans-Peter Mock, Caroline Müller, Bernd Weisshaar and Ulf-Ingo Flügge

The Plant Journal (2007) 50, 888-901
doi: 10.1111/j.1365-313X.2007.03095.x

The R2R3-MYB transcription factor HAG1/​MYB28 is a regulator of methionine-derived glucosinolate biosynthesis in *Arabidopsis thaliana*

Tamara Gigolashvili, Ruslan Yatsusevich, Bettina Berger, Caroline Müller and Ulf-Ingo Flügge

The Plant Journal (2007) 50, 888-901
doi: 10.1111/j.1365-313X.2007.03133.x
Regulation of the Glucosinolate and Sulfur Limitation (SLIM1 factor)

Marayuma-Nakashita et al, 2007
The Aliphatic Glucosinolates (from Met)- Subcellular Location of Enzymes

Schuster et al., 2007
The Aliphatic Glucosinolates (from Met)- Subcellular Location of Enzymes

- BCAT (aminotransferase acting on Met) cytosol

- MAM and other chain elongation pathway enzymes are located in plastids

- Metabolism of Met derivatives in the ER and interface with cytosol
The Glucosinolates Metabolon

Grubb and Abel, 2006
Cyanogenic Compounds from Plants
Cyanogenic Compounds

- Plants ability to produce cyanide (or cyanogenesis) is known for a long time.

- Structure of only about 60 cyanogenic compounds have been published (in 2002).

- These (N-containing) compounds are either cyanogenic glycosides (glycosides of α-hydroxynitiles/ cyanohydrins).

Or: a few cyanogenic lipids.
Cyanogenic Glycosides

- Based on the general formula:

\[
\text{R}_1 \text{C}_6\text{H}_{12}\text{O}_6 \text{CN}
\]

- The sugar residue is almost always D-glucose joined by an alpha-D-glucosyl linkage

- \( \text{R}_1 \) is either an aliphatic or aromatic group and \( \text{R}_2 \) is mainly H
Cyanogenic Glycosides

Dhurrin

Amygdalin

cyanohydrin

Linamarin

Leptaustalin

Prunasin

Taxiphyllin
Cyanogenic Glycosides

- Classified according to the AMINO ACID source of the $R_1$ group

- Valine, Isoleucine, Leucine, Phenylalaalanine, Tyrosine
Cyanogenic Glycosides Catabolism

- A beta-glycosidase will often produce the cyanohydrin (aglycon) and a sugar

- A second type of enzyme (hydroxyynitrile lyase) will catalyse the dissociation of the cyanohydrin to a carbonyl compound and HCN (hydrogen cyanide)
Cyanogenic Glycosides Catabolism

Beta-glycosidase

Lyase

Cyanogenic glycoside

Cyanohydrin

Ketone or aldehyde
Linamarin Catabolism (from Valine)

Linamarin → Linamarase → Glucose → Acetone cyanohydrin

CH₃–C–C≡N

CH₃–C–C≡N

Linamarin

C₆H₁₁O₅

C₆H₁₂O₆

OH

Acetone cyanohydrin

pH > 5

Hydroxynitrile lyase (HNL)

CH₃–CO–CH₃ + HCN

Acetone

Hydrogen Cyanide
Cyanogenic Glycosides & HCN

- Hydrogen cyanide HCN, (cyanohydric acid or prussic acid)

- A powerful poison (to a wide spectrum of organisms), volatile colorless liquid with the odor of bitter almonds

- It is toxic due to its ability to link with metals (e.g. Fe++ and Mn++) that are functional groups of many enzymes

- It inhibits processes such as the reduction of oxygen in the cytochrome respiratory chain, electron transport in photosynthesis and the activity of enzymes such as catalase and oxidase
Cyanogenic Glycosides & HCN

- Prussic acid passes through the mucous membranes and the skin, but principally through the lungs, into the blood

- It blocks the process by which oxygen is released from red blood corpuscles

- Zyklon B consisted of prussic acid
Cyanogenic Lipids

- Long-chain fatty acids ($C_{18}$ or $C_{20}$) attached to the hydroxynitrile

- In the seeds of the Sapindaceae (4 types)
Cyanogenic Plants

- Prunus species
- Sorghum
- Lotus
- Apples, peaches, cherries and apricots
- Flax
- Corn
- Cassava
- Almonds
- Passion fruit
Cyanogenic Plants

- Cyanogenesis is a mechanism against predators such as herbivores (other functions are also N storage and production of antifungal compounds from their aglycons)

- Normally, substrates and enzymes are localized to different subcellular compartments

- Upon damage they are mixed
Cyanogenic Plants

- Bacteria, fungi and animals are also cyanogenic.

- Cyanogenicity is often associated with certain species but to different degree.

- Certain structural types of cyanogenic glycosides are associated with specific groups of plants.
Cyanogenic Plants

- The presence of cyanogenic compounds in food and forage plants is a problem (e.g. Cassava and Sorghum)

- Identification of cyanogenesis could be done by simple color tests (Guignard and Feigl-Anger)
Cassava (Manihot esculenta, Crantz) roots (starchy food) are the primary source of calories for more than 500 million people, the majority of whom live in the developing countries of Africa.
Cyanogenic Crop Plants- Cassava

- Plants with 20mg HCN /100 g fresh weight are considered toxic

- Many cultivars exceed this value

- Detoxication methods developed by native Indians consuming the root:
  - grating or grinding the plant, pressing the ground material to remove liquid containing HCN (thus mixing the glucosidase with its substrate), storing the product and cooking)
Cyanogenic Plants- Activity in Plants

- Lima bean accessions with different cyanogenic capacity (black-high release/ open-low release/ strips-high precursors, low beta-gal, low release)

Ballhorn and Leiberei, 2006
Cyanogenic Plants- Activity in Plants

- Cyanogenic glycosides sometimes represent a substantial proportion of the plants nitrogen

- In some cases they are converted into other metabolites once moved from seeds to cotyledons

- Plants detoxify HCN by conversion into Asparagine and beta-cyanoalanine
Dhurrin in Sorghum (*Sorghum bicolour*)

- Dhurrin - highest accumulation in seedlings

- Content varies in plant age and growth conditions

- CYP79A1, CYP71E1 and the glucosyl transferase UGT85B1 catalyse its biosynthesis from Tyrosine

(Metabolon formation?)

Busk and Muller, 2002
Engineering Dhurrin

- Introducing the entire pathway (CYP71E1/CYP79A1/sbHMNGT)

- Accumulation of 4 +/- 0.5 mg dhurrin per gram fw

- Similar to the concentration in sorghum bicolour seedlings

- Small reductions in growth

Resistance to an Herbivore Through Engineered Cyanogenic Glucoside Synthesis

David B. Tattersall, Søren Bak, Patrik R. Jones, Carl Erik Olsen, Jens K. Nielsen, Mads L. Hansen, Peter B. Høj, Birger Lindberg Møller
Engineering Dhurrin

- Introducing the P450's ONLY (CYP71E1/CYP79A1)

- Accumulation of p-hydroxybenzaldehyde and its derivatives

- More than 100 GTs of Arabidopsis cannot convert p-hydroxymandonitrile to dhurrin

- Severe phenotypes

**Resistance to an Herbivore Through Engineered Cyanogenic Glucoside Synthesis**

David B. Tattersall, 1,2 Soren Bak, 1,2* Patrik R. Jones, 1,2,4* Carl Erik Olsen, 2,3 Jens K. Nielsen, 2 Mads L. Hansen, 3 Peter B. Høj, 4,5 Birger Lindberg Møller 1,2
Engineering Dhurrin

- Introducing CYP79A1 ONLY
- The product of the CYP79A1 reaction could serve as substrate for CYP83 from the Glucosinolate pathway
- Presence of CYP71E1 prevents this interaction
Engineering Dhurrin
- Introducing the CYP's also to transgenic Tobacco

Bak et al., 2002
Engineering Dhurrin

- Flea beetle and larvae feeding
Engineering Cyanogenic Glucosides levels in Cassava

- Traditional breeding could reduce levels of Cyanogenic Glucosides but not cassava devoid of these compounds

- Valine and Isoleucine are the precursors for Linamarin and Lotaustralain

- Major amount of Cyangonic glucosides are transported from the shoot to the tubers

Cassava Plants with a Depleted Cyanogenic Glucoside Content in Leaves and Tubers. Distribution of Cyanogenic Glucosides, Their Site of Synthesis and Transport, and Blockage of the Biosynthesis by RNA Interference Technology

RNA interference

CYP79D1

CYP79D2

CYP71E orthologue

UGT85B orthologue

Figure 1. The biosynthetic pathway for the cyanogenic glucosides linamarin and lotaustralain in cassava. The enzymatic step blocked by RNAi technology is indicated.
Engineering Cyanogenic Glucosides levels in Cassava

- RNAi silencing of the two redundant CYP79D genes (300 independent transgenic lines)
- 92% reduction in cyanogenic glucoside content (Linamarin and lotaustralin) in tubers
- Acyanogenic leaves (<1% of wild-type)
Engineering Cyanogenic Glucosides Levels in Cassava

- RNAi Cassava lines with <25% of cyanogenic glucoside accumulation exhibited a morphological phenotype when grown in vitro (long internodes, barely any roots, slow growth)

- In soil the wild type phenotype was restored

- Nitrogen deficiency affected the transgenic lines more than wild-type (cyanogenic glucosides as N storage)
END OF COURSE

- Up to the end of the month (July), a mini review (3-4 A4 pages) on "The secondary to primary metabolism interface"

- Pathways and Metabolites, your choice!