Lecture #6

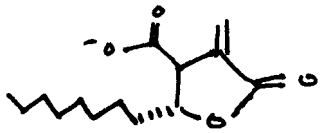
Lecture 6
2/17/04

Exam will be 2/25/04 from 7:30-9:30pm, closed book, no notes

Ref (use sequence alignments to define amino acids used in "A" domain)
Chem Biol 1999 **6**, 493-505
Chem Biol 1999 **7**, 211-14

Review- Walsh (antibiotic challenges/opportunities)
Chem Biol 2003 **3**, 124-34

Remember from last lecture (obesity)



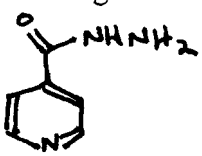
C-75 inhibitor of FAS
1) C-75 decreases FA biosynthesis
2) mice lose weight
3) Concentration MCoA increases

Hypothesis: Mouse thinks that it is in the "fed state" when [MCoA] goes up, therefore they lose weight. Somehow the change in concentration of MCoA must signal the "fed state" to the brain, probably indirectly.
Major area of current research- is MCoA a sensor? How does it communicate to the brain, the fed state?

Tuberculosis (TB)-

Infects 1 in 3 people worldwide
3 million die each year and 8 million are infected/year

1 major drug is used against TB,



Isoniazid
Cheap to make (no chiral centers)

Proposed to function by inhibiting FA biosynthesis, specifically mycolic acid biosynthesis (C_{80}) and thus the formation of the cell wall. (See Handout 2a)

Isoniazid is a pro-drug. It is activated by a catalase once it has entered the body.
-metabolized into a reactive species (handout 2a page 8)

-proposed to inhibit ER and KS (mycolic acid biosynthesis)
-no cell wall

A high percentage genes in mycobacteria tuberculosis are involved in lipid biosynthesis and degradation (this makes sense when you look at their elaborate cell walls)

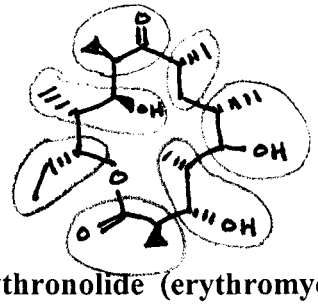
Resistance has developed to Isoniazid, mainly because of difficulty with patient compliance. This bacteria doubles every 2 weeks, so you have to take the drug for months. Patients feel better earlier, stops taking drug, and the bacteria remaining are the ones most resistant to the drug.

Problem- we have no Isoniazid replacement that is so cheap and easy to make.

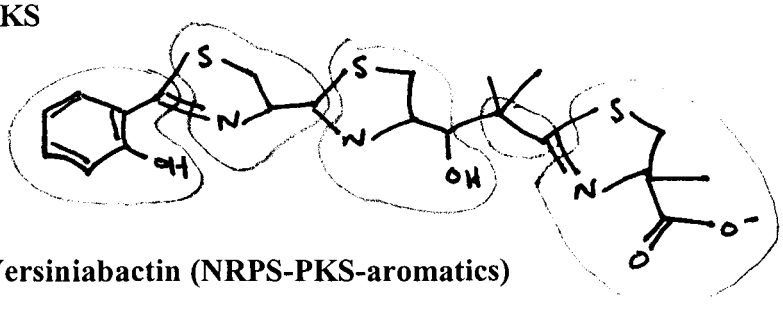
Handout 2a page 8 shows the reaction that is believed to occur- catalase reacts with Isoniazid to form a reactive species, that then forms a covalent bond to NAD. Look at structure of NAD- Isoniazid complex bound to ER enzyme...notice that the Ph's should be P.

SECONDARY METABOLISM

Examples of PKS and NRPS- after this section you should be able to look at these compounds and understand their biosynthesis. I have circled the building blocks in each molecule.

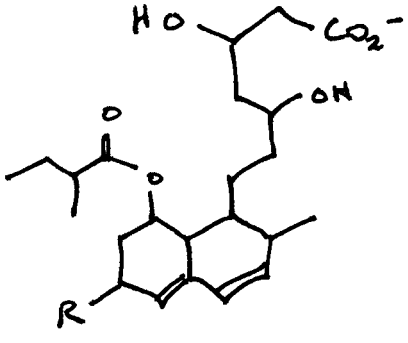


6-deoxyerythronolide (erythromycin)
PKS



Yersiniabactin (NRPS-PKS-aromatics)

Lovastatin (iterative PKS) –problem set 2 example



You will learn to do a retrosynthetic analysis and find the building blocks of these molecules.

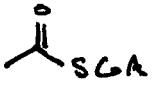
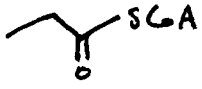
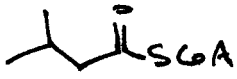
Outline of this part of module 2

A. Players – small molecules, proteins (look at PKS and NRPS in parallel)
B. Chemistry-
   -Post-translational modification, Initiation, Elongation, Decoration, Termination
C. Specific Examples-
   -Erythromycin (gain of function, deletions, replacements),
   -Iron Siderophores- Enterobactin (pulls Fe out of environment, NRPS "waiting room" model) Yersiniabactin (PKS, NRPS)-fidelity

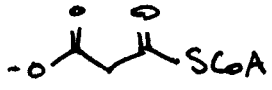
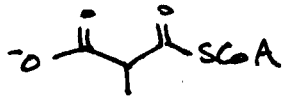
A.PLAYERS

I. small molecules

PKS

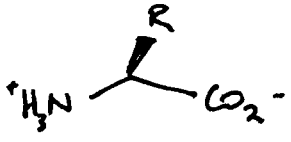
Loaders: **Acetyl CoA (ACoA)** **Propionyl CoA (PCoA)** **isobutaryl CoA (BCoA)**

Extenders: **Malonyl CoA (MCoA)** **methyl malonyl CoA (MMCoA)**

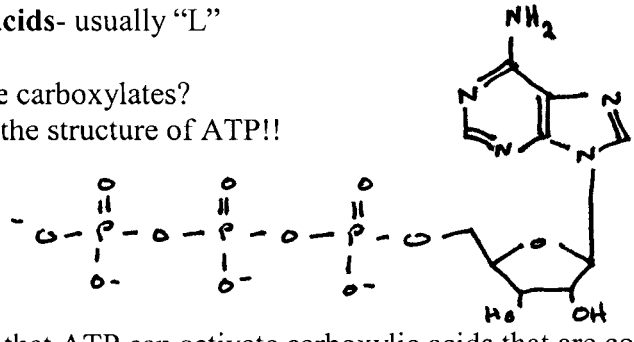
Notice these are all already activated thioesters (thioesters have the same reactivity as oxygen esters with oxygen nucleophiles, but are more reactive than oxygen esters with amines nucleophiles)

NRPS
Loaders:



20 natural amino acids- usually "L"

How do you activate carboxylates?
ATP!!! Memorize the structure of ATP!!



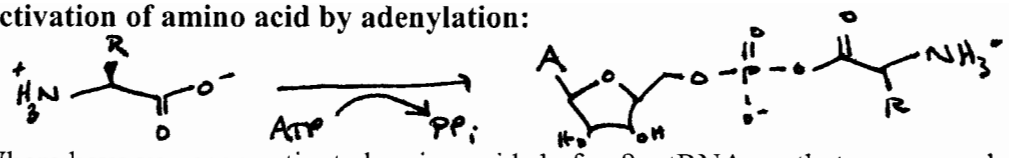
ATP

There are two ways that ATP can activate carboxylic acids that are commonly used in metabolism

i) adenylation (add AMP)
ii) phosphorylation

For NRPS, nature uses adenylation

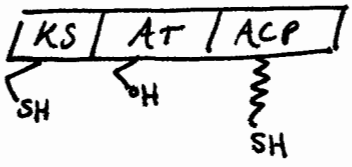
Activation of amino acid by adenylation:

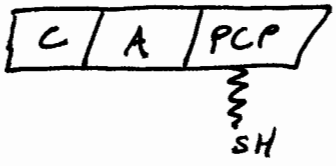


Where have you seen activated amino acids before? – tRNA synthetases use adenylation to attach amino acids to tRNA

PKS loaders are already activated thioesters- but, they have already been adenylated in order to turn them into thioesters.

II. Protein players
Definitions
Domain: small piece of protein with a single catalytic activity
Module: Minimal functional unit





PKS
KS=ketosynthase
AT=acyl transferase
ACP= acyl carrier protein

NRPS
C= condensing domain
A=activating domain (adenylates aa)
PCP= peptidyl carrier protein

ACP and PCP have both been renamed "T" domains for thiolation, because of the swinging pantetheine arm.
The **squiggle** (~~SH) will be used to indicate the phosphopantethiene swinging arm

What is the organization of the modules??
PKS
Type I- non-iterative
Every reaction is done on a new domain (for 50 reactions, you need 50 domains)
The order of the domains tells you the biosynthetic pathway
200-2,000 kDa proteins (huge!)
ex. Erythromycin

Type II –iterative
Like FAS, protein uses the same domain over and over again to catalyze a series of reactions, less defined specificity
Often involved in aromatic natural products (Lovastatin is an example, but is not an aromatic natural product)

Type I PKS are like assembly lines
KEY POINT: The template for the biosynthetic pathway is the sequence of the polypeptide. From the nucleic acid sequence, you can PREDICT the biosynthetic pathway.