Lecture #8

Lecture 8
2/20/04

Annoucements:
EXAM: weds feb 25th 7:30-9:30pm
Make-up: weds afternoon from 12-5pm in conference room
Review session: Tues 7-8:30pm

Leadley Lecture, March 3&4 at 4pm

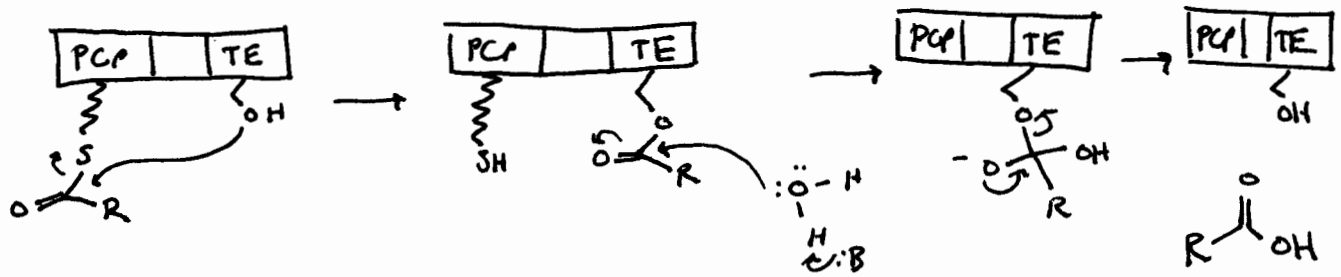
Last time, we discussed the CHEMISTRY of PKS and NRPS, including priming, initiation, elongation, and decoration. We will continue today with termination, and then SPECIFIC EXAMPLES.

B. CHEMISTRY (continued)

I. Termination

All occurs in TE domain - a super-family of proteins that look like alpha, beta hydrolases (lipases, being the best characterized)
- All of these proteins use covalent catalysis with a nucleophile. In the TE domains, the nucleophile is a serine in ser-his-asp catalytic triad (like chymotyrpsin)
-Other members of this family use cysteine or aspartates as nucleophiles.
-Structural hallmark of these enzymes is beta-strand, sharp elbow, helix motif
-Nu is located at the sharp elbow
-TE domainis usually located at the C-terminus (of either a huge protein if all domains joined on one peptide, or the last modular peptide)

The reaction occurs in two main steps,
1) transfer of finished chain to nucleophile of TE domain (S activated by H,D for attack)
2) attack of nucleophile, like H_20 on carbonyl of finished chain (through tetrahedral intermediate), to release final product from the enzyme

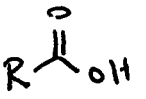
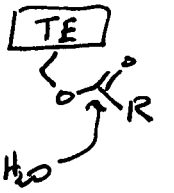
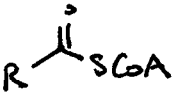
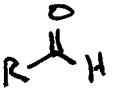
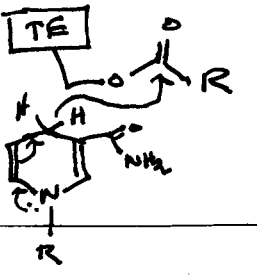


TE reaction in NRPS with H_20 as the nucleophile

~~SH = PHOSPHOPANTATHEINE ARM

R = FINISHED CHAIN

There are multiple endings possible, depending on the nucleophile that releases the product

Nucleophile	Products	Drawing
H_20 attacks, tetrahedral intermediate, hydrolysis	Carboxylic acid product 	
Hydoxyl group attacks INTRAmolecularily (or amino group)	Lactone  (or Lactam) 	
CoA	Thioester 	
Reduction with **NADH**	Aldehyde 	

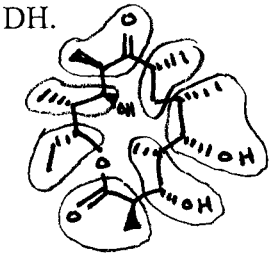
SPECIFIC EXAMPLES:

Erythromycin: $3.5 billion/year

Handout 2a, page 13 shows examples of the DIVERSITY of erythromycin products available by rearranging domains

Handout 2a, page 11: genes to make erythromycin isolated – DEBS1(alpha); DEBS2 (betal); DEBS3 (gamma).
3 polypeptides required- each 300 kDa
(without sugar decorations- 6-dEB is precursor to actual drug, decorated with sugars)

Each of the polypeptides of the protein are homodimeric [alpha$_2$-beta$_2$- gamma$_2$]
-Last step of reaction- formation of lactone, tells you immediately the end of the biosynthetic pathway. The other end must be the beginning. Go from one end to the other and look for either two or three carbond units. The two carbon units are always within the chain and the third carbon appears as an appendage. This is because of the chemistry, that is, all carbon-carbon bonds are formed between C-alpha and the carbonyl of the thiol ester.
-Extra Methyl groups indicate that structure composed of proponyl CoA and methylmalonyl CoA
(alpha carbon is activated for Caisen reaction)
The decorating domains define whether one observes a ketone, and alcohol, a double bond, or a completely saturated chain. (DH,ER,KR). For example if you see a ketone in a unit, you only need a KS, AT and ACP within that one module. On the otherhand if you see a double bond, you need a KS, AT, ACP and a KR, DH.



6-dEB with building blocks indicated

Can you make a diagram of the extending chain as it moves along the assembly line?

QUEDTION: How robust is this organization?
Can we modify protein or substrate and change the product? Look at the handout with all the erythromycin analogs that have been made as examples of the changes outline below.

a) Can we replace one domain with another?

The AT domain for erythromycin loads MMCoA, Can we replace it with a domain that loads MCoA? The answer is YES
Successfully replaced with an AT from rapamycin (another natural product) uses MCoA
How robust the replacement is requires further analysis. What is the rate of the reaction in comparison with the wild type protein? What is the overall yield of the products in comparison with the wt-products?

b) Gain of function (addition)?

Can you replace KR with several modules such as KR,DH?
The answer, again is YES
Get double bond instead of alcohol in product

c) Loss of function (deletion)?

Can you delete KR domain?
YES (genetically, by removal of domain or through site-directed mutagenesis)
Get ketone instead of alcohol in product

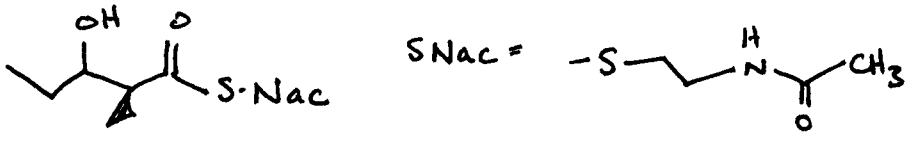
d) Can we transfer TE domain to any module? YES

Now we are only limited by the loaders in trying to make new erythromycin products (ACoA, IBCoA, PCoA)
Could you load something with a halogen, etc?? Still an active area of research

Can you find examples of products from these substitutions on page 13 of handout 2a?

Simpler Example
Science 1999, **284**, 482

Chemically synthesized (chiral) loader- dimer of propionylCoA with N-acetylcystamine thiol ester, replacing CoA (looks like first part of CoA and is easier to make and cheaper)

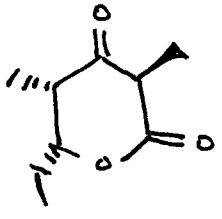


Fed this starter to module 3 of DEBS II with TE pasted onto the end

KS AT ACP TE (no decorating domains)

Got one major product! And it was cyclized!
(even though much shorter chain than normal erythromycin product, the size of the lactone ring is completely different from the wt product)

product



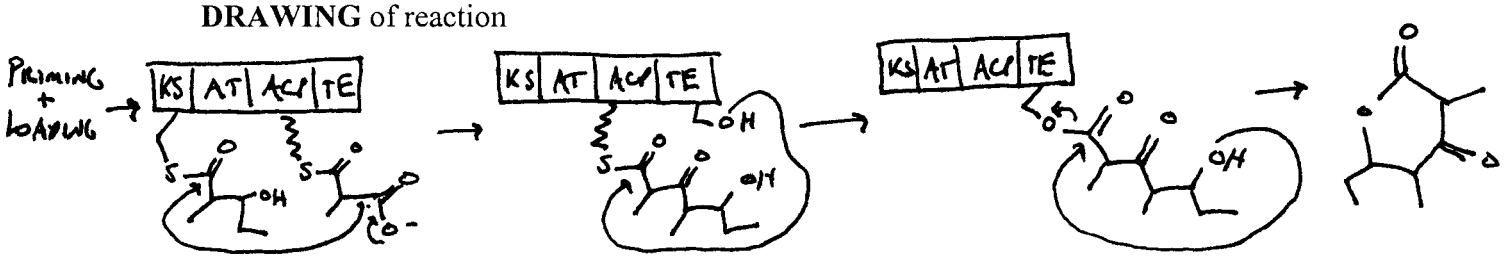
-Can load in middle of domain- after 2 reactions not 3
-Load CoA substitute
Relaxed specificity allows us to make many products

How do we get to this product?

1)Load chemically synthesized substrate on KS (either through AT domain, or directly- details are nknown)
2)Load MMCoA on ACP (probably through AT by analogy to FAS)
3)Decarboxylation
4. Form C-C bond
5)transfer to TE domain
6)Intramolecular attack by hydroxyl through a td intermediate followed by collapse to form a lactone

Release of stereospecific product

DRAWING of reaction



Page 15 of handout 2a shows the results of other experiments with chemically synthesized loaders and varied DEBs modules

The same product is observed when loading the same substrate on modules 1,2,5 (this makes sense, they all have the same domains)
Only slightly different kinetics (despite the difference in length of chain they accept in the natural reaction)

Is it more efficient to load chemically synthesized substrate or make it biosynthetically?
Page 15 Figure 8 (ABC vs. DEF) similar rate constants!

Figure 7- How important are the linker regions?