

Lecture #3

Lecture 3
2/9/04

Over the semester we will look at 4 types of molecular machines:

Fatty Acid Synthases (FAS) 180x130x75A
Ribosome 210A
Protein folding machine (like GroELGroES) 184x140A
Proteasome 150A

See CryoEM pictures in Handout 2b
Notice all that these machines are similar in size.
We will use STRUCTURE as a basis for our understanding of these molecular machines.

MODULE 2

Fatty Acid Synthase (FAS)
Will serve as a paradigm for other secondary metabolic pathways like
Polyketide synthases (PKS) and Non-ribosomal polypeptide synthases (NRPS)

Remember you are expected to read the section on FAS in a biochemistry text like
Voet&Voet.

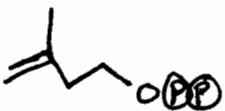
FAS enzymes catalyze a polymerization reaction
In humans C16
In microbacteria, up to C80
Where have we seen polymerization reactions before? In DNA, RNA and peptide
synthesis (Replication, transcription and translation) These examples all use a template.

FAS is a paradigm for HOMOPOLYMERIZATION reactions- polymerization of the
same substrate (not 20 different amino acids) and there is NO TEMPLATE

We will discuss how FAS accomplishes Initiation, Elongation, Termination, and Fidelity.

Other examples of biological homopolymerization reactions:

- 1) Sugar polymerization
 - Glycogen biosynthesis – start with a water soluble monomer and end with an insoluble polymer. The glycogen structures in muscle and liver are different, because the glycogen in muscle must be quickly degraded and available for use. A whole series of proteins are involved in laying down polymer and in a structure so that it can be reused.
 - Starch biosynthesis- peptidoglycans are another example
- 2) Rubber
 - formed from isopentyl pyrophosphate (also a building block for cholesterol biosynthesis. Plants make rubber better and more efficiently than chemists can in the lab.



ISOPENTYL
PYROPHOSPHATE

FAS biosynthesis

Humans make only C16 with Acetyl CoA, adding 2 carbon atoms in each elongation step
We will focus on the Human FAS

Microbacteria Tuberculosis can make C80 – mycolic acid (see page 8 of handout2a)
Long fatty acids in cell wall. How do they control chain length??

On page 2 of handout 2a, there are examples of important PKS and NRPS natural products

- 1) Avermectin B1- antibiotic for chickens- kills parasites that affect chickens living in crowded factory farm conditions (widely used drug)
- 2) Erythromycin- mainline antibiotic with little resistance (2 or 3 carbon units)
- 3) Penicillin- from NRPS- can you see the amino acids? Antibiotic that blocks cell wall synthesis
- 4) Cyclosporin- also made up of amino acids- some are unusual or decorated- this is an immunosuppressant agent
- 5) Bleomycin (not pictured)

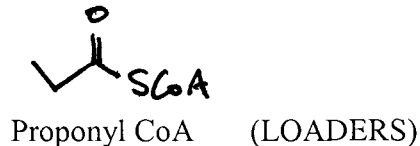
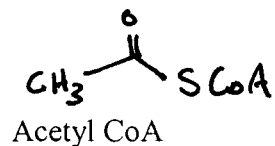
FAS reaction (mammalian)

1 acetyl CoA + 7 malonyl CoA -> CH₃(CH₂)CO₂- (C16)

Outline for discussion

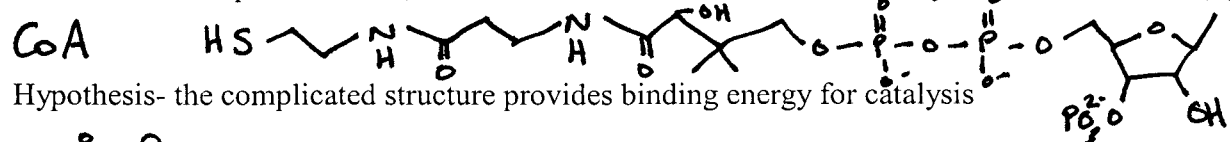
- 1)players
- 2)chemistry
- 3)structure (main focus)
- 4)chemistry as a paradigm for PKS and NRPS
- 5)medical interlude

I. PLAYERS

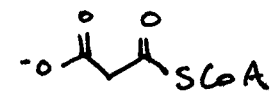


Why choose something as complicated as CoA?

From a chemical point of view, CH₃SH is all that is needed for reactivity.



Hypothesis- the complicated structure provides binding energy for catalysis



Malonyl CoA (MCoA) (EXTENDERS)

How do you put CO₂ onto acetate? BIOTIN!

3 basic ways to form carbon-carbon bonds in biochemistry

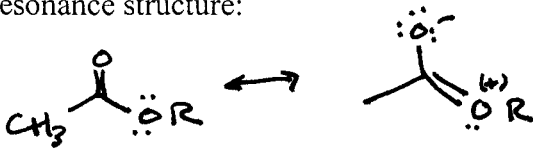
- 1) Claisen condensation (like FAS!)
- 2) Aldol
- 3) Prenyl transferase

Why has nature chosen a CoA thioester (as opposed to an oxygen ester?)

2 key reactions

- 1) acidity of a hydrogen – how easily can you form the carbanion?
- 2) Formation of c-c bond- you want a carbonyl activated for nucleophilic attack

The non-bonded e- on the oxygen of the thioester can delocalize over the carbonyl to give this resonance structure:



This reduces the + charge on the carbon of the carbonyl, making it less activated for nucleophilic attack. This also reduces the acidity of the alpha H, because there is less + charge nearby.

The energy of the orbitals in the thioester is not consistent for e- delocalization, and so the thioester is much more ketone-like. The pKa of the aH of the thioester is 4x lower than for the oxygen ester.

II. CHEMISTRY

See page 6 of Handout 2a

- a) Covalent catalysis
- b) Iterative mechanism – same set of enzymes for each addition

The iterative mechanism implies a broad specificity for substrate – the enzyme can work on C2, C4, C6 etc...

Follow the mechanism of page 6 of the Handout. The SH-pant is the “swinging arm” or phosphopantathione group. Here is a brief walk through of the general steps illustrated on the handout

AcoA binds the cys group.

MCoA binds the pant group (C2)

Decarboxylation reaction and attack of carbonanion of MCoA on AcoA

This step transfers the chain to the pant group. We now have a C4 chain

In the next few steps the chain is fully saturated

Then the saturated chain is transferred back to the cys group, and another MCoA binds the pant group.

Another cycle of elongation can then occur to form C6

Nomenclature for the different steps and enzyme domains can be found on page 3 of handout 2a

III. STRUCTURE

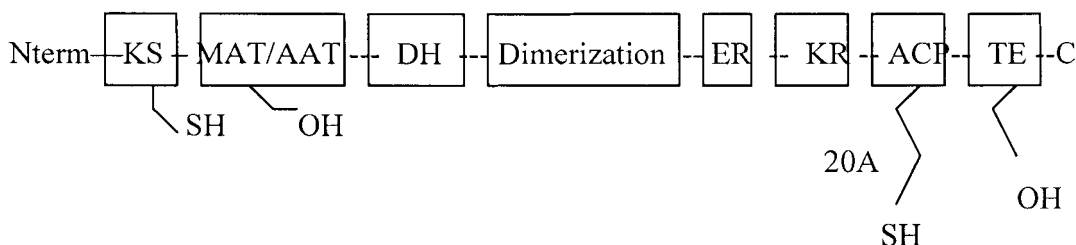
All FAS in prokaryotes and eukaryotes are composed of 7 domains (a domain is defined here as a single active site)

In E.coli (Type II FAS), all activities are on separate polypeptides- 7 different polypeptides (like ACP, KS, ER) catalyze 7 discrete reactions.

Remember, for nomenclature, see page 3 of handout 2a

Are there weak non-covalent interactions between these domains? How are these reactions organized in the cell?

In mammalian systems, the domains are linked together



The domains are connected by “linker regions”

All domains on one polypeptide

4 steps involve covalent catalysis (the substrate is covalently bound to the enzyme)

The residues involved in these 4 steps are shown cys on KS, ser on MAT/AAT, pant group on ACP and ser on TE

Do bacterial proteins have linker regions that could help the proteins interact?

Page 5 of handout 2a shows a cryoEM picture of the structure of mammalian FAS

Page 3 of handout 2a shows the head to tail structure

N-----C
C-----N

How did they determine this orientation? What experimental methods allow us to address the structure? (No Crystal structure available- too “floppy”)

Expt. Methods

1) Limited proteolysis

Do you have beads on a string, with independently folded domains?

Incubate for short time with protease (limited)

Divides enzyme into 3 domains, assay each domain

Next time, we will discuss what they found in each of these three domains, as well as other methods for addressing structure.