Lecture #10

Lecture 10
2/25/04

References
Voet& Voet, Biochemistry, p680-704
Science **232**, 34-47 (1987) -Review
Science **191**, 150-4 (1976) -Methods

From PNAS **97**, 14188 (2000)
Fidelity in PKS

Biosynthesis of Yersiniabactin (mixed PKS, NRPS)
AcetylCoA (AcoA) is present in high concentration in cell-
1)Can you misload ACoA? Yes- in vitro
2) Can it be processed by hydrolysis a requirement for the normal reaction to proceed?

Made mutants of HMWP1 (one of he genes encoding the machinery to make Yersiniabactin) to show that the entire assembly line of domains is involved in hydrolysis of ACoA

Hypothesis: AcetylCoA is hydrolyzed rapidly by a "bucket brigade," using the entire protein: HMWP1

HMWP1= KS AT MeT KR ACP C MeT_3 PCP_3 TE

Results

Construct	AcetylCoA hydrol rate	
His6-HMWP1	100%	$140min^{-1}$
(rate of hydrolysis 100x faster than of ACoA in solution w/out HMWP1)		
His6- PCP_3- TE	.24% -background level of hydrolysis	
His6- HMWP1 – TE- CH_3 (mutant Ser->Ala)	.33%	
His6- HMWP1 –(AT mutant Ser-Ala)	2.5%	
His6- HMWP1-(ACP-CH_3 mutant Ser-Ala)	7.5%	
His6- HMWP1-(PCP_3-CH_3 mutant Ser-Ala)	4.4%	

Conclusions:
PCP3 cannot pick up and hydrolyze ACoA
HMWP1 with TE mutant Ser-Ala = no hydrolysis, so the TE domain is necessary
AT,ACP and PCP domains are all necessary

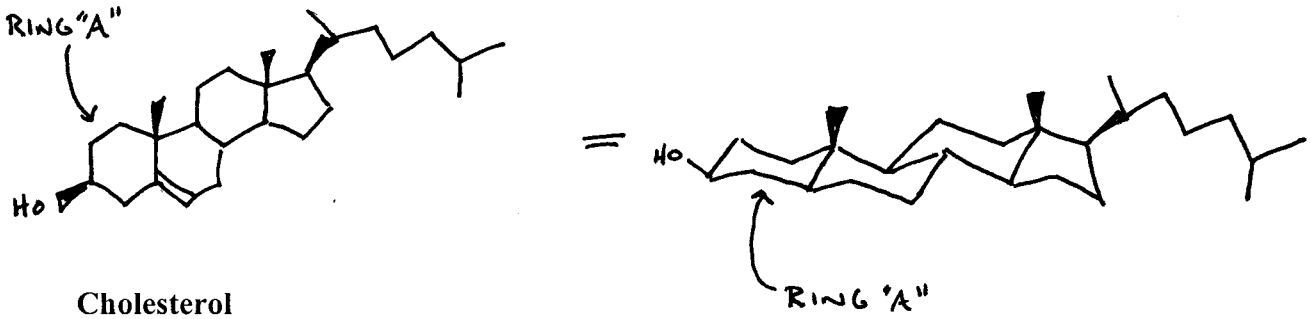
Supports hypothesis

This is an intriguing result that makes you think about the question of fidelity- how do these machines achieve fidelity?

Cholesterol Biosynthesis

I will abbreviate cholesterol as "Ch"

Brown and Goldstein won the nobel prize for their seminal research in the field



Cholesterol

Cholesterol homeostasis is key- Ch is both essential and deadly

3 Nobel Prizes have been awarded for research about Ch
1927- Wieland (structure)
1964- Bloch, Conforth, Popjack (biosynthesis)
1984- Brown and Goldstein (LDL-receptor)

Will Brown and Goldstein win another Nobel Prize?
How do you sense an insoluble metabolite?- New area of research

Ch is a rigid small molecule, hydrophobic, solubility 5 microM

Functions:
-essential constituent of membranes
-affects physical properties of membranes
-precursor to bile acids and steroid hormones (see handout 2d for structures of these)

When Ch homeostasis is not controlled it can be deadly
-deposited in arteries
-cardiovascular disease, strokes
Understanding Ch homeostasis
I Biosynthesis
II Regulation
a)LDL-receptor, receptor mediated endocytosis
b)work in progress – sterol responsive element-binding protein (SRE-BPs)
"sensor of insoluble metabolites"

Overview of stages of Ch Biosynthesis
See page 1 handout 2d

EARLY

1) 3ACoA-> HMG-CoA
2) HMGCoA reductase is major regulatory step (target of statin drugs like lovastatin), takes HMG-CoA to mevalonate (C_6), uses 2NADPH
3) Mevalonate -> isopentenyl pyrophosphate (IPP) (C_5), decarboxylative elimination, uses 3ATP

MIDDLE

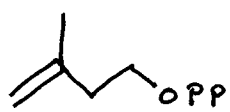
1) IPP is a major building block of natural products – made into rubber by plants (huge oligamer with 10^5 IPP)
2) isomerizes to Dimethylallyl pyrophosphate (DAPP) (used by some tRNAs)
3) IPP and DAPP are converted to Farnesyl –PP (C_{15})

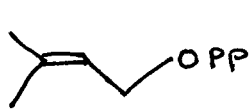
FarnesylPP is used in posttranslational modification of certain Ras proteins, dolichol (plays a central role in posttranslational modification by glycoslyation), CoQ quinones

4) Squalene synthase converts FarnesylPP to Squalene (C_{30}) (major regulated step, drug target)

LATE

1) Squalene is converted into Cholesterol
2) Ch is used to make bile acids, steroid hormones, vitamin D





Structure **Mevalonate** **Isopentenyl PP** **Dimethylallyl PP**

Formation of mevalonate (page 2 handout 2d)
1)AcCoA reversible is converted to acetoacetylCoA via a thiolase
2)HMGCoA synthase converts acetoacetylCoA + ACoA to HMGCoA
3)HMGCoA reductase (2NADPH for hydride transfer) converts HMGCoA to mevalonate

HMGCoA reductase is the rate determining step in Ch biosynthesis! Target of lovastatin-potent reversible inhibitor of HMGCoA reductase

See structure of lovastatin page 3 of handout 2d
Structure of lovastatin mimics mevalonate- targets active site by looking like the natural product
-bottom of lovastatin is a greasy hydrophobic mess-binds tightly into a binding pocket on the enzyme (common strategy in drug design)

Page 2 of handout 2d shows the conversion of mevalonate to IPP and DPP
-uses 3 ATP
-2 phosphorylations of the terminal primary OH of mevalonate
-1 phosphorylation secondary hydroxyl at C_3- makes a better leaving group
-decarboxylation to IPP (C6 is converted to a C5 unit)