Problem Set 2 Feb 18 due Feb 23

1. Pyocheln (Figure 1) is an iron chelator produced when bacteria are in iron-limited environments. The *P. aeruginosa* genome sequencing project revealed two gene clusters, pchEF and pchDCBA required for pyochelin biosynthesis. The domain organizations of PchD, PchE and PchF are shown in Figure 2. The three proteins PchD, PchE and PchF have been purified to homogeneity using the histidine tagging procedure (Figure 3).

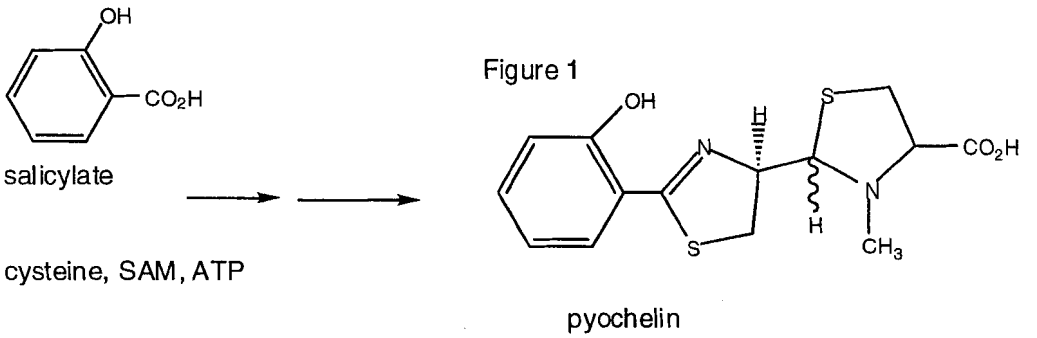
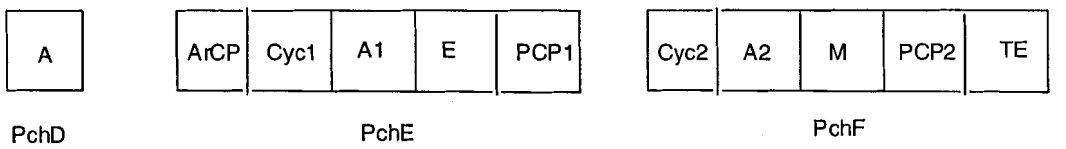


Figure 1

pyochelin

Figure 2

Domain Organization of the PchDEF biosynthetic genes



M = methyl transferase E = epimerase

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You are given the following information about this system.

a. The three proteins purified from *E. coli* when incubated with salicylate, cysteine, ATP and S-adenosylmethionine (SAM) gave no detectable product.
b. Incubation of PchE and PchF with [^{3}H]-CoA (coenzymeA) and Sfp (a phosphopantetheinyl transferase) gave radiolabeled PchE and PchF. Furthermore, a repetition of the experiment in (a) resulted in formation of pyochelin as monitored by HPLC in comparison with an authentic standard.
c. Incubation of cysteine, ATP, and [γ-^{32}P]-inorganic pyrophosphate with either PchE or PchF resulted in the incorporation of ^{32}P label into ATP (see Figures 4B and 4C).
d. A similar experiment with salicylate gave no radiolabel incorporation into ATP with either PchE or PchF, but incorporation of [γ-^{32}P]-inorganic pyrophosphate into ATP was observed with PchD (Figure 4D).
e. One additional experiment incubated [^{14}C] salicylate, cysteine, and ATP along with PchE, F and D and monitored the fate of the label relative to PchE and PchF (Figure 5).

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Figure 4: Rate of incorporation of [γ]-^{32}P-PPi into ATP to make [^{32}P]-ATP as described above.

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Questions: i. Based on what we have learned in class about NRPS and the information given above, propose a pathway for the biosynthesis of pyochelin.

ii. Provide an explanation for the data in (a) and (b) that is consistent with your answer in part i.

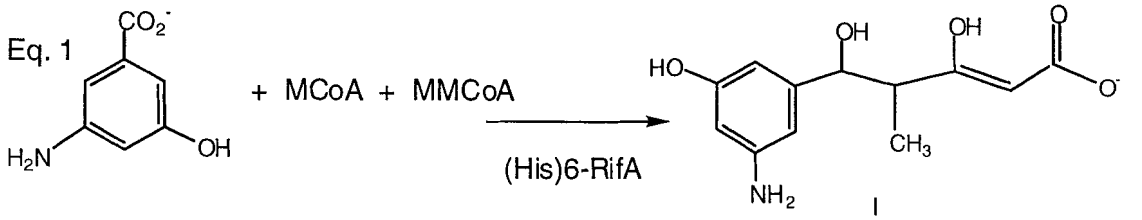
iii. Clearly show the roles of ATP in the biosynthesis of pyochelin and explain how the data in Figure 4 B through D support this role.

iv. Show how the data in Figure 5 provide further support for your mechanism in i.

v. Propose a role for S-adenosylmethionine.

2. Rifamycin is an antibiotic used in the treatment of tuberculosis. The synthase involved in the biosynthesis of rifamycin consists of a core of five multifunctional proteins called RifA, B, C, D, E and F. RifA is the N-terminal protein component. RifA was expressed with a (His)6-tagged tail and purified to homogeneity. The C-terminus of RifA was genetically engineered to possess a TE domain. Incubation of 3-amino-5-hydroxybenzoic acid, malonylCoA (MCoA) and methylmalonylCoA (MMCoA) with the (His)6-RifA protein gave I (Eq. 1).

Eq. 1

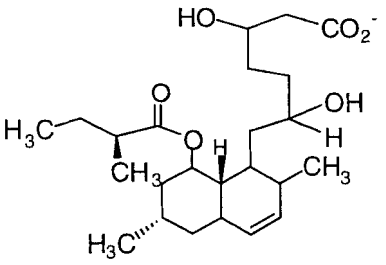


3-amino-5-hydroxybenzoate

I

i. Given the generic rules for PKS and NRPS machines, what does the fact that RifA is the N-terminal module tell you about the sequence of the biosynthetic steps catalyzed by RifA in the pathway?
ii. Post-translational modification of domains within PKS and NRPS modules has been essential for obtaining proteins that are active in making natural products such as rifamycin. Show the domains and how they must be modified (describe the chemistry) to generate active RifA so I can be made.
iii. What is the chemical transformation required for the activation of 3-amino-5-hydroxybenzoate as a precursor to I?
iv. Draw a cartoon diagram (as in the article by Keating and Walsh) of the domains and their order within the RifA module required for the biosynthesis of I. [Note, some of you may find it easier to answer v. before iv.]
v. Propose a biosynthetic pathway for the conversion of starting materials to I in Eq. 1. You may use ATP or redox cofactors if you think they are required.

Extra Problem (shows you the complexities of biosynthesis and tougher than you will be expected to solve)
Aspergillus is a fungus that makes lovastatin, a potent inhibitor of HMGCoA reductase (rate determining step in cholesterol biosynthesis). Variations of this drug make billions of $$ for drug companies as cholesterol lowering agents. There are three genes (lovb, lovc and love) required for the biosynthesis. Lovb also called LNKS is an iterative nonaketide synthase and contains almost all of the information required to make dihydromonacolin L an intermediate on the pathway to lovastatin.



Lovastatin

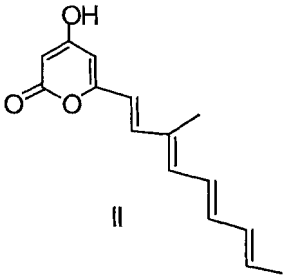
LNKS, lovb

KS AT/MAT DH MeT KR ACP X

dihydromonacolin L, I

There are a number of unusual features associated with this pathway. One is that the construction of the ring system requires a Diels Alder reaction. If this proposal is correct, this would represent the first example of such a reaction.

The second unusual feature of lovB is that it appears to be missing an ER domain which appears to be supplied by one of the other two gene products in the entire biosynthetic pathway for lovastatin. If one expresses only lovb, then one obtains several products, one of which is shown below (II).



II

If all three gene products are expressed together one can find lovastatin and also I.

Propose a biosynthetic pathway to get from the starting materials to I, using a Diels Alder type reaction and the fact that an ER domain can be supplied by another gene product in the pathway.