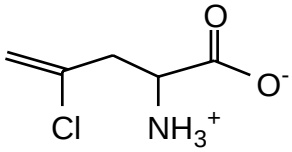
Problem Set 7

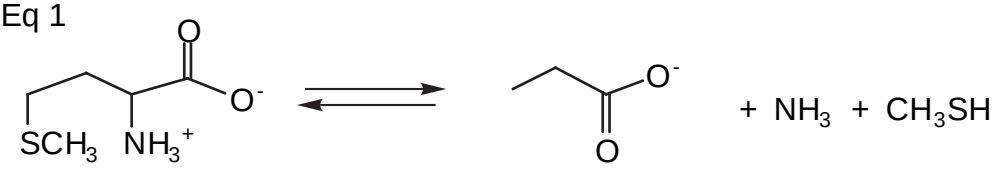
1. 2. L-2-Amino-4-chloro-4-pentenoic acid (L-ACP) is a natural product isolated



L-ACP

from fruit bodies of *Amanita pseudoporphilia* that inhibits the growth of bacteria, both *E. coli* and *Pseudomonas aeroginosa*. Extensive studies revealed that the target of this inhibitor is L-methionine γ-lyase. This enzyme catalyzes the conversion of L-methionine to α-ketobutyrate, methanethiol and ammonia (Eq. 1).

Eq 1



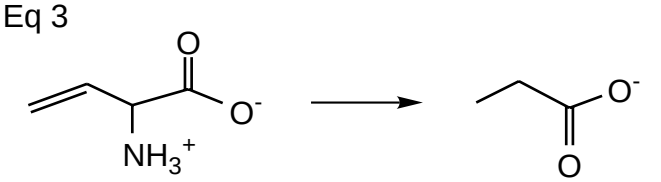
L-methionine α-ketobutyrate

The following information has been obtained about this protein:

1. The enzyme catalyzes the rapid exchange in D_2O of both the α and β hydrogens of straight chain L-amino acids such as alanine and α-aminobutyrate, that are not susceptible to elimination. Deuterated amino acids in both cases are obtained.

2. The enzyme also catalyzes the conversion of vinyl glycine to α-ketobutyrate (equation 3).

Eq 3



vinylglycine α-ketobutyrate

3. The enzyme is inhibited in a time dependent fashion by L-ACP. When [^{14}C]-ACP is incubated with the protein, a peak of radioactivity coelutes with the protein through a Sephadex G-25 column (a material that separates proteins from small molecules). The stoichiometry of the reaction is 4 moles of [^{14}C] inhibitor per tetramer of lyase.

4. The structure of the enzyme from a thermophile has been solved to 2.2 Å in the presence of propargylglycine. It is deposited in the folder marker ps 7 under pdb IE5E.

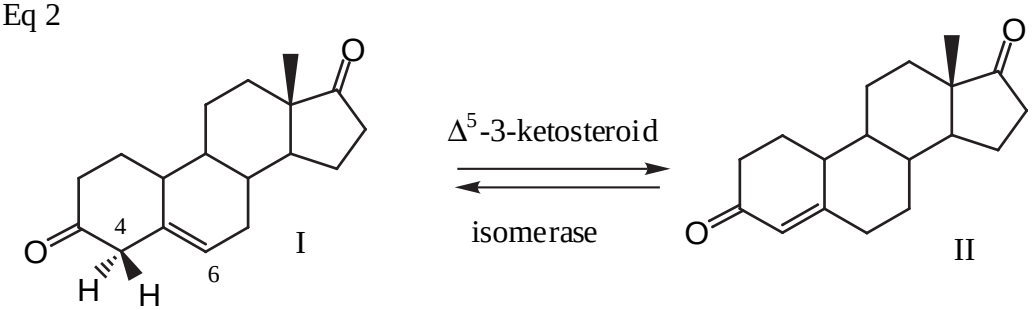
Questions: 1. Draw a flat diagram of the active site and indicate the residues that might be involved in general acid and base catalysis and the distances of these residues to amino acid side chains to which proton transfer occurs.

2. Propose a mechanism for this enzyme showing postulated roles for you identified general acid and base catalytic groups. Indicate how the information in parts 1 and 2 can be accommodated within your proposed mechanism.

3. Based on your proposal for the normal reaction, provide an explanation for the mode of inhibition by L-ACP. Design an experiment that would test your mechanistic proposal for inhibition. Clearly state the question being addressed and any problems or ambiguities that might arise from your experimental design.

2. The enzyme Δ^5-3-ketosteroid isomerase catalyzes the isomerization of Δ^5-3-ketosteroids to the conjugated Δ^4-3-ketosteroids (Eq. 2), accelerating the rate of this process by a factor of $10^{9.5}$ over the corresponding non-enzyme catalyzed reaction. In the past few years the mechanism of this reaction has been intensely investigated and the following experimental information has been obtained.

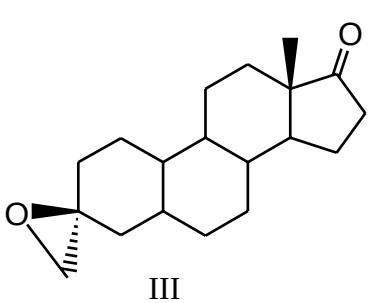
Eq 2



1. The x-ray structure of the isomerase in the absence of any ligands has been published and the NMR structure in the presence of 19-nortestosterone hemisuccinate has been solved. The NMR structure is deposited in ps 7 (pdb. 1BUQ.

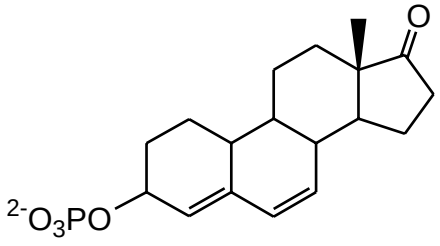
2. Incubation of [4-β-^{2}H]-I with enzyme resulted in the production of 100% of [6-β-^{2}H]-II with a ^DV of 6.1, when the reaction was run in H_2O. Deuteriation of the C-6 position of I produced no kinetic isotope effect on the reaction.

3. Incubation of the isomerase with a [^{14}C]-steroidal 3-β-oxirane, III, showed time dependent inactivation of the enzyme. The enzyme is not inactivated by the 3-α-oxirane. The inactivated protein was cleaved with trypsin and the labeled peptide isolated. The peptide was then treated with NaB3H4 and the peptide hydrolyzed with acid to give a [^{3}H]-labeled homoserine residue. The pH profile on the inactivation exhibited an apparent pKa of 4.7. This profile is very similar to the pH profile on kcat and kcat/Km for the normal isomerization process.



III

steroid 3-β oxirane



IV

Questions: 1. Draw a flat structure of the active site ligand and amino acid side chains that might be important in catalsis. Indicate the appropriate distances.

2. Propose a mechanism for the ketoisomerase. Show how each piece of evidence in 1 through 3 above can be accomodated by your proposal. Use the residues that you have identified in 1. in your mechanims.

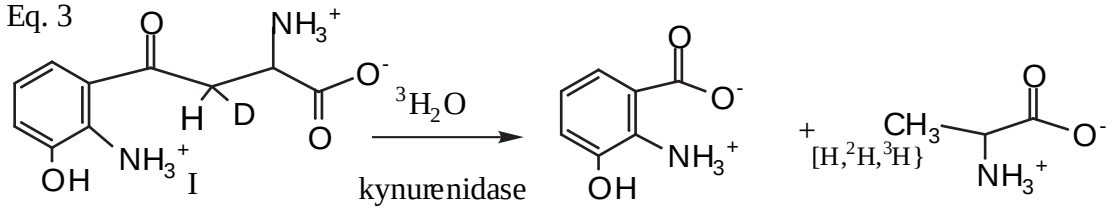
3. To test for a putative intermediate in this pathway, compound IV was synthesized and incubated with either alkaline or acid phosphatases. Is this a good experiment? Given your mechanism what might you anticipate the results of such an experiment to be?

Questions: 1. What cofactor might be involved in this transformation? Propose a mechanism that could account for the experimental data in parts 1 and 2. Show clearly how this data is accomodated within your proposal.

2. Propose a mechanism for the inactivation of SAH hydrolase by V.

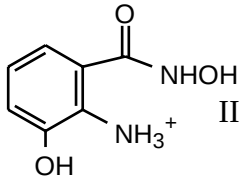
Problem Set 7 The exam is open notes, but **not** open book. You are not allowed to discuss the problems with your classmates.
Due date: Saturday May 16th. There will be a folder on my door Blg 18 Room 480B. If you have any questions about the examine, contact me by e-mail: stubbe@mit.edu.
3. Kynurenidase is an unusual pyridoxal phosphate (PLP) requiring enzyme involved in the degradation of tryptophan. The reaction catalyzed by the enzyme is shown in Eq. 3.

Eq. 3



A few experiments have been carried out that are mechanistically informative:

a. If hydroxylamine (NH_2OH) is added to the reaction during the course of



II

turnover, the hydroxamte II can be isolated.

b Starting with prochirally labeled [H, ^2H]-I and running the reaction in ^3H_2O, alanine with a chiral methyl group is obtained.

Questions 1. Propose a mechanism for this PLP requiring enzyme.

2. Succinctly describe how your mechanism accommodates the data in a and b.

3. Describe the method by which the investigators might have established that the alanine product contains a chiral methyl group.