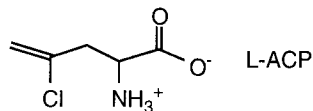
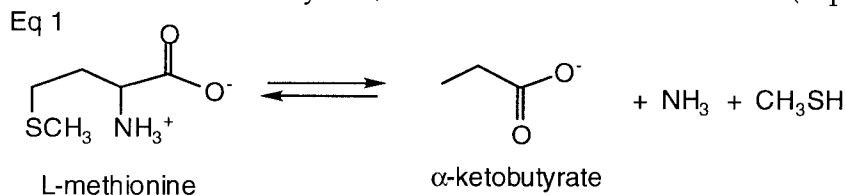


Problem Set 7 PLP and NADH chemistry

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



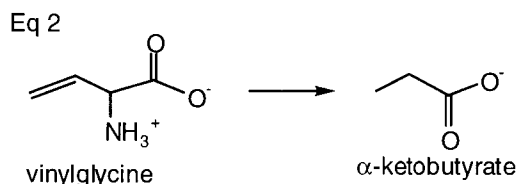
from fruit bodies of *Amanita pseudoporphyria* that inhibits the growth of bacteria, both *E. coli* and *Pseudomonas aeruginosa*. Extensive studies revealed that the target of this inhibitor is L-methionine γ -lyase. This enzyme catalyzes the conversion of L-methionine to α -ketobutyrate, methanethiol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



The following information has been obtained about this protein:

1. The enzyme catalyzes the rapid exchange in D₂O 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 vinylglycine to α -ketobutyrate (Eq. 2).



3. The enzyme is inhibited in a time dependent fashion by L-ACP. When [¹⁴C]-ACP is incubated with the protein, a peak of radioactivity co-elutes 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 [¹⁴C] 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.

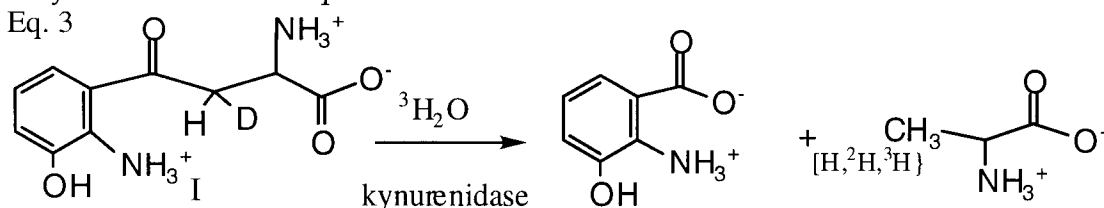
Questions: 1. Draw a flat diagram of the active site and indicate the residues that might be involved in general acid and base catalysis and the distances of these residues to amino acid side chains to which proton transfer occurs.

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

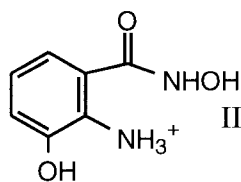
and any problems or ambiguities that might arise from your experimental design.

2. Kynureninase 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.



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 turnover, the hydroxamate II can be isolated.

b. Starting with prochirally labeled $[\text{H}, ^2\text{H}]\text{-I}$ and running the reaction in $^3\text{H}_2\text{O}$, 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.