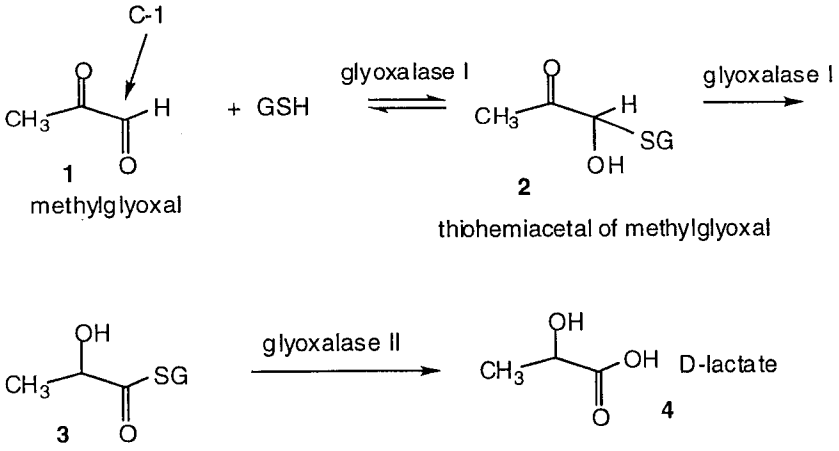
Problem Set 6 Chemistry 5.50 Nov 18 due Nov 25

Isotope effects, stereochemistry genomics

1. Glyoxylase I catalyzes the conversion of the thiohemiacetal of methylglyoxal (**2**) to the thioester of D-lactate **3**. The enzyme (glyoxalase I) also catalyzes the formation of **2** from methylglyoxal and glutathione. Compound **3** is hydrolyzed to compound **4** (D-lactate) by a second enzyme, glyoxalase II.



The following information has been obtained about this system.

i. Incubation of [1-^{3}H]methylglyoxal (**1**) with glyoxalase I results in no transfer of tritium to the solvent. If the reaction of methylglyoxal is run in $^{3}H_2O$, NO ^{3}H is found in the lactoyl thioester **3**.

ii. The mechanism of the reaction has been probed by use of a substrate analog fluoromethylglyoxal (FCH_2COCHO). Incubation of fluoromethyl-glyoxal and GSH with glyoxalase I results in the production of pyruvyl-glutathione ($CH_3COCOSG$) and F^- loss as well as fluorolactoylglutathine ($FCH_2CHOHCOSR$). Elimination of fluoride can be monitored using a F^- electrode (μM detection).

iii. If FCH_2COCDO (1-deuterated fluoromethylglyoxal) is utilized in place of the protonated material, the partitioning of the production of pyrvuyl-glutathione to fluorolactoylglutathione increases from 1:1 in the case of ^{1}H material 3:1 in the case of the ^{2}H material.

Questions a. Propose two chemically reasonable mechanisms for the glyoxylase I reaction. Both of these mechanisms have chemical precedent.

b. Use the information given in i., ii., and iii. and show how it can eliminate one of your two mechanisms. If you only have one mechanism, then show how each piece (i, ii, iii) of information can be accommodated by your mechanism.

2. This problem focuses on mandelate racemase, briefly presented in class as a member of the enolase superfamily of proteins.

i. The structure of a mandelate racemase mutant (D270N) has been placed in the folder labeled problem set 6 (pdb. 1MRA). Pull up the structure in the presence of the inhibitor (S)-atrolactate and draw a picture of the active site with groups in the nearby regions that might be involved in catalysis.

ii. You are also given the following information:

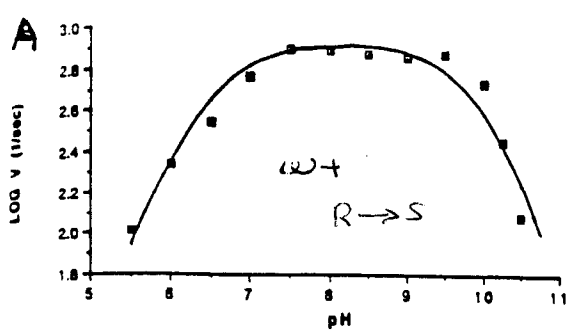
Table 1 Summary of Solvent Hydrogen Incorporation into Substrates and Products
R-or S- [α-^{1}H] mandelate in D_2O

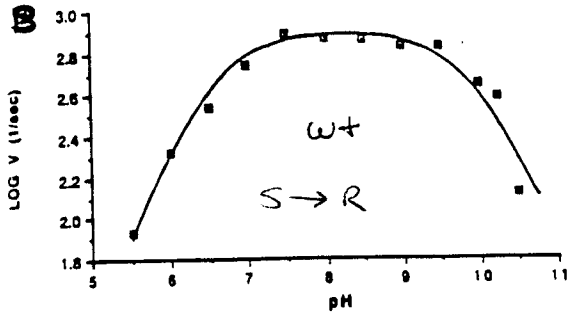
direction	% turnover	% H in product	% D in substrate
R—>S	5.3 %	1.8%	0.2%
S—>R	5.1%	3.0%	3.5%

Now repeat the experiment using [α-^{2}H]-mandelate in H_2O

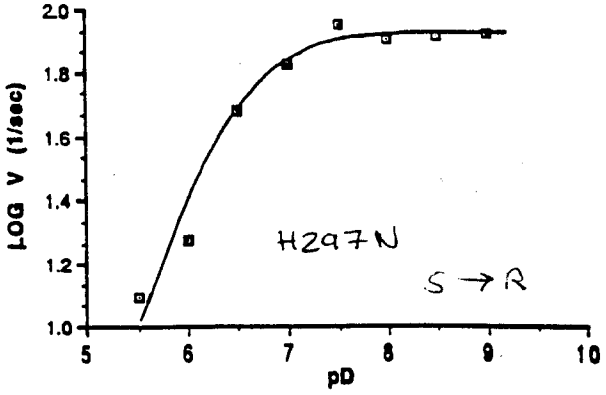
Direction	% turnover	% D in product	%H in substrate
S—>R	6.6%	2.0%	2.1%

3. pH Rate profiles (on k_{cat}) have been carried out on mandelate racemase. The following data has been obtained for the wild-type enzyme in the R—>S (A) and the S—>R direction (B).





iii. The pH profile was repeated on a site directed mutant of mandelate racemase in which H297 is converted into N and the results for the S—>R direction are shown:



Questions:

a. Based on the structure, would you expect a single residue to function as the base/acid catalyst or two different residues to function as the base/acid for the racemization? Propose a mechanism clearly indicating what residues are involved in this racemization reaction. Which residues are involved in stabilization of the transition state of the reaction?

b. How can you rationalize the exchange data in Table 1, specifically the "washin or lack thereof" of solvent into the starting substrate? Clearly explain how this data supports your proposal in question a, drawing the stereochemistry of the active site relative to the S-mandelate isomer.

c. Provide an explanation for the observed pH rate profiles with the wild-type racemase? Is it consistent with your model in part b?

d. What does the study with the H—>N mutant of racemase tell you about your mechanism in parts a and b? Are these results unusual? What insight does the active site structure give you that allows you to readily rationalize these results?

e. As we discussed in class, mandelate racemase is proposed to be part of the enolase superfamily of proteins. Find the sequence of mandelate racemase from pseudomonas putida from the ncbi web site and use this sequence to run a Blast search. The report that comes back gives you proteins that are, to differing extents, homologous to mandelate racemase. What are these proteins? If the function of the protein is known, examine what is known about the mechanism of the reaction that it catalyzes. Is it reasonable that these enzymes are sequence homolous to mandelate racemase when you compare the chemistries? Pull up the sequence for enolase and compare it to mandelate racemase. Would you ever have predicated that these proteins are related?