Determination of the Stereochemistry of the Fumarase Induced

Conversion of Fumarate to Malate- A Discovery Based Lab

This project will involve carrying out the fumarase catalyzed conversion of fumarate to malate followed by the study *of* the reaction mixture by proton NMR. Through the measurement of the proton- proton vicinal coupling constant of deuterated version malate, the stereochemistry of the enzymatic reaction will be determined, i.e., whether the addition of water is syn or anti. Please work in groups of three or at least pairs if there are numbers that don't work. Only work with people on your side of the lab if you can.

To prepare for the project, please consider the following:

Do the best you can with this. Realize a lot will be covered in laboratory lecture and the lab is two weeks long.

**Go to laboratory lecture. During lecture take notes and work on the worksheet that will be provided.

**In regard to the lab notebook, you should have for the first week, a brief introduction that includes the general purpose of the experiment, the broad importance of the experiment and the reference of the procedure and the procedure is given below. That is all that is needed.

The following reading/investigating is recommended before coming to lab.

1. *Review the reaction in question by consulting any Biochemistry textbook in the library, e.g., Stryer, Lehninger, etc, etc. The conversion of fumarate to malate is part of the krebs citric acid cycle.

2. *Write out the structure of fumarate and consider the syn and anti addition of deuterated water to it. Write the products of this reaction in their most favored conformers. What is the torsional (dihedral) angle between the hydrogens in the deuterated malate? If you struggle with this, there will be time in lab to work on this part.

3. *Read up on the Karplus relationship (how coupling constants are related to proton-proton torsional angles) . There is a paper on our current Moodle page that relates NMR to Biology. In it there are some very useful Karplus relationships. This should be adequate preparation for this lab.

4. *What do you expect the final NMR to look like given the fact that the reaction reaches **an** equilibrium meaning that both starting material and products are present. Simulate this spectrum if you can. If you struggle with this there will be time during lab to work on this simulation.

5. *The reaction must be run in buffer in order to preserve the activity of the pH sensitive enzyme. How would you make up a 0.01 M, pH = 7.3 potassium phosphate buffer? You need to use the Henderson-Hasselbalch equation here. Again, this issue will be covered in class. If you struggle bring your issues to lab.

Procedure:

**Obviously you will have to carry out the fumarate to rnalate reaction. It is easily set up by mixing 1 mL of phosphate buffer (D_2O is used as the solvent for this buffer) with 0.1 gram of disodium fumarate and 10-15 microliters (this will be specified on your lab day) of fumarase in a clean, new vial. Add the components of the reaction in the order given. A TA or instructor will be helping with the measurement of these quantities. The instructor or TA will review the use of the Eppendorf pipet and the syringes used for the addition of the enzyme and buffer, respectfully. In this course we try not to start over or waste expensive materials. Please tell us if anything goes wrong and we will work together to salvage the lab. The reaction mixture can rest in your locker in a capped vial during the reaction time. Please label this vial. Next week you will run an NMR of the active reaction to establish the stereochemistry of the addition reaction. What we are doing will make more sense after lab lectures and doing the reading above.

Please be very mindful of the fact that the deuterated buffer and the enzyme are very, very expensive. You will be measuring the buffer with a syringe - no waste and the enzyme with an Eppendorf pipet - no waste. You have to be very careful and we will be going over this with you individually. Sorry for the redundancy. If you are curious, look up what would happen if you drink D_20 rather than water.

Also, you will note that the materials in the course will be distributed in a very orderly fashion. The material in question, if liquid, will be in a central dispensing hood. It will be sitting on a label taped to the bench. There will be a labeled syringe, graduated cylinder or Eppendorf pipet for each material. Please use the provided delivery method. Take your time and think about it, read labels and put things back. If it is a solid, the material will be distributed by the balances. Similarly, there will be a labeled spatula prepared for each material and a label on the bench for the material. Again, put things back where you found them. Accidents happen and mistakes are made. If anything is not making sense or you have doubts about what you did, just ask. If anything goes wrong just ask. We are pretty good at salvaging and troubleshooting. The lab is not about performance, but learning. Many reactions can be run in spite of errors.

Completion of the provided worksheet (much of which will be completed in lecture and in lab) constitutes completion of the lab. One worksheet is due per student in your TAs folder by the week after the lab is completed as indicated on the schedule for the lab (this is the location of all due dates). Please do not make this into a long ordeal where you are rewriting, etc. It is designed to be fast. It is expected that all members of the group will have a strong knowledge of all the above areas. Emphasize the learning part of this exercise. If you do not understand the worksheet please ask.