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 NMR. Through the measurement of the vicinal coupling constant of 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.

**In regard to the lab notebook, you should have for the first week, an 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 can be done in preparation, but are optional.

1. *Review the reaction in question by consulting any Biochemistry textbook in the library, e.g., Stryer, Lehninger, etc, etc.

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? This does not have to be done ahead of lab lecture. We will work on the worksheet in lecture and in lab.

3. *Read up on the Karplus relationship (how coupling constants are related to torsional angles) , V. M. Absorption Specctroscopy of Organic Molecules. This reading is on the noodle site. Then read the other paper I have posted on noodle that is on using NMR to study biology. You can find the best Karplus equations in this paper.

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. This is not required and will be worked on in class.

5. *The reaction must be run in buffer in order to preserve the activity of the pH sensitive enzyme. You may be making your own buffer depending on how many students take on this project. 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. It is ok if you save this for class.

**Obviously you will have to carry out the fumarate to rnalate reaction. It is easily set up by

mixing 15 units of fumarase (about 10 microliters - see what is posted in lab) with 0.1 gram of sodium fumarate in

1 mL of phosphate buffer (D_2O is used as the solvent for this buffer). An NMR will be measured the day the reaction is run and anywhere from a day to a week from starting the reaction. The reaction mixture can sit in your locker in a capped vial during the reaction time.

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 Eppindorf pipet - no waste. You have to be very careful and we will be going over this with you individually.

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 mailbox by the end of lab, during the third week of the semester. 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 after completing the exercise.

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