Biochemistry Lab

MDH-CS Project Design Worksheet

Use this document to help your understanding of the background of the specific interaction between MDH and CS. When done, it will support your design of a hypothesis for the MDH-CS research problem and along with the other documents on protein-protein interactions and protein interaction assays, form a complete background and method development for your project. Like the other guides and worksheets, this is an ungraded handout. Write your answers in a separate entry in your laboratory notebook.

First read the MDH-CS Interaction review and read through the papers linked on the class website. Then, with your group, discuss and answer each of the following questions:

1) For background information, describe the role of MDH in mitochondrial metabolism. In these notes and from the initial handout, explain the thermodynamics of both MDH and CS in the TCA "forward" direction. And reviewing from biochemistry, describe why these enzymes should be shuttled/substrate channeled using thermodynamic reasoning (i.e. coupled reactions).

2) Review the Goward and Nicholls paper and describe the chemical mechanism of MDH. Look up the reaction of Citrate Synthase from your biochem textbook.

3) There are two isoforms of human MDH, MDH1 and MDH2. If you don't remember what an isoform or isozyme or isoenzyme is, please look up now. Which isozyme MDH1 or MDH2 is mitochondrial and which is cytosolic? Look up the website (https://www.uniprot.org) and search for MDHM_HUMAN. What other information on the enzyme can you find? Search for human CS, what other interesting information can you find from there?

4) First understand how do the authors of the Tompa et. al. JBC 1987 paper determine interactions using anisotropy? Then review figure two. Describe the observations and conclusions from that figure.

5) From the MDH-CS review handout, click on the link of the Manley, Yu, Datta, Ackerson and Spivey ABB, 1987 paper. Also review the results described in the Shatalin et. al Biochemistry 1999 paper found in the linked paper 3. Do the authors of these two papers come to the same conclusions about both MDH1 and MDH2 interacting with CS as does the Tompa paper? Please consider the implication of your answer.

6) Linked papers 4,5 and 6 are from the same research group. They used crosslinking to determine where MDH and other proteins including CS interact.
   a. What does lysine crosslinking mean and how is this used with mass spectroscopy to determine where MDH and other proteins interact?
   b. Review the summary from your MDH-CS review of the three linked papers. What is the over combined view of the three papers as determined by the MDH-CS review of these three papers?
   c. The authors show different docking of MDH and CS depending on the protein source. What are the three types of proteins the authors used to crosslink MDH-CS?
   d. Are there differences in the residues of MDH that bind to CS for each enzyme source?
   e. If the authors show some of the lysines are close enough to CS to interact and crosslink, doesn't that mean only lysine is important in interaction between MDH and CS? Defend your answer.
   f. The authors investigated the key amino acid residues interacting with MDH by site directed mutagenesis. What amino acids were mutated to what residues? How does this change the interactions in the interface of the two proteins? What did they find?
   g. Did the authors experimentally examine for the key residues of MDH that interact with CS?

7) Review questions 4 and 5, then look at the alignment of both MDH1 and MDH2. If both MDH1 and MDH2 have the same amino acids at or near crosslinked lysines from MDH, does that mean this region of MDH is responsible for isozyme specific binding? What would you expect to observe in MDH-CS interactions where the primary structure are very different between MDH1 and MDH2?

8) Review post-translational modification. In specific, remember what a protein kinase and phosphatase does. What are the amino acids involved in kinase/phosphatase reactions? What are the changes of the non-covalent intermolecular forces of the amino acids that become phosphorylated? Remembering that some of the amino acids are possibly phosphorylated in the interface, how would this impact potential protein-protein interactions?

9) Does phosphorylation automatically support or inhibit protein-protein interaction? Are there unique phosphorylation sites on MDH1 that do not exist on MDH2? Are there phosphorylation sites in common? What might this mean for controlling the interaction between MDH-CS?