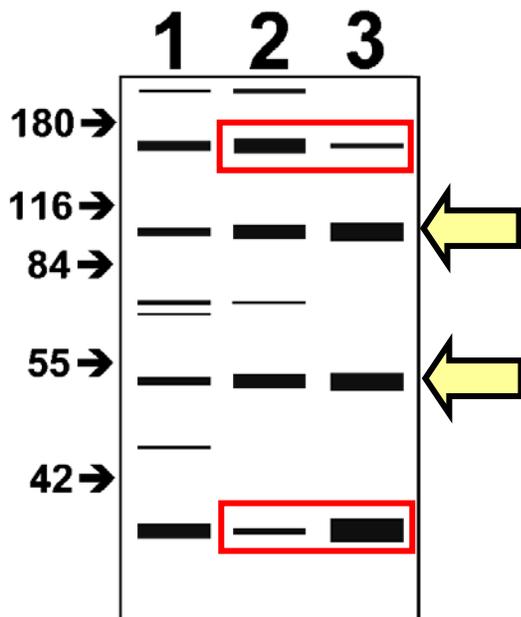


HOMEWORK #6 KEY

1. Identify the protein band(s) on the gel which are most likely to be responsible for the enzyme activity? (You can give approximate sizes or indicate directly on the gel with arrows, asterisks, circles, etc.) Explain your answer.



The arrows denote 2 bands of approximately 100 kDa and 50 kDa that are enriched by both gel filtration and isoelectric focusing as compared to the original starting material. None of the other bands are enriched by both methods.

2. What predictions, if any, can you make about subunit composition of the enzyme? Explain your answer.

The native molecular mass as determined by gel filtration is 300 kDa. The denatured polypeptides have masses of 100 kDa and 50 kDa and the 100 kDa and 50 kDa proteins are of approximately equal abundance. This suggests the protein consists of 2 subunits of the 100 kDa polypeptide and 2 subunits of the 50 kDa polypeptide.

3. How close to being completely purified will the enzyme be if the gel filtration and isoelectric focusing are carried out as sequential steps? In other words, how many contaminating bands and how much would you still expect in a preparation in which the two procedures were carried out sequentially (i.e., either subject the 300 kDa gel filtration peak to isoelectric focusing or subject the pH 6.9 isoelectric focusing fraction to gel filtration)? You can indicate on the figure the contaminating bands or draw a figure of the expected gel.

There are 2 polypeptides in addition to the 100 kDa and 50 kDa polypeptides that are found in both preparations (boxed in red in above figure). An approximately 150 kDa

polypeptide is enriched slightly by gel filtration, but decreased by isoelectric focusing and an approximately 35 kDa polypeptide which is enriched by isoelectric focusing, but decreased in gel filtration. Since neither method completely eliminates either of these proteins you would expect both to be present in a preparation in which the two techniques were performed sequentially. However, both the 150 kDa and 35 kDa polypeptides would be expected to be of lower amounts in the final preparation. In other words, the final preparation would look similar to lane 3 above except there would be much less of the 35 kDa polypeptide.

4. Assuming that the fold-purification and yields are similar if the steps are done sequentially as compared to being done separately, what would be the expected specific activity following combined gel filtration and isoelectric focusing?

The fold-purification following gel filtration is approximately 2 ($=1595/812$). The fold-purification for the isoelectric focusing is approximately 2.4 ($=1933/812$). The expected fold-purification for combining these techniques would be the product of the two or 4.8. Therefore, you would expect a specific activity of approximately 3900 units/mg protein (4.8×812).

5. Which technique would you carry out first? Why?

In the absence of other information I would do the isoelectric focusing first. Isoelectric focusing requires a low ionic strength and gel filtration is usually carried out in high salt concentrations to minimize interactions with the matrix. Therefore, if the gel filtration was carried out first, it would be necessary to remove the salt before doing the isoelectric focusing. The ampholytes from the isoelectric focusing will not affect the gel filtration. In fact, the gel filtration step will also function in removing the ampholytes.