

Answer Key - Quantitative Question from Lecture #8

(a) Because 98.5% of the initial activity (100 units) was lost upon passage of the phosphatase over the column, ion exchange has either (i) removed an essential activator of this enzyme, or (ii) dissociated the enzyme into its constituent subunits. So, Fr. #3 probably contains either (i) the intact phosphatase in a fraction that happens to still contain a little bit of the activator, or (ii) it represents the elution position of a small amount of residual native enzyme that was not dissociated into its constituent subunits.

(b) The results of the fraction-mixing experiments suggest that possibility (ii) above is the likely explanation. Namely, the phosphatase is composed of two different subunits that have been dissociated by competition for binding with ion exchanger and one polypeptide eluted in Fr. #8 and the other eluted in Fr. #12. In other words, the fact that mixing Fr. #8 with Fr. #12 yields very robust phosphatase activity suggests that each of these two fractions contains a different separated subunit of the enzyme and that these two subunits can reassociate and reconstitute the functional phosphatase.

(c) The fact that combining Fr. #8 and Fr. #12 together yields 250% more phosphatase activity than was measurable in the initial crude cell extracts indicates that the crude extract contained an inhibitor and that ion exchange chromatography removed the inhibitor, or at least separated the inhibitor away so that it is not present in either Fr. #8 or Fr. #12.

(d) There would be little or no detectable activity again (except maybe a tiny bit in Fr. #3) because competition with the ion exchanger would cause dissociation of the phosphatase into its constituent subunits again.

(e) In general, the media / beads used for size exclusion chromatography are inert and uncharged and do not interact with the proteins being subjected to filtration. So, one would expect that the phosphatase would not dissociate, and therefore robust activity of the intact enzyme would be detected in a single peak.

(f) This situation is related to (c) above. If the inhibitor passed through the column during its loading and washing, and hence is not present in any of the 20 eluted fractions, or if the inhibitor stayed stuck on the column because it was not eluted into any of the 20 fractions even at the highest salt concentration of the gradient that is typically used to elute ion exchange columns, then mixing all 20 fractions together should give 250 units of phosphatase activity. If, however, the inhibitor is present in one or more of the 20 fractions, when you mix them all together, you should get only 100 units of phosphatase activity (or maybe a bit higher because the volume of the 20-fraction mix is probably larger than that of the initial starting extract applied to the column and therefore the inhibitor will be present at a lower concentration and thus may be less effective).