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Homework #7 Key

The basic questions is why are there 2 proteins detected in the non-denaturing gels when only a single protein/polypeptide is detected in SDS gels. The similarity in size between the protein detected in SDS gels and the faster migrating (i.e., smaller) protein in the non-denaturing gels suggest that they are the same. Therefore, the question can be refined to why is the 270 kDa protein only detected in the non-denaturing system.

The Coomassie blue stained gels indicate that the preparation is not highly purified in that many non-relevant bands are detected. However, Coomassie-blue staining bands corresponding to the proteins detected by immunoblotting are apparent.

There are numerous possible explanations for the results, but they can be generalized into 3 broad categories:

1. Technical problems with electrophoresis systems.

   The lack of detection of the 270 kDa protein in the SDS gels might be due to the 10% acrylamide concentration and the lack of resolution, or the 270 kDa protein may have been inefficiently transferred out of gel matrix to the membrane.

   This is relatively easy to test by carrying out the SDS gel electrophoresis on 6% acrylamide gels.

   Similarly, one could propose that the 270 kDa protein did not stick to the membrane (eg., due to excessive SDS) and did not bind to the membrane. However, this tends to be more of a problem with small molecular weight proteins (<15 kDa).

2. Quarternary structure or protein complexes.

   The antibody could be specific for the 60 kDa protein which is a subunit of a larger protein complex or binds specifically to another protein(s) such that the size of the complex is approximately 270 kDa. This explanation would also require that there be an equilibrium between the monomer form and the multimeric form or stoichiometric considerations, since two distinct sizes are detected simultaneously. The interactions could be simple protein-protein interactions or involve di-thiol (S-S) bonds.

   A simple test for this explanation is to excise the 270 kDa band from the non-denaturing gel and subject it to SDS gel electrophoresis. If the 60 kDa protein is part of a protein complex then a 60 kDa protein should be found on SDS gels. Similarly, a two-dimensional gel with the non-denaturing gel in the first dimension and a SDS gel in the second dimension would give similar results. Testing for di-thiol bonds would involve electrophoresing the proteins on gels with and without β-mercaptoethanol.

   Other analyses, such as peptide mapping, N-terminal sequencing, gel filtration and immunoprecipitation, could also be used to resolve this issue (see below).

3. Conformational dependent epitope

   The antibody could be non-specific and recognize two distinct proteins and the 270 kDa protein has a conformational dependent epitope which is not recognized by the antibody after denaturation or reduction by β-mercaptoethanol.

   This explanation is the most difficult to directly test and confirm since the lack of detection is a negative result. One could excise the band from the non-denaturing gel as discussed above and subject it to SDS gel electrophoresis and analyze by Commassie blue staining. A band of approximately 270 kDa which stained with the dye, but not with the antibody, might be found if there were enough protein recovered.

   Similarly the protein(s) recognized by the antibody could be purified by immunoprecipitation or affinity chromatography on an antibody column and analyzed by electrophoresis and Coomassie blue protein staining. The number of protein bands and their sizes might help resolve the issue.

   The other approach would be to try other separation techniques and see if the same phenomenon is observed. The most obvious is gel filtration since it also separates by size. The goal would not necessarily be to confirm that the 270 kDa protein has a conformational dependent epitope, but to accumulate multiple forms of supporting evidence.

   One could also do peptide mapping or N-terminal sequencing of the 60 and 270 kDa proteins to determine if they are distinct proteins or the same protein. The interpretation of these results in the case of a complex composed of different polypeptide subunits might be complicated due to a mixture of proteins though.