SDS and NATIVE Gels in Protein Analysis

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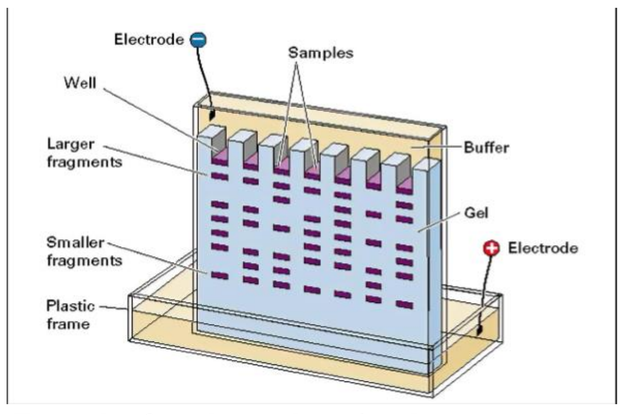
Background and Aim

This research project explores the principles and applications of gel electrophoresis under denaturing (SDS PAGE: **S**odium **D**odecyl **S**ulfate **P**oly **A**crylamide **G**el **E**lectrophoresis) and non-denaturing (Native PAGE) conditions. SDS and Native PAGE are two important lab techniques for protein analysis. SDS PAGE denatures proteins by surrounding them with uniform negative charges thereby enabling separation solely based on differences in molecular weights. In contrast, Native PAGE maintains proteins in their natural folded state, enabling separation based on a combination of sizes, shapes, and charges. Detection in SDS PAGE reveals individual subunits of protein complexes, while Native PAGE provides information about proteins without breaking them down to their individual subunits. This study highlights differences in sample preparation, gel composition, electrophoresis behavior, and detection outcomes. It offers important insights into the purity of proteins, their identification and interactions with other proteins, and shows how these methods can be exploited to address specific biological questions including the preparation of protein complexes for structural studies.

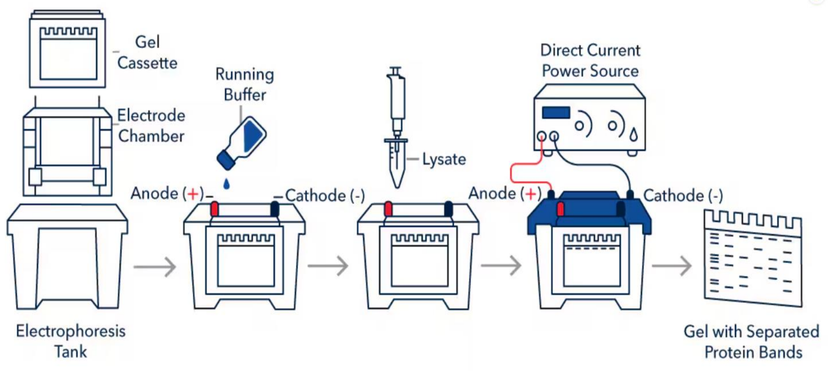
Materials and Methods

Preparation of ferritin samples requires to load 2.5 µl of sample, 5 µl of bromophenol blue dye. Beta-mercapto-ethanol breaks disulfide bonds, further unfolding the proteins. Samples are then loaded into wells with a glycerol buffer placed in the electrophoresis tank used to prevent spillage into other wells. A protein ladder with known molecular weights serves as a reference. After running the gel at 140V for 40–45 minutes, it is stained with Coomassie Blue and destained to visualize and compare protein bands. Ferritin samples are purified using an AKTA Go system.



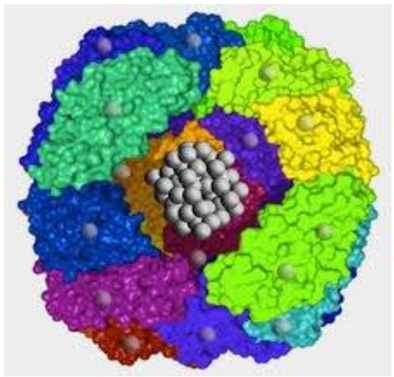
Example of an electrophoresis gel

Coomassie Blue

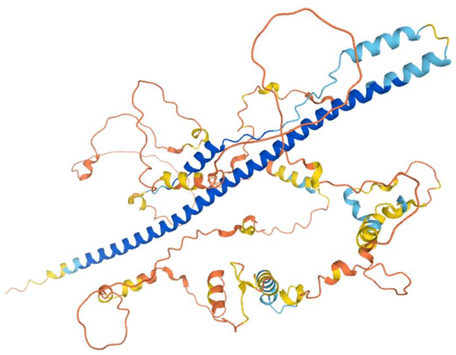


Electrophoresis equipment and step-by-step process

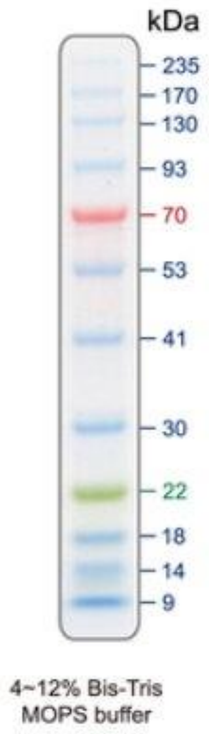
Results

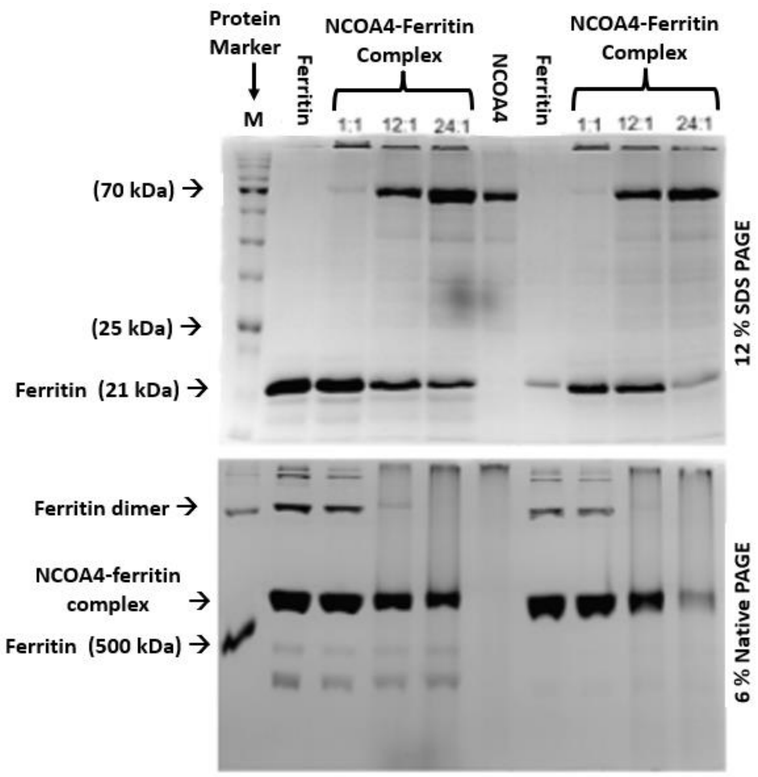


The ferritin molecule (MW 500 KDa)



The NCOA4 protein (MW 70 KDa)





Native and SDS-PAGE of the NCOA4-ferrprotein samples are obtained from a Ni-NTA chromatography column. The protein marker is shown on the left of the gels and indicated by M on the SDS-gel.

Discussion

1) Our results show a protein-complex formation between NCOA4 and ferritin, as evidenced by the upward mobility of the protein-complex band on native PAGE.
2) No difference in the interaction between NCOA4 and ferritin is observed under different conditions (LB medium rich in iron, and M9 medium poor in iron), suggesting that iron does not plan a role in the formation of the NCOA4-ferritin complex.
3) On SDS PAGE, the two protein bands (one at 21 kDa and the other at 70 kDa) suggest that a complex has formed, and that the reducing and denaturing conditions of the SDS PAGE led to the dissociation of the complex.
4) Both gels (SDS and native) demonstrate a high purity of our protein samples, as evidenced by the single band appearance for ferritin and NCOA4.
5) Further experiments and binding studies are required to determine the binding stoichiometry between NCOA4 and ferritin.

Conclusion

Our results demonstrate high purity for the two recombinantly expressed proteins, NCOA4 and ferritin. Our data show that NCOA4 and ferritin form a stable complex independent of iron levels, as shown by native and SDS PAGE analyses. Further studies are needed to determine the exact binding stoichiometry of this interaction.

References

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2) Westermeier, R. (2016). *Electrophoresis in practice : a guide to methods and applications of DNA and protein separations* (Fifth edition.). Wiley-VCH Verlag.
3) Pelin Kilic, Sema Karabudak, Begum Cosar, Busra Nigar Savran, Merve Yalcin (2024). *Residual protein analysis by SDS–PAGE in clinically manufactured BM-MSC product. Electrophoresis* 2024, 45 (17-18), 1606-1617.

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