Title: Optimizing cell lysis for human NF-kB p50/RelA protein yield

Abstract:

The RelA/p50 heterodimer is a member of the NF-κB family, which regulates inflammation and immune responses and is activated in cancers and autoimmune disorders. Targeting it could be a therapeutic treatment for these diseases specifically. The main objective of this research is to compare the function of the NF-κB RelA/p50 between the human (H. sapiens) and mouse (M. musculus) homologs to evaluate the mouse model effectiveness for immune response studies. We successfully recombinantly expressed in *E. coli* and purified the target proteins of both organisms. Their presence was confirmed by SDS-PAGE analysis, but the extraction process removed a significant amount of target protein which ended up in the pellet fraction. We performed additional experiments to improve protein extraction from *E. coli* by changing sonication cycle numbers and probe tip positions at constant duration and power levels. As a result, the amount of protein production decreased on SDS-PAGE gels while a distinct froth developed. This can be a signal that denaturation and aggregation processes occured. This research shows that protein extraction through sonication requires additional study because it remains difficult to achieve proper cell disruption without risking protein unfolding and aggregation. The development of sonication methods needs to focus on creating optimal buffer solutions and extraction techniques that preserve protein structure for binding and structural studies that could reveal RelA/p50 regulatory mechanisms and guide future therapeutic research.