| Affiliation | Professor |
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| **Name** | Dr. Abdulrahman M. Al-Senaidy |
| **Project's Title** | Renaturation studies of yeast alcohol dehydrogenase |
| **Brief Introduction (max 300 words)** | During protein unfolding and refolding, protein aggregation is a often observed due to nonspecific interactions between the hydrophobic regions of the polypeptide chains (Jaenicke 1998; Lilie, Schwarz et al. 1998). Usually protein aggregation reduces the biological activity and leads into insoluble forms. To suppress the non-native interactions and enhance solubility during protein folding studies, low molecular weight solubility enhancers are added, known as chemical chaperones (Lange and Rudolph 2009). It was found that different class of chemical chaperones is able to suppress protein aggregation in both native and denatured protein molecules (Hailu, Foit et al. 2012). Inhibition or deceleration of protein aggregation is crucial for protein purification and curing protein misfolding related diseases such as neurodegenerative diseases (Alzheimer's, Parkinson's , Huntington's etc.), cystic fibrosis, diabetes, certain types of cancer (Sloan, Fillmore et al. 2009).<br><br>Yeast alcohol dehydrogenase (YADH) (EC 1.1.1.1) is a NAD+ dependent oxidoreductase. YADH is (150 KDa) tetrameric protein, made up of 4 identical or very similar subunits. Each subunit binds with two divalent metals (zinc atom). One zinc atom is essential for the activity while another zinc atom required for the structure and stability of the YADH (Veillon and Sytkowski 1975; Magonet, Hayen et al. 1992). Alcohol dehydrogeases are industrial enzymes. It's application studied in wine and beer production and employed in a number of biotechnological processes such as bioconversion of organic wastes into ethanol (Lortie, Fassouane et al. 1992). Therefore, a robust refolding method with high yield and reproducibility will enhance its applicability.<br><br>In this study, condition for YADH refolding will be optimized and chemical chaperones assisted refolding of YADH will be evaluated. The results will shed light on the optimum condition for YADH refolding and role of different chemical chaperones on the reactivation and refolding of YADH. |
| **Methodology (max 300 words)** | Purity of commercial YADH will be analyzed by SDS-PAGE. If required, YADH will be further purified. 6mg/ml YADH will be denatured at room temperature for 0.1 to 2 hours in 6M GdmCl or 8M Urea, 5 mM EDTA, 10 mM phosphate buffer pH 7.0. Completeness of unfolding will be determined by estimating residual biological activity or intrinsic fluorescence or secondary structure by Far UV CD studies. After completeness of unfolding, pH will be lowered to ~4.0 by adding drop wise acetic acid and dialyzed extensively to remove EDTA and NAD against 4M GdmCl or 6 M Urea pH 4.0 at 4 oC. Protein concentration will be quantified by Bradford assay. Aliquote will be made and stored at -80oC .<br>Refolding of denatured YADH will be done by dilution method at room temperature. To achieve high yield of refolded YADH, following |

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| | parameters will be optimized.<br>1- Effect of Zn on the refolding of metal-free denatured YADH<br>2- Effect of NAD concentration on the refolding<br>3- Screen pH from 6-11at optimum concentration of zinc and NAD<br>4- Effect of temperature<br>5- Effect of YADH concentration<br>6- Refolding kinetics<br>7- Effect of chemical chaperone (Arginine) under optimum condition<br>8- Effect of chemical chaperone (Proline) under optimum condition<br>9- Secondary structure characterization of refolded YADH by Far UV CD<br>10-Tertiary structure characterization of refolded YADH by intrinsic fluorescence. |
| **The project is applicable within (months)** | 8 |
| **References (max 300 words)** | 1- Hailu, T. T., L. Foit, et al. (2012). "In vivo detection and quantification of chemicals that enhance protein stability." Anal Biochem.<br><br>2- Jaenicke, R. (1998). "Protein self-organization in vitro and in vivo: partitioning between physical biochemistry and cell biology." Biol Chem 379(3): 237-243.<br><br>3- Lange, C. and R. Rudolph (2009). "Suppression of protein aggregation by L-arginine." Curr Pharm Biotechnol 10(4): 408-414.<br><br>4- Lilie, H., E. Schwarz, et al. (1998). "Advances in refolding of proteins produced in E. coli." Curr Opin Biotechnol 9(5): 497-501.<br><br>5- Lortie, R., A. Fassouane, et al. (1992). "Displacement of equilibrium in electroenzymatic reactor for acetaldehyde production using yeast alcohol dehydrogenase." Biotechnol Bioeng 39(2): 157-163.<br><br>6- Magonet, E., P. Hayen, et al. (1992). "Importance of the structural zinc atom for the stability of yeast alcohol dehydrogenase." Biochem J 287 ( Pt 2): 361-365.<br><br>7- Sloan, L. A., M. C. Fillmore, et al. (2009). "Small-molecule modulation of cellular chaperones to treat protein misfolding disorders." Curr Opin Drug Discov Devel 12(5): 666-681.<br><br>8- Veillon, C. and A. J. Sytkowski (1975). "The intrinsic zinc atoms of yeast alcohol dehydrogenase." Biochem Biophys Res Commun 67(4): 1494-1500. |