

Refolding of Horseradish Peroxidase (HRP) in the Presence of Metal Cofactor & Ionic Liquid

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Recently, the importance of microorganisms as a bio-factory for the production of recombinant protein has been emphasized. However, production of recombinant protein via microorganisms often leads to the accumulation of inactive protein in the form of protein aggregates called inclusion body (IB). As such, protein refolding process is essential to convert such inactive IB into the protein of active form. Peroxidase is one of the most well-known hemoproteins which catalyze the oxidation of numbers of chromogenic hydrogen donors. Peroxidase contains four disulfide bonds and two different types of metal center which provide a stabilizing effect on protein structure and function. In this study, two different metal cofactors (Ca²⁺ & heme) and [EMIM][Cl] were employed to enhance HRP refolding yield. HRP refolding yield remarkably increased up to over three times by adding hemin and calcium chloride into refolding buffer compared to that in conventional urea-containing refolding buffer. Additional [EMIM][Cl] into heme and calcium cofactor-containing refolding buffer resulted in a further increase in HRP refolding yield up to 80%.