Purification during expression : Purification of nascent erythropoietin by its receptor in a cell-free protein synthesis system

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Abstract

The expanding applications of the cell-free protein synthesis system include not only the production of cytotoxic, regulatory or unstable proteins but also the molecular interaction detection, structure and function analyses and high- throughput screenings. To assess the potential use of molecular interaction as a purification method, we examined the protein-protein interaction (cytokine- receptor) in a cell-free protein synthesis system. We cloned erythropoietin(Epo) and erythropoietin receptor into pK7 and pET23b, respectively. The genes were coexpressed and the complex was purified with immobilized metal affinity chromatography (IMAC) in a cell-free protein synthesis system simultaneously. Elution from the receptor yielded tag-free nascent erythropoietin. Our novel approach offers a very facile, rapid and cost-effective purification method.

References

1. Muronetz V. (2001), Use of protein-protein interactions in affinity chromatography, *J. biochemical and biophysical methods* **49**, 29-47.
2. Stiege W. (1995), The potentials of the in vitro protein biosynthesis system, *J. biotechnology* **41**, 81-90.
3. Kevin W. Harris (1992), Ligand binding properties of the human erythropoietin receptor extracellular domain expressed in *Escherichia coli*, *J. biological chemistry* **267**, 15205-15209.