
IDENTIFICATION AND QUANTITATION OF NEWLY SYNTHESIZED PROTEINS IN ESCHERICHIA COLI

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Quantitative analysis of protein synthesis and degradation rates will give new insight in regulation of their concentrations in the cell. Determination of synthesis rates of new proteins by measurement of their transient concentrations upon perturbation of growth conditions is a daunting challenge. Sensitive and selective detection of newly formed proteins on a proteome wide scale is hampered by a pool of pre-existing ones.

Recently, we presented a method to identify and quantify several hundreds of newly synthesized proteins in *Escherichia coli* upon pulse labeling cells with the methionine analogue azido homo alanine (azhal). Azhal contains an azide group that is inert under biological conditions in the cell but provides a reactive moiety for selective isolation and separation of labeled proteins. For the first 30 minutes after inoculation, a methionine-auxotrophic strain grows equally well on azhal as on methionine. Upon a pulse of 15 minutes and digestion of total protein, azhal-labeled peptides were isolated by a retention-time shift between two reversed phase chromatographic runs. The retention time shift in our diagonal chromatographic approach is induced by a reaction selective for the azide group in labeled peptides using tris-(2-carboxy-ethyl)-phosphine. Selectively modified peptides are identified and quantified by reversed phase liquid chromatography and on-line tandem mass spectrometry¹. Our method enabled for the first time quantification of newly formed proteins upon temperature perturbation of *E. coli* cells on a proteome wide scale.

In this study the relative amounts of proteins synthesized by *E. coli* for 10 minutes immediately after a switch from aerobiosis to anaerobiosis were determined, by our new pulse-labeling method. The majority of up-regulated newly made proteins detected after the anaerobic switch are involved in glycolysis and pathways aimed at avoiding glycolysis grinding to a halt by a redox-imbalance in the cell. Collation of the levels of total proteins with levels of newly synthesized proteins shows that the early proteomic changes are predominantly the result of an increase in the rate of translation rather than a decrease in the rate of protein degradation. Comparison with micro-array data suggests that the early increase of glycolytic enzymes and proteins of the phosphotransferase system is primarily regulated at the level of translation rather than at the level of transcription.

- 1 Kramer, G.; Sprenger, R. R.; Back, J.; Dekker, H. L.; Nessen, M. A.; van Maarseveen, J. H.; de Koning, L. J.; Hellingwerf, K. J.; de Jong, L.; de Koster, C. G., Identification and quantitation of newly synthesized proteins in *Escherichia coli* by enrichment of azidohomoalanine-labeled peptides with diagonal chromatography. *Mol Cell Proteomics* 2009, 8, (7), 1599-611.