

Summary

Identification of differential proteins in an *E.coli* based cell-free *in vitro* translation system

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High resolution two-dimensional gel electrophoresis (2-DE) and subsequent analysis of differential proteins by peptide fingerprint is an important tool to identify changes in complex protein mixtures. We used 2-DE to investigate the early abrogation of protein biosynthesis in an *E.coli* based *in vitro* translation system. We identified twenty one differential proteins by comparing protein patterns between start and end of the translation.

In order to obtain those results various methodical problems concerning the sample preparation had to be resolved. This involved removal of low molecular weight components and amplification of the weak spots typical for most differential proteins in our system. Therefore all samples were desalted, fractionated and concentrated by reversed phase HPLC.

Among the differential proteins regulatory proteins, substrate binding proteins, protein repair enzymes, RNA binding proteins of unknown function and proteins directly involved in translation were detected. From such observations, conclusions can be drawn about the general state, regulatory mechanisms and undesired side reactions in the *E.coli* lysate.

For example starting from the identification of the putrescine/spermidine periplasmatic transport protein as a differential protein the state and influence of these two polyamines on the protein biosynthesis could be determined. Moreover we could obtain an important information through analysis of the general state of the polyamines. Concentration of agmatine, a degradation product of the amino acid arginine and a substrate for the synthesis of putrescine and spermidine, increased and reached a peak shortly before the translation stopped. We found that the protein synthesis remained constant shortly after the maximum of the agmatine concentration was reached. Further an observation by Kim and Swartz (2000), who described a massive decrease of the concentration of arginine during *in vitro* translation, can be explained now.

The time course of the reactions in our system implies, that those reactions are part of a regulatory network. Interesting in this respect is the differential protein HtrA, which can act as a protease or a chaperone, and which is a part of the apoptosis machinery in eukaryotic cells.