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Poster Presentation

Recombinant protein production in cell-free systems: strategies for improving yield and functionality

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Background

One of the major obstacles of the post-genome era is the efficient production of proteins for structural and functional analysis. To solve this problem cell-free protein biosynthesis is an attractive tool. It is fast, easy to handle and adaptable to the requirements of the synthesized proteins.

Results

Our highly productive cell-free protein synthesis system based on *E. coli* lysates yields up to several hundred micrograms protein per milliliter in a 1 h batch reaction. Recently we developed an advanced version of this system, which we call the "Recycling System". This system comprises two or more cycles, each subdivided in a protein synthesis and a subsequent recycling phase. The recycling step saves high molecular weight components such as template, ribosomes and transcriptional/translational factors, whereas low molecular weight reaction byproducts (e.g. inhibitory inorganic phosphate) are removed.

Since this recycling technology leads to an accumulation of the target protein in consecutive synthesis cycles, it is well suited for preparative approaches such as the synthesis of native or labeled proteins for structural research (NMR, X-ray). Furthermore recycling can be used for lysate programming, i.e. in the first synthesis step proteins can be expressed, which are beneficial for the production of a target protein in the following synthesis step(s). As we demonstrate here, this strategy could be successfully applied to the preparation of chaperone-enriched lysates for functional protein production.

Finally we present alternative strategies for enhancing functionality of cell-free synthesized proteins such as the addition of folding catalysts, specific modification of reaction conditions, cotranslational application of a multifactorial matrix and posttranslational reactivation.

Conclusion

Our data clearly show, that cell-free protein biosynthesis is a versatile platform for production of functional proteins in analytical and preparative scale.

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