Microbial Cell Factories

Poster Presentation

Nonclassical inclusion bodies in *Escherichia coli*

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Background

Formation of inclusion bodies (IBs) during over-expression of recombinant proteins in *Escherichia coli* is of common occurrence. The target protein inside inclusion bodies is usually misfolded and a series of denaturation/renaturation steps is necessary for isolation of biologically active protein. Purification protocol must be optimized case by case. Due to low efficiency of most denaturation/renaturation procedures a lot of trials have been performed to obtain soluble and properly folded target proteins inside *E. coli* cytoplasm with the aim of increasing the yield of the target protein.

In our laboratory we are exploring another approach based on our recent observation, that Granulocyte Colony-Stimulating Factor (G-CSF), when produced in *E. coli* at lower temperature (around 25°C), forms inclusion bodies containing large amount of correctly folded protein precursor [1]. Such inclusion bodies have remarkably different properties than the classical inclusion bodies therefore we proposed the term "nonclassical inclusion bodies" (ncIBs). Here we would like to expose some typical characteristics of the ncIBs.

Most of our studies were performed on G-CSF but similar results were also found for His7dN6TNF-α(His-tagged, N-terminally truncated form of tumor necrosis factor) and GFP (green fluorescent protein) indicating that the phenomenon of ncIBs is of broader occurrence.

Results

Nonclassical inclusion bodies have some interesting properties. They are more fragile and more soluble in mild detergents than classical inclusion bodies. Methods often used for disruption of bacterial cells during the isolation of IBs, such as enzymatic lysis, sonication and high pressure homogenization were compared to check whether bacterial cell disruption method has any influence on mechanical stability and solubility of IBs.

Enzymatic lysis of bacterial cells appears to be mild enough disruption method not influencing the integrity of IBs and the supernatant of the lysed sample does not contain any target protein. As judged from SDS-PAGE analysis, enzymatic treatment does not assure complete release of the cytoplasmic material, including the majority of soluble host proteins, although under the optical microscope only IBs are observed in a highly clustered form. Another drawback of this method is that the enzyme (lysozyme) diffuses into the pores of ncIBs and stays there entrapped, which can represent a problem during protein isolation.

On the other hand, homogenisation and sonication, methods usually used for bacterial cell disruption, enable total release of IBs, but they seem to be quite harmful for ncIBs. Surprisingly, a noticeable disassembling and partial or sometimes complete solubilization of ncIBs was observed. Therefore, optimization of sonication and homogenisation for isolation of ncIBS from bacterial cells is necessary.

Nonclassical IBs are very soluble in classical washing solutions containing detergents (e.g. 1 % deoxycholate, octylglucoside or Triton X-100). Even washing of studied ncIBs (G-CSF, GFP, His7dN6TNF-α with lower detergent concentrations, e.g., 0.1 % deoxycholate results in significant protein loss.

Another interesting property of ncIBs, initially observed at macro level during centrifugation, is irreversible contraction when the buffer pH is reduced from neutral (pH 7) to acidic (pH 4). This was further confirmed by electron microscopy and the size of ncIBs at various pH was determined. Preliminary results show that inclusion bodies have rather porous structure, which seems to depend on the buffer pH. The smaller size of the pores at acidic pH are probably the cause for less efficient extraction of the target protein from IBs at low pH. As a consequence of contraction, one would also expect the increase of density, which was actually qualitatively confirmed in centrifugation experiments.

Probably the most important and useful property of ncIBs is large amount of properly folded target protein or its precursor. The target protein can be extracted in non denaturing conditions using low concentration of mild detergents or even simple buffers.

Conclusion

Nonclassical IBs compared to classical are characterized by higher fragility, higher solubility, irreversible contraction at acidic pH and most importantly, a high amount of correctly folded target protein or its precursor. When employing classical isolation procedure on preparative scale the described properties can lead to substantial loss of the target protein. However, on the other hand, using these properties as an advantage for the development of simplified cost-effective downstream processes, without denaturing solvents and with no need for renaturation, poses a challenge for the future.

References

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