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Poster Presentation

Low growth temperatures improve the conformational quality of aggregation prone recombinant proteins in both soluble and insoluble *E. coli* cell fractions

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Background

Protein aggregation is a major bottleneck in the bacterial production of recombinant proteins. Among others, induction of gene expression at suboptimal growth temperatures (for instance, below 37°C in *Escherichia coli*), has been repeatedly observed as a convenient procedure to enhance the solubility of aggregation-prone proteins and to minimize inclusion body formation. However, the effect of low growth temperatures in protein features, other than mere solubility, has been rarely explored.

Results

In this work, we have determined the folding status and functionality of an engineered GFP variant when produced at 37, 30 and 16°C. The strain used was BL21 (DE3) and the plasmid pET21-b(+). Gene expression was induced by IPTG using different production times for each temperature: 2 h at 37°C, 5 h at 30°C and 16 h at 16°C, which are equivalent to the same biomass increase.

After induction, fluorescent microscopy analysis was carried out, and the cellular extract was fractioned in order to analyse either the soluble or insoluble fraction using fluorimetry. The amount of recombinant GFP protein was calculated by Western Blot to obtain the specific fluorescence emission. Plasmidless BL21 (DE3) was used as a negative control. Fluorimetry analysis showed that fluorescence emission of the soluble fraction was approximately five times higher at 16°C than at 37°C. On the other hand, fluorescence emission of the insoluble fraction was approximately 1.5 times higher at 16°C than at 37°C. However, when these fluorescence values are related to the quantity of protein (Figure 1), the results of the soluble fraction are similar, but surprisingly in the insoluble fraction an increased fluorescence emission (10 times higher) is observed at 16°C with respect to 37°C.

Conclusion

As expected, solubility of a recombinant GFP was largely improved at low temperatures. On the other hand, and very interestingly, the quality of GFP, as reflected by its specific fluorescence emission, was largely enhanced at 16°C not only in the soluble cell fraction but also in the residual inclusion bodies. This intriguing observation indicates that the physicochemical conditions governing protein folding act in parallel on both soluble and aggregated protein forms. Therefore, protein misfolding and aggregation are clearly not coincident events, what strongly supports the hypothesis that incorrect folding is not a straightforward cause of protein deposition.

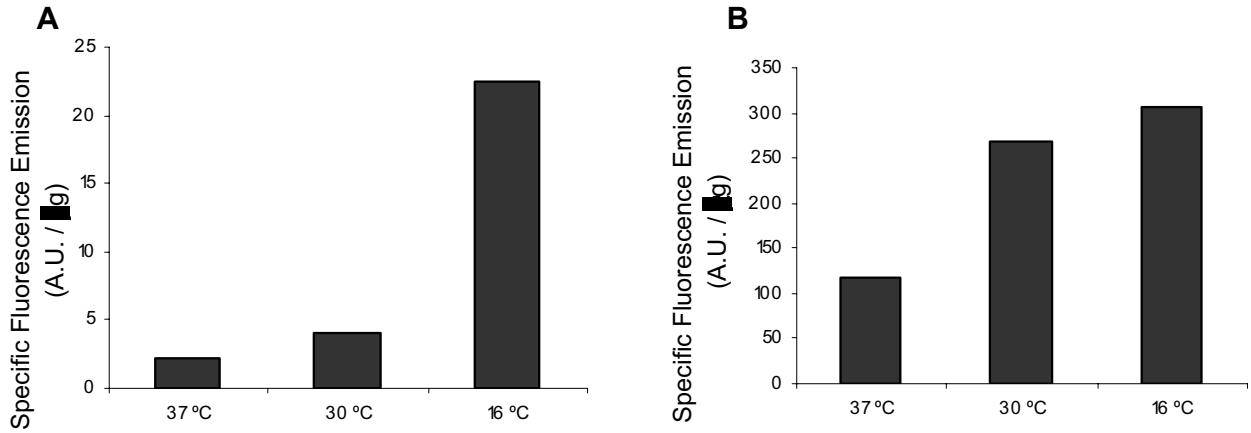

Figure 1
Specific Fluorescence Emission of the engineered GFP variant. Insoluble cell fraction (A) and Soluble cell fraction (B).

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