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Performance of beta-galactosidase inclusion bodies in enzymatic bioprocesses

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Background

Inclusion body formation is a common event during bacterial over-expression of recombinant genes. This phenomenon represents a great matter of concern in biotechnology, because it has restricted the spectrum of proteins marketed in this field. In a previous work, we have observed that recombinant enzymes produced in bacteria are not completely inactivated when deposited as inclusion bodies [1] and that aggregation as inclusion bodies does not necessarily split protein population into active and inactive fractions. Therefore, we decided to further explore and fully characterize the behaviour of purified beta-galactosidase inclusion bodies in presence of substrate, during a small-scale bioprocess.

Results

In this work we have analysed the state of the inclusion bodies formed by an engineered *E. coli* beta-galactosidase fused to the aggregation-prone foot-and-mouth disease virus (FMDV) VP1 capsid protein (VP1LAC). Inclusion bodies were resuspended in Z buffer and incubated at 37°C in agitation in presence of ONPG substrate (and in its absence as internal control). Under these conditions, the beta-galactosidase embedded in inclusion bodies efficiently hydrolyses ONPG (Figure 1A), while no product appears in absence of the substrate (Figure 1B). To quantify the activity remaining in inclusion bodies and that eventually present in the soluble fraction, during the incubation of this aggregates with ONPG, samples were taken at two points (t2 min and t30 min) and after centrifugation, the supernatant and the pellet were used for a second enzymatic analysis with CPRG as substrate (Figure 2) [1,2]. The amount of protein was also quantified in both soluble and insoluble fractions at the times chosen (Table 1).

The results obtained, comparing the samples incubated with and without ONPG (named control in Figure 2), suggest that the presence of substrate in the suspension might positively influence the solubilisation of the aggregated protein.

Conclusion

We could conclude that, interestingly, when an enzyme aggregated as inclusion bodies is incubated with its substrate, part of this protein might be spontaneously solubilised in a process that seems to be eventually favoured by the presence of substrate. Moreover, this soluble protein shows considerable enzymatic activity that is a major contributor to the enzymatic process initiated by inclusion bodies.

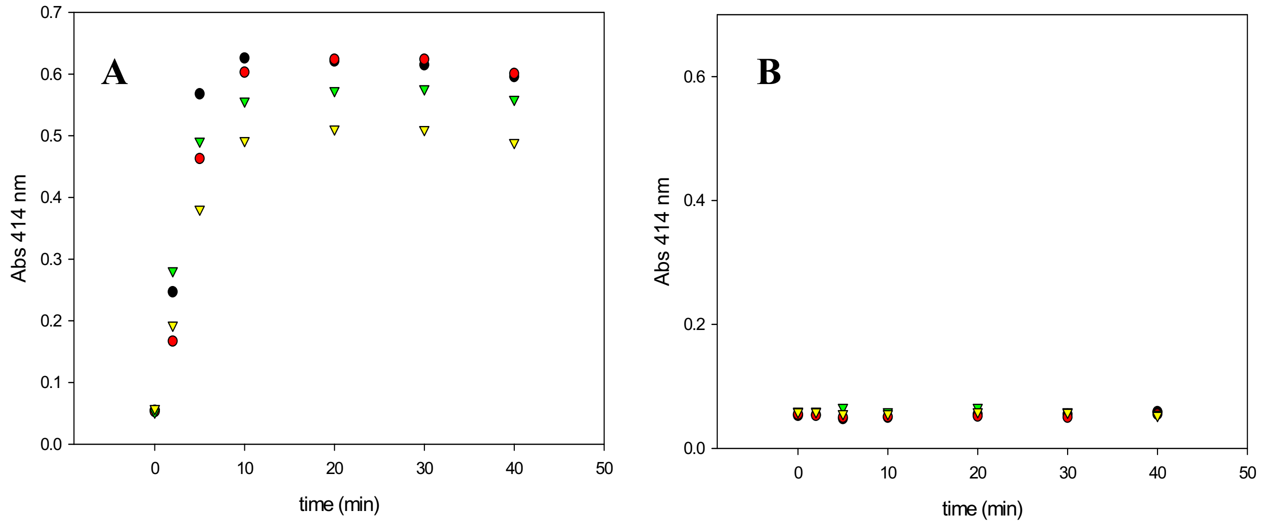

Figure 1
A. Product formed by inclusion bodies (quadruplicate) through ONPG hydrolysis as determined at 414 nm. B. Control (inclusion bodies without ONPG).

Table 1:

Protein (%)	Inclusion bodies		Soluble	
	t2 min	t30 min	t2 min	t30 min
samples	100	28	<1	72

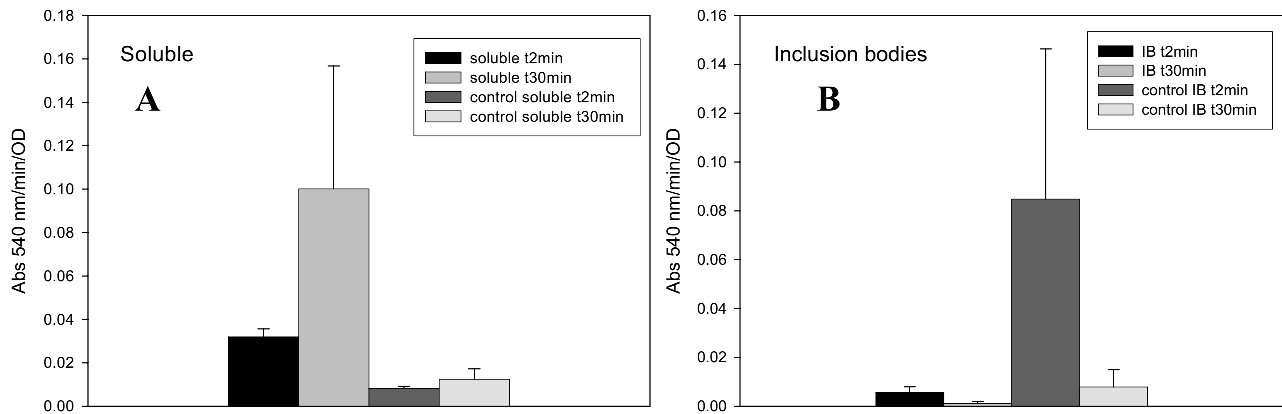

Figure 2
A. Product formed by soluble fraction (A) and inclusion bodies (B) through CPRG hydrolysis as determined at 540 nm.

Acknowledgements

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References

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