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Poster Presentation

DnaK-J are limiting for proper recombinant protein folding only at low production rates and when the physiological heat-shock stress response is not triggered

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Background

It is known that the bacterial production of recombinant, misfolding-prone proteins triggers the heat shock response, as observed through the monitoring of several marker genes [1]. On the other hand, the co-production of selected chaperones along with the recombinant protein has been largely explored as a strategy to minimize aggregation of the product, with rather unpredictable and not always consistent results [2-4]. The reasons for the limited success of this approach could lie in suspected although not proven different folding requirements of specific protein species and the occurrence of differential bottlenecks in their in vivo folding pathways. Moreover, it has not been discarded that the protein-induced heat-shock response could eventually eclipse the rise of functional plasmid-encoded chaperones resulting from co-production. To explore this possibility and to offer more light on the functional mechanics of recombinant protein folding we have quantitatively determined the intracellular levels of DnaK under different conditions, during the production of a misfolding-prone GFP variant.

Results

The production in *E. coli* MC4100 of an engineered GFP protein (VP1GFP), controlled by the Trc promoter (in pTVP1GFP) and triggered by 1 mM IPTG results in an important level of aggregation, with VP1GFP occurring in both the soluble and insoluble cell fractions at similar extents (42.2 % ± 1.6 and 57.8 % ± 1.6 respectively). When the producer strain carried pBB535 [5] (containing both IPTG-inducible $P_{A1/lac\text{-}O1}$ controlled *dnaK-J* chaperone genes) as a second plasmid, the addition of IPTG promotes the co-expression of both the chaperone gene set and the *vp1gfp* gene. However, this does not result in any detectable shift in the fractioning of VP1GFP, which still occurs in the insoluble cell fraction in an important level (48.2 % ± 3.6). In the same line, the specific fluorescence of VP1GFP is not modified by *dnaK-J* co-expression in both soluble (143.1 ± 31.5 fluorescence units/μg VP1GFP versus 125.4 ± 29.7) and insoluble (26.2 ± 6.7 versus 30.6 ± 5.4 fluorescence units/μg VP1GFP) cell fractions. Also, the total fluorescence determined by OD unit is not significantly affected by coexpression (378.0 ± 20.1 fluorescence units/ml of culture · OD_{550} versus 315.0 ± 8.5 fluorescence units/ml of culture · OD_{550}).

The amounts of DnaK were determined in all cases by means of Western blot analysis as indicative of the chaperone set production, and the plasmidless MC4100 strain and MC4100/pBB535 were added as controls for this experiment. As observed in Figure 1, the levels of DnaK were slightly lower in presence of pTVP1GFP than in its absence (MC4100/pBB535 versus MC4100/pTVP1GFP/pBB535), probably due to a gene dosage effect of plasmid

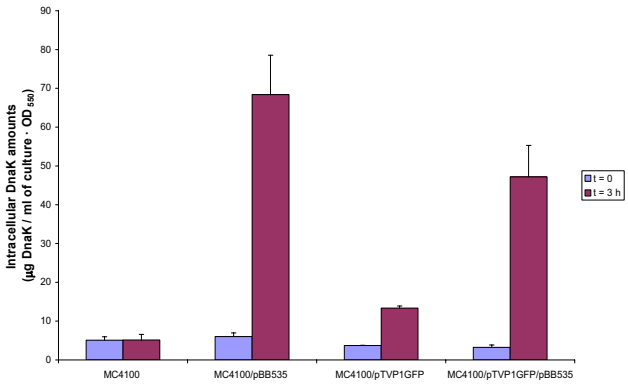

Figure 1
Estimation of the intracellular amounts of DnaK in the different strains used in this work, under 1 mM IPTG induction.

coexistence as previously suggested for other plasmid sets [6]. However, DnaK amounts in MC4100/pTVP1GFP/pBB535 upon IPTG addition were still three-fold higher than in MC4100/pTVP1GFP. In this last case, the heat shock response promoted by the production of VP1GFP itself, although clearly detectable, only doubled the intracellular levels of the chromosomal-encoded DnaK present in plasmidless MC4100 (Figure 1). This clearly indicates that the VP1GFP-mediaded induction of the heat shock response is not eclipsing the phenotypic effect of additional DnaK-J amounts provided by an encoding plasmid.

Interestingly, at low IPTG doses (0.02 mM), the levels of DnaK cannot be distinguished between MC4100/pTVP1GFP/pBB535 and MC4100/pTVP1GFP (not shown), but the specific fluorescence of VP1GFP is almost doubled in presence of pBB535 (Figure 2).

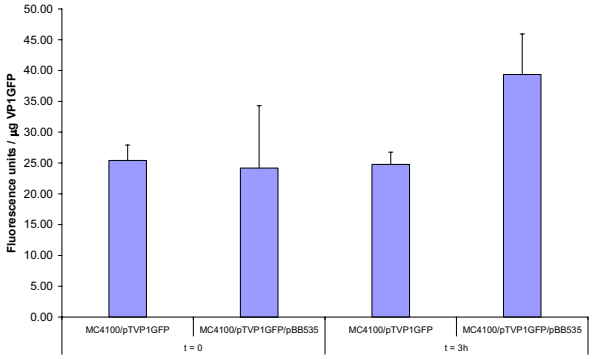

Figure 2
Specific fluorescence of the soluble VP1GFP in the different strains used in this work, under 0.02 mM IPTG induction. Insoluble VP1GFP was not fluorescent.

Conclusion

DnaK-J are limiting for the proper folding of low amounts of VP1GFP, in absence of any endogenous heat-shock response. However, under strong production conditions, a physiological heat-shock response is triggered and an additional income of DnaK-J does not promote any detectable effect on VP1GFP protein quality. This indicates that the DnaK-J levels reached as a response to protein production are high enough to offer a sufficient supply of such chaperones. Therefore, the proper folding of VP1GFP under these conditions is probably restricted by a limiting factor other than DnaK-J, probably a heat-shock product or other cell element whose levels remain modest during recombinant protein production.

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