Must we live with inclusion bodies?

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PROTEIN FOLDING

IN VIVO

Must we live with inclusion bodies?

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High expression levels of recombinant proteins do not necessarily mean that the protein must be in inclusion bodies (IBs). This talk will describe some of the more successful solutions to the problem of expressing proteins in an active form in *E. coli*.

Many groups prefer to express proteins in an insoluble form, primarily because of the advantages listed in **Table 1**. The disadvantages are often ignored.

Advantages of IBs	Disadvantages
High expression level> reduced fermentation costs	Refolding shifts problems and costs downstream.
Production can be monitored with PAGE or immunoblotting	Production cannot be monitored directly by activity.
Cytoplasmic proteins are washed away, simplifying purification. "95%" purity can be quickly achieved	The other "5%" contaminants are hydrophobic, poorly soluble membrane proteins and cell wall fragments
The major contaminants are oligomers and misfolded or proteolyzed forms of the desired protein	Separation of multiple forms of the same protein is the most difficult purification step.
The pL promotor with T induction often yields protein where other systems fail	If the protein does not refold well, another expression system will still be needed.

When to use inclusion bodies: The most successful IB purification schemes have been for small flexible proteins. IB purifications are often favoured by university labs with no access to fermentors, as large amounts of protein can be produced in shaker flask culture. Increasing cell density per liter does not improve economics, as the limiting factor in the purification is the volume required for refolding. IBs are also useful for the production of factors toxic for bacteria that are not producible by secretion (eg., histones). In addition, no successful system for producing membrane proteins in a soluble form has been described.

The difficulties of refolding larger proteins economically have proved prohibitive. Animal cell cultivation was more economical for production of tPA than production in IBs because the purification costs were so high (Datar, Cartwright, & Rosen, MS in prep.).

Integration of upstream and dowstream processing The choice of protein expression system should not be left to the fermentation group. Their emphasis is usually on rapid and cheap production of large amounts of protein, with little regard to the downstream purification. It is important to keep all options open at the beginning of the cloning, because once a process is running, it is hard to change anything. Although protein purification is complex, it should be thought of as a stream that starts at the fermentor and ends in vials of purified product.

The only valid yield figure for a process is the amount of usable protein produced per liter of culture fluid. As simple as this definition sounds, real yield figures tend to be elusive. Several groups, often in different cities, take part in the purification. True costs per mg protein are also difficult to calculate at the lab scale as labor costs are often underestimated.

Secretion systems In some cases, the desired protein is too toxic or sensitive to proteolysis to be expressed intracellularly in a soluble form. Secretion from *E. coli* has been very successful for heterologous proteins that are secreted in their original environment, including human growth hormone,[1] "humanized" antibodies,[2] and bacterial[3] and mammalian[4] RNases. Secretion from other bacterial species, yeasts, and fungi is also possible.

Yields in secretion systems can be increased by growing the cells to high densities and by using host strains selected for low protease production. Optimizing the growth temperature, reducing air bubbling and adding protease inhibitors or detergents to the medium can also help to increase protein in the supernatant.

Expressing soluble proteins intracellularly.One of the easiest and most generally applicable method to increase the protein in the soluble fraction is to reduce the E. coli growth temperature.[5] **Table 2** lists a few proteins that are more soluble when produced in *E.coli* grown at 30°C or less.

Protein:	Promotor:	Reference:
Mammalian:		
human Interferon-α2	T7, colE1, Amp	Reference 5
human Interferon-γ	pTrp	"
murine Mx	pTrp	"
human Interferon-β	pTrp	Mizukami,et al.(1986) Biotech. Letters **8**,605
human transforming growth factor-β	pTrp	Seow, et al.(1989) Gene 83,117-129.
rabbit muscle glycogen phosphorylase	pTrplac hybrid, T7	Browner,et al. (1991) Prot. Eng. **4**:351
murine cAMP-dependent protein kinase (catalytic subunit)	T7	Slice, L.W. & Taylor, S.S. (1989) J. Biol. Chem. 264,20940
Plant:		
radish 3-OH-3-methyl glutarylcoenzyme A reductase	T7	Ferrer et al. (1990) FEBS Let. 266,67-71.
rice lipoxygenase L-2	T7	Shirano,Y.& Shibata,D. (1990) FEBS Let271,128
yeast α-glucosidase P1	tac-hybrid	Kopetzki,et al. (1989) Mol. Gen. Genet.216,149
Bacteria:		
T4 DNA polymerase	pL, ptac	Lin,et al.(1987) Proc. Natl. Acad.Sci.84,7000
Ricin A-chain	pL,pTrp, lacUV5	Piatak,et al. (1988) J.Biol. Chem. 263, 4837,Ready et al.(1991) Prot. Struc. Func. Gen. 10, 270
Diphtheria toxin & fusion proteins	trc	Bishai et al. (1987) J. Bacteriol. 169, 5140
P22 tailspike	phage promotor	Haase-Pettingell, C.A. and King, J. (1988) J. Biol. Chem. 263, 4977
Subtilisin E (secretion system)	tandem lpp/lac-OmpA	Takagi,et al.(1988) Bio/Technology 6, 948

Changes in the protein sequence. In some cases, one might consider altering the protein sequence to obtain a protein with better solubility characteristics. For example, discrete changes in human hemoglobin make a more stable protein for use in blood substitutes, and the solubility of insulin preparations can be altered by changing residues at the oligomer interface. The *in vivo* and *in vitro* aggregation tendencies of a protein do not necessarily correlate.

Certain proteins remain soluble under all growth conditions. Several examples of a single amino acid change rendering a protein more[6] or less[7] likely to form IBs have been found.

In conclusion, there are now many ways to express large amounts of protein in a soluble form in *E. coli*. We can for the most part control the solubility of proteins produced, so we no longer need to live with IBs unless we want to.

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