Inhibition of protein synthesis in Escherichia coli by expression of RNAs containing multiple ribosome binding sites

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Abstract

Biologically synthesized poly(α,L-glutamic acid) (PLGA) can be chemically modified to form monodisperse poly(γ-benzyl α,L-glutamate) (PBLG). This material shows rare smectic ordering where macromolecular rods organize into highly-ordered layers. Analysis of PBLG has been hindered by low level biosynthesis of PLGA. An unusual feature of PLGA expression is that its accumulation is inversely related to the levels of its mRNA. This phenomenon has been investigated with the objective of improving the bioproduction of PLGA. PLGA was expressed as a C-terminal fusion with dihydrofolate reductase (DHFR) in *E. coli* strain BL21, carrying an IPTG-inducible DHFR-PLGA gene fusion in the pQE15 plasmid. In exponentially growing cells, accumulation of DHFR-PLGA was optimal at 0.01 mM IPTG and decreased at higher concentrations of IPTG. However, maximal DHFR-PLGA accumulation occurred in cells grown to saturation with no IPTG induction. It appears, therefore, that the accumulation of DHFR-PLGA is optimal in nongrowing cells translating low levels of DHFR-PLGA mRNA over long periods of incubation. Overexpression of both *E. coli* $tRNA^{Glu}$ and the glutamyl-tRNA synthetase did not improve DHFR-PLGA production. *In vivo* incorporation of [^{35}S]-methionine was inhibited >95% by induction of either translatable or untranslatable PLGA constructs, but induction of the corresponding anti-sense constructs was not inhibitory. Sucrose gradient centrifugation analysis showed that expression of PLGA RNA resulted in nearly complete depletion of free 30S ribosomal subunits and the appearance of new complexes in the polyribosome region of the gradient. These new complexes were enriched in 16S rRNA but also contained 23S rRNA, and unlike normal polysomes, they were resistant to breakdown in the presence of puromycin. These results support the conclusion that multiple internal ribosome binding sites in the PLGA coding sequence inhibit translation of both DHFR-PLGA and cellular proteins by sequestering ribosomal subunits in nonfunctional complexes on the PLGA mRNA. ^

Subject Area

Molecular biology

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