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From the Bench

Rheostatic Control of Protein Expression Using Tuner Cells

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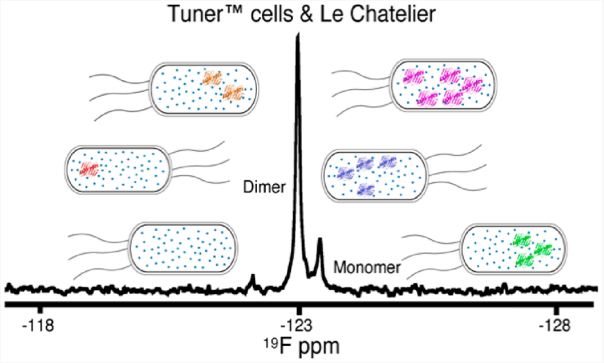
ABSTRACT: We assessed the ability of two strains of *Escherichia coli*, BL21 (DE3) and Tuner (DE3), to express a variant of the B1 domain of protein G, which forms a side-by-side dimer, by using fluorine-labeling and ^{19}F nuclear magnetic resonance spectroscopy. BL21 cells express the protein in a binary, all-or-none, manner, where more cells express the protein at a high level with an increasing inducer concentration. Tuner cells express the protein in a rheostatic manner, where expression increases across all cells with an increasing inducer concentration.



Many expression systems exploit the *lac* operon, whose inducer, isopropyl β-D-thiogalactoside (IPTG), acts in a stochastic manner in commonly used *Escherichia coli* strains such as BL21 (DE3).[1] "Stochastic" means that, in such cells, plasmid-driven protein expression is either "on" or "off." That is, low IPTG concentrations induce protein synthesis in a small fraction of cells, and high IPTG concentrations induce protein synthesis in all cells.

Tuner (DE3) *E. coli* cells are different. They contain a deletion (Δ*lacZY*) of lactose permease, which should make all cells equally permeable to IPTG.[1,2] This deletion should result in homogeneous protein expression that can be controlled, rheostat-like, by varying the IPTG concentration, but we are unaware of a direct test of this feature.

To test the potential rheostatic nature of expression in Tuner cells, we exploited Le Chatelier's principle; a system at equilibrium reacts to change in a way that counteracts the change. Specifically, we tested the effect of high and low IPTG concentrations on a monomer−dimer equilibrium in Tuner (DE3) and BL21(DE3) cells using a variant of the B1 domain of protein G (GB1, UniProt ID P06654), whose sole tryptophan at position 43 can be easily labeled with fluorine.[3] The A34F variant of GB1 forms a thermodynamically stable dimer.[4] The monomer and dimer exhibit unique ^{19}F chemical shifts and are in slow exchange on the chemical shift time scale.[5] The idea is that increasing GB1 concentrations in cells will affect the monomer dimer equilibrium.

MATERIALS AND METHODS

A pET11a plasmid containing the GB1 A34F mutant was used for protein expression.[5] The plasmid was transformed into BL21 (DE3) (Novagen) or Tuner (DE3) (Novagen) cells by heat shock. A new transformation was performed every 3 weeks. Following overnight incubation at 37 °C, a single colony was used to inoculate 5 mL of Luria broth supplemented with 100 μg/mL ampicillin (final concentration). The culture was incubated with shaking at 37 °C at 225 rpm (New Brunswick Scientific Innova I26). After 6−8 h, 500 μL of the culture was used to inoculate 100 mL of supplemented M9 media. This 100 mL culture was shaken at 37 °C overnight and added to 100 mL of fresh supplemented M9 minimal media. The culture was incubated at 37 °C, and its optical density at 600 nm (OD_{600}) was monitored (Bio-Rad Spectra Plus). When the OD_{600} reached 0.45, 12 mg of 6-fluoroindole (Sigma-Aldrich) dissolved in 250 μL of dimethyl sulfoxide was added.[3] The culture was shaken for an additional 45 min, after which protein expression was induced by adding IPTG to final concentrations of 25 or 1000 μM. After 45 min, chloramphenicol (50 μg/mL final concentration) was added to halt the expression.

The cells were pelleted at 2000*g* for 20 min and washed three times with an in-cell NMR buffer [200 mM HEPES, 100 mM bis-tris propane, 150 μg/L ampicillin, and 50 μg/mL chloramphenicol dissolved in 10% D_2O (pH_{read} 7.8)]. The pellet was resuspended in 250 μL of in-cell NMR buffer. Experiments were conducted with a Bruker Avance III HD spectrometer equipped with a QCI cryoprobe operating at a ^{19}F Larmor frequency of 470 MHz. Spectra comprised 32 768 points, 512 scans, an acquisition time of 0.9 s, and a sweep

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width of 20 ppm. Data were processed with TopSpin Version 3.6.1.

^{19}F NMR spectra of supernatants from cell slurries were acquired to assess GB1 leakage.[6] After each experiment, the cells were gently pelleted at 2000*g*, and a spectrum of the 2-fold diluted supernatant was acquired. Leakage was not observed. After the in-cell experiment, the cells were lysed and pelleted, and a lysate spectrum was obtained.[7]

The protein was purified and buffer spectra acquired.[8] The monomer and dimer peaks were integrated to give their relative populations at five GB1 concentrations using serial dilution. The data were fit to yield a $K_{D\rightarrow M}$ at 298 K and pH 7.5 of ~20 μM.

RESULTS AND DISCUSSION

The spectrum of A34F GB1 in buffer exhibits two peaks, one for the monomer and one for the dimer (Figure 1A). As required by Le Chatelier's principle, the fraction of dimer increases with an increasing GB1 concentration (Figure 1A).

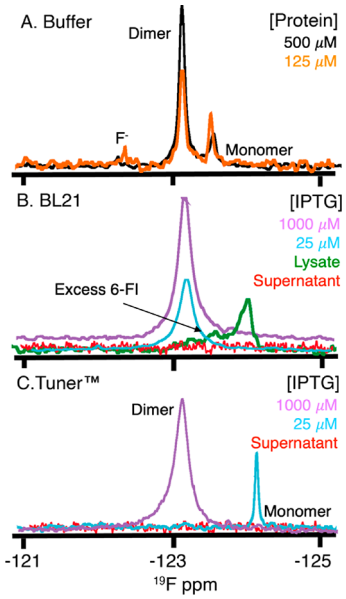

Figure 1. ^{19}F NMR spectra acquired at 298 K of A34F GB1 in (A) buffer at pH 7.5, (B) in BL21 cells, and (C) in Tuner cells. The resonance from F^- can be seen in panel A. The resonance from excess 6-fluoroindole (6-FI) can be seen in panels B and C.

Next, we examined the effect of IPTG concentration on the expression of the variant in Tuner (DE3) and BL21 (DE3) cells. The stochastic nature of expression in BL21 (DE3) cells is shown by the observation that the signal increases at the higher IPTG concentrations, but only the dimer is observed at both concentrations (Figure 1B). The observation of the only dimer is consistent with the fact that the intracellular concentration of GB1 under these conditions is approximately 2 mM per cell.[9] In other words, GB1 is highly expressed in a small fraction of cells at a low IPTG concentration and highly expressed in all cells at a high IPTG concentration. As a control, cells were then lysed, and the lysate was diluted 4-fold to confirm that the monomer is present (Figure 1B).

The rheostatic tunability of Tuner (DE3) cells is indicated by the observation that only a monomer is detected at a low IPTG concentration, and only a dimer is detected at a high IPTG concentration (Figure 1C). Expression increases with IPTG concentration in Tuner cells, and both monomer and dimer are observed at intermediate IPTG concentrations (Figure S1). That is, in Tuner cells, the IPTG concentration controls GB1 concentration across all cells, and Le Chatelier's principle requires that a higher intracellular GB1 concentration results in more dimer.

The dimer resonance has a larger width-at-half-height compared to the monomer in cells (Figure 1) and lysates (Figure S1) compared to buffer. This increase may arise from dimer-specific attractive interactions in the cellular milieu that are absent in buffer. We are testing this and other ideas via surface amino acid changes.[10]

In summary, our results show that Tuner (DE3) cells can be used to vary protein expression in *E. coli* in a homogeneous, rheostatic manner. In future studies, we will quantify the equilibrium constant for dissociation of the dimer in cells and assess the effect of interactions between the dimer and the intracellular milieu.[10]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.biochem.9b01101.

^{19}F NMR spectra (PDF)

Accession Codes

Controlling protein expression of A34F GB1, UniProtKB P06654.

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Notes

The authors declare no competing financial interest.

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