Analytical Biochemistry 518 (2017) 139–142

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Assessing translational efficiency by a reporter protein co-expressed in a cell-free synthesis system

Yu Jin Park [1], Kyung-Ho Lee [1], Dong-Myung Kim [*]

Department of Chemical Engineering and Applied Chemistry, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon, 34134, South Korea

ARTICLE INFO

Article history:
Received 1 November 2016
Accepted 27 November 2016
Available online 29 November 2016

Keywords:
Cell-free protein synthesis
Translational efficiency
Co-expression
Assessment of gene expression

ABSTRACT

We demonstrate the use of a cell-free protein synthesis system as a convenient tool for assessing the relative translational efficiencies of genes. When sfGFP was used as a common reporter gene and co-expressed with a series of target genes, the intensities of sfGFP fluorescence from the co-expression reactions were highly correlated with the individual expression levels of the co-expressed genes. The relative translational efficiencies of genes estimated by this method were reproducible when the same genes were expressed in transformed *Escherichia coli*, suggesting that this method could be used as a universal tool for prognostic assessment of translational efficiency.

1. Introduction

Because they are not restricted by the constraints of cell viability and structural integrity of cellular membranes, cell-free synthesis systems enable instant expression of proteins from various forms of genetic material [1–4]. This unique feature of cell-free protein synthesis makes it an ideal technique for rapid and parallel expression of proteins. However, as is also the case in studies of cell-based protein production, quantitative analysis of gene expression in a cell-free protein synthesis system is limited by the paucity of proper means to measure the levels of expressed proteins. Although a few methods can generate signals reflecting protein accumulation, such as translational fusion of reporter proteins [5], addition of fluorescence-generating tags [6], and incorporation of radioactive amino acids [7,8], each of these methods suffers corresponding drawbacks: requirement for genetic modification of the target genes, high cost of reagents, or the use of hazardous radioactive materials. These methods also require multiple time-consuming steps for the treatment of reaction samples prior to quantification of the expressed proteins.

In an effort to develop simpler methods for determining the translational efficiency of genes, we devised a technique that measures the fluorescence from a reporter protein as an indicator of the translational efficiency of target proteins co-expressed in a cell-free synthesis system. Because the transcripts of target and reporter genes must compete for a finite pool of ribosomes when they are co-expressed in the same reaction mixture, we predicted that ribosome loading of the reporter mRNA would be negatively affected by the relative loading efficiency of the target mRNA. Assuming that the translation rate of mRNA is largely determined at the initiation stage, which is governed by ribosome loading efficiency, we predicted that the fluorescence of the reporter protein would be inversely proportional to translation rates of the target genes. We tested this assumption by comparing the fluorescence of the superfolder green fluorescence protein (sfGFP) co-expressed with a series of genes with different translational efficiencies. sfGFP fluorescence measured during the co-expression reactions was almost linearly correlated with the reciprocal of the translational efficiency of the target genes, quantified separately. These results demonstrate that our strategy of sfGFP co-expression represents a straightforward method for predicting the expression levels of genes programmed in a cell-free protein synthesis system, without requiring complicated and time-consuming experimental manipulations. Moreover, the relative expression levels of recombinant proteins estimated by this method were reliably reproduced when the same genes were expressed in transformed cells, justifying the use of this approach as a universal tool for rapidly evaluating the translational efficiencies of specific gene constructs.

2. Materials and method

2.1. Materials

ATP, GTP, UTP, CTP, creatine phosphate, creatine kinase, and *Escherichia coli* (*E. coli*) total tRNA mixture (from strain MRE600)

* Corresponding author.
E-mail address: dmkim@cnu.ac.kr (D.-M. Kim).
[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.ab.2016.11.019
0003-2697/

were purchased from Roche Applied Science (Indianapolis, IN, USA). L-[U-^{14}C]leucine (11.9 GBq/mmol) was obtained from Amersham Biosciences (Uppsala, Sweden). DNA oligomers were provided by Integrated DNA Technologies (Coralville, IA, USA) at 25-nmol scale. Unless otherwise stated, all other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used without further purification. *E. coli* S12 extract was prepared from strain BL21-Star (DE3) as previously described [9].

2.2. Cell-free synthesis and protein quantification

The standard reaction mixture for cell-free protein synthesis consisted of the following components in a final volume of 15 μL: 57 mM HEPES–KOH (pH 8.2); 1.2 mM ATP; 0.85 mM each of CTP, GTP, and UTP; 2 mM DTT; 0.17 mg/mL *E. coli* total tRNA mixture; 0.64 mM cAMP; 90 mM potassium glutamate; 80 mM ammonium acetate; 12 mM magnesium acetate; 34 μg/mL L-5-formyl-5,6,7,8-tetrahydrofolic acid (folinic acid); 1.0 mM each of all 20 amino acids; 2% polyethylene glycol 8000; 67 mM creatine phosphate; 3.2 μg/mL creatine kinase; 10 μM L-[U-^{14}C]leucine (11.9 GBq/mmol); 6.7 μg/mL DNA; and 4 μL of S12 extract. Cell-free protein synthesis reactions were conducted in a water bath at 30 °C. Cell-free synthesized proteins were quantified by measuring trichloroacetic acid-precipitated radioactivity using a liquid scintillation counter (Wallac 1410, PerkinElmer, Waltham, MA, USA), as described previously [9]. In the reactions for assessing protein expression, identical concentrations of the plasmids (Table S1) encoding target protein and sfGFP (6.7 μg/mL) were added to the reaction mixture, and fluorescence of co-

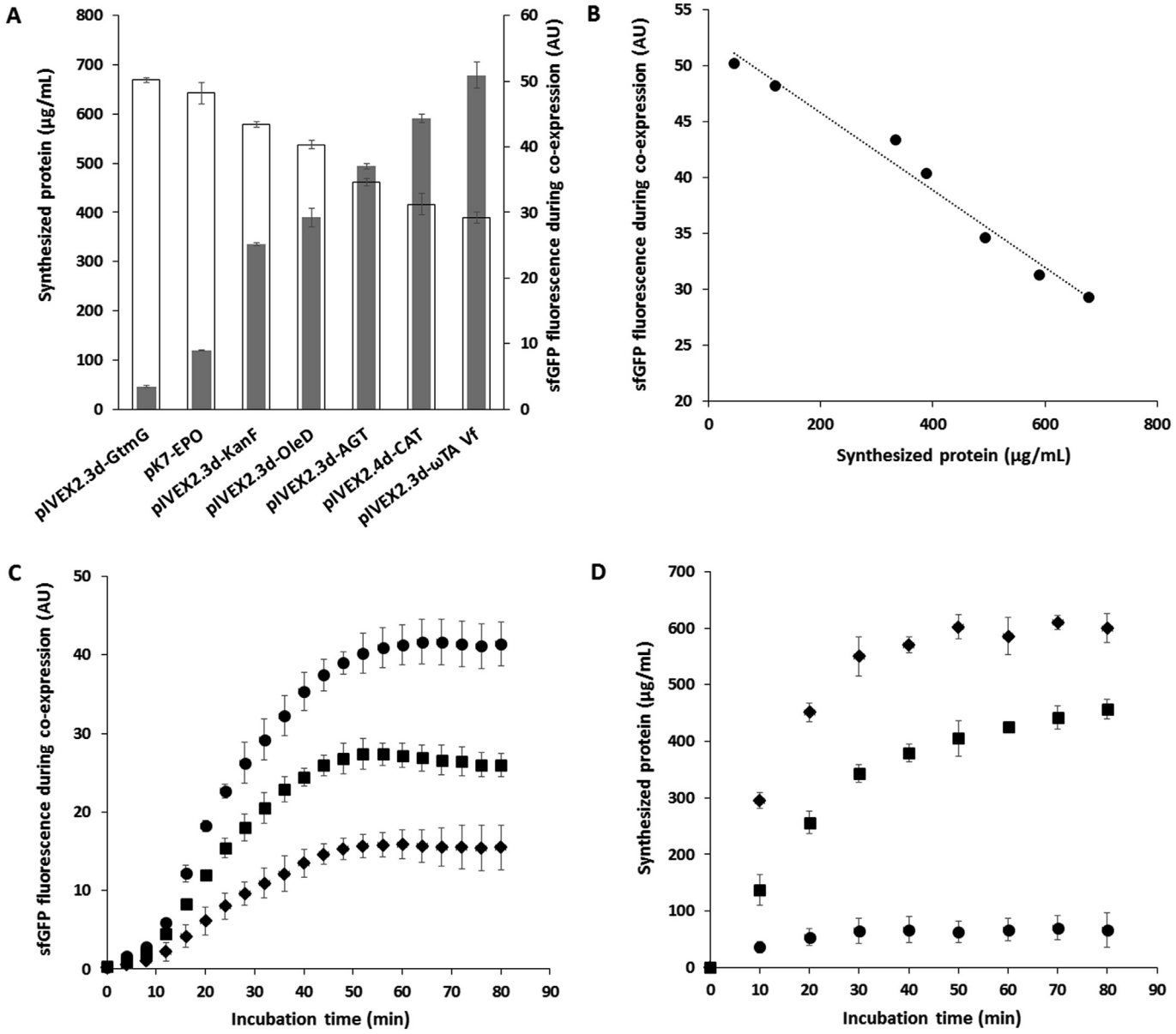

Fig. 1. Co-expression of different proteins with a common reporter protein (sfGFP). (A) Indicated plasmids were co-incubated with pK7-sfGFP in the same reaction mixture for cell-free synthesis. After 2 h for incubation, sfGFP fluorescence in the reaction mixture was measured as described in Materials and Methods (blank bars). Measured sfGFP fluorescence was compared with the amounts of individual proteins expressed in separate reactions (filled bar). (B) Statistic analysis showed a good correlation between the individual expression level and sfGFP fluorescence from the co-expression reactions (R^2 value = 0.945). The Pearson's correlation coefficient was −0.972 (P-value < 0.05). (C) Plasmids pIVEX 2.3d-GmtG, pIVEX 2.3d-OleD, or pIVEX2.3- ωTA Vf were co-expressed with the pK7-sfGFP in the same reaction mixture. During the incubation of the reaction mixtures, sfGFP fluorescence from the reaction mixture was measured every 4 min. (D) The three plasmids were separately incubated and the expressed proteins were quantified by measuring TCA-precipitated radioactivity of ^{14}C leucine in the assay samples withdrawn during the reactions.