Application of a Light-Inducible Metabolic Switch for Enhancing Bio-Production in Microorganisms

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Research objective

The production of useful compounds using microorganisms is important for achieving a sustainable society. Thus far, much effort has been dedicated to changing metabolic flux distributions to enhance target production through static pathway modifications. To further improve productivity, it is highly desirable to fine-tune the fluxes to achieve an ideal state. Although chemically inducible systems are widely used for metabolic regulation, these chemicals cannot be removed from the broth, it is difficult to perform flux fine-tuning. Furthermore, many useful compounds compete for the formation of biomass, and these are produced with repressing cell growth. However, cellular activity gradually decreases during the stationary phase. Once metabolism switches to the production mode by adding inducers, it becomes difficult to return to the growth mode. To overcome these issues, we developed a metabolic switch to alter flux distribution by light irradiation using an optogenetic tool (CcaS/R). Because light can irradiate cells from outside the vessels, it allows for the fine-tuning of flux with on-off regulation. The optogenetic switch may also contribute to enhanced production by repeating the production and growth modes.

In the present study, a light-inducible switch was applied to 1,3-propandiol (1,3-PDO) production in *Escherichia coli*. We developed an engineered strain that could change the metabolic state between growth and 1,3-PDO production modes using green and red lights, respectively (Fig. 1). The effects of flux control on 1,3-PDO production were also evaluated. Furthermore, because the metabolic network has many branches, orthogonal switches are necessary to widely alter the flux distributions. We developed another optogenetic switch using a blue-light-inducible transcription factor and regulated the flux ratios at the two branches.

Methods

During 1,3-PDO production, triosephosphate isomerase (TPI) expression is regulated by CcaS/R. After deleting *tpiA* from the MG1655(DE3) strain, we introduced genes encoding CcaS/R. *tpiA* is expressed under the control of the *cpcG2* promoter which is regulated by CcaS/R. To implement the 1,3-PDO synthesis pathway, *dhaB* and *gdrAB* derived from *Klebsiella pneumoniae* were introduced. The cells were inoculated into 20 mL of synthetic

medium containing glycerol, at an initial OD_{600} of 0.05, and incubated at 37°C and 150 rpm with LED light irradiation. 1,3-PDO was measured using high-performance liquid chromatography.

In multidimensional flux control, the flux ratio between the Embden-Meyerhof-Parnas (EMP), pentose phosphate (PP), and Entner-Doudoroff (ED) pathways is regulated by CcaS/R and EL222. Because a switch for controlling the flux ratio between the EMP and PP pathways has already been developed using CcaS/R, we constructed another switch to control the flux ratio between the PP and ED pathways using EL222. The MG1655(DE3)*ΔpgiΔgntR* strain was used as the parental strain. The EL222 binding sequence was inserted into the appropriate regions of the target gene promoters for up and down regulations. The cells were inoculated into 1 mL of synthetic medium containing [1-^{13}C] glucose at an initial OD_{600} of 0.1 in a 24-well plate. The plates were then incubated at 37°C and 500 rpm under each light condition. The flux ratio was determined based on the ^{13}C-enrichment of proteinogenic amino acids.

Results

1. Production of 1,3-PDO using a light-inducible metabolic switch

TPI expression was induced and repressed under green and red light, respectively. Thus, the constructed strain is expected to grow well under green light and produce 1,3-PDO under red light (Fig. 1B). To verify whether the TPI switch functioned properly, we first cultured the cells under continuous light conditions (Fig. 1C). As expected, growth was faster under green light than under red light. No differences in 1,3-PDO production were observed under green and red light.

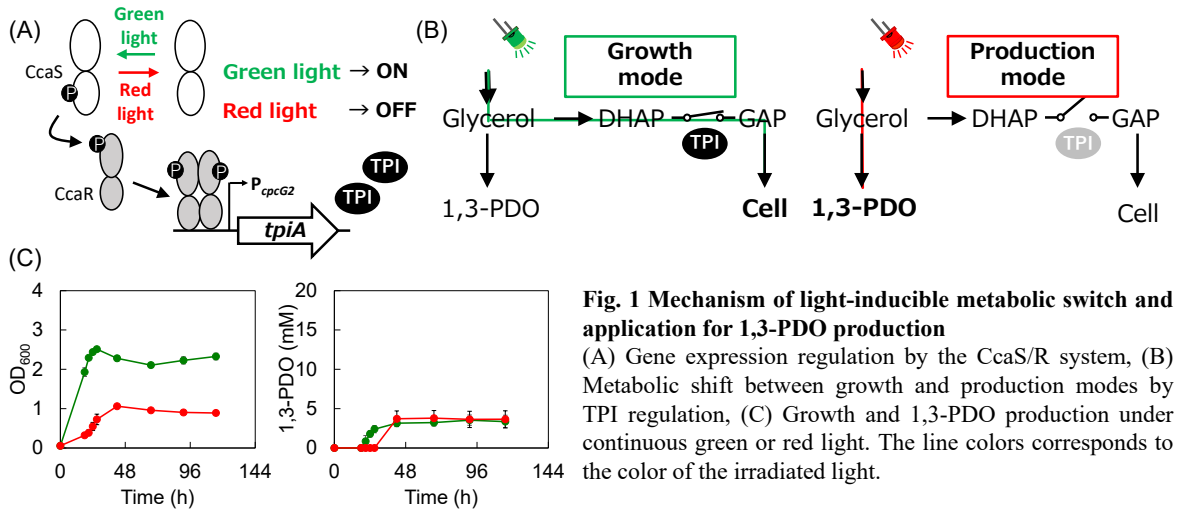

Fig. 1 Mechanism of light-inducible metabolic switch and application for 1,3-PDO production
(A) Gene expression regulation by the CcaS/R system, (B) Metabolic shift between growth and production modes by TPI regulation, (C) Growth and 1,3-PDO production under continuous green or red light. The line colors corresponds to the color of the irradiated light.

Next, we initially irradiated the cells with green light to facilitate growth and then changed to red light to alter the metabolism from growth to production modes (Fig. 2). Compared with the continuous green light condition, the cells grew to a similar OD_{600} and produced 1.6 times the amount of 1,3-PDO under red light. Furthermore, the effect of periodic light (1 min green

and 9 min red) during the stationary phase on 1,3-PDO production was evaluated. 1,3-PDO production was 2.3 times higher than that under the condition that initially facilitated growth with green light and switched to red light (Fig. 2). However, because 1,3-PDO was mainly produced during the early phase, recovery was not achieved. Growth reached a plateau at a certain level, even under continuous green light conditions, suggesting a shortage of essential nutrients. In the future, we will reconsider the components of the medium and aim to achieve efficient 1,3-PDO production by optimizing the periodic light patterns.

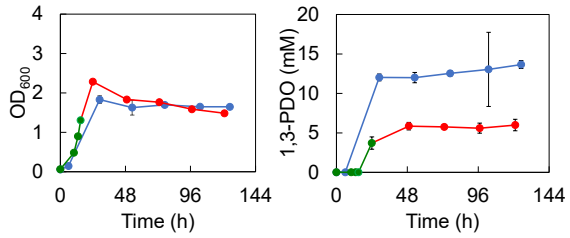

Fig. 2 Fermentation profiles with changing light during the stationary phase
For the data shown with green and red lines, the growth was initially induced by green light. After the OD_{600} reached 1, the green light was changed to red light to shift the metabolism to production mode. The line colors correspond to the color of the irradiated light. For the data shown with blue lines, after inducing the growth by green light similarly, periodic light (1 min green and 9 min red) was irradiated.

2. Control of flux distribution with two branches by multicolor irradiation

To manipulate the flux distribution by light irradiation, we developed a metabolic switch using another optogenetic tool that responds to light wavelengths different from those used by the CcaS/R system. The flux ratios of the three glycolytic pathways were regulated using green/red-light-responsive CcaS/R and blue-light-responsive EL222 (Fig. 3A). First, we aimed to control the flux ratio between the PP and ED pathways using blue light irradiation. After blocking the EMP pathway, the *gnd* and *edd* promoters were engineered for up- and downregulation, respectively, by EL222 under blue light. The flux ratios under dark and blue light conditions are shown in Fig. 3B. The PP and ED fluxes increased under dark and blue light conditions, respectively, suggesting that the blue-light-responsive metabolic switch functioned correctly. A previously developed CcaS/R-based switch for controlling the flux ratio between the EMP and PP pathways was implemented (Fig. 3C). The EMP flux increased under green light and the sum of the PP and ED fluxes increased under red light. Under both red and blue light, the PP flux decreased and the ED flux increased.

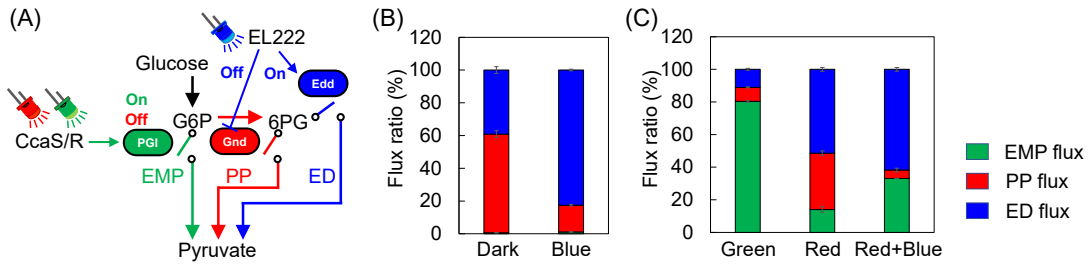

Fig. 3 Control of glycolytic flux ratio using CcaS/R and EL222 by multicolor irradiation
(A) Scheme of flux rewiring of glycolytic pathways by two optogenetic tools, (B) Flux ratios under dark and blue light conditions in a PGI-deletion strain with repressed PP flux and inducing ED flux by EL222, (C) Flux ratios when EL222 and CcaS/R are combined to control three glycolytic pathways.

Conclusion

The metabolic state of 1,3-PDO-producing *E. coli* can be altered to growth and production modes using a light-inducible metabolic switch. Furthermore, the flux ratios at the two branches of the glycolytic pathways were successfully regulated by multicolor light irradiation.