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Regulatory transcription factor (CooA)-driven carbon monoxide partial pressure sensing whole-cell biosensor

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ABSTRACT

We designed and constructed a whole-cell biosensor capable of detecting the presence and quantity of carbon monoxide (CO) using the CO regulatory transcription factor. This biosensor utilizes CooA, a CO-sensing transcription regulator that activates the expression of carbon monoxide dehydrogenase (CODH), to detect the presence of CO and respond by triggering the expression of a GUS reporter protein (β-glucuronidase). The GUS reporter protein is expressed from a CO-induced CooA-binding promoter (P_{cooF}) by CooA and enables the effective colorimetric detection of CO. An *Escherichia coli* strain used to validate the biosensor showed growth and GUS activity under anaerobic conditions; this study used the inert gas (Ar) to create anaerobic conditions. The pBRCO biosensor could successfully detect the presence of CO in the headspace. Moreover, the GUS-specific activity of pBRCO according to the CO strength as partial pressure followed Michaelis-Menten kinetics ($R^2 = 0.98$). It was confirmed that the GUS-specific activity of pBRCO increased linearly up to 30.39 kPa ($R^2 = 0.98$), and thus, a quantitative analysis of CO concentration (*i.e.*, partial pressure) was possible.

1. Introduction

Carbon monoxide (CO) modulates cellular processes and signaling pathways in biological systems [1]. CO has been demonstrated to affect cellular functions such as inflammation, cell growth, metabolism regulation, and apoptosis [1,2]. A deeper understanding of how CO is recognized and consumed within cells is essential for elucidating the mechanisms involved in oxidative stress, inflammation, and cellular metabolism [3,4]. In addition, CO is a multifunctional molecule with diverse applications in energy conversion, C1 chemistry, and industrial biotechnology [5]. CO is the main component of synthesis gas (syngas), and is recognized as a valuable resource to be converted to multi-carbon chemicals by either chemical or biological reactions, reducing greenhouse gas emissions and increasing chemical conversion efficiency [5,6]. In biological CO conversion reactions, microorganisms such as acetogens and methanogens metabolize CO through the Wood-Ljungdahl Pathway [6,7].

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Some microbes possess transcription factors that recognize specific molecules as analyte and regulate the transcription of related genes. CooA, a CO-sensing transcription regulator, was initially reported in the phototropic bacterium *Rhodospirillum rubrum* [8,9]. CooA is a heme-containing protein that binds CO in its heme pocket, causing a conformational change *via* a hinge that allows it to bind to DNA and activate the expression of carbon monoxide dehydrogenase (CODH) [10]. The heme group in CooA is unique in that it contains a histidine residue coordinated to the iron atom, allowing the selective binding of CO to other gases [10]. The CooA protein is thought to exist in two different states: an inactive form that cannot bind DNA and an active form that can bind DNA and activate the transcription of CODH [10,11]. Hence, it would be feasible to establish analyte (*i.e.*, CO) detection system based on regulatory transcription factor (*i.e.*, CooA), indicating that the development of a CO-sensing whole-cell biosensor is possible by utilizing CO-sensing transcriptional regulatory mechanisms and reporter proteins [12]. A CooA-based CO-sensing mechanism allows cells to recognize and respond to the presence of CO and trigger the expression of a GUS reporter protein (β-glucuronidase) [13,14,15]. As a visual indicator of CO exposure, CO-sensing whole-cell biosensors have the potential to elucidate intracellular regulatory mechanisms directly involving CO [16]. This type of biosensor, which incorporates a living cell, provides an accurate representation of a cell's complex and dynamic environment and allows investigation of the interplay between CO and cellular processes [13,17]. In particular, the occurrence of CO sensing and regulation of regulation intensity could be identified. Therefore, the development and validation of a whole-cell biosensor capable of detecting the presence and concentration of CO are important for advancing our understanding of its impact on cellular processes and its potential applications in both CO-related biology and bioconversion processes.

In this study, we developed a whole-cell biosensor based on a CO-sensing transcriptional regulatory mechanism that cooperates with reporter proteins. CO has various effects on biological metabolism; however, identifying CO-sensing mechanisms using living cells is useful for several reasons [18]. First, it provides insights into how microorganisms sense and respond to CO, including the genes and pathways involved in CO utilization. This knowledge may inform the development of more efficient and effective CO-utilizing microorganisms [19]. Second, identifying CO-sensing transcriptional regulatory mechanisms is crucial for developing CO-sensing biosensors with potential applications in industrial biotechnology, environmental monitoring, and human health [13]. Finally, by understanding how microorganisms sense and respond to CO, it is possible to develop more effective strategies for using CO as a renewable energy source by controlling and optimizing CO utilization in microorganisms [20]. As far as we know, there is not any study reported using the CO-sensing transcriptional regulator in microbial whole-cell.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Rhodospirillum rubrum KCTC1372 was grown anaerobically at 30 °C with shaking (200 rpm) in *R. rubrum* no-light CO (RRNCO) medium supplemented with 15 mM fructose as the carbon and energy source [21].

E. coli DH5α was grown under aerobic or anaerobic conditions in Luria-Bertani (LB) medium (Becton, Dickinson, and Company, USA). CO partial pressures (0, 5.07, 10.13, 20.26, 30.39, 50.65, 70.91, and 101.3 kPa) was added to the anaerobic LB to form the substrate. Erythromycin (300 μg/mL) was added to screen for transformants [14,22].

2.2. Plasmid construction

pELMKO and pECPH2::*uidA* were used to confirm negative and positive GUS activities, respectively (Table 1) [14]. pELMKO was constructed by modifying pECas9 [14]. To construct pECas9V2, multiple cloning sites (MCS), F-MCS_pECas9, and R-MCS_pECas9, were annealed via sequential reaction at 95 °C for 5 min and then at 25 °C for 1 h. The assembled fragment was then introduced into pECas9 through the *Spe*I site. To construct pELMKO, the pIP404 replicon, pMB1 replicon, and em^R from pECas9V2 were amplified

Table 1
Information on plasmids and primers.

Plasmids	Relevant characteristics	Source
pECPH2:*uidA*	pIP404 *ori*, pIP404 rep, em^R, pMB *ori*, H2, *uidA*	[14]
pECas9	pIP404 *ori*, pIP404 rep, em^R, pMB *ori*, H2, Cas9	[14]
pECas9V2	pIP404 *ori*, pIP404 rep, em^R, pMB *ori*, H2, Cas9, MCS	This study
pELMKO	pIP404 *ori*, pIP404 rep, em^R, pMB *ori*, MCS	This study
pBRCO	pIP404 *ori*, pIP404 rep, em^R, pMB *ori*, H2, *cooA*, P_{cooF}, *uidA*	This study
Primers	**Sequences (5'→3')**	**Source**
F-MCS_pECas9	CTA GTA TCG ATC GTC TAG ACT CGA GAT GCA TCC CGG GCC TGC AGG ACC GGT TTA ATT AAG GGC CCG TCG AC	This study
R-MCS_pECas9	CTA GGT CGA CGG GCC CTT AAT TAA ACC GGT CCT GCA GGC CCG GGA TGC ATC TCG AGT CTA GAC GAT CGA TA	This study
F_pELMKO	GTC GGA TCC CAA AAA AAT TTC CAA TAA T	This study
R_pELMKO	TTT GGA TCC GGC GAG GAA AAA ATA AGA G	This study
KP001	AAG GGC CCG GCA TCC GTT TAA AGT	This study
KP002	CCT TAA TTA AGA GCG CGG GTA GGC CTG	This study
KP003	AAG GGC CCA ATC CTT TTG CCA TTT ACA AAA CC	This study
KP004	GGT TAA TTA ACC CAC TCT AAG CCA CAA ACA CGC C	This study

*Underlined: priming sequences. Non-underlined: 5′-overhang with/without restriction enzyme recognition sites. MCS: multiple cloning site.

using the primers F_pELMKO and R_pELMKO, and the amplified fragment was assembled using the *Bam*HI site.

The biosensor in which the H2 promoter, *cooA*, P_{cooF}, and *uidA* were introduced for CO sensing was named pBRCO [9,14,23] The *cooA* and P_{cooF} sequences were also found in *R. rubrum*. pBRCO was constructed by modifying the pECPH2:*uidA* plasmid. The *cooA* sequence combined with the H2 and P_{cooF} sequences were ordered as synthetic double-stranded DNA fragments (gBlocks, Integrated DNA Technologies, Coralville, Iowa, USA). The gBlocks were amplified using KP001 and KP002, and the amplified fragment was assembled using the *Apa*I and *Pac*I sites. The *uidA* gene was amplified using KP003 and KP004, and the amplified fragment was assembled using the *Nhe*I and *Pac*I sites.

2.3. GUS activity assay

E. coli DH5α transformants were cultured in 10 mL LB medium. Cells were centrifuged (15,520×*g*) for 5 min at 4 °C and then resuspended in 400 μL of sodium phosphate buffer (50 mM, pH 7.0) [15] To produce permeabilized cells, 25 μL of an acetone-toluene (9:1 v/v) solution was added, and the mixture was incubated at 37 °C for 40 min. For the quantitative GUS activity assay using 4-nitrophenyl β-*d*-glucuronide (4-NPG), 200 μL of a GUS buffer (50 mM sodium phosphate (pH 7.0), 10 mM β-mercaptoethanol, 1 mM EDTA, 0.1% Triton X-100), and 20 μL of GUS substrate (10 mg/mL of 4-NPG in 50 mM sodium phosphate) were added to 50 μL of a cell suspension [14,15]. The mixture was incubated at 37 °C, and the reaction was stopped by adding 200 μL of a stop buffer (200 mM Na_2CO_3); then, 200 μL of the mixture was added to a 96-well plate (SPL Life Sciences, Republic of Korea) [14,15]. Absorbance was measured at 405 nm using a microplate reader (Epoch, BioTek, USA) at 10-min intervals for up to 30 min [14,15]. One unit (U) was defined as 1 μmol of 4-NPG ($\varepsilon_{405} = 18\ mM^{-1}\ cm^{-1}$) per minute [14].

3. Results and discussion

3.1. Construction of CO-sensing whole-cell biosensors using the GUS reporter gene

We presented the design and construction of a whole-cell biosensor capable of detecting the presence and level of CO using colorimetric analysis (Fig. 1). The proposed biosensor is based on utilizing CooA, a dimeric CO-sensing transcription factor and activator of *R. rubrum*, as the core component. CooA is a CODH transcription factor and a key enzyme involved in CO metabolism. As CO induces CooA, it binds to a specific DNA transcription factor binding site (TFBS) in the P_{cooF} region and activates the transcriptional levels of downstream genes [8]. To enhance the sensitivity of the cellular biosensing system, a reporter protein coupled with the identified promoter P_{cooF} was inserted into the biosensor to express activated CooA (Fig. 1A) [8]. The GUS reporter protein was chosen because it has been extensively utilized in various assays and has been demonstrated to retain its activity even in anaerobic and CO-rich environments [14]. Moreover, it also enables the effective colorimetric detection of CO, even in autofluorescent strains [14,15]. In addition, GUS protein expression can be evaluated qualitatively and quantitatively through colorimetric analysis and introduced into the biosensor (Fig. 1A and B).

As mentioned, we used the transcription factor CooA, the *cooF* promoter (P_{cooF}), and the GUS reporter protein to construct a genetically regulated whole-cell-based biosensor system that exhibited a CO-sensing transcriptional regulation mechanism and named it pBRCO. The pECPH2::*uidA* vector was used as the backbone for introducing CooA, P_{cooF}, and the GUS reporter protein. Here, the previously obtained H2 promoter was introduced for the constitutive expression of CooA, whereas P_{cooF} was introduced to express the GUS reporter protein, ensuring that their individual transcription and protein expression did not interfere with each other [14]. The DNA-binding motif of TFBS was introduced into P_{cooF}, enabling CooA to bind to P_{cooF} using CO as a substrate and activate its transcription. Conversely, pBRCO exhibited low GUS activity in the absence of CO. The presence of CO substrates and their transcriptional regulation were confirmed through qualitative and quantitative assays of GUS activity (Fig. 1C).

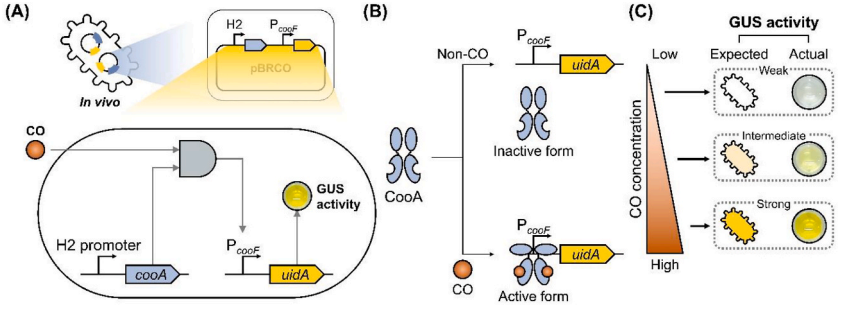

Fig. 1. Schematic of the CO-sensing whole-cell biosensor. (A) CO-sensing mechanism of the biosensor pBRCO. CO regulates GUS activity through CooA in the intracellular environment. (B) CO-sensing and GUS regulation. The presence of CO affects the resulting form of CooA and the expression of GUS reporter protein. (C) The presence of CO leads to increased GUS activity and yellow coloration when 4-NPG is used as a substrate. The strength of the color reaction corresponds to the expression level of the GUS reporter protein. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2. Optimizing the culture conditions to evaluate the CO-sensing whole-cell biosensor

To determine the cultivation conditions for evaluating the CO-sensing whole-cell biosensor pBRCO, we examined the growth and metabolic inhibition ofE. coli DH5α transformed with the GUS constitutive expression vector pECPH2::*uidA* under anaerobic conditions with CO. In the absence of genes encoding CooA and P_{cooF}, the ability of the GUS reporter protein to show sufficient activity to transfer CO-induced CooA and P_{cooF} signals must be confirmed [14,15]. Therefore, the extent of growth and metabolic inhibition caused by anaerobic and CO conditions was confirmed by comparing the growth rate and GUS-specific activity in pECPH2::*uidA*--transformed strains. To confirm the potential effect of nitrogen (N_2), anaerobic conditions were established by purging with N_2 or inert Ar gas (101.3 kPa). Next, CO was fed at strength of 0, 5.07, 10.13, 20.26, 30.39, 50.65, 70.91, and 101.3 kPa in the headspace with an N_2 or Ar balance (Fig. 2) [22].

No significant difference in growth rate was observed between N_2- and Ar-based conditions (Fig. 2A, B, 3A). Although GUS-specific activity decreased by 9.08% under Ar conditions compared to N_2 conditions, no significant difference in growth levels was observed between the two conditions. Moreover, metabolic inhibition barely occurred under Ar conditions (Fig. 3B). Incubation of the strain with Ar was thought to have no significant inhibitory effect on GUS activity. Therefore, further reactions for pBRCO evaluation were analyzed based on the results obtained under Ar conditions. Although growth inhibition was not confirmed under Ar/CO conditions (Fig. 3C), GUS-specific activity was inhibited by extent of CO concentration; and a similar pattern was observed under N_2 conditions (Figs. 3D, 4A and 4B). Compared to the no CO group, GUS-specific activity decreased gradually as follows by 6.56% to 24.72% (at 0 to 101.3 kPa) (Fig. 3D). The decrease in GUS-specific activity at 20.26 kPa was 19.18%, which was higher than the 17.74% decrease observed at 30.39 kPa, but the difference was not significant (*p-value* 0.3). Furthermore, since GUS-specific activity gradually decreases under N_2 conditions, it is anticipated that there will be no significant effect under Ar conditions (Fig. 4B). A linear decrease in GUS-specific activity was detected in the range of 0–30.39 kPa atmospheric CO content. Moreover, the pECPH2::*uidA*-transformed strains showed GUS-specific activity at all CO concentration ranges. After exceeding 30.39 kPa of CO partial pressure, GUS-specific activity decreased due to the CO-induced inhibition of metabolism but showed sufficient activity to be applied to pBRCO.

3.3. Confirming the feasibility of the CO-sensing whole-cell biosensor through GUS-specific activity analysis

After constructing pBRCO, the feasibility of the CO-sensing whole-cell biosensor was demonstrated by analyzing its GUS-specific activity in response to CO level variation (Fig. 5). Next, transcriptional regulation was confirmed by observing differences in GUS activity under various CO conditions. We validated the transformation of pBRCO into *E. coli* DH5α cells, which lack the CO-sensing mechanism present in CooA and have low GUS activity. The transformed strains were grown under anaerobic conditions pressurized 30.39 kPa CO and then GUS activity was confirmed. The results showed that pBRCO specifically displayed a yellow coloration and higher GUS-specific activity under CO conditions, whereas no activity was observed in the negative control (pELMKO) (Fig. 5A). In contrast, the positive control (pECPH2::*uidA*) displayed constant GUS activity regardless of the CO conditions (Fig. 5A). These results demonstrate the successful operation of pBRCO and its potential for CO sensing applications.

The operation and effectiveness of pBRCO were verified by analyzing its GUS-specific activity in response to different CO concentrations. Its GUS-specific activity was measured at different CO partial pressures (0, 5.07, 10.13, 20.26, 30.39, 50.65, 70.91, and 101.3 kPa) (Figs. 3 and 4). The positive control (pECPH2::*uidA*) showed a decrease in GUS-specific activity with an increase in CO strength. The negative control (pELMKO) showed a similar trend, which was also observed in the N_2 condition (Figs. 4C and 5B). In contrast, pBRCO showed an increase in GUS-specific activity as the CO concentration increased, with a linear increase up to 30.39 kPa CO pressure (Fig. 5B). Specifically, pBRCO demonstrated a 41.52% increase in GUS-specific activity at 5.07 kPa CO, 64.37% (10.13 kPa), 110.57% (at 20.26 kPa), 148.89% (at 30.39 kPa), 183.54% (at 50.65 kPa), 188.94% (at 70.91 kPa), and 214.25% (at 101.3 kPa), respectively (Fig. 5B). These results indicate that pBRCO can quantify CO strength, confirming the viability of the CO-sensing whole-cell biosensor pBRCO as a tool for detecting CO levels.

The feasibility of quantifying CO concentration through pBRCO was assessed through GUS-specific activity analysis. The GUS-

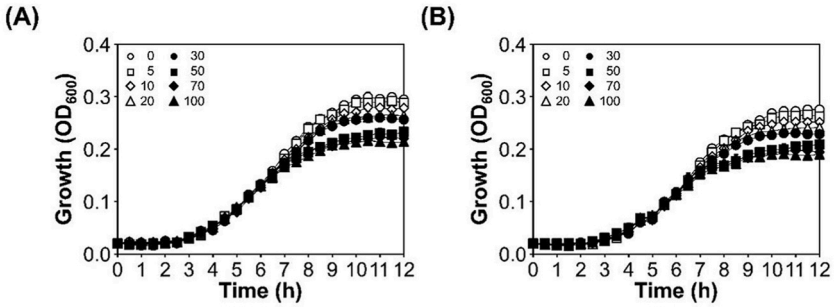

Fig. 2. Growth profile of pECPH2::*uidA*-transformed *E. coli* DH5α under anaerobic CO conditions. (A) Growth curve of the pECPH2::*uidA*-transformed strain showing the optical density at 600 nm (OD_{600}) under N_2 conditions. (B) Growth curve of the pECPH2::*uidA*-transformed strain showing the optical density at 600 nm (OD_{600}) under Ar conditions. All measurements were repeated three times.

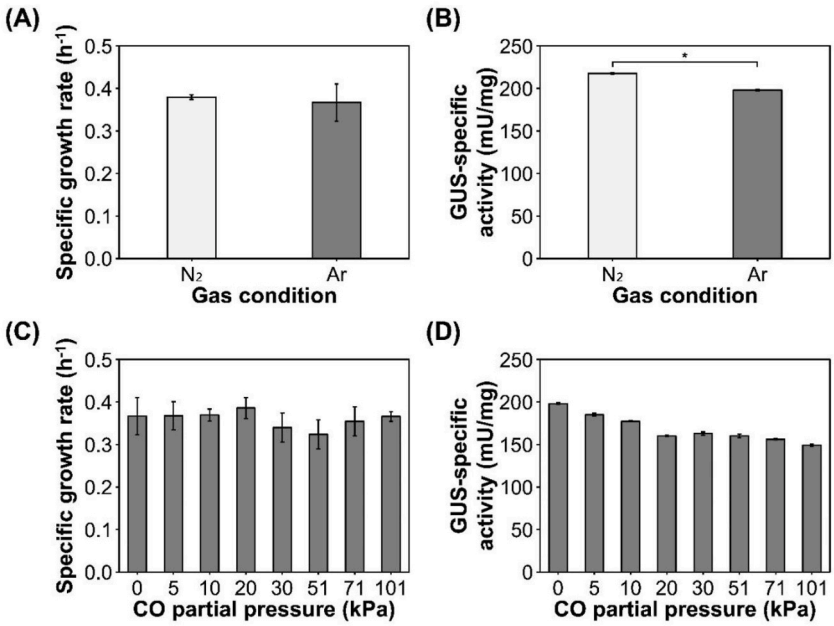

Fig. 3. Characterization of the growth and metabolic inhibition levels of pECPH2::*uidA*-transformed *E. coli* DH5α under anaerobic CO conditions. (A) Specific growth rate under N_2 and Ar conditions. (B) GUS-specific activity under N_2 and Ar conditions. Growth in an Ar atmosphere showed a decrease in GUS-specific activity of 9.08% compared to that in N_2. (C) Specific growth rate for each CO concentration under Ar conditions. (D) GUS-specific activity for each CO concentration under an Ar atmosphere. All measurements were repeated three times.

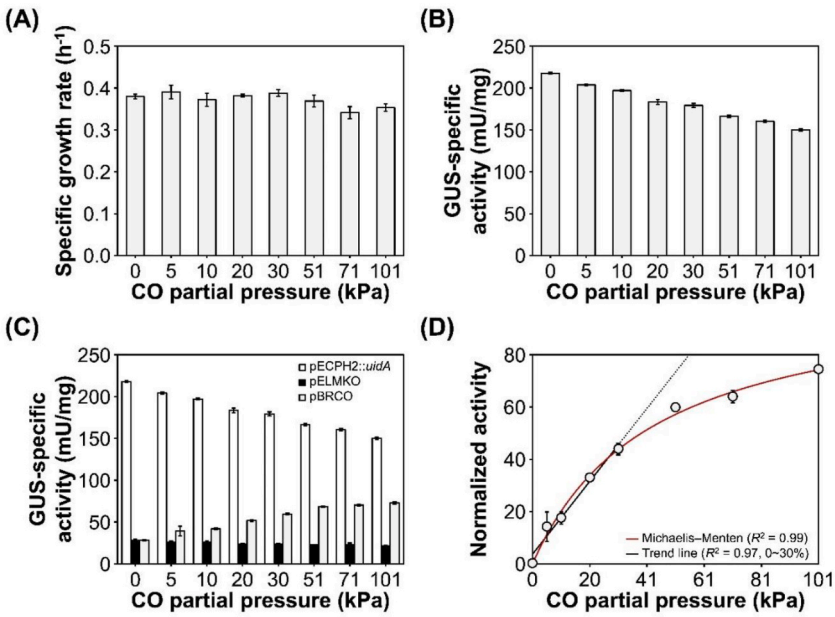

Fig. 4. Characterization of the growth and metabolic inhibition levels of pECPH2::*uidA*-transformed *E. coli* DH5α under anaerobic CO conditions with confirmation of CO-sensing whole-cell biosensor operation. (A) Specific growth rate for each CO concentration under N_2 conditions. (B) GUS-specific activity for each CO concentration under N_2 conditions. (C) GUS-specific activity of the positive control (pECPH2::*uidA*), negative control (pELMKO), and pBRCO in each CO concentration under N_2 conditions. (D) GUS-specific activity of pBRCO normalized based on the reduction rate of the GUS-specific activity of pECPH2::*uidA* after subtracting the GUS-specific activity of pELMKO in each CO concentration under N_2 conditions. The normalized GUS-specific activities of the pBRCO followed Michaelis-Menten kinetics ($R^2 = 0.99$) and increased linearly from 0 to 30.39 kPa atmospheric CO content ($R^2 = 0.97$). All measurements were repeated three times.

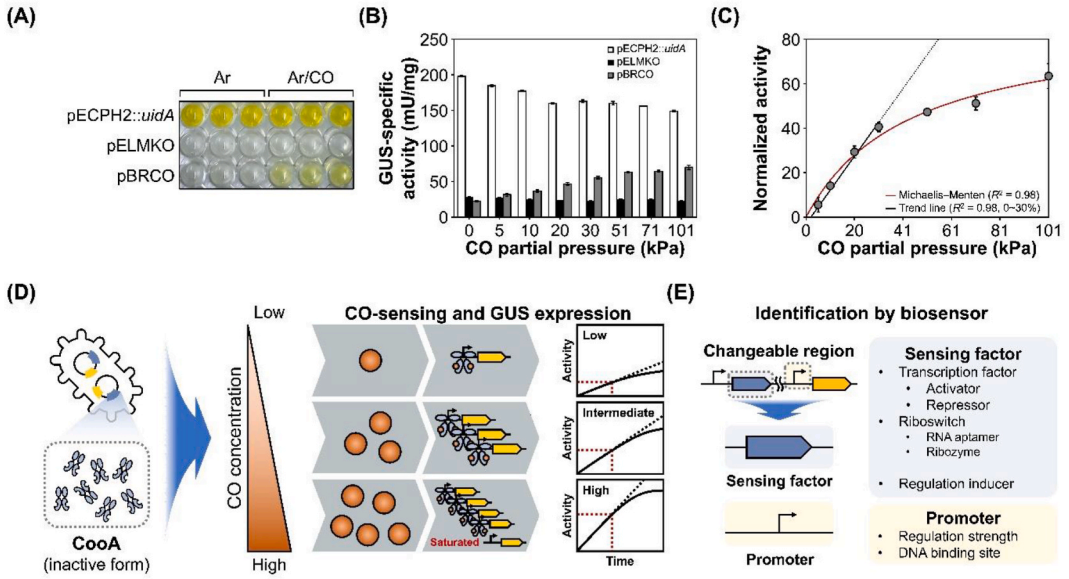

Fig. 5. Confirmation of the operation of the CO-sensing whole-cell biosensor according to CO level. (A) Confirmation of GUS activity through the 4-NPG substrate under Ar and 30.39 kPa CO conditions. The pBRCO has a yellow coloration specific to the CO condition. (B) The GUS-specific activity of the positive control (pECPH2::*uidA*), negative control (pELMKO), and pBRCO in each CO concentration under Ar conditions. (C) The GUS-specific activity of the pBRCO normalized based on the reduction rate of the GUS-specific activity of pECPH2::*uidA* after subtracting the GUS-specific activity of pELMKO in each CO concentration under Ar conditions. The normalized GUS-specific activities of the pBRCO followed Michaelis-Menten kinetics ($R^2 = 0.98$) and increased linearly from 0 to 30.39 kPa atmospheric CO content ($R^2 = 0.98$). (D) With pBRCO, the presence of CO can be qualitatively and quantitatively confirmed for GUS activity. (E) Novel regulators, including transcription factors and riboswitches, regulatory strength, and the binding sites of the factors can be identified through modification of the sensing factor region of the biosensor. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

specific activity of pBRCO was normalized to the reduction rate of the GUS-specific activity of pECPH2::*uidA* after subtracting the GUS-specific activity of pELMKO. The normalized GUS-specific activity of pBRCO was then plotted against same range of CO partial pressure (0, 5.07, 10.13, 20.26, 30.39, 50.65, 70.91, and 101.3 kPa); a similar pattern was observed under N_2 conditions (Figs. 4D and 5C). The GUS-specific activity of pBRCO followed Michaelis-Menten kinetics with a high degree of correlation ($R^2 = 0.98$), and the activity increased linearly up to 30.39 kPa ($R^2 = 0.98$). These findings confirmed that CO sensing using pBRCO could accurately quantify up to 30.39 kPa of CO partial pressure (Fig. 5C). The concentration of the CO substrate is the gas ratio in the headspace, not the dissolved state that participates in the actual reaction. Moreover, the GUS specific activity was normalized, so it is difficult to accurately derive the Michaelis-Menten kinetics parameters. Assuming sufficient CO substrates are provided, saturation is expected to occur in CooA, which directly senses CO, or in the GUS reporter protein expressed *via* P_{cooF}.

In this study, we successfully constructed a CO-sensing whole-cell biosensor, pBRCO, incorporating a CO-sensing transcriptional regulatory mechanism. Using pBRCO, the presence of CO could be qualitatively and quantitatively determined (Fig. 5D). Furthermore, by modifying the CooA and P_{cooF} regions, pBRCO could be used to identify other CO-sensing-related regulatory factors, such as novel CO-sensing mechanisms that have not yet been discovered, and even riboswitches (Fig. 5E). The structural characteristics of this biosensor containing reporter proteins induced by transcription factors and TFBS can be used to identify transcriptional regulatory mechanisms by introducing novel transcription factors, binding regions, and inducers.

4. Conclusion

In this study, we successfully constructed a CO-sensing whole-cell biosensor based on the CO-sensing transcription factor CooA in *R. rubrum* and the GUS reporter gene. The biosensor pBRCO was designed to exhibit CO-sensing transcriptional regulation by utilizing the CooA, P_{cooF}, and GUS reporter proteins. The GUS reporter protein was selected for its insensitivity to CO and ability to provide colorimetric data. Growth evaluation of the pECPH2::*uidA*-transformed strain under anaerobic conditions revealed no significant difference in growth rate between N_2 and Ar-based conditions; however, its GUS-specific activity was lower in an Ar atmosphere than in N_2. In addition, it was confirmed that the growth of the strains and quantification of GUS activity were possible at all CO concentrations. Verification of pBRCO was confirmed through its GUS-specific activity. The GUS-specific activity of pBRCO followed Michaelis-Menten kinetics with a high correlation ($R^2 = 0.98$), and the activity increased linearly up to 30.39 kPa of CO partial pressure ($R^2 = 0.98$). These results confirm that CO sensing *via* pBRCO can be linearly quantified up to 30.39 kPa, indicating that CO molecules are a limiting factor in such conditions. As we believe that this type of whole-cell biosensor is not affected by the condition and type of gas phase, it can be operated in several conditions for identify the novel regulatory mechanisms. The biosensor developed

in this study is a promising tool for advancing our understanding of CO-related biology and bioconversion processes. Based on this biosensor, we anticipate that it has the potential to be used to identify CO-sensing-related regulation factors and novel CO-sensing mechanisms. According to the structural characteristics of this biosensor, it can be used to identify novel CO-related transcriptional regulatory mechanisms.

Author contribution statement

Byeongchan Kang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hyeryeong Lee: Analyzed and interpreted the data; Wrote the paper.

Soyoung Oh: Conceived and designed the experiments; Wrote the paper.

Ji-Yeon Kim, In Seop Chang: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Young-Joon Ko: Conceived and designed the experiments.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] H.P. Kim, S.W. Ryter, A.M.K. Choi, Co as a cellular signaling molecule, Annu. Rev. Pharmacol. Toxicol. 46 (2006) 411–449, https://doi.org/10.1146/annurev.pharmtox.46.120604.141053.

[2] M. Sun, H. Yu, K. Zhang, S. Wang, T. Hayat, A. Alsaedi, D. Huang, Palladacycle based fluorescence turn-on probe for sensitive detection of carbon monoxide, ACS Sens. 3 (2018) 285–289, https://doi.org/10.1021/acssensors.7b00835.

[3] S.W. Ryter, L.E. Otterbein, Carbon monoxide in biology and medicine, Bioessays 26 (2004) 270–280, https://doi.org/10.1002/bies.20005.

[4] H. Kang, B. Park, S. Oh, D. Pathiraja, J.-Y. Kim, S. Jung, J. Jeong, M. Cha, Z.-Y. Park, I.-G. Choi, I.S. Chang, Metabolism perturbation caused by the overexpression of carbon monoxide dehydrogenase/Acetyl-CoA synthase gene complex accelerated gas to acetate conversion rate of *Eubacterium limosum* KIST612, Bioresour. Technol. 341 (2021), 125879, https://doi.org/10.1016/j.biortech.2021.125879.

[5] S. Fujimori, S. Inoue, Carbon monoxide in main-group chemistry, J. Am. Chem. Soc. 144 (2022) 2034–2050, https://doi.org/10.1021/jacs.1c13152.

[6] A. Kaithal, M. Hölscher, W. Leitner, Carbon monoxide and hydrogen (syngas) as a C1-building block for selective catalytic methylation, Chem. Sci. 12 (2021) 976–982, https://doi.org/10.1039/D0SC05404F.

[7] S.W. Ragsdale, Enzymology of the wood–ljungdahl pathway of acetogenesis, Ann. N. Y. Acad. Sci. 1125 (2008) 129–136, https://doi.org/10.1196/annals.1419.015.

[8] Y. He, T. Gaal, R. Karls, T.J. Donohue, R.L. Gourse, G.P. Roberts, Transcription activation by CooA, the CO-sensing factor from *Rhodospirillum rubrum*: THE interaction between CooA and the C-terminal domain of the α subunit of RNA polymerase*, J. Biol. Chem. 274 (1999) 10840–10845, https://doi.org/10.1074/jbc.274.16.10840.

[9] D. Shelver, R.L. Kerby, Y. He, G.P. Roberts, CooA, a CO-sensing transcription factor from *Rhodospirillum rubrum*, is a CO-binding heme protein, Proc. Natl. Acad. Sci. USA 94 (1997) 11216–11220, https://doi.org/10.1073/pnas.94.21.11216.

[10] M.K. Chan, CooA, CAP and allostery, Nat. Struct. Mol. Biol. 7 (2000) 822–824, https://doi.org/10.1038/79559.

[11] W.N. Lanzilotta, D.J. Schuller, M.V. Thorsteinsson, R.L. Kerby, G.P. Roberts, T.L. Poulos, Structure of the CO sensing transcription activator CooA, Nat. Struct. Mol. Biol. 7 (2000) 876–880, https://doi.org/10.1038/82820.

[12] M. Koch, A. Pandi, O. Borkowski, A.C. Batista, J.-L. Faulon, Custom-made transcriptional biosensors for metabolic engineering, Curr. Opin. Biotechnol. 59 (2019) 78–84, https://doi.org/10.1016/j.copbio.2019.02.016.

[13] G. Femerling, S. Gama-Castro, P. Lara, D. Ledezma-Tejeida, V.H. Tierrafría, L. Muñiz-Rascado, C. Bonavides-Martínez, J. Collado-Vides, Sensory systems and transcriptional regulation in *Escherichia coli*, Front. Bioeng. Biotechnol. 10 (2022), https://doi.org/10.3389/fbioe.2022.823240.

[14] J. Jeong, J.-Y. Kim, B. Park, I.-G. Choi, I.S. Chang, Genetic engineering system for syngas-utilizing acetogen, *Eubacterium limosum* KIST612, Bioresour. Technol. Rep. 11 (2020), 100452, https://doi.org/10.1016/j.biteb.2020.100452.

[15] O. Johnsborg, D.B. Diep, I.F. Nes, Structural analysis of the peptide pheromone receptor PlnB, a histidine protein kinase from *Lactobacillus plantarum*, J. Bacteriol. 185 (2003) 6913–6920, https://doi.org/10.1128/JB.185.23.6913-6920.2003.

[16] L. Yuan, W. Lin, L. Tan, K. Zheng, W. Huang, Lighting up carbon monoxide: fluorescent probes for monitoring CO in living cells, Angew. Chem. Int. Ed. 52 (2013) 1628–1630, https://doi.org/10.1002/anie.201208346.

[17] G.S. Hossain, M. Saini, R. Miyake, H. Ling, M.W. Chang, Genetic biosensor design for natural product biosynthesis in microorganisms, Trends Biotechnol. 38 (2020) 797–810, https://doi.org/10.1016/j.tibtech.2020.03.013.

[18] S.Y. Lee, D.-Y. Lee, T.Y. Kim, Systems biotechnology for strain improvement, Trends Biotechnol. 23 (2005) 349–358, https://doi.org/10.1016/j.tibtech.2005.05.003.

[19] S.K. Ainala, E. Seol, B.S. Sekar, S. Park, Improvement of carbon monoxide-dependent hydrogen production activity in *Citrobacter amalonaticus* Y19 by over-expressing the CO-sensing transcriptional activator, CooA, Int. J. Hydrog. Energy 39 (2014) 10417–10425, https://doi.org/10.1016/j.ijhydene.2014.04.148.

[20] S. Zhang, F. Guo, W. Yan, Z. Dai, W. Dong, J. Zhou, W. Zhang, F. Xin, M. Jiang, Recent advances of CRISPR/Cas9-based genetic engineering and transcriptional regulation in industrial biology, Front. Bioeng. Biotechnol. 7 (2020) 459, https://doi.org/10.3389/fbioe.2019.00459.

[21] O. Revelles, N. Tarazona, J.L. García, M.A. Prieto, Carbon roadmap from syngas to polyhydroxyalkanoates in *Rhodospirillum rubrum*, Environ. Microbiol. 18 (2016) 708–720, https://doi.org/10.1111/1462-2920.13087.
[22] D.L. Gee, W. Duane Brown, The effect of carbon monoxide on bacterial growth, Meat Sci. 5 (1981) 215–222, https://doi.org/10.1016/0309-1740(81)90004-8.
[23] M.-C. Hoffmann, Y. Pfänder, M. Fehringer, F. Narberhaus, B. Masepohl, NifA- and CooA-coordinated *cowN* expression sustains nitrogen fixation by *Rhodobacter capsulatus* in the presence of carbon monoxide, J. Bacteriol. 196 (2014) 3494–3502, https://doi.org/10.1128/JB.01754-14.