

Modeling the Light- and Redox-Dependent Interaction of PpsR/AppA in *Rhodobacter sphaeroides*

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ABSTRACT Facultative photosynthetic bacteria switch their energy generation mechanism from respiration to photosynthesis depending on oxygen tension and light. Part of this transition is mediated by the aerobic transcriptional repressor PpsR. In *Rhodobacter sphaeroides*, the repressive action of PpsR is antagonized by the redox- and blue-light-sensitive flavoprotein AppA which results in a unique phenotype: The repression of photosynthesis genes at intermediate oxygen levels and high light intensity, which is believed to reduce the risk of photooxidative stress. To analyze the underlying mechanism we developed a simple mathematical model based on the AppA-dependent reduction of a disulfide bond in PpsR and the light-sensitive complex formation between the reduced forms of AppA and PpsR. A steady-state analysis shows that high light repression can indeed occur at intermediate oxygen levels if PpsR is reduced on a faster timescale than AppA and if the electron transfer from AppA to PpsR is effectively irreversible. The model further predicts that if AppA copy numbers exceed those of PpsR by at least a factor of two, the transition from aerobic to anaerobic growth mode can occur via a bistable regime. We provide necessary conditions for the emergence of bistability and discuss possible experimental verifications.

INTRODUCTION

Purple nonsulfur bacteria are remarkably versatile in their growth capabilities. Under aerobic conditions, they generate energy via respiration using molecular oxygen as the final electron acceptor. However, when the oxygen tension drops below a certain threshold these bacteria derive energy through photosynthesis using light as an alternative energy source. In *Rhodobacter sphaeroides* the transition from aerobic to anaerobic growth is mediated by three major regulatory systems which induce the formation of components of the photosystem at the transcriptional level: The PrrB/PrrA (RegB/RegA) two-component system (1,2), the anaerobic activator FnrL (3), and the aerobic repressor PpsR (CrtJ) (4,5). Unlike the first two systems, which are global gene regulators, PpsR is specifically involved in the regulation of photosynthesis (PS) genes such as *bch* (bacteriochlorophyll synthesis), *crt* (carotenoid synthesis), *puc* (pigment-binding proteins of the light harvesting complex II), and *puf* (polypeptides of the reaction centers) (6,7).

The oxygen tension serves as the major regulatory signal for the expression of PS genes. Typically, three growth regimes are distinguished according to the amount of dissolved oxygen in the growth medium (8,9): Aerobic ($\approx 200 \mu\text{M}$), semiaerobic ($\approx 100 \mu\text{M}$), and anaerobic/low oxygen ($\leq 3 \mu\text{M}$). Under aerobic conditions, PpsR represses gene transcription by cooperative binding to two palindromic sites in its target promoters (10,11). This binding is stimulated by oxygen causing the formation of an intramolecular disulfide bond between two redox-active cysteine

residues (5,12). As oxygen tension drops, the disulfide bonds are reduced to thiol groups, lowering the DNA binding affinity. As a result, the repressor molecule dissociates from the DNA, thereby allowing the transcription of PS genes under anaerobic conditions even in the absence of light (13).

The effect of light on PS gene transcription depends on both the oxygen tension and the light quality, i.e., the wavelength of the incident photons. Under semiaerobic conditions the expression of photosynthesis genes such as *puf* and *puc* is highly repressed in *R. sphaeroides* under strong blue light (450 nm) illumination (14), presumably to avoid the accumulation of toxic reactive oxygen species in the simultaneous presence of oxygen and light. This light-dependent repression is mediated by the blue-light- and redox-sensitive protein AppA which antagonizes the repressive action of PpsR in the dark. Because an AppA-homolog does not seem to exist in other purple bacteria, the phenotype related to the blue-light response under semiaerobic conditions is unique to *R. sphaeroides* (8).

First evidence for an interaction between AppA and PpsR came from the observation that an AppA null mutant is impaired in photosynthetic growth whereas a secondary PpsR null mutant relieves this effect (15,16). In addition, *ppsR* gene expression was found to be generally unaffected by growth conditions, suggesting that AppA acts as a modulator of the PpsR activity at the posttranscriptional level. Moreover, in the presence of AppA, the apparent DNA binding affinity of PpsR is significantly decreased (11), indicating that AppA interferes with DNA binding of PpsR and is required for a full induction of PS genes.

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In vitro experiments showed that AppA inhibits the DNA-binding activity of oxidized PpsR by two mechanisms (11,17):

1. By reducing a disulfide bond in PpsR; and
2. By a blue-light-dependent sequestration of PpsR proteins into transcriptionally inactive complexes.

Based on this core mechanism, several phenomenological models were proposed to explain the effect of oxygen and blue light on the regulatory properties of the PpsR/AppA circuit (8,11,18,19). However, it is unclear whether these two interactions are sufficient to generate the experimentally observed behavior, especially the PS gene repression under high light illumination in the semiaerobic regime. It is also unknown whether the AppA-mediated antirepression of PpsR activity has further beneficial effects compared to more simple regulatory mechanisms in other purple bacteria.

As a first step toward a more quantitative understanding of the regulatory capabilities of the AppA/PpsR system, we have developed a simple mathematical model based on the structural knowledge of the interactions between AppA and PpsR. Because none of the kinetic parameters are known from experiments we introduce dimensionless quantities to assess the relative importance of individual reaction steps for the steady-state behavior of the system. We find that high light-induced repression of PS genes can indeed emerge at intermediate oxygen concentrations, provided that PpsR is reduced on a much faster timescale than AppA and if the reduction of PpsR occurs in an effectively irreversible manner. The model further predicts that the transition from aerobic to anaerobic growth regime could occur via a bistable switch which, to our knowledge, has not been observed experimentally yet. We provide necessary conditions for the occurrence of bistability and discuss possible experimental verifications.

METHODS

Model for the interaction between AppA and PpsR

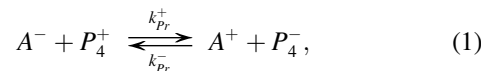
AppA is a flavoprotein which contains a FAD-binding domain in its N-terminal region (denoted as BLUF for blue-light sensing using flavin adenine dinucleotide). With FAD noncovalently attached to the BLUF domain, it can act as a blue-light sensor (11,20,21). In addition, AppA contains a cysteine-rich C-terminal domain which is believed to be involved in the oxidation/reduction of PpsR (11). Moreover, recent studies identified a heme-binding domain in the C-terminal part of the AppA protein (19,22), which suggests that AppA, with heme bound as a cofactor, can act as a redox sensor depending on the redox status of the bound heme. Together, this gives AppA the unique capability to regulate the transcriptional activity of PpsR in a light- and redox-dependent manner (8).

Reduction of PpsR by AppA

According to in vitro measurements by Masuda and Bauer (11), AppA exerts its antirepressive action on PpsR in two stages (Fig. 1). At the first stage, the reduced form of AppA (A^-) reduces a disulfide bond in oxidized PpsR (P_4^+), which occurs independently of the light conditions. The molecular mechanism of this two-electron transfer is not yet clear. Redox titration experiments have shown that both PpsR and AppA have two redox-active

thiol groups that can form intramolecular disulfide bonds with a similar midpoint potential of approximately -320 mV at pH 7.0 (23). This suggests that the equilibrium constant for the electron transfer is close to 1. However, the experiments by Masuda and Bauer (11) indicate that AppA and PpsR do not represent a standard redox couple because they could not observe an inverse electron transfer from reduced PpsR to oxidized AppA.

To investigate both possibilities, we model the electron transfer between AppA and PpsR as a reversible reaction of the form



where k_{pr}^+ and k_{pr}^- are second-order rate constants. The equilibrium constant $K_{eq} = k_{pr}^+/k_{pr}^-$ is related to the difference between the midpoint potentials of the dithiol/disulfide couples in PpsR and AppA as

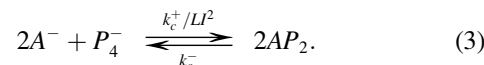
$$\Delta E_m = E_m^{P_4^+/P_4^-} - E_m^{A^+/A^-} = \frac{RT}{2F} \ln K_{eq}. \quad (2)$$

The factor related to the universal gas constant R and the Faraday constant F has a value of $RT/2F \approx 13$ mV at room temperature ($T = 298$ K).

Complex formation between AppA and PpsR

At the second level of regulation, the reduced form of AppA can form a complex with reduced PpsR. Experiments based on size exclusion chromatography have revealed that, in the complex, one AppA molecule is associated with two PpsR monomers corresponding to half of a PpsR molecule, which exists as a stable tetramer in solution (11). The same study showed that complex formation is inhibited by high intensities of blue-light irradiation ($LI = 900 \mu\text{mol}/\text{m}^2 \text{ s}$). However, a subsequent study found that AppA responds to blue light over several orders of magnitude down to $0.2 \mu\text{mol}/\text{m}^2 \text{ s}$ (24). Other experiments indicate that light absorption induces a structural change in the BLUF domain of AppA (25), which results in interactions with its C-terminal part, thereby causing the dissociation of PpsR (19).

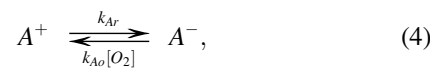
To keep the number of state variables and unknown parameters as small as possible, we do not distinguish between light-excited and nonexcited forms of AppA. Instead, the light-dependent complex formation between AppA and PpsR is modeled in an effective manner as



This effective description takes into account the observed 2:1 stoichiometry as well as the light-dependent inhibition of the complex (AP_2) formation between AppA and PpsR (11). In Eq. 3, k_c^+/LI^2 and k_c^- denote an effective third-order rate constant and a second-order rate constant, respectively. In the Supporting Material, we show how the inverse quadratic dependence of the forward rate on the light irradiance arises from a more detailed description of the complex formation through an underlying multistep process. This analysis also reveals how k_c^+ and k_c^- are related to the kinetic parameters of the multistep process.

Redox regulation of AppA

To implement the redox-sensing capabilities of AppA, we follow the model proposed by Han et al. (19), according to which AppA utilizes heme as a cofactor, bound to its C-terminal domain, to sense the cytosolic redox conditions. Consequently, we assume that AppA exists in two interconvertible states according to the scheme



where A^+ and A^- correspond to an oxidized and a reduced heme cofactor, respectively. This is consistent with the light-sensing reaction in Eq. 3

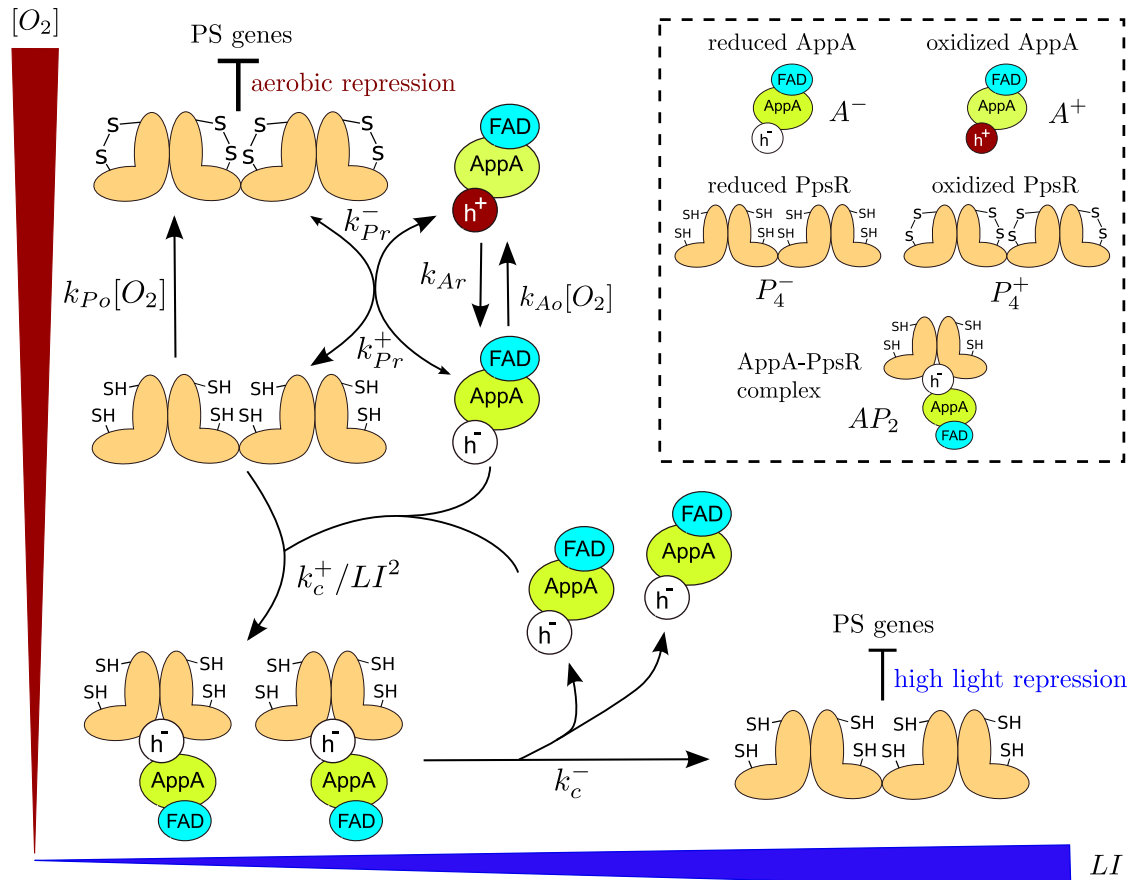


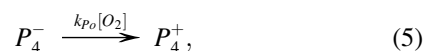
FIGURE 1 Model for the interaction between AppA and PpsR as a function of the oxygen concentration $[O_2]$ and blue-light irradiance LI (adapted from the literature (11,17,19)). The tetramers with intramolecular disulfide bonds (S-S) and thiol groups (SH) denote the oxidized and the reduced form of the PpsR repressor, respectively (see the legend in the dashed frame). The AppA protein has an FAD and a heme cofactor attached where h^+ and h^- denote the oxidized and reduced form of the heme cofactor, respectively. Both the oxidized and the reduced form of PpsR act as a repressor of photosynthesis genes, but with different strengths as indicated by the line thickness.

because AppA is only responsive to light when the bound heme is in its reduced state and heme binding is known to increase the association constant with PpsR (19). It was also suggested that the reduction of the heme cofactor could affect the electron flow from AppA to PpsR which is consistent with the reaction in Eq. 1. However, it is still unclear how AppA is reduced in the first place because its midpoint potential is probably much more negative than that of the cytosol.

Due to these uncertainties in the molecular redox-sensing mechanism of AppA, we simply assume in Eq. 4 that, in the absence of oxygen, the heme cofactor in AppA is constitutively reduced by some unknown agent with first-order rate constant k_{Ar} , whereas the oxidation of the heme occurs proportional to the oxygen concentration. Hence, $k_{Ao}[O_2]$ is a pseudo-first-order rate constant.

Reoxidation of PpsR

If the electron transfer from AppA to PpsR in Eq. 1 was indeed effectively irreversible ($k_{Pr}^- \ll k_{Pr}^+$), as suggested by the experiments of Masuda and Bauer (11), PpsR would have to be reoxidized through an AppA-independent mechanism. To account for this possibility, we assume that PpsR is reoxidized proportional to the oxygen concentration as



where $k_{Po}[O_2]$ is a pseudo-first-order rate constant.

Model equations

Assuming mass-action kinetics for the reactions in Eq. 1 and Eqs. 3–5 we arrive at the following set of ordinary differential equations:

$$\begin{aligned} \frac{d}{dt}[A^-] &= k_{Ar}[A^+] - k_{Ao}[O_2][A^-] - k_{Pr}^+[A^-][P_4^+] \\ &\quad + k_{Pr}^-[A^+][P_4^-] - 2\left(\frac{k_c^+}{LI^2}[A^-]^2[P_4^-] - k_c^-[AP_2]^2\right) \\ \frac{d}{dt}[P_4^-] &= k_{Pr}^+[A^-][P_4^+] - k_{Pr}^-[A^+][P_4^-] - k_{Po}[O_2][P_4^-] \\ &\quad - \left(\frac{k_c^+}{LI^2}[A^-]^2[P_4^-] - k_c^-[AP_2]^2\right) \\ \frac{d}{dt}[AP_2] &= 2\left(\frac{k_c^+}{LI^2}[A^-]^2[P_4^-] - k_c^-[AP_2]^2\right). \end{aligned} \quad (6)$$

In addition, we assume that the total amounts of PpsR and AppA molecules are conserved according to

$$\begin{aligned} [P_4^+] + [P_4^-] + \frac{1}{2}[AP_2] &= [P_T] \quad \text{and} \\ [A^+] + [A^-] + [AP_2] &= [A_T], \end{aligned} \quad (7)$$

such that the expressions in Eq. 6 become a closed system for the reduced forms of AppA and PpsR as well as for the complex AP_2 . For PpsR, this assumption seems to be justified as its expression levels were found to be largely independent of the growth conditions (16). However, the regulation of AppA expression is not known, so we will investigate how the steady-state behavior of the system in Eq. 6 depends on the ratio $[A_T]/[P_T]$. Because we focus on the mechanism of interaction between AppA and PpsR (and to be consistent with the assumption of constant total amounts of AppA and PpsR), we also neglect dilution terms due to cell growth in the expressions in Eq. 6.

Equations 6 and 7 contain six unknown kinetic parameters and two parameters for the total amounts of AppA and PpsR proteins. Because none of these parameters is, to our knowledge, known experimentally, we will introduce dimensionless quantities to reduce the number of free parameters. In addition, this allows us to assess the relative importance of individual reaction steps for the steady-state behavior of the system. Specifically, if we measure concentrations in terms of the total protein concentrations as

$$\begin{aligned} x_1 &= \frac{[A^-]}{[A_T]}, & x_2 &= \frac{[P_4^-]}{[P_T]}, & x_3 &= \frac{[AP_2]}{[P_T]}, \\ x_4 &= \frac{[P_4^+]}{[P_T]}, & x_5 &= \frac{[A^+]}{[A_T]}, \end{aligned} \quad (8)$$

the expressions in Eq. 6 become

$$\begin{aligned} \frac{d}{d\tau}x_1 &= 1 - x_1(1 + O) - \frac{x_3}{\gamma} - \frac{2\delta}{\gamma} \left(x_1^2 x_2 - I^2 \frac{x_3^2}{\gamma^2} \right) \\ &\quad - \frac{\beta}{\gamma} \left[x_1 \left(1 - x_2 - \frac{x_3}{2} \right) - \frac{x_2}{K_{eq}} \left(1 - x_1 - \frac{x_3}{\gamma} \right) \right] \\ \frac{d}{d\tau}x_2 &= \beta \left[x_1 \left(1 - x_2 - \frac{x_3}{2} \right) - \frac{x_2}{K_{eq}} \left(1 - x_1 - \frac{x_3}{\gamma} \right) \right] \\ &\quad - \alpha O x_2 - \delta \left(x_1^2 x_2 - I^2 \frac{x_3^2}{\gamma^2} \right) \\ \frac{d}{d\tau}x_3 &= 2\delta \left(x_1^2 x_2 - I^2 \frac{x_3^2}{\gamma^2} \right), \end{aligned} \quad (9)$$

where time (τ) is measured in units of $1/k_{Ar}$ whereas the other parameters are summarized in Table 1. The initial conditions have to be chosen such that the conservation relations

$$\begin{aligned} x_4 &= 1 - x_2 - \frac{x_3}{2} > 0 & \text{and} \\ x_5 &= 1 - x_1 - \frac{x_3}{\gamma} > 0 \end{aligned}$$

are obeyed. Note that the factor 1/2 in front of x_3 results from the stoichiometric factor of 2 in Eq. 3. Hence, x_3 can vary in the interval $[0, 2]$ whereas all other variables vary in the interval $[0, 1]$.

The two main parameters in this study are the oxygen concentration and the light irradiance. They are measured in units of $K_O = k_{Ar}/k_{Ao}$ and $K_L = (k_c^+[P_T]/k_c^-)^{1/2}$ as $O = [O_2]/K_O$ and $I = LI/K_L$, respectively.

TABLE 1 Definition of the parameters in the expressions in Eq. 9

$\alpha = \frac{k_{Po}}{k_{Ao}}$	$\beta = \frac{k_{Pr}^+[A_T]}{k_{Ar}}$	$\gamma = \frac{[A_T]}{[P_T]}$	$\delta = \frac{k_c^+[A_T]^2}{LI^2 k_{Ar}}$	$K_{eq} = \frac{k_{Pr}^+}{k_{Pr}^-}$
$O = \frac{[O_2]}{K_O}$	$I = \frac{LI}{K_L}$	$K_O = \frac{k_{Ar}}{k_{Ao}}$	$K_L = \left(\frac{k_c^+ P_T}{k_c^-} \right)^{1/2}$	

A_T and P_T denote the total amounts of AppA and PpsR, respectively.

Steady states for $K_{eq} \gg 1$

If the reduction of PpsR by AppA in Eq. 1 is effectively irreversible ($K_{eq} \gg 1$), the steady states of the expressions in Eq. 9 are given by

$$\begin{aligned} x_3 &= \gamma x_1 \frac{\sqrt{x_2}}{I} \quad \text{with} \\ x_1 &= \frac{1 - \frac{\alpha}{\gamma} O x_2}{1 + O + \frac{\sqrt{x_2}}{I}}, \end{aligned} \quad (10)$$

where x_2 is determined by the nonnegative roots of the fifth-order polynomial

$$p_5(y) = f_\infty(y) - f_\beta(y) = 0, \quad y \equiv \sqrt{x_2}. \quad (11)$$

Here, f_∞ and f_β are given by

$$f_\infty(y) = \left(1 - \frac{\alpha O}{\gamma} y^2 \right) p_3(y), \quad (12)$$

$$f_\beta(y) = \frac{\alpha O}{I\beta} y^2 (I(1 + O) + y)^2. \quad (13)$$

In Eq. 12, $p_3(y)$ denotes the third-order polynomial

$$\begin{aligned} p_3(y) &= (1 - y^2)(I(1 + O) + y) - \frac{\gamma y}{2} \left(1 - \frac{\alpha O}{\gamma} y^2 \right), \\ &= I(1 + O)(1 - y^2) + y \left(1 - \frac{\gamma}{2} \right) - y^3 \left(1 - \frac{\alpha O}{2} \right). \end{aligned} \quad (14)$$

Note that f_∞ is independent of β while f_β is inversely proportional to it, hence

$$\lim_{\beta \rightarrow \infty} p_5(y) = f_\infty(y).$$

In general, the fifth-order polynomial in Eq. 11 can admit, at most, five real roots corresponding to five possible stationary states of Eqs. 9 and 10. However, for them to be biologically meaningful one has to require that they fall within the interval $(0, 1)$ due to the scaling in the expressions in Eq. 8. From the structure of the polynomials p_5 and p_3 , one can derive some simple conclusions about the possible number of positive steady states of Eqs. 9 and 10. For example, from the Eqs. 11–14, it is obvious that $p_5(0) > 0$ and $p_5(1) < 0$. Hence, by continuity, p_5 must have at least one positive root in the interval $(0, 1)$ independent of all other parameter values.

In later applications, we are mostly interested in the case when $\beta \gg 1$. In that case, the roots of p_3 closely approximate those of p_5 because f_β in Eq. 11 can be neglected. By Descartes' rule, p_3 has precisely one positive root if $\alpha O \leq 2$ because the coefficients in p_3 (Eq. 15) exhibit only one sign change (counted in consecutive order in y). On the other hand, p_3 exhibits two sign changes if $\alpha O > 2$. In that case, p_3 can have either two positive roots or none. Hence, $\alpha O > 2$ is necessary for p_5 to have three positive roots altogether. A closer analysis indicates that $\alpha O > \gamma > 2$ is an additional necessary condition for all three roots to fall within $(0, 1)$ when $\beta \gg 1$.

For the later interpretation of the results, it is also important to note that the steady-state values of the expressions in Eq. 9, as defined by Eqs. 11–15, only depend on γ and the three parameter combinations

$$\begin{aligned} a_1 &= \alpha O, \\ a_2 &= \beta I, \\ a_3 &= I(1 + O). \end{aligned} \quad (16)$$

Hence, if we report a certain behavior of the system for a particular set of the four parameters α , β , O , and I it is clear from the expressions in Eq. 16 that the same behavior also exists for another set of (positive) parameters α' , β' , O' , and I' , as long as the constants a_1 , a_2 , and a_3 retain their numerical values.

Meaning of the parameters

The parameter γ in Table 1 compares the ratio between total amounts of AppA and PpsR proteins, and the parameters α and β can be interpreted in terms of the relative timescales for the oxidation and reduction of PpsR and AppA, respectively. For example, α compares the timescale for the oxidation of reduced PpsR (Eq. 5) with that for the oxidation of reduced AppA (Eq. 4) at a given oxygen concentration. Large values of α mean that reduced PpsR is oxidized faster than reduced AppA. Similarly, β compares the timescale for reduction of oxidized PpsR (Eq. 1) with that for the reduction of oxidized AppA (Eq. 4). Consequently, large values of β indicate that PpsR is reduced on a faster timescale than AppA.

RESULTS

In most of what follows, we will assume that the electron transfer from AppA to PpsR in Eq. 1 is effectively irreversible ($k_{pr}^- \ll k_{pr}^+$) as suggested by the observations by Masuda and Bauer (11). Note that this corresponds to the limit $K_{eq} \rightarrow \infty$ in the expressions in Eq. 9. In selected cases, we will show how a finite value of the equilibrium constant would modify the behavior of the system.

Because the steady-state behavior of the expressions in Eq. 9 is qualitatively different depending on the ratio between total copy numbers of AppA and PpsR ($[A_T]/[P_T] < 2$ or $[A_T]/[P_T] \geq 2$), we will consider both cases separately. Whenever possible, the results will be related to the behavior of the system expected from current experimental knowledge.

The case $[A_T]/[P_T] < 2$

For convenience, we consider the case where the total amounts of AppA and PpsR proteins are equal ($\gamma = [A_T]/[P_T] = 1$). If, in addition, the timescales for reduction and oxidation of both molecules are equal ($\alpha = \beta = 1$, compare to Table 1), the steady-state levels of reduced PpsR (P_4^-), oxidized PpsR (P_4^+), and the AppA-PpsR complex (AP_2) change monotonously with oxygen concentration (Fig. 2). Under aerobic conditions ($O = [O_2]/K_O \gg 1$), PpsR is mostly oxidized and the levels of reduced PpsR and the AppA-PpsR complex are low (Fig. 2, A and B). This is in agreement with the repressive action of oxidized PpsR under aerobic conditions irrespective of the light intensity.

Under low oxygen levels ($O \ll 1$) and, specifically, under anaerobic conditions ($O = 0$) it depends on the light irradiance whether PpsR is mostly in its reduced form or associated with AppA in a complex (Fig. 2 C). This is in agreement with the general idea that, under high light conditions ($I = LI/K_L \gg 1$), photosynthesis (PS) genes are suppressed due to the action of reduced PpsR, while under low

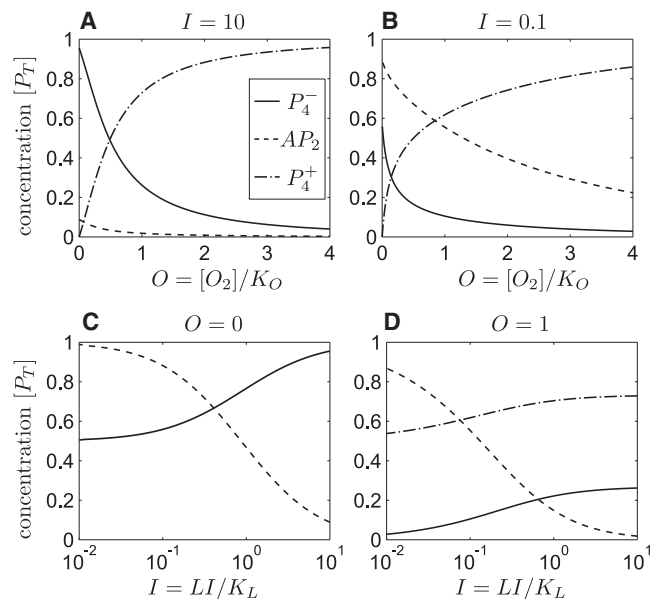


FIGURE 2 Steady-state behavior of reduced PpsR (P_4^-), oxidized PpsR (P_4^+), and the AppA-PpsR complex (AP_2) as a function of (A and B) the oxygen concentration $O = [O_2]/K_O$ for different values of the light irradiance ($I = LI/K_L$) and (C and D) the light irradiance for different oxygen concentrations. Note that under anaerobic conditions ($O = 0$), the concentration of P_4^+ is zero (C). Parameters: $\alpha = \beta = \gamma = 1$, $K_{eq} = \infty$ (compare to Table 1).

light conditions ($I \ll 1$) PS genes are induced because AppA sequesters PpsR molecules into transcriptionally inactive complexes (11,17). However, from Fig. 2 C it is apparent that not all PpsR molecules can be sequestered by AppA if both proteins are present in equal amounts ($\gamma = 1$) because two AppA molecules are required to bind one PpsR molecule. Hence, even under low light conditions, half of the PpsR molecules were still free to bind DNA, which would prevent an efficient induction of PS genes.

At intermediate oxygen levels ($O \sim 1$) there is a significant amount of free oxidized PpsR (P_4^+) under both low light ($I \ll 1$) and high light ($I \gg 1$) conditions (Fig. 2 D). Hence, PS genes would be repressed largely independent of the light irradiation—in contrast with the specific repression of PS genes observed experimentally under high light conditions (14,24). This suggests that if the rates for reduction and oxidation of PpsR and AppA are all equal ($\alpha = \beta = 1$) the phenomenon of high light repression of PS genes at intermediate oxygen concentrations cannot be explained.

High light PS gene repression in the semiaerobic regime

When the rate of PpsR reduction is significantly increased compared to the rate of AppA reduction ($\beta \gg 1$), a maximum in the steady-state curve of reduced PpsR (P_4^-) develops at $O \sim 1$ if the light irradiance I is sufficiently large (Fig. 3). The exact position of this peak depends on the parameter α (see Fig. S1 in the Supporting Material). When

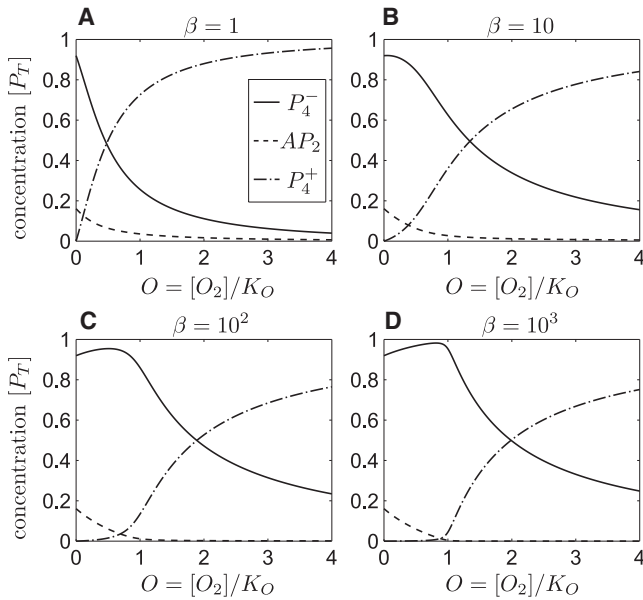


FIGURE 3 Development of a peak in the oxygen-dependent steady-state curve of reduced PpsR (P_4^-) as the relative rate ($\beta = k_{Pr}[A_T]/k_{Ar}$) between the reduction of PpsR and that of AppA increases. (A) $\beta = 1$. (B) $\beta = 10$. (C) $\beta = 10^2$. (D) $\beta = 10^3$. Parameters: $I = 5$, $K_{eq} = \infty$ and $\alpha = \gamma = 1$.

P_4^- reaches its maximum, the concentration of the AppA-PpsR complex (AP_2) is low (Fig. 3 D) and, consequently, PS genes would be effectively repressed at intermediate oxygen levels by the reduced form of PpsR. This suggests that the nonmonotonic dependence of P_4^- on the oxygen concentration could provide a rationale for the specific repression of PS genes in *R. sphaeroides* in the semiaerobic regime under high light conditions. Indeed, as the light irradiation decreases, the maximum of P_4^- at intermediate oxygen concentrations disappears (see Fig. S2).

Under high light conditions, the P_4^- concentration at completely anaerobic conditions ($O = 0$) is only slightly lower compared to the maximum at intermediate oxygen concentrations (Fig. 3 D). Hence, PS genes would still be largely repressed in that regime by reduced PpsR. Such a phenotype has recently been observed in PrrB knock-out experiments (9). Compared to wild-type cultures, where the repressive action of the AppA/PpsR system is normally counteracted by the PrrB/PrrA two-component system, these experiments revealed that, in the absence of the sensor kinase PrrB, photosynthesis genes are repressed by blue light to almost the same extent as under semiaerobic conditions, suggesting that the nonmonotonic dependence of P_4^- on the oxygen concentration can also account for this phenotype.

Next, we investigate how peak formation in the steady-state curve of P_4^- depends on the reversibility of the electron transfer from AppA to PpsR in Eq. 1. As the backward rate for the reduction of PpsR by AppA increases (K_{eq} decreases), the maximum in P_4^- becomes smaller and eventually disappears (Fig. 4) when the forward and the backward rates are equal

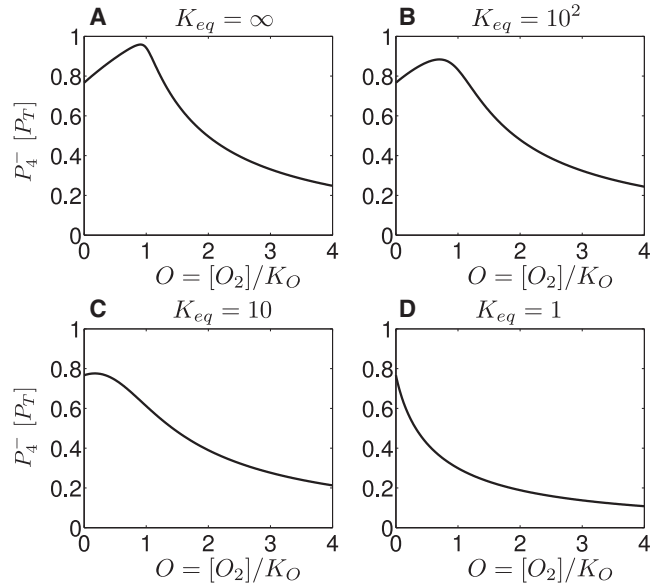


FIGURE 4 Disappearance of the peak of reduced PpsR (P_4^-) at intermediate oxygen levels ($O = [O_2]/K_O \sim 1$) as the backward rate in Eq. 1 increases such that the equilibrium constant $K_{eq} = k_{Pr}^+/k_{Pr}^-$ approaches unity. (A) $K_{eq} = \infty$. (B) $K_{eq} = 10^2$. (C) $K_{eq} = 10$. (D) $K_{eq} = 1$. Parameters: $\beta = 10^3$, $\alpha = 1 = \gamma$, and $I = 1$.

($K_{eq} = 1$). This suggests that the observed phenotype of high light repression of PS genes at intermediate oxygen levels is not compatible with an equilibrium constant close to 1. It also supports the view that AppA and PpsR are not in redox equilibrium in vivo (23), in agreement with the observation that the electron transfer between AppA and PpsR is effectively irreversible (11).

The case $[A_T]/[P_T] \geq 2$ and the possibility of bistability

A simple consequence of the stoichiometry of the reaction in Eq. 3 is that an efficient sequestration of PpsR molecules into AppA-PpsR complexes can only occur if the protein copy numbers of AppA exceed those of PpsR by at least a factor of two ($\gamma \geq 2$). Under these conditions, almost all PpsR molecules are complexed with AppA molecules under low light irradiation in the anaerobic regime (Fig. 5). As a result, the concentration of free reduced PpsR drops significantly, which would result in an effective PS gene induction under low light conditions.

Another interesting phenomenon emerges if, in addition to $\gamma > 2$ and $\beta \gg 1$, the ratio between the rate of reoxidation of PpsR and that of AppA is sufficiently large ($\alpha O > 2$): Under these conditions the transition from the anaerobic to the aerobic growth regime can occur via a bistable switch at intermediate oxygen levels (Fig. 6 A). Here, two stable stationary states (solid lines) coexist in a region ($O \approx 0.6 \dots 1$) which is bounded by two saddle-node bifurcations. At low values of the oxygen concentration, almost all PpsR is complexed with AppA while the concentration of both

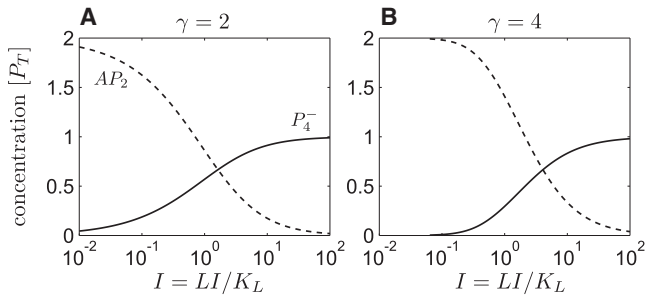


FIGURE 5 Increasing the ratio between total amounts of AppA and PpsR proteins ($\gamma = [A_T]/[P_T]$) increases the amount of reduced PpsR (P_4^-) that can be sequestered into AppA-PpsR complexes (AP_2) under low light ($I \ll 1$) conditions. (A) $\gamma = 2$. (B) $\gamma = 4$. Parameters: $O = [O_2]/K_O = 0$, $\beta = 10^3$, $K_{eq} = \infty$ and $\alpha = 1$.

reduced and oxidized PpsR is low (Fig. 6 B). In that regime, PS genes would be effectively transcribed.

However, upon increasing the oxygen concentration beyond the saddle-node bifurcation at $O \approx 1$, there would be an abrupt change in the expression levels of PS genes, because the concentration of free PpsR molecules (P_4^- and P_4^+) jumps to large values while AP_2 levels significantly decrease. In the other direction, when coming from high oxygen concentrations, PS genes would remain repressed until O_2 levels decrease beyond the second saddle-node bifurcation at $O \approx 0.6$, where almost all PpsR is again sequestered into inactive complexes leading to hysteresis.

Regions and conditions for bistability

Because the steady states only depend on the parameter combinations αO , βI , and $I(1 + O)$ (compare to the expressions in Eq. 16), a change in α and β can always be compensated by an appropriate change in O and I (as long as all parameters remain positive) without compromising the ability to generate bistability. For example, if the oxygen

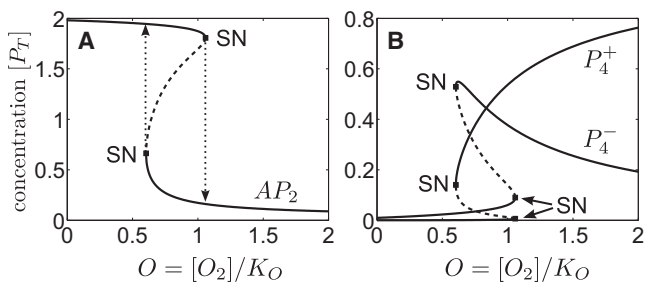


FIGURE 6 One-parameter bifurcation diagrams showing how the number of steady states changes as a function of the oxygen concentration. (A) AppA-PpsR complex (AP_2); (B) reduced PpsR (P_4^-) and oxidized PpsR (P_4^+). In the region between the two saddle-node bifurcations (SN), three stationary states coexist and the system exhibits hysteresis (indicated by dotted lines): Upper and lower branches denote stable steady states (solid lines) while the middle branch (dashed line) corresponds to an unstable steady state. Parameters: $\beta = 10^3$, $\alpha = 10$, $\gamma = 4$, $K_{eq} = \infty$ and $I = 0.1$.

concentration and the light irradiance are fixed at the values used in Fig. 6, there is a whole region in the two-parameter plane spanned by α and γ (Fig. 7 A) or α and β (Fig. 7 B) where bistability (gray-shaded region) can occur. Together, these figures suggest that bistability can only emerge for $\gamma > 2$ if there is a suitably large timescale separation between the oxidation of PpsR and AppA as well as between the reduction of PpsR and AppA ($\beta \gg 1$). Similar as for the peak formation in Fig. 4 we find that decreasing the equilibrium constant for the electron transfer from AppA to PpsR (Eq. 1) compromises the ability of the system to generate a bistable response (see Fig. S3).

A necessary condition for a reaction network to exhibit bistability is the presence of a sufficiently strong positive feedback mechanism (26), although such a feedback mechanism might be difficult to identify by pure visual inspection of the network structure (27). The situation for the AppA/PpsR network shown in Fig. 1 is quite similar, as it does not contain any apparent positive feedback loops. However, it is well known that dead-end complex formation (28) and sequestration of signaling molecules (29) can result in a bistable system response. Hence, the light-dependent complex formation between AppA and PpsR could represent a potential source of bistability in that system. Indeed, when we plot the dissociation rate of the AP_2 complex against the steady-state concentration of reduced AppA (see Fig. S4), a strong positive feedback becomes apparent as increasing amounts of reduced AppA lead to an even higher production of reduced AppA through the dissociation of the AP_2 complex.

DISCUSSION AND CONCLUSIONS

The facultative photosynthetic bacterium *R. sphaeroides* utilizes the blue-light- and redox-sensitive flavoprotein AppA to regulate the activity of the aerobic transcriptional repressor PpsR, which is specifically involved in the regulation of photosynthesis genes. As a result of this protein-protein interaction, *R. sphaeroides* exhibits a unique phenotype: The blue-light-dependent repression of PS genes under semiaerobic conditions (14).

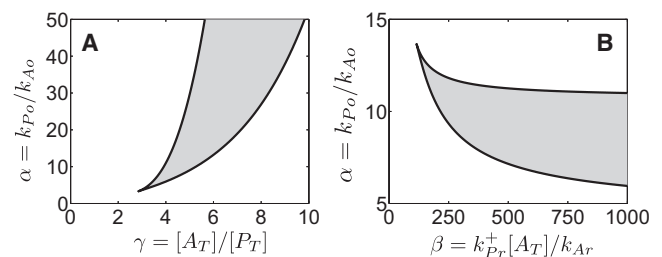


FIGURE 7 Regions of bistability projected on different two-parameter planes: (A) α versus γ for $\beta = 10^3$ and (B) α versus β for $\gamma = 4$. Two stable steady states and one unstable steady state coexist in the gray-shaded region, which is bounded by two saddle-node bifurcations (solid lines). Parameters: $O = 1$, $I = 0.1$, $K_{eq} = \infty$.

Here, we have investigated how this phenotype arises from the molecular interactions between AppA and PpsR. For this purpose, we have developed a simple mathematical model based on the structural knowledge of the AppA/PpsR interactions (Fig. 1), in particular, the AppA-mediated reduction of PpsR (Eq. 1) and the light-dependent complex formation between the reduced forms of AppA and PpsR (Eq. 3). This core mechanism was augmented by a redox-sensing reaction for AppA (Eq. 4) and an oxygen-dependent reoxidation of PpsR (Eq. 5). The idea was to analyze the kinetic requirements for these processes that could lead to a PS gene repression at intermediate oxygen concentrations and, thereby, provide a mechanistic basis for the understanding of the AppA/PpsR system in *R. sphaeroides*.

PS gene repression under semiaerobic conditions

Our results suggest that the phenomenon of high light repression of PS genes under semiaerobic conditions can be related to the development of a maximum in the concentration of reduced PpsR at intermediate oxygen concentrations (Fig. 3), which occurs only if the light irradiance is sufficiently large (Fig. S2). Our simulations indicate two additional requirements for peak formation to occur: First, the rate of reduction of PpsR has to be much larger than that for the reduction of AppA ($\beta \gg 1$); and, second, the equilibrium constant for the electron transfer reaction from AppA to PpsR must be sufficiently large ($K_{eq} \gg 1$).

The latter requirement agrees with the observation of Masuda and Bauer (11), according to which the electron transfer from AppA to PpsR is effectively irreversible. However, the requirement $K_{eq} \gg 1$ seems to contradict experiments by Kim et al. (23), which suggest that the midpoint redox potentials of AppA and PpsR are equal ($K_{eq} = 1$). The authors argued that protein-protein interactions between AppA and PpsR could shift the midpoint potential of one or both proteins, which could favor the electron transfer from AppA to PpsR. From Fig. 4 we can estimate that a significant peak formation in reduced PpsR requires an equilibrium constant of $K_{eq} \gg 10$. According to Eq. 2 this would result in a shift of the midpoint potential difference between PpsR and AppA of at least $\Delta E_m = 30$ mV (at $T = 298$ K). Based on the observation that heme binding to AppA increases the association rate between AppA and PpsR (19), it is conceivable that heme is also involved in mediating protein-protein interactions between AppA and PpsR, which could explain such a shift in the midpoint potential.

Possibility of bistability in the AppA/PpsR system

The steady-state analysis of the expressions in Eq. 9 has shown that the experimentally observed interactions between AppA and PpsR are, in principle, sufficient to explain the high light repression of PS genes under semi-

aerobic conditions. In addition, we found that the network structure of the AppA/PpsR system is such that it can potentially exhibit bistable behavior which would result in a hysteretic switchlike induction of PS genes as a response to changing redox conditions in the environment (Fig. 6). Based on the root structure of the fifth-order polynomial in Eq. 11, we have derived necessary conditions for the emergence of multiple steady states which can be summarized as $\beta \gg 1$ and $\alpha O > \gamma > 2$.

This shows that bistability, similar to peak formation at intermediate oxygen concentrations, requires a timescale separation between the reduction rates of PpsR and AppA ($\beta \gg 1$). However, bistability also requires that PpsR can be efficiently sequestered by AppA molecules ($\gamma > 2$) and that reoxidation of PpsR occurs on a faster timescale than reoxidation of AppA corresponding to the shaded region in Fig. 7 A.

The prediction of bistability in the AppA/PpsR system is somewhat surprising, as, to our knowledge, no hysteretic behavior has been reported yet for PS gene expression in *R. sphaeroides*. However, given that the kinetic requirement $\beta \gg 1$ is also essential for the specific PS gene repression in the semiaerobic regime, we expect this condition to be generally valid. The condition $\gamma > 2$ should also be fulfilled because otherwise an efficient sequestration of PpsR molecules, as it is necessary for the induction of PS genes under anaerobic conditions, would not be possible (Fig. 2 C). Hence, measurement of the remaining kinetic parameter $\alpha = k_{Po}/k_{Ao}$ would give a first indication whether bistability could be observable in the AppA/PpsR system. Alternatively, measurement of the relative rates of reduction and oxidation of AppA ($K_O = k_{Ar}/k_{Ao}$) could be used to estimate α because both parameters determine the semiaerobic regime in our model. For example, a value of $K_O = 500 \mu\text{M}$ would correspond to a value of $\alpha = 5$ according to Fig. S1 B.

Bistability is a common phenomenon in biology (26,30), and it has also been observed in sugar uptake systems of *Escherichia coli* using single cell measurements (31,32). Similar measurements of PS gene expression patterns in *R. sphaeroides* could provide for an independent indication for the existence of bistability in the AppA/PpsR system. In the bistable regime, the transition from the noninduced to the induced state is often driven by random molecular fluctuations leading to a coexistence of induced and noninduced cells (31,32). As a result, a fraction of the cell population would already derive energy from photosynthesis while the remaining fraction still performed respiration. As in other bacteria, such a heterogeneity in gene expression patterns could be an advantageous survival strategy for a population in the face of unforeseeable environmental fluctuations (33–35).

SUPPORTING MATERIAL

Four figures, additional text, and 12 equations are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495\(11\)00459-0](http://www.biophysj.org/biophysj/supplemental/S0006-3495(11)00459-0).

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