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Kinetic properties of the blue-light response of stomata

(gas-exchange method/pulse induction/kinetic model/photoreceptor/*Commelina communis* L.)

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ABSTRACT **The stomatal response to blue light was analyzed with gas-exchange techniques in *Commelina communis* L. leaves by using high-fluence-rate short pulses. Pulses of blue light were given under a background of high-fluence-rate red light, which maintained photosynthesis at near saturation and stomatal conductance at a steady state. A single blue light pulse of 1–100 sec induced an increase in stomatal conductance, which peaked after 15 min and then returned to the initial steady-state level within 50–60 min after the pulse. The response could be repeatedly induced in the same leaf. Red light pulses on a red background did not induce any comparable response. The stomatal response quantified by integrating the conductance increases after pulse application approached saturation with increasing pulse duration ($t_{1/2}$ ≈9 sec with 250 μmol·m^{-2}·sec^{-1} of blue light). After a saturating pulse, sensitivity to a second pulse was restored slowly. This recovery response, quantified from the conductance increases caused by the two pulses, approached saturation with a $t_{1/2}$ of ≈9 min. These results were used to test a model in which a molecular component in the sensory transduction process is considered to exist in two interconvertible forms, *A* and *B*. If *B* is the physiologically active form inducing stomatal opening, then *A* is the inactive form. The *A* to *B* conversion is a light-induced reaction and the *B* to *A* conversion is a thermal reaction. Rate constants for these reactions were estimated from single- and double-pulse experiments (at a fluence rate of 250 μmol·m^{-2}·sec^{-1}, k_1 = 0.075 sec^{-1}; thermal rate constant k_d = 0.0014 sec^{-1}), allowing the calculation of steady-state concentration of *B* under continuous irradiation. The calculated values accurately predicted the steady-state stomatal conductances under continuous blue light.**

Blue light (BL) responses of plants include phototropism, phototaxis, induction of carotenoid synthesis, and regulation of leaf movement and circadian rhythms. It is currently thought that flavins are the photosensing pigments in many of the BL-sensitive responses (1, 2). The precise nature of the photoreceptor and the primary photoreactions, however, remain unknown.

In addition to the responses related to photosynthesis, a BL-specific response has been implicated in the photocontrol of stomatal movement (3–6). Several characteristics of this BL response point to its potential as a model system for the study of the early sensory reactions. The essential sequence of the sensory transduction takes place in a single cell, the guard cell (7, 8); in contrast, many other BL responses in higher plants are multicellular and involve complex cell-to-cell interactions (e.g., phototropism). The BL response of guard cells modulates ion fluxes including uptake of K^+ as the major osmoticum. There exists a close relationship between guard cell K^+ content and guard cell volume or stomatal aperture (9). These properties provide a basis for the quantitative analyses of the early sensory reactions by measuring stomatal movement.

Many of the BL responses can be induced by a short pulse of BL (10–13). In previous work with stomata, a rapid transient increase in transpiration in *Avena* leaves caused by a BL pulse as short as 3 sec has been reported (14). In the present study, we have characterized kinetic properties of the stomatal response to BL by using pulse stimulation. The responses were quantified in attached leaves of *Commelina communis* by using gas-exchange techniques. Background irradiation with high-fluence-rate red light (RL) was used to saturate and thereby minimized the component of the stomatal response to BL depending on photosynthesis in guard cells and the underlying mesophyll cells. The obtained results allowed us to test a kinetic model, which predicted the steady-state values of stomatal conductance under continuous BL.

MATERIALS AND METHODS

Plants of *C. communis* were grown from seed in pots of soil (Goldstrike potting soil, Master Nurserymen's Assoc., Concord, CA) in a greenhouse. Plants were watered daily, fertilized weekly with a solution of Spoonit (Plantsmith, Mountain View, CA) at ≈3 g/liter, and used for experimentation when 4–6 weeks old.

Stomatal conductance to water vapor and rates of CO_2 assimilation were simultaneously measured at 90- or 120-sec intervals with a steady-state gas-exchange system (Armstrong Enterprises, Palo Alto, CA). The main characteristics of the instrument were described by Field *et al.* (15). Intercellular CO_2 concentrations were calculated from the values for photosynthetic rates, stomatal conductance, and ambient CO_2 concentrations (15). Computer-assisted data acquisition allowed real-time measurements and computations. Two attached leaves from different plants were enclosed in the gas-exchange cuvette. Leaf temperature was kept constant at 25 ± 0.5°C; vapor pressure deficits ranged between 0.8 and 1.2 kPa, and ambient CO_2 concentration remained between 320 and 350 ppm.

The gas-exchange system was placed in a dark room. The leaves in the cuvette were continuously irradiated with RL (500 μmol·m^{-2}·sec^{-1}) at an angle of ≈45° to the adaxial surface of the leaf. When stomatal conductance reached steady state under RL (2–3 hr after the start of irradiation), experimental treatments with BL began. The BL was given normal to the adaxial surface of the leaf.

RL was obtained by passing light from a Sylvania 300-W lamp (PAR56/2MFL) through a wide-band hot mirror (Optical Coating Laboratory, Santa Rosa, CA), a Kodak 1A red filter (50% cutoff at 645 nm), and a layer of Cinemoid (no. 5A). BL was obtained by passing light from a General

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Abbreviations: BL, blue light; RL, red light.

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Electric Gemini 300 projection lamp through an OCLI wide-band hot mirror, a 5-cm-thick water bath with running tap water, and a Rohm and Haas 2424 blue Plexiglas filter (maximal transmittance, 470 nm; half-band width, 100 nm). An electric shutter was used to control the duration of irradiation. Light sources were placed in metal boxes equipped with an air-cooling system. Fluence rates were measured with a Li-Cor quantum meter.

RESULTS AND DISCUSSION

Stomatal Response to a Single Pulse of BL. Under continuous RL irradiation, *Commelina* leaves showed steady-state values of stomatal conductance, CO_2 assimilation rates, and calculated intercellular CO_2 concentrations ranging between 0.15–0.25 mol·m^{-2}·sec^{-1}, 10–13 μmol·m^{-2}·sec^{-1}, and 200–260 ppm, respectively. A pulse (1–100 sec) of BL (250 μmol·m^{-2}·sec^{-1}) given under these conditions resulted in an increase in stomatal conductance that peaked at ≈15 min, with conductance values returning to the baseline level within 50–60 min after the pulse (Fig. 1*a*). Baseline levels often changed slowly during the course of an experiment (e.g., see Fig. 4), but these long-term changes did not affect the measurement of the responses to pulses. The onset of the response could be clearly resolved within 2–3 min, with earlier conductance measurements being uncertain because of thermal transients at the humidity sensor resulting from the operation of the temperature control device of the gas-exchange cuvette. Response to a BL pulse could be repeatedly induced in the same leaf. A pulse of RL (30 or 100 sec, 500 μmol·m^{-2}·sec^{-1}; Corning 2-61 glass filter plus no. 5A Cinemoid) applied against the same background RL used with BL pulses, induced no detectable conductance changes, indicating that the response observed with a BL pulse is not a consequence of direct stimulation of photosynthesis by BL.

Time-course graphs of conductance (conductance curves) obtained with BL pulses of various durations (fluence rate, 250 μmol·m^{-2}·sec^{-1}) showed that the magnitude of the response increased with pulse duration up to ≈30 sec; from 30 to 100 sec, no further increase was apparent. The shapes of the conductance curves were nearly identical when different pulse durations were used, with the peaks occurring at about the same time. The conductance response to a given pulse was quantified by integrating the area enclosed between the conductance curve (upper curves in Fig. 1) and the extrapolated baseline. Responses to different pulse durations were expressed as values relative to the response induced by a saturating BL pulse (250 μmol·m^{-2}·sec^{-1}; pulse duration, 50 or 100 sec). A plot of these relative responses showed a $t_{1/2}$ of ≈9 sec. The natural logarithm of the differences between the relative response values and the saturation value (=1) was plotted as a function of pulse duration (Fig. 2). The points fit a straight line reasonably well, suggesting that the response approached saturation exponentially; a hyperbolic function, however, cannot be strictly excluded.

Reciprocity for the pulse-induced conductance response was tested. In one set of experiments, a fluence of 1.25×10^3 μmol·m^{-2} was applied in 5 or 48 sec; in another, 2.5×10^3 μmol·m^{-2} was applied in 10 or 96 sec. The higher fluence provided approximately half-saturation. No significant difference in the magnitude of the response between two different pulse durations was apparent for either fluence (data not shown).

Stomatal Response to Two Pulses of BL. The single-pulse experiments showed that a 50-sec pulse of BL at 250 μmol·m^{-2}·sec^{-1} saturated the pulse-induced response and a 100-sec pulse (equivalent to two consecutive 50-sec pulses) did not cause any detectable further increases in conductance. However, if sufficient time elapsed between two pulses, an increase in the total area below the conductance curve became apparent. This increase occurred even if the second pulse was applied before the response to the first pulse was completed (Fig. 1*b*). Subtraction of the conductance curve resulting from the response to a single 50-sec pulse from that resulting from the response to two sufficiently separated pulses yielded a curve essentially identical to that resulting from the single pulse (Fig. 1*c*). The curve in Fig. 1*c* was interpreted as the specific response to the second pulse.

The responses to two pulses separated by increasing time intervals were quantified by measuring the area enclosed between the conductance curve and the extrapolated baseline. The value obtained for each treatment was plotted relative to the response to a single 50-sec pulse. As shown in Fig. 3, the value increased with time between pulses, approaching saturation with $t_{1/2}$ ≈9 min. The natural logarithm of the difference between the relative response values and the saturation value (=2) were plotted as a function of time between pulses (Fig. 3 *Inset*). The plot approximately fits a straight line. The intercept of the regression line was very close to zero, indicating that the response to the second pulse appeared immediately or within an unresolvable time after the first pulse.

Kinetic Model. In the light of the results from pulse experiments described above, the following kinetic model for the BL response was examined. The model postulates that a molecular component of the phototransduction process can exist in two interconvertible forms or states, A and B. The conversion of A to B occurs as a result of photoexcitation of the sensory pigment, and the conversion of B back to A is a thermal reaction. B is the physiologically active form and

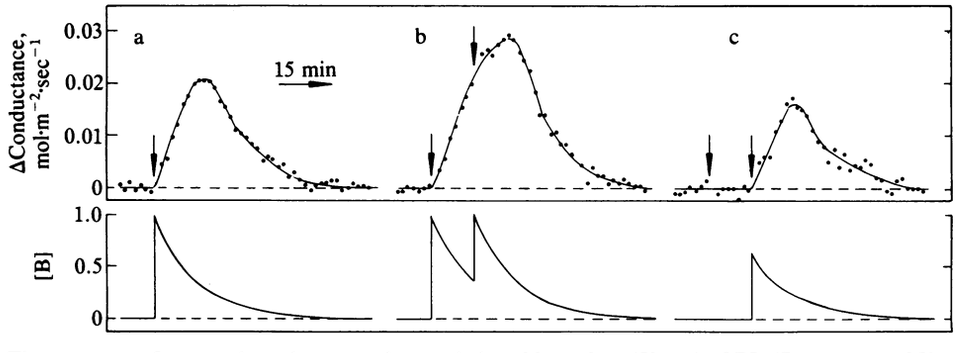

FIG. 1. (*Upper*) Time courses of stomatal conductance changes induced by pulses (50 sec) of BL (fluence rate, 250 μmol·m^{-2}·sec^{-1}) on a background of continuous RL (500 μmol·m^{-2}·sec^{-1}). (*a*) Response to a single pulse. (*b*) Response to two pulses separated by 12 min. (*c*) Subtraction of the single-pulse response from the two-pulse response. Values are the increase in conductance above the baseline obtained with background RL. Arrows indicate the time at which BL pulses were applied. (*Lower*) Theoretical calculated changes in the relative concentration of B occurring during the conductance changes described above. B is the physiologically active form in the kinetic model, $A \underset{k_d}{\overset{k_1}{\rightleftharpoons}} B$.

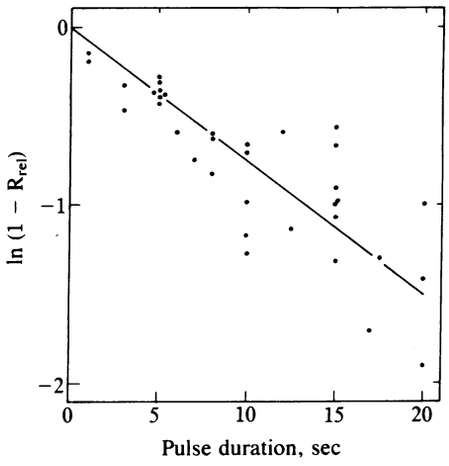

FIG. 2. Relationship between stomatal response and BL-pulse duration (250 μmol·m^{-2}·sec^{-1}). Background irradiation with RL was as described in Fig. 1. The response in stomatal conductance was quantified by measuring the area enclosed between the conductance curve and the extrapolated baseline (see Fig. 1*a*). The natural logarithm of the values obtained by subtracting the relative response (R_{rel}) from the saturating response (=1) is plotted as a function of pulse duration. The standard linear regression analysis yielded a line with slope = −0.065, intercept = −0.13, and r = −0.84 (n = 35). Since the response with 0 pulse duration is 0—i.e., $\ln(1 - R_{rel}) = 0$, the regression line passing through zero on the y axis was also obtained by using the formula, $b = \frac{\Sigma x_i y_i}{\Sigma x_i^2}$. The slope of this line was −0.075.

mediates a dark reaction that, through further dark-reaction steps, induces stomatal opening. A is the inactive form. Schematically, the reaction can be written as $A \underset{k_d}{\overset{k_1}{\rightleftharpoons}} B$, where k_1 and k_d are first-order rate constants for light-induced and thermal reactions, respectively. Similar models for the BL-dependent response have been suggested previously (16, 17; see also ref. 18).

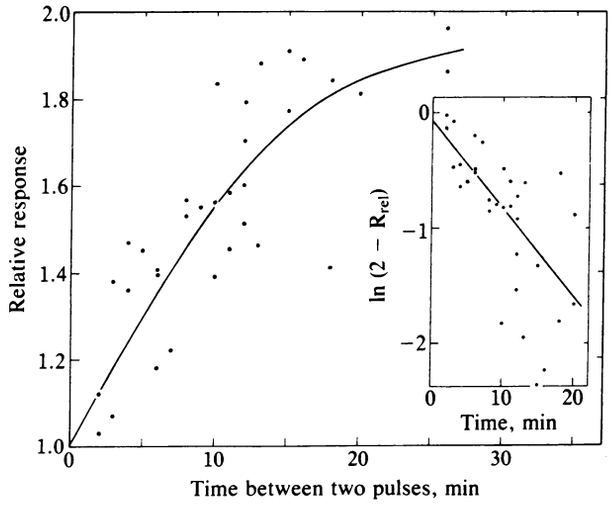

FIG. 3. Relationship between stomatal response and time intervals between two BL pulses (duration of each pulse, 50 sec; fluence rate, 250 μmol·m^{-2}·sec^{-1}). Background RL was as described in Fig. 1. The response to two pulses was quantified by measuring the area enclosed between the conductance curve and the extrapolated baseline (see Fig. 1*b*). Response magnitudes relative to those produced by a single pulse are shown. Time between two pulses was measured from the onset of each pulse irradiation. (*Inset*) The natural logarithm of the values obtained by subtracting the relative response (R_{rel}) produced by two pulses from the saturating response (=2). Slope = −0.083; intercept = −0.067; r = −0.67 (n = 32).

We assume that k_d is very small so that conversion of B back to A during pulse stimulation is negligible (the estimated k_d value supports this assumption; see below). Since the decay of B (i.e., conversion back to A) proceeds by a first-order reaction, the integrated concentration of B over time after pulse stimulation is proportional to the maximum concentration of B produced by the pulse stimulation. Assuming that the increase in conductance obtained by integrating the conductance increment over the baseline is proportional to the integrated concentration of B, and hence the maximum concentration of B, we can obtain a k_1 value of 0.075 sec^{-1} for a fluence rate of 250 μmol·m^{-2}·sec^{-1}, from the slope of the regression line in Fig. 2. The validity of the reciprocity law shown in the pulse-induced response indicates that k_1 is a linear function of the fluence rate up to at least 250 μmol·m^{-2}·sec^{-1}. Therefore, k_1 for a given fluence rate (X μmol·m^{-2}·sec^{-1}) can be estimated by 0.0003·X·sec^{-1}.

The model indicates that after a full conversion of A to B by a saturating pulse, the conversion of B back to A proceeds via first-order kinetics. Since the integrated concentration of B resulting from the second pulse parallels the concentration of A present at the time of second-pulse stimulation, the kinetics of the increment in the integrated area of the conductance curve by the second pulse (Fig. 3) reflects the kinetics of B to A conversion. The k_d value estimated from the slope of the regression line in Fig. 3 (*Inset*) is 0.083 min^{-1} (0.0014 sec^{-1}). This analysis assumes that A to B conversion is completed immediately after pulse stimulation. This is supported by the result that the intercept of the regression line is very close to zero.

Calculated changes in the relative B concentration after the application of one or two saturating BL pulses are shown in Fig. 1 (*a* and *b*) together with time courses of conductance changes. Fig. 1 also depicts the relationship between the conductance change and the B concentration change induced by the second pulse. The relationship between the change in conductance ensuing from the second pulse, which was estimated by subtraction, and the theoretical change in B concentration also obtained by subtraction is identical to that obtained with a single pulse.

Stomatal Response to Continuous BL: A Test of the Model. The model indicates that under continuous BL irradiation, the light-induced and thermal reactions occur simultaneously, resulting in photostationary concentrations of B. The available rate constants made it possible to calculate the photostationary concentration of B for different fluence rates of BL,¶ allowing us to compare the conductance response to continuous BL with the calculated levels of B.

As stated previously, a pulse of RL at a fluence rate of 500 μmol·m^{-2}·sec^{-1} given over the background RL did not cause any stomatal response, but this additional RL given continuously induced a gradual increase in conductance. This increase continued for at least 45 min, by which time the conductance increased by 15%. There was, however, a fast increase (≈10%) in the rate of CO_2 assimilation upon the additional RL irradiation, which occurred before stomatal conductance was enhanced. This indicated that the conductance increase in response to the additional RL was a result of direct enhancement of photosynthesis. A similar increase in the CO_2 assimilation rate was observed with continuous high-fluence-rate BL (e.g., 250 μmol·m^{-2}·sec^{-1}) applied over the background RL. Since such an effect of BL invalidates

¶In the kinetic model, $A \underset{k_d}{\overset{k_1}{\rightleftharpoons}} B$, described in the text, $d[B]/dt = k_1[A] - k_d[B]$. The concentration of B at time t, $[B]_t$, after the onset of irradiation ($[A] + [B] = 1$, $[B]_0 = 0$) is given by $[B]_t = k_1/(k_1 + k_d)[1 - e^{-(k_1+k_d)t}]$, which is an integrated form of the above equation. Under steady-state conditions, $k_1[A] = k_d[B]$; when $[A] + [B] = 1$, $[B] = k_1/(k_1 + k_d)$.

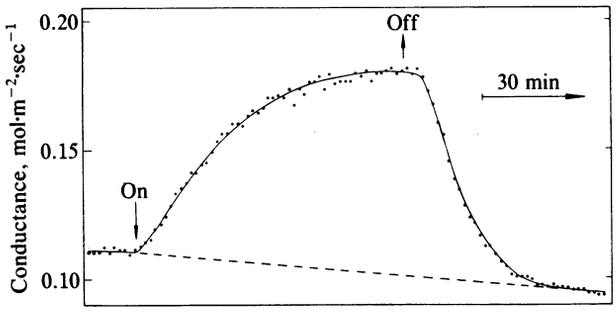

FIG. 4. Time course of the change in stomatal conductance induced by continuous BL (25 μmol·m^{-2}·sec^{-1}). Background RL was as described in Fig. 1. BL was turned on and off at the times indicated by arrows.

the measurements of the BL-specific response, fluence rates <25 μmol·m^{-2}·sec^{-1}, which did not cause any fast increases in photosynthetic rate, were used to study stomatal responses to continuous BL.

The stomatal response to continuous BL was quantified by measuring the increments in steady-state conductance values obtained by adding different fluences of BL to the background RL. With the fluence rates tested, new steady states were attained within 1.5 hr after addition of BL (Fig. 4). In experiments conducted with different leaves on different days, the conductance response at a test fluence rate was obtained as a value relative to that at a standard fluence rate of 25 μmol·m^{-2}·sec^{-1}. When values were normalized at the fluence of 25 μmol·m^{-2}·sec^{-1}, the steady-state conductance responses closely matched the theoretical steady-state concentrations of B (Fig. 5).

Fig. 6 compares the decrease in stomatal conductance after the end of continuous BL. There was a lag of ≈5 min before the conductance started to decrease while no comparable lag was observed in the opening response to a BL irradiation (Figs. 1 and 4). This lag may reflect some complex regulation mechanism taking place during or after BL irradiation. The relative time courses of the conductance decrease after steady-state conductances were reached under two different fluence rates of BL, were nearly identical (Fig. 6), suggesting that all the physiological changes reflected in stomatal closure are linearly related to the increments in steady-state conductance induced by continuous BL. Fig. 6 also illustrates the theoretical changes in relative concentration of B. The conductance decrease after the lag paralleled the predicted decrease in B concentration, in essential agreement with the kinetic model.

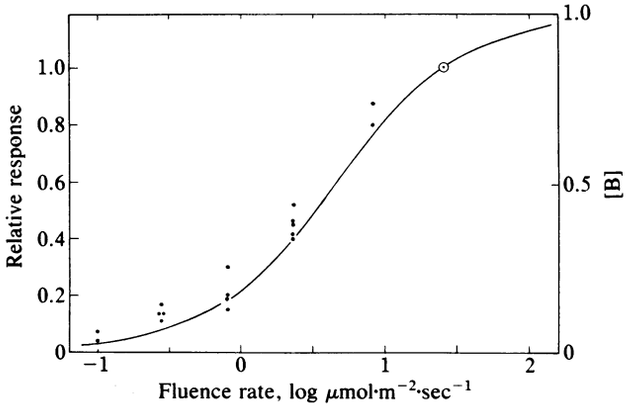

FIG. 5. Steady-state conductance responses to continuous BL as a function of fluence rates. Background RL was as described in Fig. 1. The magnitude of the response was obtained from the difference between the steady-state conductance measured under continuous BL and the conductance estimated from the extrapolated baseline (see Fig. 4). The response is plotted relative to that measured with 25 μmol·m^{-2}·sec^{-1}. Solid line, theoretical curve of relative steady-state concentrations of B. For calculation of B see ¶ footnote in text.

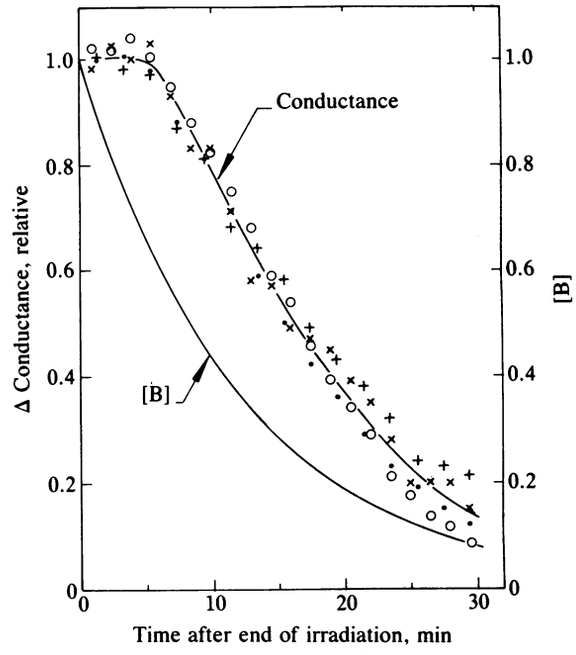

FIG. 6. Time courses of conductance changes occurring after the end of continuous BL. Background RL was as described in Fig. 1. The conductance values above the baseline (dashed line in Fig. 4) are shown. Values are relative to the maximal conductance increment obtained at the steady-states under continuous BL. The fluence rate of BL was 25 μmol·m^{-2}·sec^{-1} (●, means of nine measurements; ○, means of three measurements) or 2.3 μmol·m^{-2}·sec^{-1} (+, means of two measurements; ×, means of three measurements). Calculated changes in theoretical B concentration are also shown. The B concentration plotted is relative to the steady-state concentration under BL.

Further Implications of the Results. The kinetic model does not specify the precise nature of the components A and B, but it implies that the B form exerts a catalytic role in a reaction and is not used as a substrate (see ref. 18). The catalysis of the reaction could be a direct action of B, or an indirect one resulting, for instance, from the activation of an enzyme by B. It is possible that the postulated molecular components are interconvertible forms of the photoreceptor (or a molecular complex with the photoreceptor moiety) as previously assumed (16, 17). They could also be interconvertible forms of a molecule (or molecular complex) separated from the photoreceptor, with photoexcitation of the latter resulting in the A to B conversion through one or more reaction steps. Several classes of reactions are consistent with the A to B conversion postulated by the model (19). With respect to the B to A conversion, we assumed it to be a thermal reaction, although in a similar model invoking a photochromic photoreceptor (20, 21), it has been postulated to be driven by both light and thermal energy. Our results cannot distinguish between these two possibilities because of the continuous RL background used in these experiments. If the B form of the BL photoreceptor absorbs light predominantly in the RL region, the rate constant for the B to A conversion would remain virtually unchanged under irradiation with different fluence rates of BL.

The empirical analysis presented in this paper provides an approach for the study of the stomatal response to light and could be valuable for further elucidation of the mechanisms of the BL responses in plants.

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