

A minimal model of light-induced circadian rhythms of nitrate reductase activity in leaves of barley

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Lillo, C. and Ruoff, P. 1984. A minimal model of light-induced circadian rhythms of nitrate reductase activity in leaves of barley. – *Physiol. Plant.* 62: 589–592.

Observed circadian rhythms of nitrate reductase (NR) (EC 1.6.6.1) activity in leaves of barley (*Hordeum vulgare* L. cv. Herta) under continuous light conditions are described by a simple kinetic model. The oscillatory mechanism has been decomposed into the negative and positive feedback loops which are necessary according to present theories of chemical oscillating systems. Our results indicate that the decrease of NR activity in darkness can be considered as a reversible unimolecular conversion of the active form of NR into an inactive form, forming a negative stabilizing feedback loop. The light-induced increase of NR activity is related to a positive destabilizing feedback loop. In our treatment this process is represented as an autocatalytical reaction.

Additional key words – Damped oscillations, feedback loops, *Hordeum vulgare*.

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Introduction

Circadian rhythmicity of NR activity has recently been found in leaves of young barley seedlings (Lillo 1984). The circadian increase and decrease of NR activity continued for 3 to 4 days under continuous high intensity light. However, in continuous darkness, NR activity decreased in a two-step process and no increase of NR activity was observed.

In this system no attempt has yet been made to decompose the oscillatory mechanism into the respective negative and positive feedback loops which are necessary according to present theories of chemical oscillators (Nicolis and Portnow 1973, Franck 1978). We have investigated the switch on/off behaviour of NR activity along these lines. The results indicate that the switch off mechanism in darkness can be considered as a reversible unimolecular conversion of the active form of nitrate reductase into an inactive form, forming a negative stabilizing feedback loop relative to NR activity. On the other hand, the light-induced increase of NR activity is closely related to a positive destabilizing feedback loop. In our treatment this process is represented as an auto-

catalytical reaction. Combination of the light-induced activation of NR and the inactivation reaction recorded in darkness, which is assumed also to be present under light conditions, results in oscillatory behaviour as found by experiment.

Abbreviation – NR(A), nitrate reductase (activity).

Materials and methods

Plant material

Barley seeds (*Hordeum vulgare* L. cv. Herta) were germinated and grown as previously described (Lillo 1983). The plants were grown under fluorescent lamps (Philips 33 white, 65 W m⁻², 400–700 nm) and given a light:dark 8:16 h regime. The first leaf was harvested 8 days after sowing.

Extraction and assay of NR (EC 1.6.6.1) activity

Leaf material, 1 g, was homogenized in a buffer at pH 7.5 containing 50 mM K⁺-phosphate, 1% (v/v) casein, 1 mM EDTA, 14 mM 2-mercaptoethanol and 0.1% (v/v) Triton X-100 (Lillo 1984). NR activity was tested as

Received 16 April, 1984; revised 19 June, 1984

previously described, using NADH as the electron donor (Lillo 1983).

Method of calculation

The equations of the kinetic model were transformed into the corresponding set of ordinary differential equations which were solved by numerical integration. The calculations were performed on a CDC CYBER 170 computer using the program package DARE P (Wait and Clarke 1976, Skjeldestad 1977).

Results and discussion

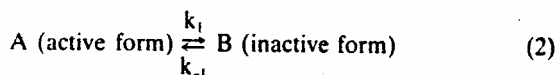
Decay mechanism of NR activity in darkness

In a previous report, Lillo and Henriksen (1984) observed that when plant seedlings with high NR activity were transferred to darkness NR activity was found to decrease according to the following relationship (Fig. 1):

$$\log \left(\frac{\text{NRA} - \text{NRA}^\infty}{\text{NRA}^0 - \text{NRA}^\infty} \right) = -\alpha \cdot t \quad (1)$$

where NRA denotes nitrate reductase activity at any time t , while NRA^0 and NRA^∞ are initial NR activity at $t = 0$ and NR activity after a long time (2 h or more), respectively. α denotes a constant.

This empirically-found relationship may be deduced by assuming that the active form of NR, here denoted simply as A, decomposes reversibly to an inactive form B:



Further we assume that NR activity is proportional to the concentration of the active form of NR, which we denote as C_A , i.e.,

$$\text{NRA} = \text{constant} \cdot C_A \quad (3)$$

In eq. 2, k_1 and k_{-1} denote the rate constants of the forward and reverse reactions, respectively. From this equation we obtain the rate expression

$$-\frac{dC_A}{dt} = \frac{dC_B}{dt} = k_1 \cdot C_A - k_{-1} \cdot C_B \quad (4)$$

At equilibrium, when $t \rightarrow \infty$, we have

$$k_1 \cdot C_A^\infty - k_{-1} \cdot C_B^\infty = 0 \quad (5)$$

with the equilibrium constant K defined by

$$K = \frac{C_B^\infty}{C_A^\infty} = \frac{k_1}{k_{-1}} \quad (6)$$

From this it follows, using the relation $C_B^\infty = C_A^0 - C_A^\infty$, that

$$C_A^\infty = \frac{k_{-1}}{k_1 + k_{-1}} \cdot C_A^0 \quad \text{and} \quad C_B^\infty = \frac{k_1}{k_1 + k_{-1}} \cdot C_B^0 \quad (7)$$

Replacing C_B in eq. 4 by $C_B = C_A^0 - C_A$, we get

$$\begin{aligned} \frac{dC_A}{dt} &= k_{-1} \cdot (C_A^0 - C_A) - k_1 \cdot C_A = -(k_1 + k_{-1}) \cdot \\ &\left(C_A - \frac{k_{-1}}{k_1 + k_{-1}} \cdot C_A^0 \right) = -(k_1 + k_{-1}) \cdot (C_A - C_A^\infty) \end{aligned} \quad (8)$$

where the final expression (8) follows from eq. 7. Finally we integrate eq. 8, i.e. calculate

$$\int_{C_A^0}^{C_A} \frac{d(C_A')}{C_A' - C_A^\infty} = - \int_0^t (k_1 + k_{-1}) dt' \quad (9)$$

which is identical to the expression in eq. 1, assuming eq. 3 to be correct.

The sum of the rate constants k_1 and k_{-1} is therefore identical to the slope of the graph in the inset of Fig. 1, while the value of $1/k_1$ is given by the zero time tangent of the C_A -time curve in Fig. 1 (Schmid and Sapunov 1982).

It should be stressed that so far light activation of NR has not been accounted for by this model, and accumulation of the active form during light exposure is

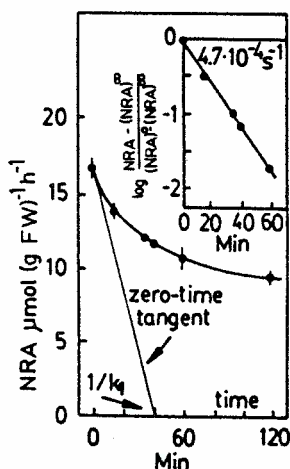


Fig. 1. Decrease of NR activity in darkness. The plants were transferred to darkness at the end of the photoperiod on day 8, thereafter kept in continuous darkness. Each point represents the mean of 4 samples, \pm SE is indicated by vertical bars. The zero-time tangent determines k_1 ($= 1/40 \text{ min}^{-1} = 1.5 \text{ h}^{-1}$), while the slope of the straight line in the inset determines $k_1 + k_{-1}$.

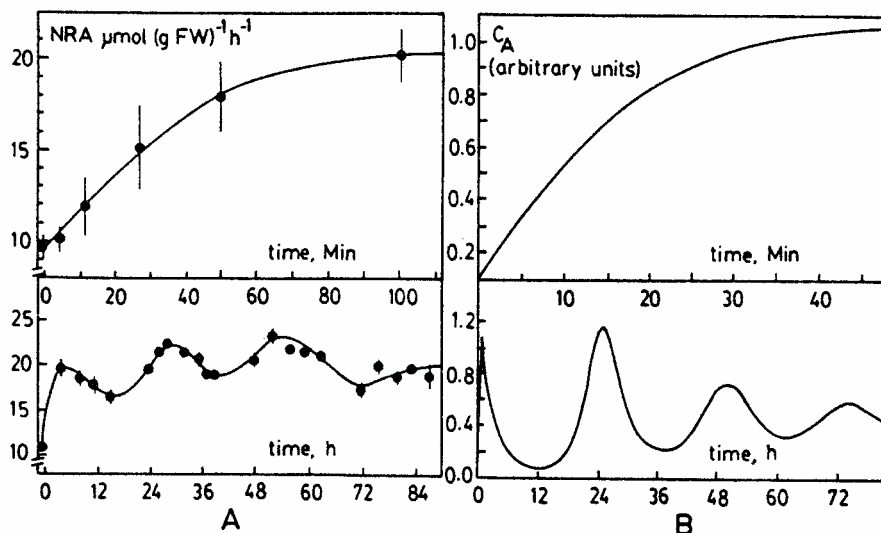


Fig. 2. A (upper part). Increase of NR activity after transferring the plants to light after the 16 h dark period. Each point represents the mean NR activity of 2 samples. Vertical bars indicate the spread. A (lower part). The oscillations of NR activity during a 88 h continuous light period. After the 16 h dark period the plants were transferred to continuous light (time = 0). Each point represents the mean of 6 samples. \pm SE is indicated. B. Calculated increase and circadian rhythmicity of A (the active form of NR) found by numerical integration of eq. 15 a-c. Initial values: $C_A = 0.1$, $C_B = 15.0$, $C_C = 7.0$ (arbitrary units).

therefore not necessarily caused by transforming the inactive form B into A in the single step reaction of eq. 2.

Increase of NR activity in light

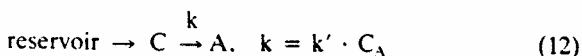
When NR activity in darkness has reached the lower plateau corresponding to NRA^* or C_A^* , plants transferred to light show a rapid increase of NR activity and finally damped circadian rhythms set in (Fig. 2A).

For oscillations to occur, present reaction kinetic theories about chemical oscillating systems require the existence of a fast destabilizing positive feedback loop, i.e. reaction steps which increase the formation of A, and a slow negative feedback loop which decreases the amount of A, where A is the oscillating compound. This is the principle of "antagonistic feedback" (Franck 1978).

While eq. 2 is considered as the negative feedback loop, we do not yet know the mechanistic form of the positive feedback control. This step may either occur by backward activation (eq. 10) or by forward inhibition (eq. 11) (Franck 1978)



In our treatment, we represent this step by an autocatalytical process



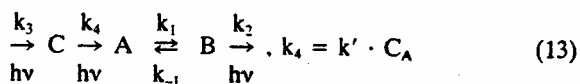
where the rate constant k is proportional to C_A , and C is formed from a certain reservoir.

As noted by Lotka more than 70 years ago (Lotka 1910), processes described by autocatalytical reactions as in eq. 12, may lead to damped oscillatory behaviour.

The model

Equation 2 has been used to explain the decrease of NR activity in darkness. However, we assume that this reaction also occurs under light conditions.

Combination of reaction 2 (which is the negative feedback loop) and reaction 12 (the positive feedback loop) defines our model



In order to avoid an extreme build-up of B (the inactive form of NR), B is assumed to be removed by a light-induced reaction. Reaction 13 generates the following rate equations:

$$\frac{dC_A}{dt} = k' \cdot C_A \cdot C_C - k_1 \cdot C_A + k_{-1} \cdot C_B \quad (14a)$$

$$\frac{dC_B}{dt} = k_1 \cdot C_A - k_{-1} \cdot C_B - k_2 \cdot C_B \quad (14b)$$

$$\frac{dC_C}{dt} = k_3 - k' \cdot C_A \cdot C_C \quad (14c)$$

Before numerical integration was performed, eq. 14 a-c were transformed into a dimensionless time scale $T = k' \cdot t$, which gives the final form of the differential equations used in the calculations:

Tab. 1. Values of rate constants.

Rate constant	Value, h ⁻¹
k_1	1.50
k_{-1}	0.19
k_2	0.84
k_3	0.62
k'	0.12

$$\frac{dC_A}{dT} = C_A \cdot C_C - K_1 \cdot C_A + K_{-1} \cdot C_B \quad (15a)$$

$$\frac{dC_B}{dt} = K_1 \cdot C_A - K_{-1} \cdot C_B - K_2 \cdot C_B \quad (15b)$$

$$\frac{dC_C}{dT} = K_3 - C_A \cdot C_C \quad (15c)$$

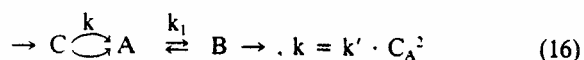
where $K_i = k/k'$, $i = -1, 1, 2, 3$.

Table 1 shows the rate constants used in the calculations and Fig. 2B shows the increase of NR activity and oscillatory behaviour observed in the model. While k_1 and k_{-1} were determined experimentally (Fig. 1), the other rate constants are still variable unknown parameters. Thus, the 24 h period length observed in the experiment is still not a natural consequence of the presented model. To be formally in agreement with the experiment, the remaining rate constants have been chosen so that a 24 h period length is obtained.

A comparison of Fig. 2A and B shows that this simple model can account for the main features of NR activity observed in darkness and under light conditions. However, the model presented by eq. 13 is only able to produce damped oscillations, because k_4 in eq. 13 is only proportional to C_A (Hanusse 1972). On the other hand, when the rate constant k_4 is proportional to C_A^2 (or higher powers of C_A) then it is theoretically possible to obtain sustained undamped oscillations (Tyson and Light 1973).

By performing some minor modifications to eq. 13,

we found the following model to generate undamped limit-cycle oscillations (results not shown)



where, parallel with the autocatalytic step with the rate constant k , an uncatalyzed step was included. However, since in the experiments performed so far, only damped oscillations have been observed, up to now we have not seen any need to extend the model to a limit cycle oscillator. We consider this simple model a useful starting point, since it can be easily extended or modified as new experimental facts arise.

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