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A simple model describing the kinetics of the xanthophyll cycle

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Abstract

A new kinetic model of the xanthophyll cycle is proposed. The model is based on the assumption that the light-dependent interconversion of the so-called available and unavailable violaxanthin constitutes the rate-limiting process of the cycle at intermediate, non-saturating light intensities. This assumption, together with the known properties of violaxanthin de-epoxidase, explains all specific features of the experimental facts.

Keywords: Xanthophyll cycle; Violaxanthin; Zeaxanthin

1. Introduction

Light-dependent interconversions of violaxanthin and zeaxanthin in chloroplasts were discovered by Sapozhnikov et al. in 1957 [1]. Subsequent investigations [2–5] have led to the conclusion that these transformations constitute a transmembrane cycle with the scheme shown in Fig. 1. One of the enzymes of the cycle, violaxanthin de-epoxidase has been purified [2,6]. It is a water-soluble enzyme with optimum pH of 5 [2]. The enzyme catalyzes violaxanthin de-epoxidation *in vitro* solely in the presence of membrane lipids, especially monogalactosyldiacylglycerol [6]. The de-epoxidation occurs only only after the thylakoid lumen has been acidified by the light-driven electron transport in tylakoid membranes because of the acidic pH optimum of the de-epoxidase. Moreover, a larger pool of violaxanthin is available for de-epoxidation at higher light intensities [2–5]. The physiological function of the xanthophyll cycle remained unknown for a long time. On the basis of our measurements of the photoprotective activity of violaxanthin and zexanthin in liposomes and emulsions [7,8], we postulated that the cycle has a photoprotective role. It seems to use, that after the

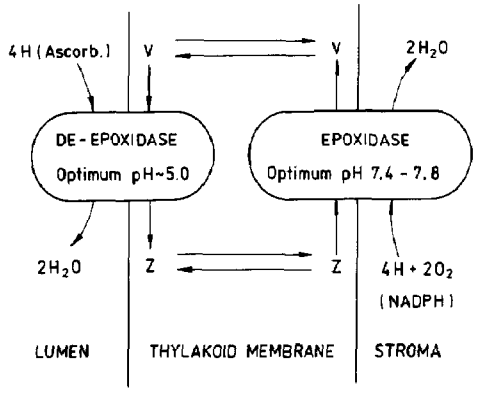

Fig. 1. Scheme of the xanthophyll cycle. V – violaxanthin, Z – zeaxanthin.

series of publications by B. Demmig and coauthors [9,10 and references therein] there is no doubt about a close relation of the cycle to the phenomena of photoproduction and photoinhibition. The proposal by Demmig et al. [9,10] that zeaxanthin promotes the heat dissipation of the absorbed radiation energy has recently been questioned [11]. As is said above, the de-epoxidation of violaxanthin occurs in the light, after the acidification of thylakoid lumen. However, illumination of the leaves which does not saturate the CO_2 assimilation results only in a transitional increase in the zeaxanthin level. The steady-state level of zeaxanthin starts to grow only during-saturated photosynthesis [3]. According to an analysis of the kinetics of the xanthophyll cycle [12] the presence of the reducing agent for the violaxanthin de-epoxidation is the main limiting factor of the steady-state level of zeaxanthin. At low light intensities the de-epoxidation of violaxanthin proceeds as long as the small pool of a reducing agent is not exhausted. The lack of a constant inflow of the reducing agent makes the continuous functioning of the de-epoxidase impossible. Only under saturating illumination the reducing agent might be produced in quantities sufficient for the constant functioning of de-epoxidase and, consequently, for the increase in the steady-state level of zeaxanthin in thylakoids.

Since the rate of the xanthophyll cycle reactions even at saturation is much smaller than the rate of the primary photosynthetic processes [3,13], it seems rather improbable to us that the xanthophyll cycle is controlled by the products of these processes.

In our model, dependence of the reactions rate constants and violaxanthin availability on illumination sufficiently explain transitional changes and steady-state level of zeaxanthin concentrations after illumination.

2. The kinetic model

The scheme of the kinetic model is shown in Fig. 2. We divide xanthophylls which participate in the violaxanthin cycle into three pools: zeaxanthin (Z, x), violaxanthin available for de-epoxidase (V_1, y) and violaxanthin unavailable

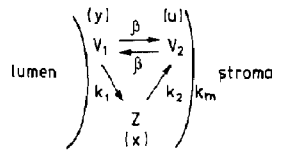

Fig. 2. Scheme of the xanthophyll cycle reactions in the discussed kinetic model. V_1 and V_2 stand for the violaxanthin pools available and unavailable; x, y, u – the relative pigment concentrations for the pools Z, V_1 and V_2, respectively; k_1 – rate constant of de-epoxidation; k_2 – rate constant of epoxidation; k_m – Michaelis' constant; β – rate constant of the V_1, V_2 interconversion.

for de-epoxidase (V_2, u). The cycle includes two essentially irreversible enzymatic reactions and, for this reason, it does not satisfy detailed microbalance. Such an assumption does not contradict the second law of thermodynamics because each of the enzymatic steps of the cycle is coupled with redox reactions which constitute the source of free energy. Reducing compounds are delivered by light-driven reactions of photosynthesis with much higher rate than the rate of the xanthophyll cycle reactions. The following simplifying assumptions have been made: (1) Antheraxanthin, the intermediate product of both the enzymatic reactions has been omitted. This allows us to use a lower order of differential equations. (2) The rate constants (β) of V_1 and V_2 interconvertions are the same in the two directions to reduce the number of free parameters. (3) The sum of concentrations of the three xanthophylls is constant. This assumption is a good approximation [9]. (4) In principle, zeaxanthin should be represented by two interconversible pools Z_1 and Z_2 (cf. Fig. 1). However, in order to obtain qualitative features of the observed kinetics [3] we had to accept that $Z_1 \rightleftharpoons Z_2$ interconversion proceeds at a much higher rate than other processes of the cycle. This is why we decided to represent zeaxanthin as one pool and to reduce in this way the number of free parameters. (5) We describe de-epoxidation of violaxanthin as a simple monomolecular reaction instead of using the Michaelis–Menten kinetics. This assumption let us avoid one additional free parameter. (6) We describe zeaxanthin epoxidation as a process with Michaelis–Menten light-independent kinetics.

According to the above assumption, the concentrations of the reagents can be described by the following equations:

$$\left.\begin{aligned}\frac{dZ}{dt} &= k_1 V_1 - \frac{k_2 Z}{k_m + Z}\\ \frac{dV_1}{dt} &= \beta(V_2 - V_1) - k_1 V_1\end{aligned}\right\} \quad (1)$$

with a conservation of mass law

$$Z + V_1 + V_2 = C, \quad (2)$$

where C is the constant sum of all three pools. The variable V_2 can be eliminated from eqs. (1) on the basis of (2). Let us introduce new, dimensionless variables:

$$x = \frac{Z}{C},\ y = \frac{V_1}{C},\ u = \frac{V_2}{C},\ \tau = \frac{k_2}{C}t \quad (3)$$

and new dimensionless kinetic parameters:

$$k = \frac{k_1 C}{k_2},\ l = \frac{\beta C}{k_2},\ m = \frac{k_m}{C} \quad (4)$$

Adopting the above notations, eqs. (1) and (2) can be written in dimensionless form as

$$\left.\begin{aligned}\frac{dx}{d\tau} &= -\frac{x}{m + x} + ky\\ \frac{dy}{d\tau} &= -lx - (2l + k)y + l\end{aligned}\right\} \quad (5)$$

$$x + y + u = 1 \quad (6)$$

Only the values between 0 and 1 have physical meaning for the new variables x, y and u. It was established that the rate of violaxanthin de-epoxidation and violaxanthin availability increases with the increasing intensity of light [3,4]. According to this conclusion, we suppose that the relative rate constants k and l are higher at higher light intensities.

3. Solutions to the model

In the dark, when violaxanthin de-epoxidase is inactive ($k = 0$), the system can reach an equilibrium state at the following concentrations:

$$x_D = 0,\ y_D = 0.5,\ u_D = 0.5 \quad (7)$$

The above values follow immediately from eqs. (5) and (6) if we put in (5) $k = 0$, $dx/d\tau = 0$ and $dy/d\tau = 0$. At non-vanishing values of k, that is in illuminated leaves or chloroplasts, there is no equilibrium. However, $dx/d\tau$ and $dy/d\tau$ vanish in steady-state, when concentrations of the xanthophylls are given by the following relations:

$$\bar{x} = 0.5\left\{1 - m - \frac{2}{k} - \frac{1}{l} + \left[\left(1 - m - \frac{2}{k} - \frac{1}{l}\right)^2 + 4m\right]^{0.5}\right\} \quad (8)$$

$$\bar{y} = \frac{\bar{x}}{k(m + \bar{x})}, \text{ and} \quad (9)$$

$$\bar{u} = 1 - \bar{x} - \bar{y} \quad (10)$$

It can be seen from (8) that at a given constant m, the steady state level of zeaxanthin ($\bar{x}$) depends on the value of parameter $a = 2/k + 1/l$. So, in order to obtain higher values of $\bar{x}$, one has to increase both rate constants k and l. For example, if $m = 0.1$ and $l = 0.1$ the steady-state level of zeaxanthin will rest equal to approximately 0.01 even at very high values of k. In such a case it is possible to obtain only a transitional increase of the zeaxanthin level.

In Fig. 3, there are shown computer solutions of equations (5) at different values of the rate constants k and l. The time is counted from the moment when the light was turned on. Consequently, we accepted (7) as initial conditions for

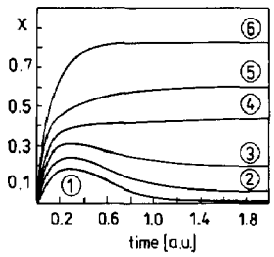

Fig. 3. Computer simulation of the time course of the zeaxanthin level according to the eq. (5). The curves correspond to $m = 0.1$ and to the following values of the kinetic parameters k and l: (1) $k = 3.5$, $l = 0.1$; (2) $k = 5$, $l = 0.5$; (3) $k = 7.5$, $l = 1$; (4) $k = 10$, $l = 2$; (5) $k = 15$, $l = 3$; and (6) $k = 20$, $l = 10$. Time units correspond to τ as definied by relation (3).

the solutions presented in Fig. 3. We tried to reproduce distinct maxima of zeaxanthin concentration in roughly the same place on the time-scale [3] and the possibility of the complete conversion of violaxanthin into zeaxanthin [10]. It appeared that both the experimental facts cited above can be reproduced at a small enough value of relative Michaelis' constant m. This is why we have put $m = 0.1$. The values of k and l increase in the sequence shown by numbers at the corresponding curves in Fig. 3 and curves with higher indices should correspond to those obtained experimentally (e.g. [3]) at higher light intensities. De-epoxidation of violaxanthin to zeaxanthin takes place on the illumination. In the case of curves (1–3), this process has a high rate in comparison with the rate of conversion of the pool of the unavailable violaxanthin (V_2 or u) into that accesible for de-epoxidase (V_1 or y). Initially the pool of zeaxanthin (x) grows and the pool of available violaxanthin diminishes. At the same time new-formed zeaxanthin is transformed into unavailable violaxanthin (u). The decrease of the pool of available violaxanthin results in decreasing rate of de-epoxidation. This, at zeaxanthin epoxidase still active, leads to the decrease of zeaxanthin concentration to a low steady-state level. So, in our model, the transitional increasing of zeaxanthin concentration occurs under conditions when the overall rate of the cycle is controlled by the transformation of the so called unavailable violaxanthin (u) into the available one (y). Maybe it would be useful to note that for curves 1-6 in Fig. 3 the rate constant of de-epoxidation (k) changes from 3.5 to 20 and the constant 1 from 0.1 to 10. In the same units the effective rate constant of zeaxanthin epoxidation at very small x amounts to $1/m$, that is to 10.

4. Changes in the absorbance at 505 nm (ΔA_{505})

There is a well established correlation between the reactions of the xanthophyll cycle and changes in chloroplast absorption at 505 nm (ΔA_{505}) [14,15]. These absorbance changes have been used for monitoring de-epoxidase activity and violaxanthin availability. The kinetic model discussed here gives a possibility to interpret these changes. One of the absorption maxima of aggregated forms of xanthophylls is localized at 505nm [16,17]. Both monomeric violaxanthin and zeaxanthin reveal only weak absorption at 505 nm. Our investigations [18,19] suggest that among caroteniods zeaxanthin has the strongest tendency to aggregate in one-component and mixed monolayers at the water–air interface. In view of this, we consider the zeaxanthin pool (Z or x) as a pool of aggregated xanthophyll which absorbs at 505 nm, at least to a large extent. The pool of unavailable violaxanthin (V_2 or u) is expected to be the second pool absorbing at 505 nm. So, we can assume that the following approximate relation holds:

$$\Delta A_{505} = \alpha[x + u - u(0)], \tag{11}$$

where α is the proportionality coefficient depending on the molar absorptivities of aggregated violaxanthin and zeaxanthin at 505 nm. The relation (11) together with (6) and (7) gives

$$\Delta A_{505} = \alpha(0.5 - y). \tag{12}$$

It follows from (12), (9) and (8) that the steady-state value of

$$\overline{\Delta A}_{505} = \alpha \frac{k/l + 2\bar{x}}{2 + k/l} \tag{13}$$

Thus, the $\overline{\Delta A}_{505}$ can be a proper measure of the steady-state zeaxanthin level only at $k \ll l$, that is at a much lower rate of the violaxanthin de-epoxidation than the rate of the interconversion of the two violaxanthin pools. In the opposite case ($k \gg l$) the value of $\overline{\Delta A}_{505}$ can be saturated even at a very low zeaxanthin level ($\bar{x}$). Siefermann-Harms [3] concluded that the whole thylakoid violaxanthin becomes available at light intensities lower than the intensity saturating the rate of the de-epoxidation. In view of eq. (13) we should treat such a statement with a dose of prudency so far as it is based on ΔA_{505} measurements.

On the other hand, the initial rate of the ΔA_{505} increase is a proper measure of the rate of the de-epoxidation. As can be inferred from eqs. (12), (5) and (7)

$$\frac{\mathrm{d}}{\mathrm{d}\tau}(\Delta A_{505})\Big|_{t=0} = \alpha \frac{\mathrm{d}y}{\mathrm{d}\tau}\Big|_{t=0} = 0.5\ \alpha k \tag{14}$$

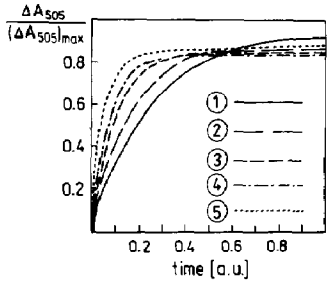

Fig. 4. Absorbance changes at 505 nm calculated according to eqs. (12) and (5). Time units and values of the kinetic parameters as in Fig. 3.

the initial value of the time derivative of the absorbance of the sample at 505 nm is directly proportional to the rate constant of violaxanthin de-epoxidation (k). Figure 4 presents time course of ΔA_{505} calculated according to eqs. (12) and (5). The curves clearly demonstrate the features discussed above. Besides that, it can be seen that the curves $\Delta A_{505}(\tau)$ (1–3) have no distinct maxima in contrast to the curves $x(\tau)$ (1–3) in Fig. 3 corresponding to the same values of kinetic parameters k and l.

Within the framework of our kinetic model, changes in the absorbance at 505 nm are determined by the pool of the available violaxanthin (V_1 or y). Increases in the absorbance at 505 nm correspond to decreases in V_1. After turning off the light, the pool V_1 would be restored by diminishing the pool V_2 or, indirectly, by the pool Z and we should observe decreasing absorbance at 505 nm.

5. Conclusions and final remarks

Our model is consistent with experimental results if the following assumptions are true: (1) Transition of violaxanthin from the unavailable to available pool is the rate limiting process of the xanthophyll cycle at intermediate, non-saturating light intensities. (2) The zeaxanthin epoxidase has a small Michaelis' constant. Our interpretation of the changes in the absorbance at 505 nm offers an explanation for the lack of transient maxima in ΔA_{505} in spite of the occurrence of such maxima in zeaxanthin concentration.

We should mention that qualitatively the same results could be obtained if the zeaxanthin epoxidation were treated as a simple monomolecular reaction. In such a case, however, the rate constant of epoxidation should be light-dependent and should decrease at the increasing intensity of light.

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