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THE INFLUENCE OF ATP/NADPH RATIO ON DISTRIBUTION OF REDUCTIVE PENTOSE PHOSPHATE CYCLE METABOLITES AS A POSSIBLE CAUSATIVE FACTOR OF ENHANCEMENT EFFECT, CHROMATIC TRANSIENTS AND SPECTRAL CHANGES OF QUANTUM YIELD OF PHOTOSYNTHESIS

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A simple kinetic model for the reductive pentose phosphate cycle is suggested and analyzed. The changes in ATP/NADPH ratio caused by light wavelength alterations have been shown to result in the specific redistribution of metabolites in the cycle. The redistribution permits an explanation of the kinetic patterns of the enhancement effect and chromatic transients of photosynthesis. The spectra of relative ATP concentration have been calculated using enhancement values for green plants and cyanobacteria. Relative changes in quantum yield of photosynthesis were also calculated using data from spectra of relative ATP concentration changes. It is shown that changes in the quantum yield of photosynthesis may be fully explained by means of the spectral dependence of the relative ATP concentration in chloroplasts. It is concluded that the enhancement effect, chromatic transients and the 'red drop' of photosynthetic efficiency are not to be considered as exhaustive arguments for the Z-scheme of photosynthesis, although they do not contradict it.

Introduction

The enhancement effect of photosynthesis discovered by Emerson et al. [1], and, closely associated with it, chromatic transients caused by changes in wavelength of incident light (which Blinks was the first to observe [2]), as well as the 'red drop' of photosynthetic efficiency [3], have been found both in unicellular algae and in higher plants [4,5]. All these phenomena are usually interpreted from the point of view of a consequtive scheme of interaction of two photosystems (for references see Ref. 5).

However, the enhancement effect may not always be found in isolated chloroplasts if measured by the rate of photoreduction of exogenic $NADP^+$ with water, though in the absence of exogenic $NADP^+$ a considerable enhancement effect may be observed in O_2 evolution associated with CO_2 assimilation [6–8]. It was suggested that the enhancement was the result of a joint contribution by cyclic and noncyclic photophosphorylation for CO_2 assimilation and was not related to a noncyclic electron flow from water to $NADP^+$ [6–8].

Blinks [9] observed the opposite direction of changes in CO_2 assimilation and O_2 evolution during chromatic transients and came to the conclusion that chromatic transients were connected with different rates of NADPH and ATP formation and with corresponding changes in pool sizes of ribulose 1,5-bisphosphate and other intermediates of the reductive pentose phosphate cycle as a result of alteration of incident light wavelength.

According to Arnon [10], the 'red drop' of photosynthetic efficiency in green plants may be considered a consequence of the following phenomena: in long-wavelength light the cyclic photophosphorylation exhibits a 'red rise', while the

noncyclic one becomes limiting. As a result, the relative amount of NADPH decreases and the efficiency of CO_2 assimilation drops.

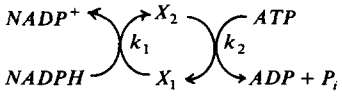
It appears, therefore, reasonable to suggest that the enhancement effects, 'red drop' of photosynthesis as well as chromatic transients, are connected with some processes in the reductive pentose phosphate cycle rather than with direct interaction of photosystems acting in series. The changes in rates of ATP and NADPH formation due to the alteration in wavelength of incident light apparently influence those processes. We have made an attempt to look into this problem by constructing a kinetic model of the reductive pentose phosphate cycle action at low light intensity. When studying the model behaviour, we observed enhancement effects, chromatic transients and could calculate action spectra of photosynthesis.

Theoretical consideration

The model of the reductive pentose phosphate cycle functioning at weak light

The CO_2 assimilation rate in the reductive pentose phosphate cycle appears to be determined at low light intensity by ATP and NADPH concentrations, the formation rates of which depend on light intensity. Only two stages of the reductive pentose phosphate cycle depending on ATP and NADPH concentrations have been taken into account. The first stage is the conversion of 3-phosphoglycerate into glyceraldehyde 3-phosphate, requiring ATP and NADPH. The NADPH concentration at weak illumination is apparently low enough, while the ATP concentration is maintained at a comparatively high level [11–13]. The general rate of the reaction on the first stage may be shown to be limited by NADPH concentration rather than by that of ATP under such conditions. The second stage is ribulose 5-phosphate phosphorylation, requiring ATP.

The reductive pentose phosphate cycle action at low light intensity may be presented as follows:



where X_1 is the electron acceptor from NADPH, X_2 is the component interacting with ATP (X_1 is the concentration of 3-phosphoglycerate; X_2 is the sum of ribulose 5-phosphate concentration and concentrations of all its precursors in the reductive pentose phosphate cycle tied together by fast reversible reactions), k_1 and k_2 are the rate constants of the reactions.

Assuming all the reactions to fit the law of mass action, the reductive pentose phosphate cycle behaviour may be described by a simple system of linear differential equations:

$$d[X_1]/dt = k_2[ATP][X_2] - k_i[NADPH][X_1] \quad (1)$$

$$d[X_2]/dt = k_i[NADPH][X_1] - k_2[ATP][X_2] \quad (2)$$

where:

$$[X_1] + [X_2] = [R] \quad (3)$$

Here [R] is the amount of all the metabolites in the reductive pentose phosphate cycle, assumed to be constant during the experiments the model describes.

It is also suggested that during X_1 and X_2 concentration changes evoked by alteration of light conditions, ATP and NADPH concentrations may be taken as stationary (the transition time to a new steady-state level of ATP and NADPH concentrations does not exceed a few seconds [11], while the transition time of metabolites in the reductive pentose phosphate cycle is at least an order of magnitude longer [14]).

Let us suppose that the stationary concentrations of ATP and NADPH may be taken as proportional to light intensity by weak monochromatic illumination. Then:

$$[NADPH] = I(\lambda)[NADPH]'(\lambda) \quad (4)$$

$$[ATP] = I(\lambda)[ATP]'(\lambda) \quad (5)$$

where $I(\lambda)$ is the intensity of incident monochromatic light at wavelength λ. The intensity of light by which some arbitrarily defined unit of photosynthesis rate is maintained may be taken as a unit of light intensity. $[NADPH]'(\lambda)$ and $[ATP]'(\lambda)$ are the stationary concentrations at the unit light intensity of monochromatic light.

Now, we can give:

$$a(\lambda) = k_1[\mathrm{NADPH}]'(\lambda)[\mathrm{R}] \quad (6)$$

$$b(\lambda) = k_2[\mathrm{ATP}]'(\lambda)[\mathrm{R}] \quad (7)$$

where $a(\lambda)$ and $b(\lambda)$ are coefficients dependent on wavelength rather than on light intensity.

The solution of Eqns. 1–3 for the case in which the ATP and NADPH pool sizes undergo abrupt transitions in a new steady state may be deduced:

$$[\mathrm{X}_1] = ([\mathrm{X}_1]_s^o - [\mathrm{X}_1]_s) \exp\{-[a(\lambda)+b(\lambda)] \times I(\lambda)(t-t_0)/[\mathrm{R}]\} + [\mathrm{X}_1]_s \quad (8)$$

where:

$$[\mathrm{X}_1]_s = b(\lambda)[\mathrm{R}]/[a(\lambda)+b(\lambda)] \quad (9)$$

$$[\mathrm{X}_1]_s^o = b(\lambda^o)[\mathrm{R}]/[a(\lambda^0)+b(\lambda^0)] \quad (10)$$

The subscript (s) indicates the stationary level and the superscript (o) indicates the levels of concentration before the light conditions are changed; t_0 is the starting time of the illumination changes.

Calculation of the rate of photosynthesis

The photosynthesis rate measured by O_2 evolution (ν) may be determined finally by the rate of NADPH oxidation on the terminal link of the electron transfer chain, i.e., by the rate of X_1 reduction:

$$\nu = k_i[\mathrm{NADPH}][\mathrm{X}_1] \quad (11)$$

The photosynthesis rate may also be expressed by the rate of CO_2 photoassimilation. It is well known that upon increasing the CO_2 concentration, the photosynthesis rate at low light intensities does not increase [5,15], i.e., the carboxylation rate is not limiting in these conditions. Therefore, the rate of CO_2 assimilation (ν_c) should be determined by the rate of ribulose 5-phosphate phosphorylation only, or in terms of our model by the rate of X_2 phosphorylation:

$$\nu_c = k[\mathrm{ATP}][\mathrm{X}_2] \quad (12)$$

Substituting the X_1 concentration values from Eqns. 8–10 into Eqn. 11 we may obtain:

$$\nu(\lambda) = \left[\frac{\nu(\lambda^0)_s a(\lambda) I(\lambda)}{a(\lambda^0) I(\lambda^0)} - \nu(\lambda)_s\right] \exp\{-[a(\lambda) + b(\lambda)] I(\lambda)(t-t_0)/[\mathrm{R}]\} + \nu(\lambda)_s \quad (13)$$

where:

$$\nu(\lambda)_s = I(\lambda)[1/a(\lambda)+1/b(\lambda)]^{-1} \quad (14)$$

$$\nu(\lambda^0)_s = I(\lambda^0)[1/a(\lambda^0)+1/b(\lambda^0)]^{-1} \quad (15)$$

Hence, the linear dependence between photosynthesis rate and light intensity is true within the limits of our model.

Using Eqns. 3 and 9, we can deduce from Eqn. 12 that the stationary rate of CO_2 assimilation and that of O_2 evolution are equal.

It is possible, using Eqn. 14, to write for the case of unit light intensity:

$$1/a(\lambda)+1/b(\lambda) = 1 \quad (16)$$

Calculation of enhancement values and chromatic transients of photosynthesis

Let us indicate by λ_1 the wavelength range of light absorbed mainly by pigment System I and by λ_2 that absorbed mainly by pigment System II. In accordance with McSwain and Arnon [6] we shall assume the NADPH synthesis rate at simultaneous illumination with two weak light beams ($I(\lambda_1)$ and $I(\lambda_2)$) to be equal to the sum of NADPH synthesis rates when each light beam is presented separately. Analogously, such an additivity may be supposed to hold true for the rate of ATP synthesis, i.e., the ATP synthesis rate depends on the amount of absorbed quanta. Hence, the absence of enhancement effect is assumed for the rates of NADPH and ATP formation.

Let us suppose that the assumption on the additiviy of the rates of formation of NADPH and ATP may be extended to their concentrations as well. Then:

$$[\mathrm{NADPH}](\lambda_1,\lambda_2) = I(\lambda_1)[\mathrm{NADPH}]'(\lambda_1) + I(\lambda_2)[\mathrm{NADPH}]'(\lambda_2) \quad (17)$$

$$[\mathrm{ATP}](\lambda_1,\lambda_2) = I(\lambda_1)[\mathrm{ATP}]'(\lambda_1) + I(\lambda_2)[\mathrm{ATP}]'(\lambda_2) \quad (18)$$

where (λ_1,λ_2) indicates simultaneous illumination with both $I(\lambda_1)$ and $I(\lambda_2)$ light beams.

The photosynthesis rate during simultaneous illumination with both beams may be derived from Eqns. 13–15 by substituting [NADPH] (λ_1,λ_2) and [ATP] (λ_1,λ_2) instead of [NADPH] and [ATP]. Using Eqns. 4 and 5, we can obtain from Eqn. 14:

$$\nu(\lambda_1,\lambda_2)_s = \left[\frac{1}{I(\lambda_1)a(\lambda_1)+I(\lambda_2)a(\lambda_2)} + \frac{1}{I(\lambda_1)b(\lambda_1)+I(\lambda_2)b(\lambda_2)}\right]^{-1} \quad (19)$$

The value of the enhancement may be calculated in accordance with Ref. 5 from the following expression:

$$E_{1,2} = \nu(\lambda_1,\lambda_2)_s/[\nu(\lambda_1)_s+\nu(\lambda_2)_s] \quad (20)$$

The magnitude of the enhancement may also be found as a factor referring to the gain of rate of photosynthesis to either light beam alone (see Ref. 5):

$$E_1 = [\nu(\lambda_1,\lambda_2)_s-\nu(\lambda_2)_s]/\nu(\lambda_1)_s \quad (21)$$

$$E_2 = [\nu(\lambda_1,\lambda_2)_s-\nu(\lambda_1)_s]/\nu(\lambda_2)_s \quad (22)$$

Substituting $\nu(\lambda)_s$ and $\nu(\lambda_1,\lambda_2)_s$ from Eqns. 14 and 19 into Eqn. 21 and introducing:

$$N = I(\lambda_2)/I(\lambda_1)$$

one can obtain:

$$E_1 = N\left[\frac{1}{a(\lambda_1)/N+a(\lambda_2)} + \frac{1}{b(\lambda_1)/N+b(\lambda_2)}\right]^{-1} - N \quad (23)$$

The E_1 value reaches the maximum (E_{1m}) when $N \to \infty$.

When going to limit, one can obtain from Eqn. 23 using Eqn. 16:

$$E_{1m} = \frac{a(\lambda_1)}{[a(\lambda_2)]^2} + \frac{b(\lambda_1)}{[b(\lambda_2)]^2} \quad (24)$$

Substituting $\nu(\lambda)_s$ and $\nu(\lambda_1,\lambda_2)_s$ from Eqns. 14 and 19 into Eqn. 22, we obtain:

$$E_2 = \left[\frac{1}{a(\lambda_1)+Na(\lambda_2)} + \frac{1}{b(\lambda_1)+Nb(\lambda_2)}\right]^{-1} - \frac{1}{N} \quad (25)$$

The E_2 value is maximal (E_{2m}) when $N \to 0$. We can obtain, therefore:

$$E_{2m} = \frac{a(\lambda_2)}{[a(\lambda_1)]^2} + \frac{b(\lambda_2)}{[b(\lambda_1)]^2} \quad (26)$$

Usually, the values of chromatic transients are determined as the photosynthesis rate changes directly after alteration in the wavelength of the incident light. As a rule, the light beams are adjusted in intensity to give equal steady-state rates of photosynthesis [2,5,15]. Then, we can deduce from Eqn. 13, if $\nu(\lambda^0)_s = \nu(\lambda_s)$, $I(\lambda^0) = I(\lambda)$ and $t_0 = t$:

$$L = \frac{\nu(\lambda)}{\nu(\lambda^0)_s} = \frac{[\text{NADPH}]'(\lambda)}{[\text{NADPH}]'(\lambda^0)} = \frac{a(\lambda)}{a(\lambda^0)} \quad (27)$$

Here L is the chromatic transient value, λ^0 is the wavelength of monochromatic light used before the conditions of illumination are changed.

Now, using the calculated values of the enhancement effect or those of chromatic transients, we can obtain the spectral dependence of relative changes of NADPH and ATP concentration. Examples of such a calculation will be shown below.

Determination of quantum yield of photosynthesis depending on ATP concentration

In terms of the model under discussion, it seems impossible to determine directly the changes in the quantum yield of photosynthesis by alterations in incident light wavelength. It is possible, however, to express such changes in quantum yield using the relative variations of ATP concentration.

It is known that two molecules of NADPH and three molecules of ATP are required in order to bind one CO_2 molecule in the reductive pentose phosphate cycle [8,14].

It is advantageous to calculate the quantum requirement for assimilation of one CO_2 molecule using the quantum flow density of incident light needed to maintain equal photosynthesis rates at any wavelength. The rates of NADPH and ATP synthesis coupled to noncyclic electron flow are constant in this case and equal to their formation

rates by the minimal quantum requirement. It appears reasonable to suggest that the increase in quantum requirement under suboptimal conditions is the consequence of formation of additional ATP which may be used in reactions not connected with CO_2 assimilation.

ATP concentration seems to be proportional to the ATP synthesis rate in conditions of low light intensity. Then, the amount of ATP molecules (ΔATP) that are additionally synthesized, as compared to the ATP formed at minimal quantum requirement, may be determined:

$$\Delta\mathrm{ATP} = \frac{k\{[\mathrm{ATP}]'(\lambda) - [\mathrm{ATP}]'(\lambda_{min})\}}{[\mathrm{ATP}]'(\lambda_{min})} \qquad (28)$$

where λ_{min} is the wavelength at which the minimum quantum requirement is achieved, k is a coefficient which is numerically equal to the number of ATP molecules synthesized in the reactions connected with the assimilation of one CO_2 molecule when the quantum requirement is minimal.

The number of light quanta required to form ATP molecules coupled to assimilation of one CO_2 molecule may be determined using Eqns. 7 and 28 as:

$$\delta(\lambda) = 2\Delta\mathrm{ATP} + \delta(\lambda_{min}) = 2k\left[\frac{b(\lambda)}{b(\lambda_{min})} - 1\right] + \delta(\lambda_{min}) \qquad (29)$$

The coefficient 2 in Eqn. 29 is used because in processes not coupled to noncyclic electron flow two quanta are required to form one ATP molecule [16]. $\delta(\lambda)$ is the quantum requirement by any arbitrary wavelength; $\delta(\lambda_{min})$ is the minimum quantum requirement.

Two extreme assumptions are considered:

(1) In noncyclic photophosphorylation two ATP molecules are formed in reactions coupled to the formation of two NADPH molecules and eight light quanta are consumed. Two more quanta are required to form one molecule of ATP in cyclic photophosphorylation [8,16]. Hence $\delta(\lambda_{min}) = 10$ and $k = 3$.

(2) Two molecules of NADPH are formed in noncyclic electron transfer coupled to four molecules of ATP formation in noncyclic photophosphorylation and eight quanta of light are consumed [14]. Then $\delta(\lambda_{min}) = 8$ and $k = 4$.

If the amount of ATP molecules formed during the synthesis of two NADPH molecules is assumed to be between the two possibilities discussed, the values of coefficients of Eqn. 29 will be intermediate as well.

Verification of the model

Enhancement effect: its nature and kinetics

The model of the reductive pentose phosphate cycle action suggested was tested for its ability to reproduce the known dependence of enhancement effect on $N = I(\lambda_2)/I(\lambda_1)$ (Fig. 1). Data of Ref. 17 are used: $E_1 = 2.88$ if $N = 4.35$, and $E_1 = 4.2$ if $N = 20$ for $\lambda_2 = 650$ nm and $\lambda_1 = 710$ nm.

Then, using Eqn. 16, it is possible to solve the system of two Eqns. 23 for E_1 and to find the values $a(650)$ and $a(710)$ (Table I). The dependence of E_1 on N was calculated using Eqn. 23. The solution of the model is close enough to the experimental data (Fig. 1).

The model suggested has been shown to describe also the dependence of $E_{1,2}$ on N [18], being calculated using the data of Ref. 15.

It is noteworthy that attempts to obtain the

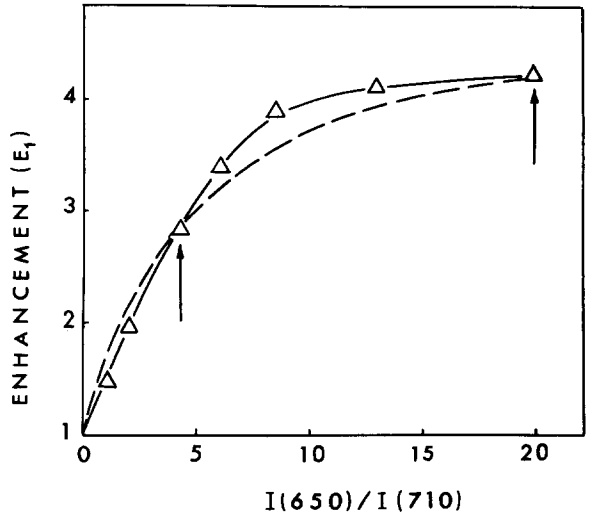


Fig. 1. Enhancement value (E_1) in *Chlorella* as dependent on light beam intensity ratio (△———△) (according to Ref. 17); - - - - -, model solution, Arrows indicate the experimental data used for calculations of the model coefficients.

TABLE I

KINETIC PARAMETERS OF THE REDUCTIVE PENTOSE PHOSPHATE CYCLE MODEL

Enhancement value (E_1) in *Chlorella* as dependent on λ [17]. The coefficients $a(\lambda)$ and $b(\lambda)$ are calculated using Eqns. 16 and 23.

λ (nm)	Enhancement (E_1) by $N = 20$ ($\lambda_2 = 650$ nm)	$a(\lambda)$	$b(\lambda)$
650	1.0	2.0	2.0
690	1.7	1.21	5.76
695	2.5	1.12	9.7
700	2.8	1.098	11.2
710	4.2	1.055	19.2

dependence of E_1 on N using the model of direct interaction of photosystems [19] have not succeeded in the description of experimental data. The dependence took the form of two intersectional straight lines, while one smooth curve was shown in the experiments [15,17].

In order to explain the nature of the enhancement effect, let us consider Fig. 2. The change in

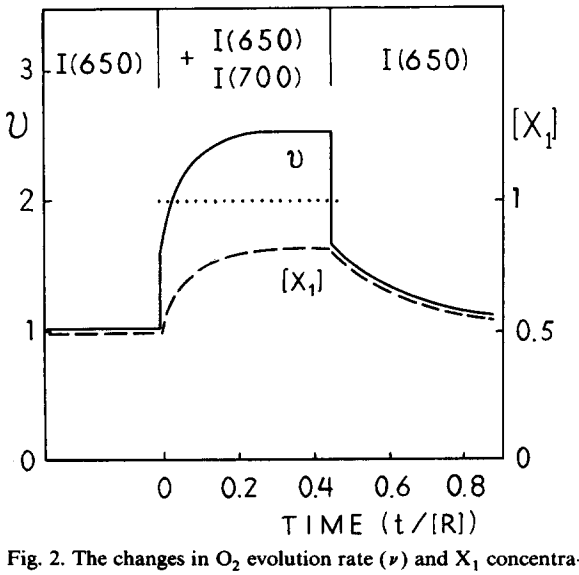


Fig. 2. The changes in O_2 evolution rate (ν) and X_1 concentration obtained in model experiment by addition of an $I(700)$ light beam to an $I(650)$ beam. The dotted line denotes the calculated sum of photosynthesis rates assuming rates of O_2 evolution caused by separate light beams added one to another. Time is expressed in arbitrary units ($t/[R]$). [R] is taken as a unit of X_1 concentration.

X_1 concentration is compared to the O_2 evolution rate by imitation of addition of complementary $I(700)$ light to the basal $I(650)$ light beam.

Every light beam is assumed to produce the arbitrarily defined unit of photosynthesis rate. X_1 concentration and O_2 evolution rate were estimated by substitution of Eqns. 17 and 18 into Eqns. 8 and 13. The coefficients were taken from Table I.

The kinetic curve of O_2 evolution at the addition of $I(\lambda_1)$ to $I(\lambda_2)$ consists of two parts. The first is a sharp increase in the rate of O_2 evolution caused by the increase in NADPH concentration after the addition of the $I(700)$ light beam. The second is a slow increase in the photosynthesis rate as a result of an increase in the concentration of X_1. The rise in X_1 concentration may be explained by the redistribution of metabolites in the reductive pentose phosphate cycle caused by additional synthesis of ATP (when $I(\lambda_1)$ was added to $I(\lambda_2)$) compared to NADPH synthesis. A similar pattern of O_2 evolution has been observed with intact plants [5]. It is evident, according to our model, that enhancement may be achieved by electron transport acceleration due to slow increase of terminal acceptor (X_1) concentration rather than as a result of a direct increase in electron flow through the interaction of photosystems.

The abolition of the enhancement of O_2 evolution associated with CO_2 assimilation when exogenous ATP is added to isolated chloroplasts [7] may also be explained. It is abvious that exogenous ATP may weaken the effect of metabolite redistribution produced by changes in the quality of light.

The proposed model explained also the peculiar decrease in the enhancement effect upon the addition of some intermediates of the reductive pentose phosphate cycle to isolated chloroplasts [20]. In this case, the cycle may be uncoupled and the characteristic redistribution of its metabolites grow weak. The spectral characteristics of enhancement effects may also be explained [18].

Spectrum of relative ATP concentration and the quantum yield of photosynthesis.

For the evaluation of the quantum yield spectrum, according to Eqn. 29, it is necessary to know the change in $[ATP]'(\lambda)$ when the wavelength of incident light is altered. The model suggested al-

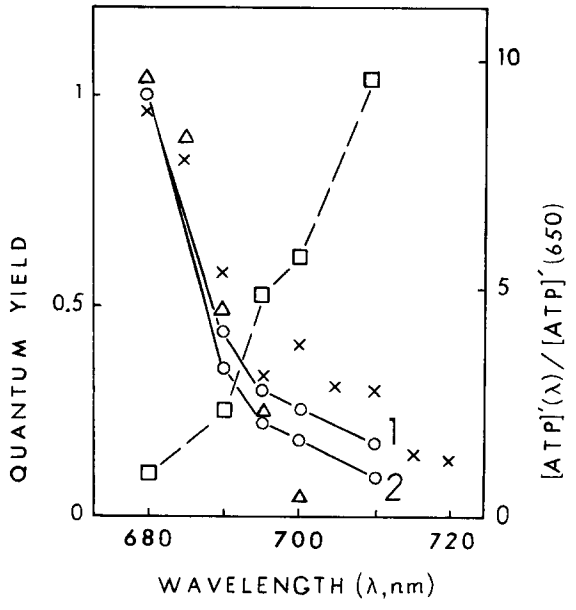


Fig. 3. The relative spectra of quantum yield of photosynthesis in *Chlorella* (normalized to 680 nm). △, according to Ref. 1; ×, according to Ref. 21; O——O, calculated from Eqn. 29 using data of Table I. Curve 1 corresponds to the assumption that by the reduction of two $NADP^+$ molecules two ATP molecules are formed. Curve 2 is that obtained when four ATP molecules are assumed to be formed. □- - - - -□, the calculated spectrum of relative ATP concentrations ($[ATP]'(\lambda)/[ATP]'(650)$), when the intensities of incident light are adjusted to give equal steady-state rates of photosynthesis for all the wavelengths tested.

lows one to determine the spectrum at relative ATP concentration when the action spectrum of photosynthesis enhancement is known.

Fig. 3 presents model solutions for spectrum of relative ATP concentration and spectral dependence of relative quantum yield of photosynthesis in *Chlorella*. The calculations have been made using Eqn. 29 and coefficients of Table I. (The quantum yield of photosynthesis has been determined as the reciprocal value of quantum requirement.) The model spectral curve of relative quantum yield of photosynthesis is reasonably conterminous to experimental data.

The useful model application may be proved in data processing on the cyanobacteria experiments (Figs. 4A, B), where both the enhancement spectrum and the action spectrum of quantum yield of photosynthesis were measured simultaneously [22].

Model solutions are compared with experimen-

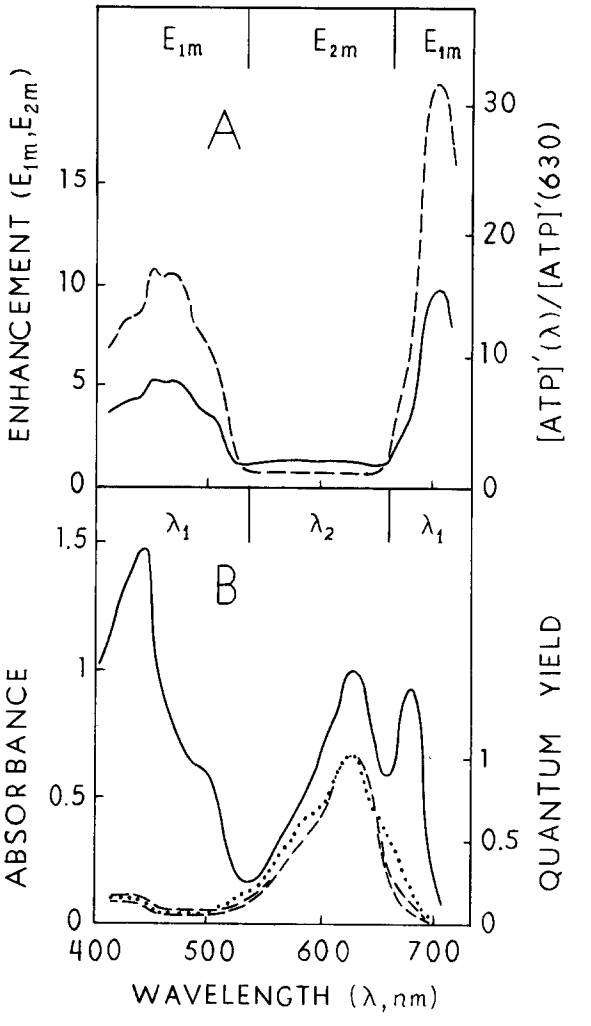


Fig. 4. (A) The spectrum of the enhancement effect in *Anacystis nidulans* (———) (generalized data of Ref. 22 for 'white' cells). The $E_{1m}(\lambda)$ values were obtained adding the weak continuous $I(\lambda_1)$ beam to the strong $I(620)$ one. the $E_{2m}(\lambda)$ values were obtained by adding the weak $I(\lambda_2)$ light to the strong $I(690)$ beam; ------, the calculated spectrum of the relative ATP concentration:

$$\left([ATP]'(\lambda)/[ATP]'(630)\right).$$

(B) The relative absorbtion spectrum (———) and the relative spectrum of quantum yield of photosynthesis (·····) for 'white' cells of *A. nidulans* [22]. Both spectra have been normalized to 630 nm. ------, the calculated relative spectrum of quantum yield of photosynthesis (normalized to 630 nm). The upper dashed line corresponds to the assumption that by the reduction of two $NADP^+$ molecules two ATP molecules are formed, the lower dashed curve is that obtained when four ATP molecules are assumed to be formed.

tal data in the following way. According to Ref. 22, $E_{1m} = 8.28$ and $E_{2m} = 1.38$ for $\lambda_1 = 690$ nm (region of PS I) and $\lambda_2 = 620$ nm (region of PS II). Then, using Eqn. 16, it is possible to solve the system of two Eqns. 24 and 26 and to find the values ($b(620) = 3.14$ and $b(690) = 92.7$. The spectrum $b(\lambda_1)$ has been calculated using Eqn. 24 and the spectrum $E_{1m}(\lambda)$. In similar way, using the $b(690)$ value the $b(\lambda_2)$ terms have been calculated (using Eqn. 26 for $E_{2m}(\lambda)$).

The maximum quantum yield of photosynthesis (i.e., the minimum of ATP concentration) is at 630 nm (Fig. 4B), that is why the spectrum of relative ATP concentration is obtained as $b(\lambda)/b(630) =$ [ATP]'(λ)/[ATP]'(630 (Fig. 4A).

The relative spectrum of the quantum yield of photosynthesis has been obtained (see Eqn. 29) per absorbed quanta. In Ref. 22 the action spectrum of photosynthesis has been found per incident quantum (Fig. 4B). That is why the calculated spectrum has been multiplied by the relative absorption spectrum (Fig. 4A). As a result, the relative spectrum of the photosynthetic quantum yield has been derived per incident quantum. This spectrum was similar to the experimental one (Fig. 4B). The calculated spectra of quantum yield of photosynthesis are alike in shape if both two and four ATP molecules are assumed to be synthesized for every two $NADP^+$ molecule reduction (Fig. 4B).

The spectra of enhancement effect and quantum yield of photosynthesis in red algae are similar to those of cyanobacteria [23]. It is reasonable, therefore, to expect that the model in question is valid for explanation of the spectral characteristics of the enhancement effect and of the quantum yield of photosynthesis in red algae as well.

Chromatic transients

The nature of chromatic transients may also be explained by the model. Fig. 5 represents the model curves corresponding to O_2 evolution rate and X_1, concentration changes by the rapid shift from $I(700)$ to $I(650)$ and vice-versa. Eqns. 8 and 13 and Table I coefficients are used.

The shape of chromatic transients observed on intact plant by O_2 evolution [2,5,15] coincides with model solutions rather well. The starting increase of O_2 evolution rate by the $I(700) \rightarrow I(650)$ transition (Fig. 5) may be explained by the increase in [NADPH]'(650) as compared to [NADPH]'(700). The decrease in O_2 evolution rate taking place afterwards is a consequence of a decrease in $[X_1]$ (cf. Eqn. 8) as a result of reduction in [ATP]'(λ)/[NADPH]'(λ) at such a transition. It is evident that the initial decrease in the rate of O_2 evolution through the $I(650) \rightarrow I(700)$ transition is a result of a decrease in [NADPH]'(700) comparing to [NADPH]'(650) and the further slow acceleration of O_2 evolution is explicable by the rise in X_1 concentration.

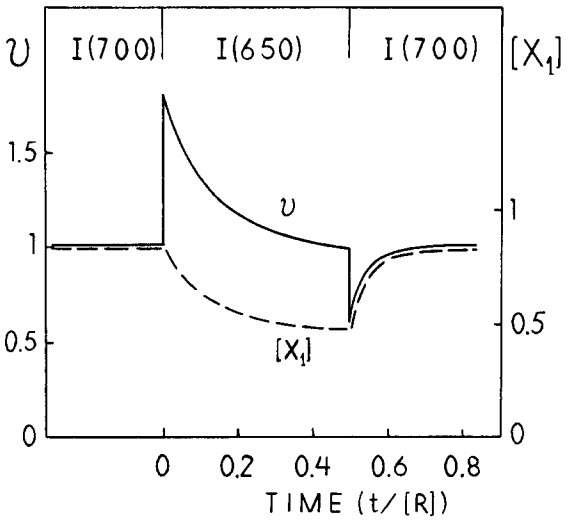


Fig. 5. Chromatic transients on the O_2 evolution rate (ν) and the X_1 concentration change obtained in a model experiment. The transients $I(700) \rightarrow I(650)$ and $I(650) \rightarrow I(700)$ are presented. The O_2 evolution rates caused by separate light beams are assumed to be constant and equal to 1. Time and $[X_1]$ scales are the same as in Fig. 2.

It is easy to explain also the results of Blinks' experiments [9] in which he discovered that chromatic transients measured by CO_2 assimilation and by O_2 evolution rates have opposite directions. According to Eqn. 16, the direction of the [ATP]'(λ) changes is opposite to that of the [NADPH]'(λ) ones, and the changes in $[X_2]$ are opposite to that of $[X_1]$ (cf. Eqn. 3). As seen from Eqn. 12, all these changes must result in opposite directions of changes in rates of CO_2 assimilation and O_2 evolution when the incident light wavelength is altered.

The model suggested permits elimination of an obvious contradiction occurring in an attempt to

explain chromatic transients by the processes taking place in the photosynthetic electron transfer chain only. Really, experimental data on the kinetics of the redox state changes of the photosynthetic electron carriers [24,25] as well as solutions of the models describing the interaction of two photoreactions in series [26,27] show that the transition time of a new steady state in the electron transfer chain when the incident light wavelength is altered does not exceed a few seconds. The chromatic transients in intact plants may run up to some minutes [2,5,15]. Hence, changes occuring in electron transport chain are of too short a duration to explain the observed chromatic effects.

It is difficult to determine exactly the duration of chromatic transients using our model, because the solutions may be found only for the time value divided by an unknown coefficient [R] (cf. Eqns. 8 and 23). But it is well known that the transition processes in the reductive pentose phosphate cycle may proceed as long as several minutes [14], in accordance with the duration of chromatic transients.

The proposed model made it possible to evaluate chromatic transients [18] using the data from Ref. 28 on the enhancement value determination. The coincidence of spectral characteristics of both chromatic transients and enhancement values E_1 and E_2 may be explained in terms of our model by the identical direction of changes in values of enhancement effects and chromatic transients when $[NADPH]'(\lambda)$ is altered [18].

Discussion

The model of the reductive pentose phosphate cycle functioning at low light intensity explains the enhancement effects, chromatic transients and spectra of quantum yield of photosynthesis quite satisfactorily. thus, the assumption of the necessity to take into account the interaction of cyclic and noncyclic photophosphorylation by CO_2 assimilation [6–8] was confirmed at the model level.

However, the observations of the enhancement effect in isolated chloroplasts measured by the rate of reduction of exogenic $NADP^+$ with water [29,30] are in contradiction with the results obtained. This contradiction may be explained by the peculiarity of the method of enhancement value measurement used in Refs. 29, 30. According to this method, for instance, continuous light ($I(\lambda_1)$ was added to modulated light $I(\lambda_2)$. The O_2 detection system responded only to the modulated production of oxygen; the direct effect of continuous light $I(\lambda_1)$ was not observed and only the enhancement of the modulated oxygen production was detected.

The addition of continuous light $I(\lambda_1)$ to modulated $I(\lambda_2)$ light shifts the stationary redox state of photosynthetic electron carriers in the direction of the predominancy of oxidized forms [31,32]. As a result, the O_2 evolution calculated on a single $I[\lambda_2)$ light flash of modulated illumination is increased [32]. But the general O_2 evolution rate caused by both modulated and continuous illumination seems not to show any enhancement of photosynthesis in isolated chloroplasts. In this case, the increase in the modulated oxygen production may be compared to the first stage of enhancement kinetics only, i.e., to the fast increase of NADPH concentration upon the addition of $I(\lambda_1)$ (cf. Fig. 2).

The slow kinetic stage responsible for the enhancement effect is not observable at the modulated illumination of isolated chloroplasts in the various experiments [29,33], because the reductive pentose phosphate cycle is uncoupled in the presence of appreciable amounts of the exogenic electron acceptor, i.e., $NADP^+$. The slow stage of enhancement kinetics may well be seen with the modulated illumination of intact plants as a gradual increase in O_2 yield per light flash [34,35] because O_2 evolution is coupled in this case to CO_2 reduction.

It should be emphasized, therefore, that the results of experiments caried out on isolated chloroplasts with the modulated illumination may not serve as a strict criterion of the presence of enhancement effects.

There was at least one problem we were not able to solve using our model. The spectrum of the enhancement effect in *Chlorella* has a pronounced maximum and minimum over the wavelength region 580–680 nm [5,15]. According to our model, the spectral distribution of ATP concentration as well as quantum yield of the photosynthesis action spectrum are expected to have corresponding extrema in this spectral region. However, the experi-

mentally determined spectrum of the quantum yield of O_2 evolution is nearly constant (less than 5% variation) over this wavelength interval [3,5]. It should be pointed out that the constancy of the quantum yield of photosynthesis in *Chlorella* within 580–680 nm is difficult to explain using the assumption of the successive action of two photosystems [5] as well.

In our model we have assumed the NADPH/$NADP^+$ ratio to be increased with light intensification. Using intact chloroplasts and protoplasts of spinach it was shown, however, that such an assumption may be accepted only when the light intensity does not exceed a few watts per square metre. When the light was more intense, the NADPH/$NADP^+$ ratio was decreased [36]. These results do not contradict our model, because of the low light intensity usually used in experiments under discussion (cf. Refs. 15, 22, 27, where only a few watts per square metre were used). We may suppose, therefore, that in such experiments the NADPH/$NADP^+$ ratio is increased by the increase in actinic light intensity.

Some conclusions may be drawn from the suggested concept concerning the significance of the redistribution of the reductive pentose phosphate cycle metabolites in the display of effects observed at low light intensity. Enhancement effects, chromatic transients and the 'red drop' of photosynthesis efficiency are considered usually as evidence of two photosystems acting in series [5,8,24]. But the explanation of all these phenomena may be given if one assumes that ATP and NADPH concentrations may change in some definite manner with alterations in incident light wavelength. No extra assumptions about the number of pigment systems or the mechanisms of interaction between the photosystems in our model were made. The photosystems and the regions of their light absorption were mentioned for the purpose of convenience only.

Thus, the action of photosystems in series should not be considered as the reason for all the phenomena mentioned above, although the Z-scheme (with the cyclic electron flow) is also sufficient to explain the increase in ATP concentration as compared to NADPH concentration in spectral region, defined as the Photosystem I absorption region. Hence, the proposed model does not contradict the Z-scheme of electron transport, or any other scheme which may result in similar action spectra of ATP and NADPH formation (cf. Refs. 8, 37).

Analysis of quantum yield of photosynthesis spectra and of spectral dependence of ATP formation in *Chlorella* and cyanobacteria shows that most light quanta absorbed are apparently effective in ATP formation.

The drop in photosynthetic efficiency in the spectral region of Photosystem I may be attributed not to the dissipation of absorbed energy, but to the usage of absorbed quanta for the formation of extra ATP molecules, which may be consumed in processes not connected with CO_2 assimilation.

As to the action spectra of the quantum yield of photosynthesis, it should be pointed out that, if along with the synthesis of two NADPH molecules even four ATP molecules are formed, it is neccessary to assume the existence of additional source of ATP (e.g., cyclic photophosphorylation) which utilizes light quanta absorbed in spectral region of pigment System I (cf. Figs. 3, 4).

It is to be noted that for the verification of the model, special experiments are desirable in which the changes in metabolite concentrations in the reductive pentose phosphate cycle would be measured together with the determination of the photosynthesis rate. There are some data on the increase of 3-phosphoglycerate concentration in leaves illuminated with light absorbed mainly by Photosystem I [38,39]. This finding is in agreement with the expected concentration rise (cf. Fig. 5). However, to show the effects in question more clearly, special experiments with very low light intensities are planned.

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