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Controlled distribution of electrons between acceptors in chloroplasts: a theoretical consideration

Leonid E. Fridlyand [a], Renate Scheibe [b,*]

[a] *Institute of Experimental Botany, Academy of Sciences of Belarus, Skorina Street 27, Minsk 220072, Belarus*
[b] *Pflanzenphysiologie, Fachbereich Biologie/Chemie, Universität Osnabrück, D-49069 Osnabrück, Germany*

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Abstract

The coupled processes of electron distribution in chloroplasts in the light are quantitatively analyzed by means of a steady-state model. The equations for electron transport to ferredoxin, nitrite reduction, cyclic electron transport, malate valve, 3-phosphoglycerate reduction, and O_2 reduction are developed. Kinetic parameters are derived from known data. Using the kinetic properties of the system and the known metabolite concentrations, the model demonstrates that photosynthetically generated electrons can be used efficiently in photosynthesis. It is shown that the modelled system can maintain the NADPH/$NADP^+$ ratio constant within a wide range of light intensities. The control coefficients for different electron fluxes have been calculated. The calculated sum of control coefficients is significantly below unity, a fact that can be explained by the existence of conserved moieties in this system. The possible role of the electron distribution in leaf cells under normal conditions and during stress is discussed.

Keywords: Electron transport; Photosynthesis; Malate valve; Mehler reaction; Mathematical model; Redox state

1. Introduction

The function of photosynthetic light and dark reactions is the maintenance of an optimal stromal ATP/NADPH ratio for flexible adaptation to changing environmental conditions such as light, temperature, or availability of CO_2. The production of ATP and NADPH is a coupled process, but if their consumption ratio does not fit to the demand of the carbon-reduction cycle, overreduction of the stromal electron acceptors will occur [1,2] and may lead to protein damage and chlorophyll bleaching. For this reason, a flexible adjustment of the electron transport under various conditions is required.

The stromal ATP/NADPH ratio is adjusted by special mechanisms which direct electrons to acceptors other than CO_2. Adjustment can be achieved by reduction of nitrite [1,3], cyclic electron flow around PS I [4,5], the reduction of O_2 [6], or by the malate valve [1,7]. However, the relative contribution of the various poising mechanisms during photosynthesis is still under debate [4,6,8].

In the present paper, an attempt is made to create

Abbreviations: ARC, anabolic reduction charge [NADPH/($NADP^+$+NADPH)]; Chl, chlorophyll; FBPase, fructose 1,6-bisphosphatase; Fd, ferredoxin; Fd_o, oxidized ferredoxin; Fd_r, reduced ferredoxin; FNR, ferredoxin–NADP reductase; MV, methylviologen; NiR, nitrite reductase; OAA, oxaloacetate; PGA, 3-phosphoglycerate; PS I, Photosystem I; PS II, Photosystem II; concentrations of compounds are represented by symbols written without brackets

* Corresponding author. Fax: +49-541-969-2265;
E-mail: scheibe@biologie.uni-osnabrueck.de

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a model which includes the main features of our current knowledge about mechanisms of the regulatory processes in the electron transport chains as electron distribution is concerned, by using a mathematical model of all these processes. However, some assumptions had to be made when experimental data were insufficient. This article is based on and follows the quantitative consideration of different aspects of photosynthetic processes inside chloroplasts as already initiated in our previous articles [9,10].

2. Model

The general structure of the model is represented in Fig. 1. The model includes the main pathways for electron consumption. The consumption of electrons by thioredoxin-catalyzed redox modification of some enzymes in chloroplasts should not exceed 2% [7], and it will not be included in this consideration.

2.1. Concept of electron pressure

The concept of electron pressure from PS I is used in this article, since we consider only electron flux from PS I, that is regulated by electron transport pressure from PS II. In this case the electron flux to Fd_o can be described by the equation

$$v_1 = N_1 k_1 (Fd_o/Fd_t) \quad (1)$$

where k_1 is some electron pressure, Fd_t is the total quantity of Fd ($Fd_t = Fd_o + Fd_r$), and (Fd_o/Fd_t) is the portion of Fd that is in the oxidized form. The coefficient N_1 stands for the quantity of electrons transported for one cycle of this reaction. Evidently, $N_1 = 1$ in this case.

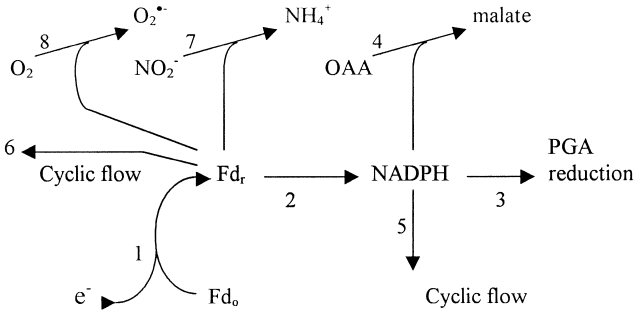

Fig. 1. General structure of the model. Numbers at reactions are used in associated flux names: (1) electron transport to ferredoxin, (2) $NADP^+$ reduction, (3) PGA reduction, (4) malate valve, (5) cyclic electron transport from NADPH, (6) cyclic electron transport from Fd_r, (7) nitrite reduction, (8) O_2 reduction.

2.2. FNR action

It is very likely that Fd is connected with FNR in one rigid complex that also includes PS I [11–14]. No information is available about kinetic rules for the electron transport between Fd and $NADP^+$ in such a complex. For this reason, some additional simplified suppositions were required. It is likely that the limiting step is the availability of $NADP^+$ for this complex, since the electron transport processes are usually fast inside a complex. Therefore, the simplest reversible one-substrate one-product Michaelis–Menten enzyme equation for such complex can be considered, since the other substrate and the other product are localized inside this enzyme complex:

$$v = \frac{V_s S/K_{ms} - V_p P/K_{mp}}{1 + S/K_{ms} + P/K_{mp}} \quad (2)$$

where v is the rate of the reaction, S and P are the substrate and product concentrations in the medium, K_{ms} and K_{mp} are the Michaelis–Menten constants for S and P, V_s and V_p are the maximum enzyme activity of forward and back reaction for the complex of the enzyme with one substrate and one product, respectively. For the case of FNR, if $V_s = V_p$, Eq. 1 can be represented as

$$v_2 = \frac{N_2 V_{2m}[(Fd_r/Fd_t)NADP^+ - (Fd_o/Fd_t)NADPH/K_2)]}{K_{m2NADP}(1 + NADP^+/K_{m2NADP} + NADPH/K_{m2NADPH})} \quad (3)$$

where $NADP^+$ is the substrate and NADPH is the product, and (Fd_r/Fd_t) and (Fd_o/Fd_t) are the relative concentrations of reduced and oxidized Fd that are in contact with FNR. Displacement of a reaction from equilibrium can be expressed in this case by the equilibrium constant (K_2). V_{2m} is the maximum enzyme activity in the forward direction for the complex of Fd_r with FNR, K_{m2NADP} and $K_{m2NADPH}$ are the Michaelis–Menten constants for $NADP^+$ and NADPH. N_2 is the quantity of the electrons which are transported for the reduction of one NADPH

molecule ($N_2 = 2$). The analogous equation was obtained in [12] by another method.

The equilibrium constant can be calculated from the redox potential difference (see for example [15]):

$$K_2 = \exp[n_e F \Delta E_2/(RT)] \quad (4)$$

where R is the gas constant, F is the Faraday constant, T is the absolute temperature, n_e is the number of electrons transferred in the reaction, and ΔE_2 is the redox potential difference.

ΔE_2 is about 80 mV as it was obtained for the two-electron transfer from reduced ferredoxin connected with FNR to bound $NADP^+$ [16]. This value is close to that which can be obtained from reduction potentials of these substances, since the reduction potential is about −0.42 for ferredoxin ox/red [14] and about −0.34 for the NADP/NADPH system at a pH close to 8 [17]. Then $K_2 = 495$ according to Eq. 4.

To determine the value of the maximal rate of electron transfer from Fd_r to $NADP^+$ we use a value such as 445 e^- s^{-1} at 7°C [18]. If we assume that an increase of the temperature by 10°C leads to an increase of the rate by 2, then the maximal rate at 25°C is about 1600 e^- s^{-1} that we can use as a value for V_{2m} for one molecule FNR.

As was mentioned above, it is very likely that Fd is connected with FNR in a rigid complex that includes PS I. Each complex can include only one molecule of each of Fd, FNR and PS I [11–14]. If some portion of Fd or FNR is outside of such complex, this means that these molecules do not take part in electron transport, although they can be measured. Evidently, in such situation we should use the concentration of PS I as a measure of the quantity of the complex and of both FNR and Fd. One PS I in spinach usually is associated with 500 molecules of chlorophyll [19]. If we assume that only 1 molecule of FNR is connected with one PS I, and then take into account the molecular weight of Chl, we obtain from these data that $V_{2m} = 6400$ μmol e^- (mg Chl)$^{-1}$ h^{-1}. The $K_{m2NADP} = 50$ μM at pH = 8 for bound FNR is taken from [20], $K_{m2NADPH} = 35$ μM from [21].

2.3. PGA reduction

As was shown by Fridlyand [22] in a theoretical consideration, the NADPH concentration does not determine the rate of PGA reduction under ambient physiological conditions. This confirms the findings of a largely constant NADPH/$NADP^+$ ratio over a broad range of light intensities [23] and upon changes in electron demand [1]. For this reason, it is likely that a change of the NADPH concentration plays a role in determining the photosynthetic rate in leaves only under low light near the light compensation point, where the NADPH concentration can be considerably decreased [9]. However, CO_2 assimilation and photorespiration also require ATP consumption. It can be assumed that, if photosynthesis is limited by electron transport, the flux of electrons to PGA reduction can be determined by the rate of ATP production that is directly connected with the rate of electron transport to Fd. However, there is some uncertainty in the H^+/e^- ratio for light-driven electron transport and consequently in the stoichiometry of ATP synthesis [4,5]. For this reason, the maximal possible rate of PGA reduction will be determined below as proportion of the total electron flux (v_1), when PGA reduction is limited by electron transport.

During photorespiration, the requirement for electrons is the same as during CO_2 assimilation [24]. For this reason, flux v_3 can be calculated as the sum of the rates of CO_2 assimilation and photorespiration.

Evidently, two possibilities should be taken into account.

(a) CO_2 assimilation is restricted by electron transport, then

$$v_3 = N_3 k_3 v_1 \quad (5)$$

where k_3 is some coefficient that will be determined in the following and can depend on the experimental conditions, $N_3 = 2$ as the number of electrons that are needed to produce one NADPH molecule to reduce one PGA molecule.

(b) There is some restriction of photosynthesis by other factors

$$v_3 = N_3 G \quad (6)$$

where G is some parameter, the value of which depends on the experimental conditions.

The rate of PGA reduction (v_3), for example, can be calculated from Eq. 8 in [9] on the basis of the known rate of CO_2 assimilation.

2.4. Malate valve

The malate-valve system, namely the conversion of excess NADPH into malate, consists of the chloroplast enzyme NADP-dependent malate dehydrogenase and the export of malate from the chloroplast to the cytosol. A mathematical model of this system has already been presented [10]. The light-dependent formation of malate is strictly regulated by the activity of the NADP-dependent malate dehydrogenase. The activation state of this enzyme is controlled by the stromal NADPH/$NADP^+$ ratio in this model.

The rate of electron transport for OAA reduction can be determined as

$$v_4 = N_4 V_{\mathrm{MDH}} \tag{7}$$

V_{MDH} is the rate of malate production that is determined by Eq. 2 from [10], $N_4 = 2$, since one NADPH molecule is necessary to reduce one OAA molecule.

2.5. Cyclic electron flow from NADPH

An antimycin A-insensitive pathway was demonstrated that is mediated by NAD(P)H-quinone oxidase and uses NADH or NADPH as substrates [25,26]. A large redox potential difference leads to the irreversibility of this reaction. However, if the quinone pool in the electron transport chain is fully reduced, it can lead to a cessation of this reaction [27] that should be taken into account in some special case that we do not consider in this article. In the general case, analogous to Eq. 2, we obtained for an irreversible reaction

$$v_5 = \frac{N_5 V_{5\mathrm{m}}\mathrm{NADPH}}{K_{\mathrm{m5NADPH}}(1 + \mathrm{NADPH}/K_{\mathrm{m5NADPH}} + \mathrm{NADP}/K_{\mathrm{m5NADP}})} \tag{8}$$

where $K_{\mathrm{m5NADPH}} = 100$ μM and $V_{5\mathrm{m}} = 36$ μmol NADPH (mg Chl)$^{-1}$ h^{-1} [25]. It was impossible to find data for K_{m5NADP} in the literature. For this reason, it was taken as K_{m2NADP}, since it is possible that FNR serves as a binding site for $NADP^+$ in this process [28]. $N_5 = 2$, since 2 electrons can be transferred upon oxidation of one NADPH molecule.

2.6. Cyclic electron flow from Fd

The antimycin A-sensitive cyclic electron flow clearly starts from reduced Fd and may be catalyzed by a specific Fd–quinone reductase [5]. Evidently, this electron transport consists of some stable multienzyme complex that includes reduced Fd, and in this situation we use the following equation:

$$v_6 = N_6 V_{6\mathrm{m}}(\mathrm{Fd_r}/\mathrm{Fd_t}) \tag{9}$$

where $V_{6\mathrm{m}}$ is some maximum cyclic electron transport from Fd_r.

According to Figs. 4 and 5A in [29], $V_{6\mathrm{m}} = 180$ μmol Fd (mg Chl)$^{-1}$ h^{-1} at $Fd_r/Fd_t = 1$. Evidently, $N_6 = 1$, since only one electron can be transported from Fd_r.

2.7. Nitrite reduction

Nitrite reduction is catalyzed by nitrite reductase (NiR) taking electrons from Fd_r that is connected with the PS I complex. The mechanism of nitrite reduction is very complex and is not yet completely understood [16]. However, the simplest equations for nitrite reduction can be built taking into account that the concentration of the end product (NH_4^+) is usually very low and that the reaction is irreversible. Then Eq. 2 can be used with $P = 0$. Furthermore, it was shown that the electron flow via nitrite reductase seems to occur with high rates even at a low redox state of Fd [1,30]. This means that this reaction is saturated at a low level of Fd_r. For this reason, the corresponding equations can be written neglecting Fd_r contents:

$$v_7 = \frac{N_7 V_{7\mathrm{m}}\mathrm{NO_2^-}}{K_{\mathrm{m7NO_2}} + NO_2^-} \tag{10}$$

where $V_{7\mathrm{m}}$ is the maximal NiR activity.

To obtain the coefficients in Eq. 10 for isolated chloroplasts; we used the data from Fig. 5 in [1] for the dependence of the rate of nitrite reduction during CO_2 fixation upon the nitrite concentrations. We recalculated these data for 25°C. As a result $V_{7\mathrm{m}} = 9.9$ μmol (mg Chl)$^{-1}$ h^{-1} at 25°C and $K_{\mathrm{m7NO2}} = 23.7$ μM. This maximum rate of nitrite reduction corresponds to about 10% of rate of CO_2 fixation in these chloroplasts (taking into account the electron consumption).

NiR catalyzes the 6-electron reduction of nitrite to ammonia, and 2 electrons from Fd are used by glutamate synthase for the reductive conversion of

2-oxoglutarate plus glutamine to glutamate [3]. As a result 8 electrons are used for nitrite reduction in the cell ($N_7 = 8$). However, since 2-oxoglutarate has to be imported from the cytoplasm, the additional 2 electrons are not used in isolated chloroplasts. In this case $N_7 = 6$.

Furthermore, in intact leaves the rate-limiting and regulated step of nitrate assimilation appears to be the initial reaction of nitrate reduction that is catalyzed by nitrate reductase in the cytoplasm and is regulated in a complex manner [31,32]. For this reason, Eq. 10 can be used only for conditions where the nitrite concentration is known.

Nitrite reduction apparently takes place only in sufficiently strong light [33]. It is possible that nitrate uptake is linked to carbohydrate supply [32]. For this reason, we suppose that in leaves, in opposite to chloroplasts, the flux v_7 is proportional to the rate of PGA reduction (flux v_3)

$$v_7 = N_7 k_7 v_3 \tag{11}$$

where k_7 is a coefficient of proportionality.

2.8. O_2 reduction

In the Mehler reaction, electrons are transferred to O_2 from Fd_r (or from PS I) in a non-enzymatic reaction and this reaction would appear to be the limiting step in the Mehler-peroxidase cycle [6]. In our model, Fd_r is the only source for O_2 reduction, and we suggest that other components of PS I are subjected to similar changes in their ratios of oxidized to reduced forms as is Fd.

The reactions of the Mehler-type oxidation by the Fd_r can be represented as first-order non-enzymatic reaction for Fd_r and as Michaelis-like reaction for O_2, since a hyperbolic curve was obtained for the dependence of this reaction on the O_2 concentration [34].

$$v_8 = \frac{N_8 V_{8m}(Fd_r/Fd_t)O_2}{K_{m8} + O_2} \tag{12}$$

where V_{8m} and K_{m8} are the maximal rate of O_2 reduction and the Michaelis constant for O_2, respectively. The K_m value for O_2 (K_{m8}) is about 10 μM for thylakoid preparations [34]. According to Asada [35] the rate of $O_2^{\bullet -}$ production in chloroplasts (V_{8m}) under optimal conditions is 30 μmol e^- (mg Chl)$^{-1}$ h^{-1}. However, no information about the Fd_r/Fd_t ratio was available for the conditions used. According to our following calculations this ratio can be about 0.75 under these conditions. Then V_{8m} can be evaluated as 40 μmol e^- (mg Chl)$^{-1}$ h^{-1}, that corresponds to 30 μmol e^- (mg Chl)$^{-1}$ h^{-1} at optimal conditions. In equilibrium with air, the O_2 concentration is about 250 μM. For this reason, in ambient conditions the rate of O_2 reduction is saturated with O_2.

The $O_2^{\bullet -}$ radicals which are produced by O_2 reduction are disproportionated by superoxide dismutase into H_2O_2 and O_2. Thylakoid-bound ascorbate peroxidase reduces H_2O_2 at the expense of ascorbate to form the monodehydroascorbate radical, which is further reduced by a thylakoid-bound monodehydroascorbate reductase. This enzyme uses reduced Fd as an electron donor to regenerate ascorbate [6]. The affinity of this enzyme for reduced Fd is about 30 times higher than the affinity of the FNR [6,36]. For this reason, we believe that the ascorbate regeneration does not limit O_2 reduction and that the quantity of electrons used to regenerate ascorbate can be added simply to the rate of O_2 reduction (coefficient N_8). As a result, $N_8 = 2$ upon the reduction of one O_2.

2.9. Model construction

The concentration changes of Fd_r and NADPH in chloroplasts as described by this model are given by a set of two differential equations, if the OAA concentration in the chloroplasts equals 0 or can be determined separately.

$$\begin{aligned} dFd_r/dt &= (v_1 - v_2 - v_6 - v_7 - v_8)/V_C \\ dNADPH/dt &= (v_2 - v_3 - v_4 - v_5)/V_C \end{aligned} \tag{14}$$

with the additional relationships

$$Fd_r + Fd_o = Fd_t$$

$$NADPH + NADP^- = N_A$$

V_C is the stroma volume in the chloroplasts, N_A is the total concentration of NADPH plus $NADP^+$. The value for $Fd_t = 0.0823$ mM was found under the following assumptions: (1) one molecule of Fd is bound in the PS I complex, (2) one complex is associated with 500 molecules of chlorophyll (see

above), and (3) the stroma volume of the chloroplasts equals 27 μl (mg Chl)$^{-1}$ [1]. $N_A = 0.332$ mM for isolated chloroplasts [1] and 0.88 mM for chloroplasts inside of cells [37].

When we take into account the malate valve, the two additional differential equations for the OAA and malate concentrations in chloroplasts from [10] were added to the system of Eq. 13.

A computer was used to obtain the numerical solutions. The fourth order Runge–Kutta method was applied to solve this system of differential equations. Calculations were performed up to the time that corresponded to a steady state. The coefficients determined above were used. It should be noted that the number of non-available parameters was high when the attempt is made to take into account the real picture of electron distribution. The parameter values used have been obtained under different conditions by different experimental procedures. Some simplifications are necessary for the simulation due to our limited knowledge. For this reason the presented model is only a semi-quantitative one.

3. Results

Only one coefficient, i.e., the electron pressure (coefficient k_1), will be initially selected for the subsequent calculations in this model. This coefficient was adopted for the conditions inside a leaf: the characteristic ARC value was taken as 0.33 as determined by Heineke et al. [37]. N_A was taken as for chloroplasts inside leaf cells (0.88 mM). The rate of net photosynthesis in spinach was taken as 200 μmol CO_2 (mg Chl)$^{-1}$ h^{-1} at normal temperature as mean ambient photosynthesis in spinach [38], which corresponds to 800 μmol e^- (mg Chl)$^{-1}$ h^{-1}. The Eq. 6 was used to determine PGA reduction and Eq. 10 for nitrite reduction. However, the NO_2^- concentration is not known for the leaf cell (see above). For a rough approximation the NO_2^- concentration was taken as 0.2 mM, that gives a rational electron consumption for this reaction and permits to compare with our data from isolated chloroplasts. The simulation of the malate valve was made as in [10] for the leaf cell. The obtained data are represented in case 1 in Table 1.

3.1. Study of the system of isolated chloroplasts

At saturating bicarbonate concentrations and during photosynthetic induction in isolated chloroplasts the CO_2 assimilation is severely limited by some processes that are not connected with the capacity to use electrons or ATP in the carbon-reduction cycle, and possibly can be explained by limitation of FBPase activity [39]. For this reason, Eq. 6 was used for PGA reduction. The characteristics of spinach chloroplasts were used as in the previous articles [9,10]. The obtained data are represented in Table 1. The corresponding fluxes are denoted as in Fig. 1. In the cases 2–5 in Table 1 the CO_2 assimilation rate was constant and corresponds to a value of CO_2 assimilation of 200 μmol CO_2 (mg Chl)$^{-1}$ h^{-1} (recalculated from [39] for 25°C). The obtained values of ARC (cases 3–5 from Table 1) were close to those obtained in experiments under similar conditions (see [39]).

The main property of the isolated chloroplast system is often the absence of the complete contents of electron acceptors, since, for example, OAA and nitrite that are imported from the cytoplasm are lacking in this system. For this reason, the isolated intact chloroplasts tend to become overreduced upon illumination, when CO_2 assimilation is the only ATP- and NADPH-consuming reaction [1]. Our calculations are in good agreement with experimental data. Case 5 from Table 1 corresponds to the situation with only CO_2 assimilation, without nitrite reduction and malate valve in action. A considerable ARC value and an increase of Fd_r can be seen at an unchanged rate of CO_2 assimilation (compare cases 2 and 5 from Table 1).

It was reported [1,30] that light-dependent CO_2 assimilation and nitrite reduction do not compete for electrons. The developed model reproduced this result. Evidently, this result arises from the limitation of CO_2 fixation in isolated chloroplasts by processes that are not connected with electron transport. For this reason, nitrite reduction can be increased with an increasing nitrite concentration up to 0.2 mM, and CO_2 fixation remains constant in this case (compare cases 2 and 3 from Table 1).

When OAA and nitrite are added at low concentrations simultaneously during steady-state CO_2

Table 1
Simulation of electron distribution in isolated chloroplasts

No.	System	NO_2^- (mM)	OAA (mM)	ARC	Fd_r/Fd_t	v_1	v_2	v_3	v_4	v_5	v_6	v_7	v_8
1	Cell	0.2	As in [10]	0.330	0.131	962.5 (100)	858.2 (89.2)	800.0 (83.1)	43.1 (4.5)	15.1 (1.6)	23.5 (2.4)	70.8 (7.4)	10.0 (1.0)
2	Chloroplast	0.2	0.2	0.332	0.142	949.5 (100)	859.8 (90.6)	800.0 (84.3)	46.2 (4.9)	13.6 (1.4)	25.6 (2.7)	53.1 (5.6)	11.0 (1.2)
3	Chloroplast	0	0.2	0.39	0.164	925.8 (100)	883.7 (95.5)	800.0 (86.4)	67.6 (7.3)	16.1 (1.7)	29.5 (3.2)	0.0 (0.0)	12.7 (1.4)
4	Chloroplast	0.2	0	0.44	0.172	916.4 (100)	819.0 (89.4)	800.0 (87.3)	0.0 (0.0)	19.0 (2.1)	31.0 (3.4)	53.1 (5.8)	13.3 (1.5)
5	Chloroplast	0	0	0.522	0.208	876.6 (100)	823.0 (93.9)	800.0 (91.3)	0.0 (0.0)	23.0 (2.6)	37.5 (4.3)	0.0 (0.0)	16.1 (1.8)
6	Chloroplast with MV	0	0	0.330	0.099	997.6 (100)	597.5 (59.9)	584.0 (58.6)	0.0 (0.0)	13.5 (1.35)	17.8 (1.8)	0.0 (0.0)	382.4 (38.3)
7	Chloroplast with $v_3 = 0$	0	0	0.989	0.771	253.0 (100)	54.4 (21.5)	0.0 (0.0)	0.0 (0.0)	54.4 (21.5)	138.9 (54.9)	0.0 (0.0)	59.7 (23.6)
8	Chloroplast with MV, $v_3 = 0$	0	0	0.973	0.327	744.9 (100)	53.0 (7.1)	0.0 (0.0)	0.0 (0.0)	53.0 (7.1)	58.9 (7.9)	0.0 (0.0)	633.0 (85.0)
9	Cell low v_3 [a]	Eq. 11	As in [10]	0.330	0.040	286.1 (100)	258.1 (90.2)	200.0 (69.9)	43.1 (15.1)	15.1 (5.3)	7.2 (2.5)	17.7 (6.	19)3.1 (1.1)

The electron fluxes are denoted by indices as in Fig. 1 (in μmol e^- (mg Chl)$^{-1}$ h^{-1}). The total electron flux from PS I (v_7) was taken as 100%, and the corresponding fractions of the total flux (v_7) are given in brackets.
$k_1 = 1107$ μmol e^- (mg Chl)$^{-1}$ h^{-1} (Eq. 1).
[a] $k_1 = 298$ μmol e^- (mg Chl)$^{-1}$ h^{-1} in case 9. See text for further explanations.

fixation, the formation of malate is slowed down. Nitrite reduction itself is not affected upon addition of OAA [1]. These results are also reflected in our model. As can be seen, the transition from conditions without nitrite (case 3) to case 2 in Table 1 leads to a decrease of OAA reduction (flux v_4), since ARC decreased.

According to data from Holtgrefe et al. [39] the addition of MV can lead to a decrease of CO_2 assimilation. The ARC value did not change in these experiments. It was assumed that the decrease of CO_2 assimilation can be determined from the decrease of FBPase activity. To simulate these experiments, the value of the maximal rate of O_2 reduction (V_{8m}) was increased by 50 times, and the rate of CO_2 assimilation was adopted so that the ARC was the same as in case 2 of Table 1. The result is represented in case 6 in Table 1. Evidently, at the same ARC the value of Fd_r is considerably lower than in case (2). This is caused by the increase of electron flux directly from Fd_r to MV. This gives us a possibility to suppose that the decrease of the FBPase activity can in fact be caused by the decrease of Fd_r, since this is accompanied by a decrease of the reduction state of thioredoxin, thus decreasing the activity of this enzyme.

In some experiments with isolated chloroplasts the light-dependent CO_2 assimilation was inhibited [27,40]. Corresponding calculations are represented in Table 1, cases 7 and 8). This case is characterized by high ARC and Fd_r content. However, if some electron flux is obtained additionally from Fd_r, for example by the addition of MV (v_8 was increased by 25 times in case 8), there is a considerable decrease of Fd_r. This leads to a depression of the cyclic electron flux (compare Fd_r and v_6 in cases 7 and 8). The decrease of cyclic electron flux upon addition of MV as obtained by Hormann et al. [40] can be explained on this basis.

3.2. Simulation of the situation in the leaf

In principle, Eq. 5 for PGA reduction could be used to simulate the dependence of photosynthesis on light intensity during limitation by electron transport. The increase of the light intensity can be modelled by an increase of the electron pressure (coefficient k_1). However, at low light processes such as light activation of different enzymes, both NADP-

dependent malate dehydrogenase and many Calvin cycle enzymes, can take place [7,14]. On the other hand, it should be pointed out that the increase of light intensity cannot increase considerably the value of the electron pressure (k_1), since feedback control of the electron transport decreases the electron pressure on PS I [23]. As a consequence of the existence of these processes and many other light-dependent reactions that were not taken into account, the obtained model cannot be used at this stage to describe the light dependence of electron transport.

However, some qualitative conclusion can be obtained from our calculations. It can be seen that the ARC level does not increase proportionally to Fd_r/Fd_t with the increase of electron pressure, and ARC is restricted to a defined level (not shown). This can be explained by the fact that increases of the rate of PGA reduction and of the Fd_r content are closely related in our model when CO_2 assimilation is limited by electron transport (Eq. 5). This means that increases of electron flux from Fd_r on $NADP^+$ correspond to an increase of the flux of NADPH into PGA reduction, and the increase of electron pressure could not lead to a corresponding increase of ARC. In these conditions, a sharp increase of OAA reduction that occurs with the increase of the ARC level above 0.3 [10], can restrict the following increase of the ARC level. It is known that with the increase of the light intensity the ARC level does not increase considerably and can be maintained at some level in leaves [23,41]. It is possible that such property is a result of the above mechanism.

The most interesting case is the decrease of the rate of CO_2 assimilation as caused by some external factor for example following stomata closure. This case was simulated in our model (Fig. 2) by the decrease of the constant level of PGA reduction (flux v_3) at constant electron pressure and initial conditions as in point 1 of Table 1. The dependence of nitrite reduction on PGA reduction was taken as in Eq. 11.

The obtained results show that with the decrease of CO_2 assimilation there is a considerable increase of the participants in pathways others than PGA reduction. Under the initial conditions 83% of the electrons that go through PS I are used for PGA reduction, and only few electrons are used for other processes (case 1 in Table 1). However, with a decrease of the rate of PGA reduction the other fluxes are increased. First, the malate valve is activated. Then the cyclic electron flow takes care of additional electrons. With an increasing redox state of Fd, reduction of O_2 occurs with significant rates (Fig. 2). The portion of PGA reduction in the total electron flux comes up to 30% with a decrease of the rate of PGA reduction by a factor of 4 (to 200 μmol e^- (mg Chl)$^{-1}$ h^{-1}). This rate corresponds roughly to the rate of electron transport into PGA reduction at the CO_2 compensation point, when the rate CO_2 of assimilation equals the sum of the rates of photorespiration and of respiration. Approximately the same part constitutes the flux through the malate valve in this situation. In this model, the redox state of Fd plays an important role in being responsible for the electron flow into the various pathways, when CO_2 is limiting at strong light.

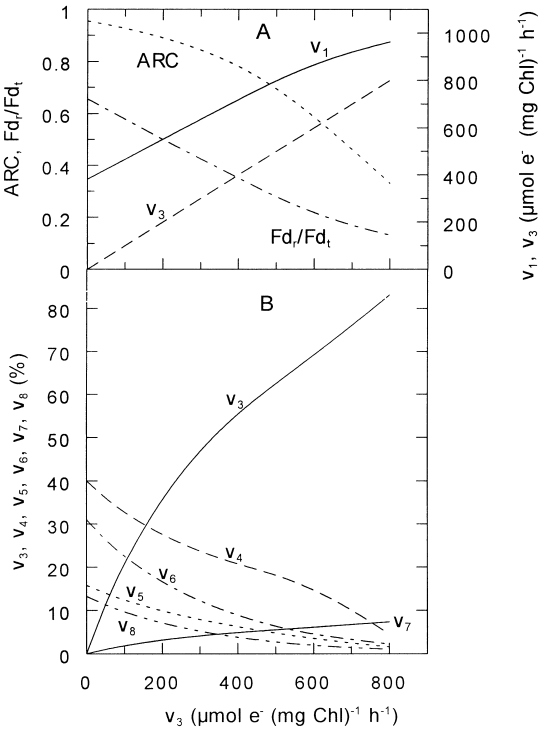

Fig. 2. (A) Calculated dependence of the rate of total electron transport (v_1), ARC and Fd_r/Fd_t on a changed rate of PGA reduction (v_3) at a constant value of electron pressure as in case 1 in Table 1. (B) The simulated dependence of the different electron fluxes (in % of v_1) (see Fig. 1 for denominations) upon the flux v_3 (PGA reduction).

However, under conditions, which indicate a

strong imbalance between the rates of ATP consumption and electron pressure, there is a build-up of a high ΔpH, because the electron flow is not linked to ATP consumption. The accumulation of protons in the thylakoid lumen then leads to feedback-control of the electron transport, either at the cytochrome *b/f* complex or through inactivation of PS II. This is accompanied by non-photochemical energy dissipation in the antenna and decreases the electron pressure on PS I [23]. This process can be taken into account in our model by a decrease of the electron pressure. The results of calculations when electron pressure was decreased in a way that the ARC value is maintained constant with the decrease of the rate of PGA reduction by 4 times are represented in case 9 in Table 1. It can be seen that the relative contributions of fluxes v_4 and v_5 are increased when compared with case 1 in Table 1, since they have constant values at constant ARC. A considerable decrease of the Fd_r/Fd_t ratio can be also obtained relative to case 1.

However, a decrease of Fd_r/Fd_t must lead to a decrease of the activity of enzymes such as FBPase (see [7]). No data could be found showing that there is a decrease of FBPase activity with a decrease of CO_2 availability. In opposite, the activities of NADP-linked malate dehydrogenase and of FBPase increase with a decrease of CO_2 availability [42]. For this reason, it can be assumed that such a large decrease of electron pressure with decrease of electron flux to PGA reduction is unlikely. However, this possibility should be taken into account.

3.3. Flux-control analysis of the electron distribution

The dependence of the flux upon the change of variables has been formalized in the metabolic control analysis [43]. The contribution that a parameter, P_i, makes to the control of flux through a pathway, J_i, is formally defined as control coefficient (C_i)

$$C_i = \frac{\Delta J_i / J_i}{\Delta P_i / P_i} \quad (14)$$

To establish the sensitivity of the model towards the parameters, each of the independent fluxes (v_3–v_8) was increased by 1% of its previous value, and a new steady state of flux of electrons (v_1) was calculated. Then C_i was calculated on the basis of Eq. 14.

Table 2 represents the calculated flux-control coefficients for different electron fluxes and for two different PGA reduction rates: at conditions as in case 1 in Table 1 and with one fourth of the initial rate of PGA reduction that can be considered as electron flux at the CO_2 compensation point. The corresponding flux can be seen in Fig. 2 at $v_3 = 200$ μmol e^- (mg Chl)$^{-1}$ h^{-1}. The control coefficients for processes other a than PGA reduction are increased in this case, excluding nitrogen reduction (v_7) that changes in parallel with PGA reduction (Eq. 11).

However, the summation of the flux-control coefficients for electron fluxes yields a value that is significantly below unity in all cases. This can be explained by the existence of two conserved moieties ($NADPH+NADP^+$) and (Fd_r+Fd_o) in the system that can decrease the sum of the flux-control coefficients [44]. In our case, ARC and Fd_r are changed in the opposite direction compared to the electron sink fluxes (v_3–v_8). For example, with the increase of some flux (v_3–v_8), ARC and Fd_r decreased at constant k_1. Their decrease leads to a reduction of other fluxes that are determined by the value of ARC and Fd_r. As a result, the increase of v_1 that is determined by the increase of some parameter (in our case the fluxes (v_3–v_8) are used as parameters) should be decreased considerably. This decreases the flux-control coefficients and thereby reduces the sum of the flux-control coefficients, too. This peculiarity of the considered system should be taken into account in attempts

Table 2
Simulated flux control coefficients of different ways of electron distribution and their sum at two different PGA-reduction rates

Rate of PGA reduction (μmol e^- (mg Chl)$^{-1}$ h^{-1})	v_3	v_4	v_5	v_6	v_7	v_8	Σ_i
800	0.550	0.026	0.010	0.015	0.048	0.007	0.657
200	0.307	0.213	0.076	0.131	0.026	0.056	0.808

Calculations were made for conditions as in Fig. 2.

to determine the flux-control coefficient in experiments.

4. Discussion

It should be pointed out that the model developed in this article is an attempt to describe the electron distribution behind PS I on the basis of the present knowledge. In the recent general model of Laisk [12] nitrite reduction was not included, and cyclic electron flow was not divided into two separate reactions. OAA reduction was only used as a mediator of pseudocyclic electron transport without consideration of its role as malate valve.

The presented theoretical calculations are in reasonable agreement with the experimental data accumulated by now, and permit evaluation of the fluxes of electrons that are used outside the Calvin cycle.

Under the simulation of normal conditions (case 1 in Table 1), the calculated rate of electron flux other than PGA reduction is about 17% of the total flux through PS I. This flux includes 7.4% for nitrate reduction. The reduction of O_2 or the malate valve, both requiring electron transport from PS II, contribute about 4.5%, and cyclic electron flow around PS I contributes 4%. However, N-assimilation includes further reductive steps other than nitrile reduction. For example, Champigny [3] calculated that about 20% of the total electron flow is required in vivo for N-assimilation in the cell. For this reason, at variance with the rate of nitrite reduction, the flux of electrons through branches different from PGA reduction can be from 10% (without nitrogen reduction) up to 30% (when this process is included).

Measurements of chlorophyll fluorescence and gas exchange have been used to identify excess electron transport from PS II under in vivo conditions. It was found for photorespiratory conditions that the photosynthetic electrons used by acceptors different from CO_2 assimilation can change from 8.5% for *Ficus* up to 25% for *Xanthium* (see Table 1 from [24]). Our results obtained with isolated intact spinach chloroplasts clearly indicate the existence of excess electrons, and we estimated that about 20% of the electrons generated in the light are in excess of the demand of the Calvin cycle [37]. These data are consistent with our consideration.

However, there are also contrasting data. For example, Valentini et al. [45] found no evidence for competitive electron sinks in leaves of *Quercus cerris*, and Loreto et al. [46] cannot find any residual electron transport in the flag leaf of wheat even though CO_2 assimilation was partially depressed by glyceraldehyde.

On the basis of this theoretical consideration it is possible to explain these contradictions by the difference in some compensation mechanisms in different species. For example, if the mechanism that decreased the electron pressure acts in such a manner that the ARC does not change with a decrease of the PGA reduction rate, then the additional electron flux to other acceptors can be low (see case 9 in Table 1).

It should be pointed out that when ARC equals 0.33, what we accept as normal physiological conditions (case 1 in Table 1), the Fd_r/Fd_o ratio calculated from Eq. 3 equals 0.001 at equilibrium. Since all obtained Fd_r/Fd_t ratios are considerably higher (Table 1), it should be assumed that this system is working in non-equilibrium conditions, when some electron flux occurs. From the above calculations, it is evident that the Fd_r/Fd_t ratio and the ARC in chloroplasts depend on the ratio between electron pressure and the rates in which the electrons are consumed.

Electron flux through the malate valve can account for about 4.5% of the total flux of electrons under normal conditions (case 1 in Table 1). A detailed consideration of this system was made in [10]; however, this consideration permits us to access the contribution of the malate valve in comparison with the other pathways. It can be seen (Fig. 2) that electron flux through the malate valve can reach about 27% at the compensation point (at a PGA-reduction rate equal to 200 μmol e^- (mg Chl)$^{-1}$ h^{-1}), and can be close to the PGA-reduction rate under these conditions where it is the main way for electron flux after PGA reduction. Similar rates were determined with isolated intact spinach chloroplasts [1]. Consequently, both the available experimental data and the calculations that were made in this article suggest that the malate valve does not act at a high rate at steady state under ambient conditions. It may be part of a more complex system of dicarboxylate shuttles as has been pointed out earlier [10]. However,

upon a restriction in CO_2 availability the malate valve can be the main sink for the electrons that do not take part in PGA reduction.

The results of our estimations presented here confirm that the photosynthetic electron transport may provide more electrons than required for CO_2 fixation. These excess electrons can be used by the 'physiological' electron acceptors (nitrite or OAA), or must be removed by poising mechanisms. Upon a beginning decrease of CO_2 availability, i.e., upon beginning decrease of the flux v_3 from 800 μmol e^- (mg Chl)$^{-1}$ h^{-1}, poising by cyclic electron transport and Mehler reaction are less effective in removing the electrons than the electron acceptors nitrite or OAA (Fig. 2).

In this work we present the theoretical considerations concerning the mechanism that controls the electron transport chain in chloroplasts. The described model is verified by a comparison with experimental results and then is used to obtain some new information about the system. However, the parameter values used have been obtained under different conditions by different experimental procedures. Because of this and due to the complexity of the system, the results obtained can often be compared with experiments only in a semiquantitative way. Taking into account this limitation, the model reflects the properties of the system and can be a useful tool for further studies of the above problem. However, subsequent experimental verification will always be necessary.

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