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Effects of Diffusion and Topological Factors on the Efficiency of Energy Coupling in Chloroplasts with Heterogeneous Partitioning of Protein Complexes in Thylakoids of Grana and Stroma. A Mathematical Model

A. V. Vershubskii, V. I. Priklonskii, and A. N. Tikhonov*

Faculty of Physics, Lomonosov Moscow State University, Moscow 119992, Russia; E-mail: an_tikhonov@newmail.ru

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Abstract—In this work, we studied theoretically the effects of diffusion restrictions and topological factors that could influence the efficiency of energy coupling in the heterogeneous lamellar system of higher plant chloroplasts. Our computations are based on a mathematical model for electron and proton transport in chloroplasts coupled to ATP synthesis in chloroplasts that takes into account the nonuniform distribution of electron transport and ATP synthase complexes in the thylakoids of grana and stroma. Numerical experiments allowed the lateral profiles of pH in the thylakoid lumen and in the narrow gap between grana thylakoids to be simulated under different metabolic conditions (in the state of photosynthetic control and under conditions of photophosphorylation). This model also provided an opportunity to simulate the effects of steric constraints (the extent of appression of thylakoids in grana) on the rates of non-cyclic electron transport and ATP synthesis. This model demonstrated that there might be two mechanisms of regulation of electron and proton transport in chloroplasts: 1) slowing down of non-cyclic electron transport due to a decrease in the intra-thylakoid pH, and 2) retardation of plastoquinone reduction due to slow diffusion of protons inside the narrow gap between the thylakoids of grana. Numerical experiments for model systems that differ with respect to the arrangement of thylakoids in grana allowed the effects of osmolarity on the photophosphorylation rate in chloroplasts to be explained.

Key words: chloroplasts, electron transport, proton transport, photophosphorylation, lateral heterogeneity, mathematical modeling

The structural and functional organization of the photosynthetic apparatus of various photosynthesizing organisms has been characterized in the literature quite comprehensively. Succession of light and dark stages of photosynthesis was determined, molecular mechanisms of key reactions of electron transport in chloroplasts were discovered, mechanisms of coupling between the processes of proton transport and ATP synthesis were elucidated, patterns of spatial structure of pigment–protein and electron-transport complexes were resolved for many photosynthesizing organisms [1-5], and structure of ATP-synthase complex was resolved [6-8]. One of the most important problems of biochemistry of photosynthesis is elucidation of mechanisms of regulation of photosynthetic processes providing maximum efficiency of photosynthetic apparatus and its adaptation to variable environmental conditions [9, 10].

* To whom correspondence should be addressed.

Mathematical modeling of the light stages of photosynthesis has long been used in analysis of light and dark stages of photosynthesis (see [11-15] and references therein). However, high degree of compartmentalization of photosynthetic processes in photosynthetic apparatus at different levels of structural organization makes it difficult to provide quantitative description of bioenergetic processes in photosynthetic systems. Heterogeneous distribution of electron transport and ATP-synthase complexes in thylakoid membranes is an intricate feature of structural and functional organization of the photosynthetic apparatus in oxygenic photosynthesizing organisms (cyanobacteria and higher plants). This feature is most clearly pronounced in chloroplasts of higher plants [16-18]. It is well known that most photosystem 2 (PS 2) complexes are localized in membranes of closely stacked grana thylakoids. In contrast to this, photosystem 1 (PS 1) and ATP-synthase complexes are localized in sites of thylakoid membranes exposed to stroma regions (inter-

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granal thylakoids and edge regions of grana thylakoids). Cytochrome *bf*-complexes are distributed homogeneously among granal and inter-granal thylakoids.

Other photosynthesizing systems are also characterized by a high degree of compartmentalization. For example, there are special systems in cells of cyanobacteria called carboxysomes. These systems have crystal-like structure surrounded by a protein envelope and contain carboanhydrase and Rubisco [18]. The function of carboxysomes is to maintain the concentration of CO_2 near Rubisco at a sufficiently high level. Specific topological features of the photosynthetic apparatus structure noted above should be taken into consideration to provide adequate description of processes of electron and proton transport and ATP synthesis in chloroplasts.

Mathematical modeling of processes of electron and proton transport with due regard to elaborate structural organization of chloroplasts can play an important role in quantitative analysis of mechanisms of regulation of energy coupling in chloroplasts. This is due to the fact that it is difficult to measure pH directly using pH-sensitive probes inside small compartments (e.g., in the narrow inter-thylakoid gap). In the preceding works [19-24] we have constructed mathematical models capable of taking into account the most important diffusion-controlled stages of non-cyclic electron transfer in class B chloroplasts and coupled processes of transmembrane proton transfer into thylakoids. A specific feature of these models was that they for the first time described the processes of transmembrane proton transfer into thylakoids taking into account buffering properties of thylakoid membrane. They also described the diffusion-controlled stages of electron and proton transport taking into account lateral heterogeneity of the lamellar system of chloroplasts.

The processes of electron and proton transport in thylakoids of two types (granal and inter-granal or stromal) were described in [22-24]. A mathematical model was constructed to describe the effect of photoinduced changes of pH inside thylakoids and in the inter-thylakoid gap on kinetics of non-cyclic electron transport in various metabolic states. This model also provided an opportunity to calculate lateral profiles of proton potential in different regimes of chloroplast activity. It was predicted within the framework of the model considered in [22-24], that the rate of non-cyclic electron transfer could be regulated at two segments of the electron-transport chain between PS 1 and PS 2. The process of the photoinduced decrease of pH in the intra-thylakoid space brings about a decrease in the rate of oxidation of plastoquinol by cytochrome *bf*-complex, whereas the rate of plastoquinone reduction at the acceptor side of PS 2 declines as a result of pH increase in the inter-thylakoid gaps.

The goal of this work was to describe the results of numerical calculations of the effect of diffusion limitations on migration of protons and effects of geometrical parameters of this system (density of thylakoid grana packing) on efficiency of energy coupling in chloroplasts. It was shown that variation of geometrical parameters of this system could have a substantial effect on the rates of electron transport and ATP synthesis, which could explain the dependence of the rate of photophosphorylation on osmotic potential of chloroplast incubation medium observed earlier.

MODEL DESCRIPTION

Geometric characteristics of the thylakoid system of chloroplasts. The spatial arrangement of electron-transport complexes in granal and inter-granal thylakoids is shown in Fig. 1. In this figure the grana thylakoid is shown as a flattened cylinder of radius a. The inter-granal thylakoid is simulated by a wider cylinder of radius b, which extends outside the grana thylakoid borders to the stroma region. The outer cylinder of radius b incorporates grana thylakoid (cylinder of radius a), which is gradually transformed into the inter-granal thylakoid. The distance between the inner surfaces of thylakoid l_i and the distance between neighboring thylakoids of grana l_0 are the geometrical parameters of the model.

Electron transport processes. The model simulates the process of linear (non-cyclic) electron transport from

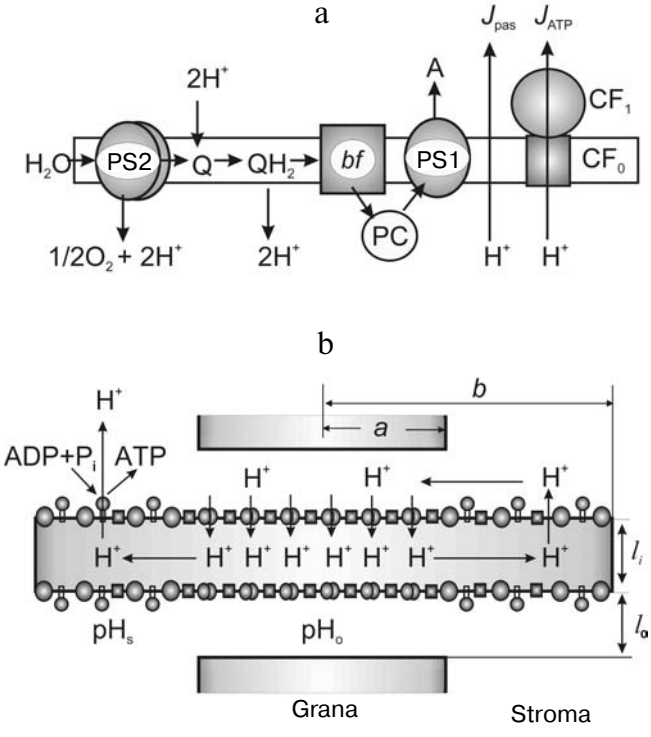

Fig. 1. Electron-transport chain (a) and pathways of proton transfer (b) simulated in model.

water to external acceptor A_{ext}. The location of electron transport and ATP-synthase complexes in the thylakoid membrane is shown in Fig. 1. It is well known that protein complexes of PS 1 and PS 2 are distributed heterogeneously between grana and inter-granal thylakoids [16, 17]. We suggested that all complexes of PS 2 and PS 1 were attributed to grana thylakoids and membranes of inter-granal thylakoids exposed to stroma, respectively. Cytochrome *b/f*-complexes are distributed homogeneously in thylakoid membranes of grana and stroma. Protein complexes of PS 1, PS 2, and cytochrome *b/f* are assumed to be immobile. Mobile electron carriers, plastoquinone and plastocyanin, which mediate electron transfer from PS 2 to *b/f*-complexes and from *b/f*-complexes to PS 1, respectively, are able to migrate in the lateral direction. Molecules of plastoquinone (Q) diffuse along the thylakoid membrane plane, whereas molecules of plastocyanin (PC) move in a relatively narrow gap of the intra-thylakoid space (Fig. 1). This model was described in more detail elsewhere [22-24].

Proton transport processes. The model simulates the processes associated with changes in the activity of hydrogen ions both in the inter-thylakoid gap and in the intra-thylakoid space. The proton transport processes occur outside thylakoids and include proton uptake from the inter-thylakoid gap coupled with plastoquinone reduction at the external (acceptor) side of PS 2 and diffusion of protons from stroma to the inter-thylakoid gap. Inside thylakoids, protons are released as a result of light-induced oxidation of water in PS 2 and oxidation of plastoquinol (QH_2) by *b/f*-complexes (Fig. 1). In addition to proton transport into thylakoids, proton release from thylakoids is also taken into account in this model. This process includes proton flow through ATP-synthase ($J^{ATP}_{H^+}(r)$) and passive leakage of protons through the thylakoid membrane ($J^{p}_{H^+}(r)$).

In the system considered in this work, hydrogen ions are able to diffuse in the aqueous medium inside the intra-thylakoid volume and in a narrow gap of the inter-thylakoid space of grana (Fig. 1). This model also takes into account the processes of interaction of hydrogen ions with proton-acceptor groups fixed at the internal and external surfaces of the thylakoid membrane ($H^+ + M^- \leftrightarrow MH$). The value of pH in stroma (pH_s) is assumed to be constant. In class B chloroplasts, condition $pH_s \approx$ const is observed because of large buffer capacity of the external medium and chloroplasts themselves [25].

Function $J^{ATP}_{H^+}(r,t)$ describing proton flow through ATP-synthase was written as follows:

$$J^{ATP}_{H^+}(r,t) = J_0 \frac{H_o(r,t)[10^{\Delta pH} - 1]}{\alpha + H_o(r,t)[10^{\Delta pH} + \beta]}. \quad (1)$$

This form of function $J^{ATP}_{H^+}(r,t)$ was chosen for reasons described in [20-24]. Variable $H_o(r,t) \equiv [H^+_o]$ corresponds to the local concentration of hydrogen ions outside thylakoids (inter-thylakoid space or stroma), ΔpH is a transmembrane pH difference ($\Delta pH(r,t) = pH_o(r,t) - pH_i(r,t)$). Coefficients α and β in Eq. (1) are determined by the value pK_A of protonated group and ratio of effective rate constants describing activity of ATP-synthase (see [24] for more detail). Function $J^{ATP}_{H^+}(r,t)$ can be used to calculate the total flux of protons through ATP-synthase, determining thereby the rate of ATP synthesis:

$$J_{ATR}(t) = \frac{k_{ATR}}{m} \cdot \frac{2\pi}{b^2 - a^2} \int_a^b J^{ATP}_{H^+}(r,t) r dr. \quad (2)$$

Coefficient k_{ATP} in Eq. (2) is a model parameter determined by the number of active ATP-synthase complexes in the stroma part of the thylakoid membrane ($a \le r \le b$, m is the stoichiometric coefficient (H^+/ATP)). Total steady-state electron flow from PS 2 to PS 1 was calculated from the following equation:

$$J_e = \frac{2\pi k_{Pc}}{b^2 - a^2} \int_a^b [P^+_{700}(r)] \{1 - [PC(r)]\} r dr, \quad (3)$$

where $[P^+_{700}(r)]$ and $[PC(r)]$ are local steady-state concentrations of oxidized forms of P_{700} and plastocyanin, respectively. The set of simultaneous differential equations describing dynamics of redox reactions of electron carriers and processes of lateral transfer of protons in the inter-thylakoid gap and in the inter-thylakoid space was suggested in [22-24].

RESULTS AND DISCUSSION

Effect of diffusion coefficient on the rate of proton uptake by thylakoids. The coefficient of diffusion of hydrogen ions D_{H^+} within the limited intra-thylakoid lumen or in the narrow gap between neighboring grana thylakoids is a model parameter that is difficult to be measured directly. There are grounds to believe that coefficient of diffusion of hydrogen ions near the membrane surface are significantly lower than in the aqueous bulk phase [26, 27]. We used the data available from the literature about kinetics of proton uptake by chloroplasts as measured with pH dyes to select a realistic value of parameter D_{H^+}. It was shown by Junge et al. [28, 29] that the rate of proton uptake mediated by PS 2 depended on the structural state of the thylakoid system. If grana thylakoids are densely packed (this case corresponds to a low value of model parameter l_0), the characteristic time of the pH change in the external medium induced by a short light pulse (this process is due to proton uptake mediated by PS 2) is $t_{1/2} \approx$ 60-100 msec, which is two orders of magnitude larger than the characteristic time of PS 2 turnover.

Relatively low rate of pH changes in the external medium can be explained by the fact that the rate of diffusion of protons in the narrow inter-thylakoid gap toward PS 2 complexes is significantly lower than in the bulk phase.

Let us consider the grounds for selection of the value of the model parameter D_{H^+}. Photoactivation of PS 2 reaction centers gives rise to a proton flow directed from stroma to the inter-thylakoid gap and caused by proton uptake at the stage of plastoquinone reduction on the acceptor side of PS 2. As noted above, the value of pH outside thylakoids (pH_s) is assumed to be constant, which can be explained by large buffer capacity of stroma. However, it is easy to calculate the number of protons $\Delta n_{H^+}(t)$, transported from stroma to the inter-thylakoid gap. We determined $\Delta n_{H^+}(t)$ as the number of protons transferred within time interval t to the inter-thylakoid gap through surface area $2\pi a l_0$. Proton flux through this area is determined by the gradient of proton concentration in the gap in the vicinity of point $r = a$, in which the gradient is equal to $\partial H_o(r,t)/\partial r|_{r=a-0}$, where $H_o(r,t)$ is the concentration of hydrogen ions. The number of protons $\Delta n_{H^+}(t)$ taken up by thylakoids within time interval t can be calculated as an integral of the proton flux through the stroma/inter-thylakoid gap border:

$$\Delta n_{H^+}(t) = 2\pi a l_0 \int_0^t D_{H^+} \frac{\partial H_o(r,t)}{\partial r}\, dt . \qquad (4)$$

Because buffer capacity of a chloroplast suspension is high, the amplitude of the light-induced changes of pH in the incubation medium of chloroplasts is usually low [25]. Therefore, it is fairly safe to assume that the value $\Delta n_{H^+}(t)$ calculated from Eq. (4) is proportional to the amplitude of the light-induced changes in pH in the incubation medium.

Kinetic curves of the proton concentration decrease in the incubation medium induced by short-term (1 msec) exposure to actinic light starting at the moment of time $t = 0$ is shown in Fig. 2. The kinetic curves shown in Fig. 2 were calculated for two values of hydrogen ion diffusion coefficient D: $0.1D_0$ and $0.02D_0$, where D_0 is the reduced diffusion coefficient of protons in the aqueous bulk phase. Proton diffusion coefficient D_{H^+} and dimensionless model parameter D_0 correlate with one another in accordance with the equation $D_0 = (\tau_0/l_0^2)\, D_{H^+}$, where constants τ_0 and l_0 are scaling factors equal to the characteristic time of the process and characteristic linear size of the system, respectively (see [24] for more detail). At $\tau_0 = 10^{-3}$ sec and $l_0 = 1$ μm, the value $D_{H^+} = 10^{-5}$ cm^2/sec corresponds to dimensionless model parameter $D_0 = 1$ (proton diffusion coefficient in water at 25°C is $9.28 \cdot 10^{-5}$ cm^2/sec [30]). The results shown in Fig. 2 were obtained assuming that proton diffusion coefficients in the inter-thylakoid gap and in the intra-thylakoid space were equal. It follows from these data that the rate of proton uptake by thylakoids substantially declines upon decreasing the proton diffusion coefficient. It was found that in the system considered in this work at $D = 0.02D_0$ the characteristic half-time of this process was $t_{1/2} = 70$ msec, which was consistent with experimentally measured kinetics of pH changes in a suspension of chloroplasts induced by a short flash of light. Indeed, it was found that in spinach chloroplasts this value was $t_{1/2} \approx 60$ msec [28], whereas in pea chloroplasts it was $t_{1/2} \approx 100$ msec [29]. Our calculations revealed that at larger proton diffusion coefficient in the inter-thylakoid gap ($D = 0.1D_0$), the rate of proton uptake by thylakoids was significantly higher ($t_{1/2} \approx 11$ msec) (Fig. 2). To compare results of theoretical calculations with experimental data, the dashed line in Fig. 2 shows the kinetics of the photoinduced absorption changes of a pH-sensitive indicator dye (Cresol Red) dissolved in the incubation medium with pea chloroplasts [29]. It follows from Fig. 2 that at the model parameter value $D = 0.02D_0$, the initial segment of the theoretical curve (time interval 0-60 msec) is a satisfactory fit to the experimental data. However, at longer time intervals, these curves diverge from each other, and the "tail" of the experimental curve is longer than that in the theoretical one. This deviation can be easily explained by the fact that within the framework of our model the processes of diffusion of hydrogen ions in

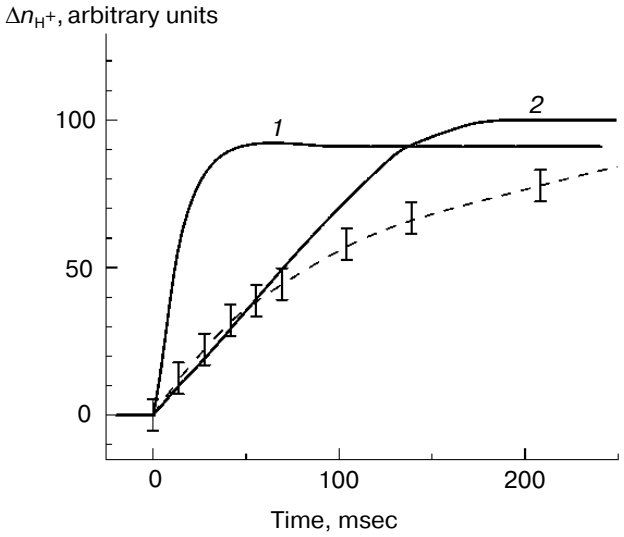

Fig. 2. Kinetics of proton uptake from surrounding medium induced in chloroplasts by a short (1 msec) pulse of light as calculated for two reduced values of proton diffusion coefficient D. Solid lines correspond to theoretical curves calculated for values of $D = 0.1D_0$ (*1*) and $D = 0.02D_0$ (*2*) (explanation in text). Dotted line corresponds to curve of photoinduced absorption changes of pH-sensitive indicator dye (Cresol Red) in suspension of pea chloroplasts excited with a short pulse of light. This kinetic curve was plotted on the basis of experimental data reported in [29] (see Fig. 2 therein, vertical dashes indicate the range of instrumental noise).

the aqueous bulk phase of surrounding medium are not taken into account [22-24]. It is also conceivable that the two-component character of the experimental kinetic curve is due to heterogeneity of the thylakoid system. However, in general, the theoretical model suggested in this work at geometrical parameters chosen above and proton diffusion coefficient $D = 0.02D_0$ provides a satisfactory description of experimentally measured kinetics of proton uptake mediated by PS 2, which was evidenced by coincidence of experimental and theoretical curves within time interval 0-60 msec. It should be emphasized that such correspondence can be obtained only assuming that the diffusion coefficient of hydrogen ions in the narrow zone near the thylakoid membrane surface is substantially lower (by a factor of ~500) than the coefficient of proton diffusion in the aqueous bulk phase. This finding is not very surprising because the gap between neighboring grana thylakoids is very small, whereas the protein molecules protruding from oppositely located membranes are in close contact with each other [16, 17].

Lateral pH profiles inside and outside thylakoids. Lateral pH profiles inside (pH_i) and outside (pH_o) thylakoids calculated for metabolic states 3 and 4 are shown in Figs. 3 and 4. These curves were obtained for different values of model parameters: inter-thylakoid gap width l_0 and proton diffusion coefficient D equal to $0.02D_0$ and $0.1D_0$ (Fig. 4). Stroma pH (pH_s) was assumed to be constant and equal to 8, initial levels of pH inside thylakoids and in the gap before photoactivation of chloroplasts were equal to pH_s. Therefore, $pH_i(0,r) = pH_o(0,r) = 8$.

It follows from Fig. 3 that profiles of intra-thylakoid $pH_i(r)$ for stationary states 3 and 4 significantly differ from one another. Under conditions of photosynthetic control (state 4), pH decreases more significantly than under conditions of intense ATP synthesis (state 3), in which protons are transferred from thylakoids to the outside through functionally active ATP-synthase complexes. State 4 is characterized by a uniform profile of the intra-thylakoid pH distribution. It follows from Fig. 3b that the extent of the pH_i decrease becomes larger upon increasing the intra-thylakoid gap width l_0. Additional efflux of protons from thylakoids to the outside in state 3 through ATP-synthase complexes located in inter-granal thylakoids gives rise to a nonuniform profile of pH_i (Fig. 3a). The extent of pH_i decrease in thylakoid grana is larger than in the intra-thylakoid stroma, which is characterized by a high rate of proton leakage to the outside through ATP-synthase complex.

The results of calculations shown in Figs. 3 and 4 also indicate that illumination of chloroplasts causes a significant increase in the value of pH_o in the gap between grana thylakoids ($pH_o \approx 9.5$-11 depending on model

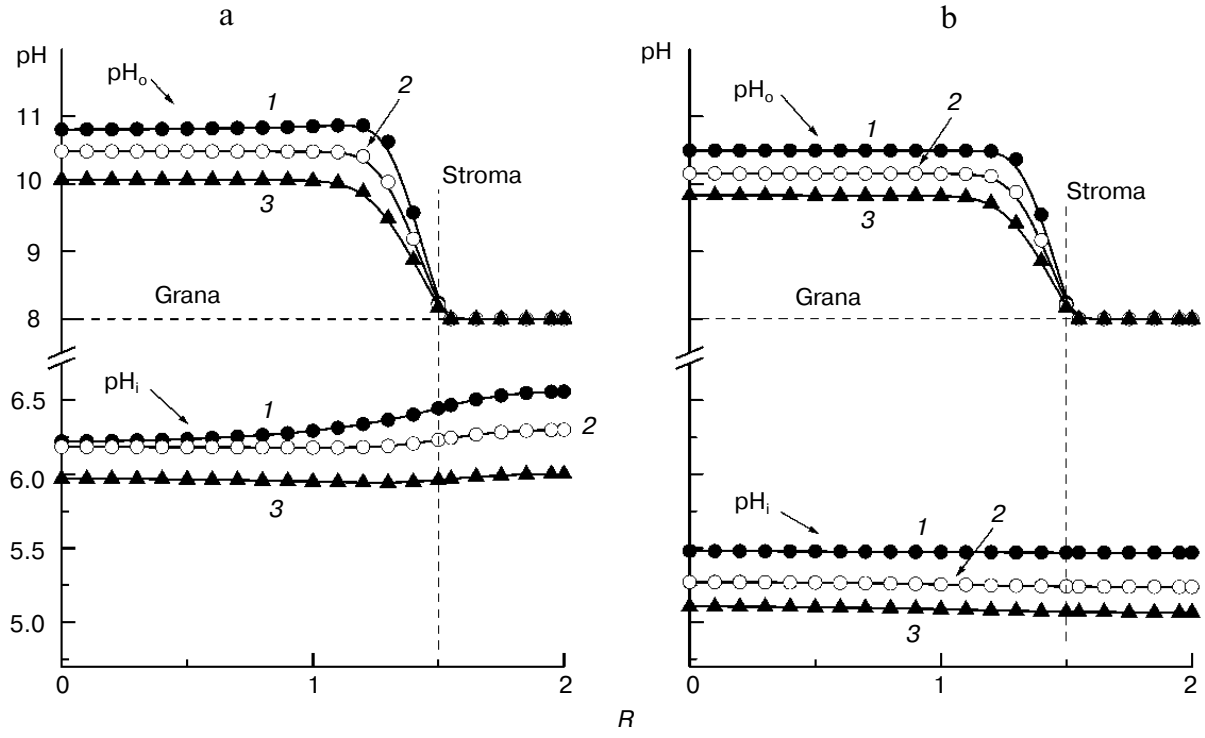

Fig. 3. Lateral profiles of pH inside (pH_i) and outside (pH_o) thylakoid in stationary states 3 (a) and 4 (b) calculated for proton diffusion coefficient $D = 0.02D_0$ and different values of model parameter l_0/l_i: 0.1 (*1*), 0.2 (*2*), and 0.4 (*3*). Dimensionless variable $R = r/a$ is a characteristic of remoteness from the center of grana.

parameters). The decrease in the activity of hydrogen ions in the intra-thylakoid gap is due to rapid uptake of protons associated with plastoquinone reduction at the acceptor side of PS 2. The proton influx from stroma under these conditions ($pH_s < pH_o$) does not compensate the light-induced decrease in the proton concentration in the gap between neighboring grana thylakoids.

It is easy to demonstrate that at pH ≈ 10-11 the number of free hydrogen ions N_{H^+} in the system of small volume V (inter-thylakoid gap) is far less than one ($N_{H^+} = 10^{-pH} \cdot V << 1$). However, it should be noted that the term "concentration" (or "activity") of hydrogen ions is still valid in this case. In this particular case, the meaning of the number of hydrogen ions (and, therefore, their concentration) is probabilistic. At $N_{H^+} < 1$, the value N_{H^+} is the probability that at moment t one unbound hydrogen ion can be found within the small volume V. It should be noted that the total number of protons in this system is large (>>1). However, the overwhelming majority of these protons is bound to buffer groups of thylakoid membrane and membrane proteins rather than associated with aqueous bulk phase. It was shown in [30] that conventional terms of chemical thermodynamics, e.g., concentration (activity) of hydrogen ions and chemical potential of hydrogen ions, could be used in thermodynamic description of small systems (for more detail see discussion in [30-32]). Therefore, it is safe to conclude that processes of proton diffusion in thylakoids and in the inter-thylakoid gap can be adequately described using mathematical apparatus based on differential equations that include concentrations of hydrogen ions as variables.

The dependence of stationary profiles $pH_o(r)$ and $pH_i(r)$ on the inter-thylakoid gap size is shown in Fig. 3. As the inter-thylakoid gap width l_0 increases (at l_i = const), the magnitude of the photoinduced increase in pH in the inter-thylakoid gap declines. This effect can be explained by increase in the volume of the inter-thylakoid gap, from which protons diffuse to plastoquinone molecules reduced at the acceptor side of PS 2. It is interesting to note that both the extent of acidification of the intra-thylakoid space and the shape of the lateral profile $pH_i(r)$ also were sensitive to the inter-thylakoid gap size. It is obvious that this dependence represents the fact that the rate of noncyclic electron transport is controlled not only by pH_i inside thylakoids (effect of pH_i on the rate of plastoquinol oxidation by cytochrome *bf*-complex [33-35]), but also by the value of pH_o. According to theoretical predictions put forward in [21-24], the total rate of electron transport in chloroplasts decreases not only upon decreasing the pH_i, but also upon increasing the pH_o, because a decrease in the concentration of hydrogen ions in the inter-thylakoid gap induces a decrease in the rate of plastoquinone reduction.

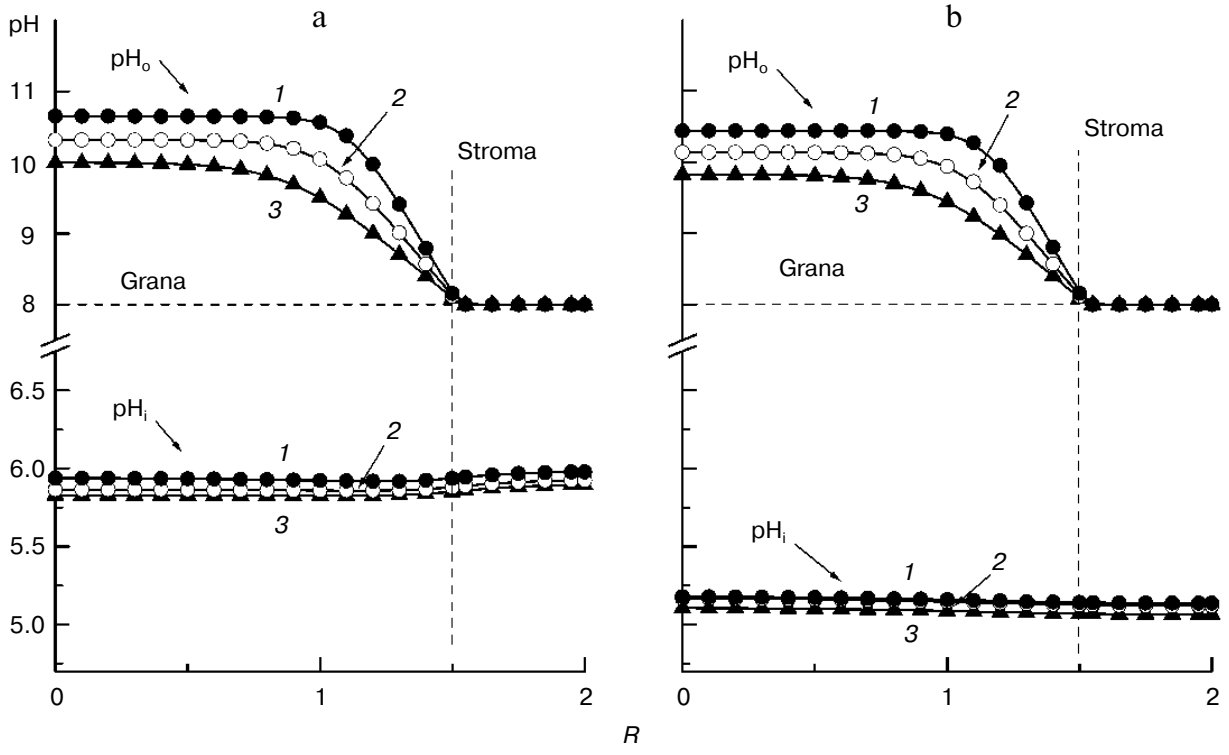

Fig. 4. Lateral profiles of pH inside (pH_i) and outside (pH_o) thylakoid in stationary states 3 (a) and 4 (b) calculated for proton diffusion coefficient $D = 0.1D_0$ and different values of model parameter l_0/l_i: 0.1 (*1*), 0.2 (*2*), and 0.4 (*3*). Dimensionless variable $R = r/a$ is a characteristic of remoteness from the center of grana.

It follows from Figs. 3 and 4 that extent of pH_o increase in state 3 is larger than in state 4. Obviously, this is due to the fact that under conditions of ATP synthesis (state 3), the rate of proton uptake by PS 2 is higher than under conditions of photosynthetic control (state 4). This result is consistent with findings that the rate of non-cyclic electron transport under conditions of ATP synthesis is higher than in state 4 [33-35]. Decrease in the rate of electron transport in state 4 is caused by the fact that pH decrease inside chloroplasts in this state is larger than in state 3 (Figs. 3 and 4).

Decrease in the rate of lateral proton diffusion inside thylakoids and in the intermembrane space is another factor causing modification of lateral profiles $pH_o(r)$ and $pH_i(r)$. The effect of proton mobility on the shape of the lateral profiles $pH_o(r)$ and $pH_i(r)$ is shown in Figs. 3 and 4 for three different values of parameter l_0. In the three cases, a fivefold increase in the proton diffusion coefficient caused modification of the shape of the profiles $pH_o(r)$ only at the grana periphery, whereas values pH_o in the central region of grana remain virtually invariable. In contrast to clearly outlined stepwise profiles typical of low values of proton diffusion coefficient, at larger rates of diffusion, values pH_o in the vicinity of point $r = a$ (border between stroma and grana regions) are changed more smoothly. It follows from comparison between Figs. 3 and 4 that in all cases an increase in parameter D caused an insignificant decrease in the value pH_i inside the thylakoid and smoothing of nonuniform profile $pH_i(r)$, typical of state 3 at low values D. This is due to the fact that increase in the rate of proton diffusion facilitates rapid leveling of proton concentration in the whole intra-thylakoid volume. On the other hand, an increase in the rate of lateral proton diffusion in the inter-thylakoid gap facilitates acceleration of electron transport in chloroplasts, thereby causing an increase in the proton pump activity and providing larger decrease in the value pH_i in thylakoids. It is also interesting to note that at large values of D the shape of the lateral profile $pH_i(r)$ is less sensitive to inter-thylakoid gap size.

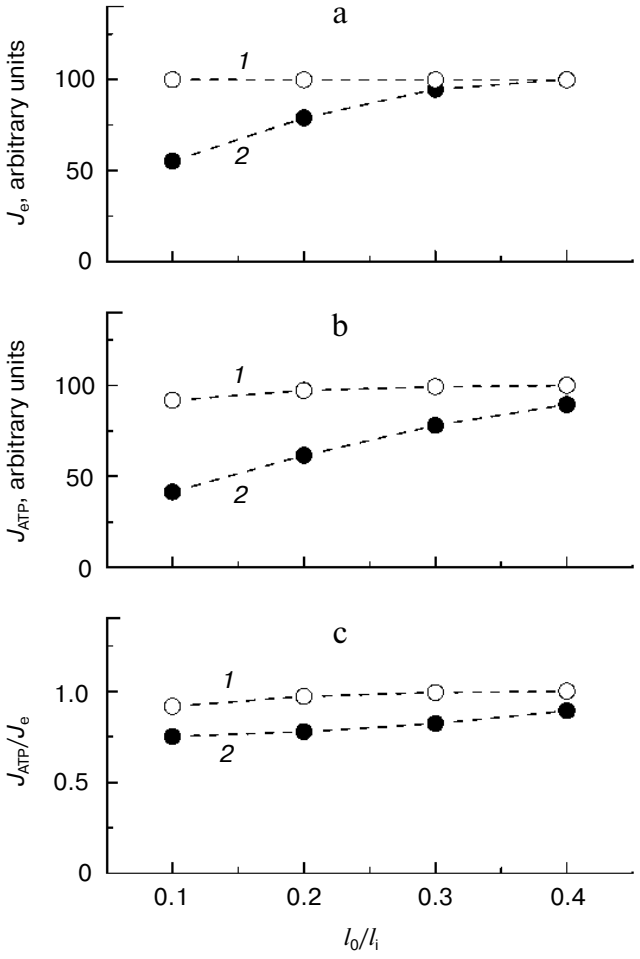

Fig. 5. Effect of geometrical parameter l_0 (at l_i = const) and proton diffusion coefficient D_{H^+} on the rate of non-cyclic electron transport J_e (a), rate of ATP synthesis J_{ATP} (b), and efficiency of photophosphorylation J_{ATP}/J_e (c). Points J_e, J_{ATP}, and J_{ATP}/J_e were calculated at different values of model parameters l_0/l_i and D ($0.1D_0$ (*1*) and $0.02D_0$ (*2*)).

Effect of proton diffusion restrictions on processes of electron transport and ATP synthesis. The dependence of stationary rate of non-cyclic electron transport (J_e), rate of ATP synthesis (J_{ATP}), and efficiency of ATP synthesis (ratio J_{ATP}/J_e), as calculated at two values of proton diffusion coefficient D, on the inter-thylakoid gap size l_0 (at the intra-thylakoid space thickness l_i = const) is shown in Fig. 5. At $D = 0.02D_0$, which corresponds to a low rate of proton diffusion, stationary rates of electron transport (Fig. 5a) and ATP synthesis (Fig. 5b) in densely packed grana ($l_0/l_i \approx 0.1$) are substantially lower than in more loosely packed stroma ($l_0/l_i = 0.4$). At a larger rate of proton diffusion ($D = 0.1D_0$), parameter l_0 exerts only a very weak effect on the rates of electron transport and ATP synthesis. However, it should be noted that for the two values of proton diffusion coefficient, efficiency of ATP synthesis (ratio J_{ATP}/J_e) only insignificantly depends on parameter l_0 (Fig. 5c).

The dependence of the rates of non-cyclic electron transport and ATP synthesis on chloroplast topology (density of thylakoid packing in grana characterized by parameter l_0) predicted from our model provides an explanation for the interesting experimental fact that the rate of photophosphorylation depends on osmotic properties of the chloroplast incubation medium. The effect of the composition of the chloroplast incubation medium (concentration of orthophosphate, buffer Mes, and sorbitol) on light-induced proton uptake and rate of ATP synthesis in class B bean chloroplasts was studied in [36]. It was found that variation of concentration of orthophosphate or Mes modified both the rate of ATP synthesis and content of protons taken up by thylakoids. This finding was not surprising because the two compounds have

buffering properties, changing thereby the extent of light-induced decrease in intra-thylakoid pH. However, it should be noted that the rate of ATP synthesis also depends on concentration of sorbitol, which does not bind protons. Under otherwise identical conditions, there was a 20-40% decrease in the rate of photophosphorylation upon increasing the concentration of sorbitol in the incubation medium (the extent of the effect depended on concentration of orthophosphate in the incubation medium). It was demonstrated in [37], that changes in osmotic properties of the incubation medium might exert an effect on internal (osmotic) volume of thylakoids. In hypotonic incubation medium, illumination of thylakoids is accompanied by their swelling [38]. Therefore, it is safe to expect that thylakoid packing in grana in this case becomes looser (in the model this corresponds to an increase in the parameter l_0). In contrast to that, in hypertonic incubation medium illumination of thylakoids is accompanied by their compaction (decrease in parameter l_0). Our model predicts that because the rate of proton transfer to the acceptor site of PS 2 is diffusion-limited, such compaction may cause a decrease in the rate of electron transport (Fig. 5a). This, in turn, causes a decrease in the rate of ATP synthesis (Fig. 5b). This effect is due to the fact that a decrease in the activity of the electron transport chain brings about a decrease in the efficiency of the proton pump, which pumps protons into thylakoids. Our calculations revealed that at relatively low rate of proton diffusion in thylakoids ($D = 0.02D_0$), the transmembrane gradient of pH, generated in inter-granal thylakoids (where ATP-synthase complexes were concentrated), in state 3 was less than in grana thylakoids (Fig. 3). As the proton diffusion restrictions became less stringent ($D = 0.1D_0$), pH profile inside thylakoids is leveled, and the value pH_i is maintained at a lower level regardless of inter-thylakoid gap width (Fig. 4). As a result, the rate of ATP synthesis at high proton diffusion coefficient is virtually insensitive to variation of the gap width l_0 (Fig. 5b).

It was suggested by many researchers [30, 32, 39, 40] that functional and morphological heterogeneity of lamellar system of chloroplasts could provide a basis of the phenomenon that values of pH in different compartments (e.g., in granal and inter-granal thylakoids) differed. In addition to small size of thylakoids, their inhomogeneity makes it difficult to measure local pH values at different sites of chloroplast. Therefore, mathematical model taking into account specific features of spatial structure of chloroplasts provides a tool for studying the effect of diffusion restrictions on pH distribution over the thylakoid membrane and the effect of topological factors (spatial organization of thylakoids) on the rates of electron transport, proton transport, and ATP synthesis in chloroplasts.

The results of our calculations demonstrated that the rate of electron transfer at the plastoquinone site of the chain could be controlled not only by the intra-thylakoid pH_i, which affects the rate of oxidation of plastoquinone by cytochrome *bf*-complex, but also by the value pH_o in the inter-thylakoid gap, which affects the rate of plastoquinone reduction. Results of numerical experimental variation of thylakoid system geometry and rate of lateral proton diffusion are consistent with a number of experimental findings. It was shown that efficiency of ATP synthesis in chloroplasts depended on specific topological features of the thylakoid (e.g., presence of spacious sites along which slow diffusion of protons occurs). The results of the calculations can explain earlier experimental findings that the rate of ATP synthesis depends on osmotic properties of a chloroplast incubation medium and, therefore, on topological factors.

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