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Towards Understanding Oscillations: A Mathematical Model of the Biochemistry of Photosynthesis [and Discussion]

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Towards understanding oscillations: a mathematical model of the biochemistry of photosynthesis

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A general model of electron transport, carbon assimilation, starch and sucrose synthesis was built on the basis of two partial models. Individual reactions were described by their $\Delta G'_0$, V_m and K_m values for substrates and products. The system of 33 differential equations was solved on a personal computer programmed in Turbo-PASCAL 3.0.

The rate of cytosolic fructose bisphosphatase (FBPase) is modelled to be dependent on the concentration of fructose 2,6-bisphosphate (F2,6BP). The synthesis of the latter is activated by inorganic phosphate and inactivated by triose phosphates. The quantum efficiency of PSII is depressed at high proton charge in thylakoids and at high redox states of the electron transport chain. One of the aims of the model was to check whether these regulatory systems could cause oscillations in photosynthesis. Transients calculated from a low to high photon flux density and from a low to high CO_2 concentration revealed an overshoot but no oscillations. Therefore, it has not been sufficiently proved whether cytosolic FBPase and PSII activity control oscillations in photosynthesis. The phosphate-limited photosynthesis is stable in cases where UDPglucose pyrophosphorylase and ADPglucose pyrophosphorylase have greater affinity for ATP (UTP) than CO_2 assimilation. In a phosphate-limited state high ΔpH is not generated, as electron transport becomes limited by the low concentration of 1,3-diphosphoglycerate.

Oscillations in photosynthesis have been a subject of interest for a few decades but the understanding of their mechanism has progressed considerably slower than the study of their phenomenology. The mechanism seems to involve a variety of feedback loops and this makes its understanding difficult. On the other hand, understanding the operation of these feedback loops means understanding the control of the photosynthetic rate, and this makes their understanding important. Quite certainly, it is hopeless to try to prove the functioning of a proposed mechanism without an adequate mathematical model. Therefore, the problem of oscillations in photosynthesis has been a powerful catalyst for the development of mathematical models of photosynthesis. Finally it is hoped that these efforts will lead to the creation of a mathematical theory of photosynthesis.

The phenomenology of oscillations in photosynthesis has been described by Laisk & Oja (1972, 1976), Laisk (1977), Walker (1976, 1981), Walker *et al.* (1983) and Sivak & Walker (1986). Oscillations occur after a transient from a light or a CO_2-limited state to a light or CO_2-saturated state, i.e., they occur when photosynthesis is internally limited. The temporary occurrence of CO_2 and O_2 exchange rates higher than the final steady-state rate suggests that under such conditions the photosynthetic rate is controlled by a feedback mechanism. A review of earlier attempts to explain oscillations in photosynthesis has been given by Laisk & Walker (1986).

A new approach to the problem appeared with the understanding of the phosphate turnover (Walker & Robinson 1978). The photosynthetic machinery consumes inorganic phosphate and produces sugar phosphates. The rate of phosphate usage in sucrose and starch synthesis determines the maximum possible rate of photosynthesis. Key enzymes in sugar and starch synthesis pathways are controlled by different sugar phosphates (activators) and P_i (inactivator) (Stitt 1987; Heldt *et al.* 1977).

This idea has been mathematically formulated by Laisk & Laarin (1983). The 'light reactions' of photosynthesis (consuming phosphate) were modelled by using the analogy of the Geiger–Muller counter of radioactive particles: after a successful photoact light reactions were closed for a certain 'dead' time. The 'dead' time was allowed to depend reciprocally on the concentration of free phosphate in the chloroplast. Enzymes that facilitated the liberation of phosphate were assumed to be activated by organic phosphates and inactivated by inorganic phosphate. This simple formulation simulated oscillations in photosynthesis in response to a stepwise change in CO_2 concentration, provided that a delay was introduced into the feedback loop of the enzyme control.

At the time when Laisk & Laarin (1983) published their work, regulatory processes governing the rate of sucrose synthesis were not understood and therefore the biochemical background of the model was explained on the basis of starch synthesis, where ADPglucose pyrophosphorylase was controlled by organic phosphates and P_i (Heldt *et al.* 1977). Later on, when studies on the fructose 2,6-bisphosphate (F2,6BP) control of the cytosolic fructose bisphosphatase (FBPase) appeared (Herzog *et al.* 1984), another model was proposed with the emphasis on sucrose synthesis (Laisk & Walker 1986). This model incorporated a rather detailed set of light reactions and an electron-transport chain, as well as the Calvin cycle, though sucrose synthesis was still depicted quite schematically. As in starch synthesis, the system showed oscillatory transients only when a delay was postulated in the response of sucrose synthesis rate to the variations of phosphate concentrations in the cytosol.

Horton & Nicholson (1987) studied the behaviour of this model and found that under a condition where sucrose synthesis was strictly limiting, damped oscillations related to starch synthesis occurred even without a special delay being introduced into the feedback loop. Though under these conditions the rate of CO_2 uptake was largely suppressed, this result still supported the hypothesis of phosphate turnover as a possible cause of oscillations.

There still remained a suspicion that the limitations of the model (Laisk & Walker 1986) in reproducing oscillations were caused by serious simplifications in the chemistry of sucrose synthesis and in the Calvin cycle. A more detailed model of the Calvin cycle, sucrose and starch synthesis was therefore built (Laisk *et al.* 1989). It incorporated a whole set of reactions of the carbon reduction cycle, plus starch and sucrose synthesis chains. Individually, the enzymatic reactions were characterized by their equilibrium constants (derived from the free energy changes), Michaelis constants (K_m) and maximum rates (V_m). The cytosolic FBPase was assumed to be inhibited by F2,6BP. The synthesis of the latter was activated at high concentrations of inorganic phosphate and low concentrations of triose phosphates in the cytosol (details are given below). From light reactions, only photophosphorylation was incorporated into the model. It was simulated as a usual enzymatic reaction with a given $\Delta G'_0$, V_m and K_m for P_i, ADP and ATP. It was assumed that, in the light, the ATPase reaction was shifted towards ATP synthesis under the influence of the electrochemical gradient across the thylakoid membranes. That gradient and the NADPH/NADP ratio were assumed to be

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constant. The main idea of the model was therefore to simulate the effect of the phosphate control of photophosphorylation, neglecting the parallel variations in the redox state of the electron transport system.

When the model was tested for oscillatory behaviour, results were negative. The cause for this was the absence of a lag in the response of F2,6BP concentration to changes in triose phosphates and orthophosphate. Its concentration started to change immediately after a change in the triose phosphate concentration. Efficient stability factors in the whole system were the quadratic dependencies of fructose 1,6-bisphosphate (FBP) on triose phosphates in the cytosol and the rate of FBPase on fructose 1,6-bisphosphate concentration (Laisk *et al.* 1989).

It is known that a fluorescence signal oscillates simultaneously with the CO_2 uptake rate (Walker *et al.* 1983). Therefore, we supposed that some control phenomena in light reactions and the electron-transport chain might be involved in oscillations. Recently Weis & Berry (1987) suggested that the PSII active centre could exist in two states, one of which had a higher, the other a lower quantum efficiency. The lower-efficiency form quenches the variable fluorescence, which is known as energy-dependent quenching (q_E). Proceeding from this idea, a model of the electron-transport chain was created (Laisk & Walker 1989) including two photosystems and the pools of the primary acceptor of PSII, plastoquinone and P_{700}, as well as ferredoxin and NADPH at the acceptor side of PSI. The electron transport was coupled to the proton transport into the thylakoid lumen. The generated ΔpH shifted the equilibrium of ATPase towards ATP synthesis. The model of the electron-transport chain was combined with a simplified model of carbon metabolism where pentose phosphates were assumed to be directly synthesized from triose phosphates and the rate of sucrose synthesis was simply postulated to be equal to the rate of CO_2 uptake.

This model was able to simulate light and CO_2 curves of photosynthesis, photochemical (q_Q) and non-photochemical (q_E) quenching and the Kautsky effect in fluorescence. Both the phosphorylation and reduction components of the assimilatory power were variable, but efforts to find oscillatory transients in the CO_2 uptake remained unsuccessful.

There remained a hope therefore that a combination of the models of carbon metabolism and the electron-transport chain would solve the problem and show us the full picture of kinetic transients in photosynthesis.

A COMPLETE MODEL OF PHOTOSYNTHESIS

The structure of a biochemically complete model of photosynthesis is shown in figure 1. As in Laisk & Walker (1989) the primary reaction section contains two photoreactions, PSII and PSI; electron transport between the two is still simplified by omitting the cytochrome *b*/*f* complex. A cyclic electron flow from ferredoxin back to plastoquinone is allowed, as is also a pseudocyclic flow to oxygen or to some other acceptor besides NADP (denoted as X^-/X redox pair). The carbon reduction cycle, starch and sucrose synthesis pathways have been described according to their known biochemical structure, as described in Laisk *et al.* (1989). In addition, it was assumed that adenylates and uridine nucleotides in the cytosol are mostly phosphorylated at the expense of the energy exported from the chloroplast in the result of the operation of a triose phosphate–PGA shuttle. The concentration of NADH in the cytosol was assumed to be constant. As in Laisk *et al.* (1989), biochemical reactions have been described by their free

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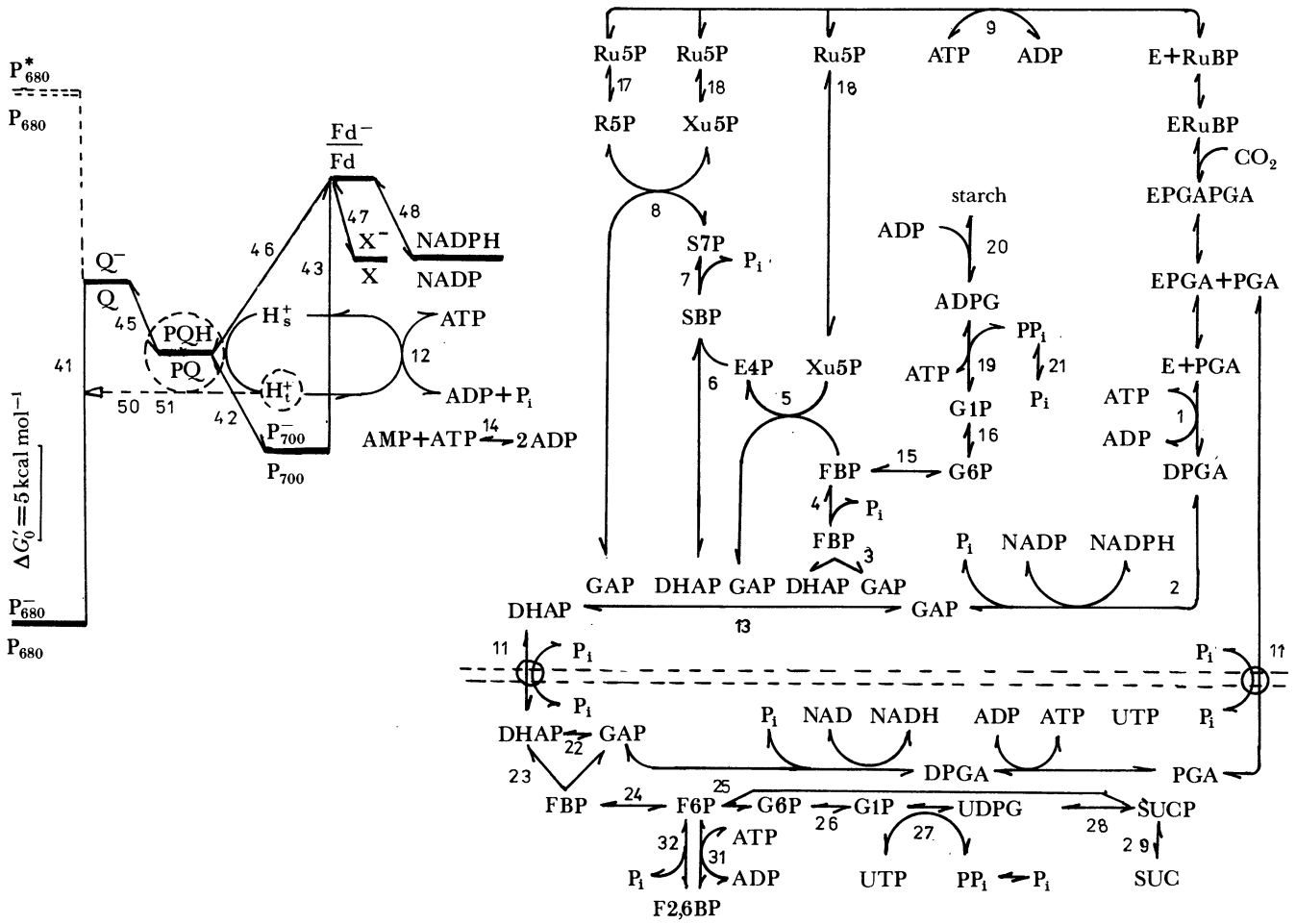

FIGURE 1. General structure of the model (abbreviations as follows). Thylakoid compartment: P^-_{680}/P_{680}, donor side of the PSII (P^*_{680}, excited state of the PSII centre); Q^-/Q, acceptor of PSII; PQH/PQ, plastoquinone, reduced-protonated and oxidized forms; P^-_{700}/P_{700}, donor side of the PSII reaction centre; Fd^-/Fd, acceptor side of PSI; NADPH/NADP, nicotinamide adenine dinucleotide phosphate, reduced and oxidized; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; P_i, orthophosphate; PP_i, pyrophosphate; H^+_s and H^+_t, hydrogen ions in stroma and thylakoids respectively. Feedback regulation of the PSII activity by H^+_t and PQH/PQ is shown by a dashed arrow. Stroma and cytosol compartments: E, ribulose 1,5-bisphosphate carboxylase; RuBP, ribulose 1,5-bisphosphate; PGA; ERuBP, enzyme-bound RuBP; PGA, glycerate 3-phosphate; EPGAPGA, two PGA molecules bound to the enzyme; EPGA, one PGA bound to the enzyme; DPGA, 1,3-diphosphoglycerate; GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetonephosphate; FBP, fructose 1,6-bisphosphate; F6P, fructose 6-phosphate; E4P, erythrose 4-phosphate; Xu5P, xylulose 5-phosphate; SBP, sedoheptulose 1,7-bisphosphate; S7P, sedoheptulose 7-phosphate; R5P, ribose 5-phosphate; Ru5P, ribulose 5-phosphate; Xu5P, xylose 5-phosphate; G6P, glucose 6-phosphate; G1P, glucose 1-phosphate; ADPG, ADPglucose; F2,6BP, fructose 2,6-bisphosphate; UDPG, uridine diphosphate glucose; UTP, uridine triphosphate; UDP, uridine diphosphate; SUCP, sucrose phosphate; SUC, sucrose; NADH/NAD, nicotinamide adenine dinucleotide, reduced and oxidized.

energy changes $\Delta G'_0$, Michaelis constants K_m and maximum rates V_m. These parameters (except V_m) were obtained from the literature. Maximum rates were adjusted so as to satisfy experimental data about the distribution of intermediates. In the case of a two-substrate, two-product reaction, the rate equation was

$$V = V_m \frac{([A][B]-[C][D]/k_E)/(K_A K_B)}{1+[A]/K_A+[B]/K_B+[C]/K_C+[D]/K_D+[A][B]/K_A K_B+[C][D]/K_C K_D}, \quad (1)$$

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where A and B are substrates, C and D are products, K_A, K_B, K_C, K_D are Michaelis constants for them, and k_E is an equilibrium constant

$$k_E = \exp(\Delta G'_0/RT). \tag{2}$$

The phosphate translocator and transketolase were described by more complicated equations because they have more reagents. Ribulose bisphosphate carboxylase (Rubisco) was described in detail including enzyme-bound RuBP and PGA pools.

The cytosolic bisphosphatase (24) was described by the following rate-equation

$$V_{24} = V_{m,24}\frac{[FBP_c]([FBP_c]-[F6P_c][P_{i,c}]k_{E,24})}{(K_{m,24}[FBP_c])^2} \div\{1+\frac{[FBP_c]^2}{(K_{m,24}[FBP_c])^2}+\frac{[F6P_c]}{K_{m,24}[F6P_c]}+\frac{[F6P_c][P_{i,c}]}{K_{m,24}[F6P_c]K_{m,24}[P_{i,c}]}+\frac{[P_{i,c}]}{K_{m,24}[P_{i,c}]}\}, \tag{3}$$

where $K_{m,24}[FBP_c] = K_{m,0,24}[FBP_c](1+[F2,6BP_c]/K_{i,24}[F2,6BP_c])$.

Here $[F2,6BP_c]$ is the concentration of fructose 2,6-bisphosphate in the cytosol (subscript c means that the concentrations of reagents are those in the cytosol compartment; for the denotions of intermediates see legend to figure 1). Equation (3) describes a sigmoidal kinetic curve with K_m being modified by the presence of F2,6BP.

The rates of synthesis and degradation of F2,6BP were assumed to be controlled by cytosolic triose phosphates and free orthophosphate, $P_{i,c}$ reciprocally. For F2,6BP kinase,

$$V_{31} = V_{m,31}\frac{1+[P_{i,c}]^2/(K_{a,31}[P_{i,c}])^2}{1+[T3P_c]^2/(K_{m,31}[T3P_c])^2}. \tag{4}$$

For F2,6BP bisphosphatase,

$$V_{32} = V_{a,32}[F2,6BP_c]/K_{m,32}[F2,6BP_c], \tag{5}$$

$$V_{a,32} = V_{m,32}\frac{1+[T3P_c]^2/(K_{m,32}[T3P_c])^2}{1+[P_{ic}]^2/(K_{a,32}[P_{i,c}])^2}. \tag{6}$$

According to these equations, the steady-state concentration of $F2,6BP_c$ is higher when $P_{i,c}$ is higher and $T3P_c$ is lower. Rates V_{31} and V_{32} determine the speed of the transient, and were chosen in such a way that the transients in the F2,6BP concentration were completed within 15–20 s (Laisk *et al.* 1989).

The kinetic behaviour of both photoreactions was modelled as for enzymatic reactions, the maximum turnover rate (V_m) of which was assumed to be determined by the frequency of attacking photons into the particular photosystem. The K_m for substrates and products (P^-_{680}, Q^-, P^-_{700}, Fd) were assumed to be very low, so that the rate of a reaction was determined by irradiance density and equilibrium conditions only. If K_m in equation (1) is allowed to approach zero, the equation will reach the form

$$V = V_m([A^-]/[A]-[B^-]/[B]k_E)/([A^-]/[A]+[B^-]/[B]). \tag{7}$$

Similar kinetics was also used to describe enzymic reactions 47 and 48. For reactions 42, 45 and 46 both versions, the first-order kinetics and equation (7), were tried, and it was found that the behaviour of the system did not differ in principle.

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To describe ATP synthesis, it was assumed that the linear transport of each electron from P_{680}^- to NADP was coupled to the appearance of two protons in the thylakoid lumen. Most of those protons were assumed to be bound to a 'buffer' (BFH, protonated and BF, non-protonated buffer forms), and the concentration of free protons in the thylakoid, $H^+_{f,t}$, was calculated from a buffer equation.

The proton gradient on thylakoid membranes bears chemical energy which shifts the equilibrium of reaction 42, so that

$$G_{42} = G_{0,42} + 0.599 \ln([H^+_{f,t}]/[H^+_{f,s}]).$$

It also shifts the ATPase (reaction 12) towards ATP synthesis, so

$$G_{12} = G_{0,12} - 0.599\, HPR \ln([H^+_{f,t}]/[H^+_{f,s}]),$$

where *HPR*, the 'hydrogen:phosphate ratio' was assumed to be 3.0 and $G_{0,12} = +7.3$ kcal mol^{-1}.

The regulation of the activity of PSII was described as a reduction–protonation of the 'light-harvesting complex' of PSII (LHCII) (we do not know the mechanism of this control, therefore the denotion LHCII simply means 'something which determines the number of quanta active at PSII'). Both high total concentration of protons in the thylakoid lumen and high reduction state of plastoquinone facilitate inactivation,

$$V_{50} = V_{m,50}\left\{[\mathrm{LHCII}]\frac{[\mathrm{LHCIIH}]}{k_{E,50}([PQH]/[PQ])([BFH_t]+[H^+_t])/([BF_t]-[H^+_t])}\right\}. \quad (8)$$

However, the 'reduced–protonated' $LHCIIH^+$ is not quenching. Only after a slow conformational change which is described as

$$V_{51} = V_{m,51}([\mathrm{LHCIIH}^+]-[\mathrm{LHCII_Q}])/[\mathrm{LHCII_T}], \quad (9)$$

where subscript T means 'total', does it become inactivated and quench fluorescence.

In table 1, calculated steady-state distributions of the pools of intermediates are given for four different situations. The first line corresponds to a high CO_2 concentration and high light intensity without severe phosphate limitation; in the second line sucrose synthesis rate has been decreased by decreasing the V_m of the SPSase $V_{m,28}$; the third line is light limited; and the fourth is CO_2 limited. As one can see, orthophosphate tends to be low in all cases, as it is efficiently trapped in hexose phosphates (HP). Evidently, a control of chloroplast FBPase not yet included in the model is necessary to prevent hexoses from rising too high. At a low CO_2 concentration hexoses are somewhat lower, because phosphate is now partly in RuBP which is at 9 mM level. At low CO_2 the electron-transport chain becomes highly reduced and the phosphorylation potential is also high, high-energy adenylphosphates (2ATP+ADP) are at 5 mM level (maximum is 6 mM).

The steady-state light curves of photosynthesis (P), photochemical (q_Q) and energy-dependent quenching (q_E) calculated from the model at a high CO_2 concentration in the presence and absence of sucrose synthesis limitation, are shown in figure 2. As can be seen, a greater limitation of the carbon metabolism causes greater q_E, but q_Q is descreased only a little. Evidently, the regulation of the efficiency of PSII stabilizes the redox state of the electron-transport chain. Only with severe CO_2 limitation does the electron-transport chain become over-reduced (table 1). The calculated light curves of q_Q and q_E are very similar to curves from

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TABLE 1. THE DISTRIBUTION OF INTERMEDIATES UNDER DIFFERENT LIMITING CONDITIONS OF PHOTOSYNTHESIS CALCULATED FROM THE MODEL

(P is the rate of CO_2 assimilation in μM s^{-1}; PFD is photon flux density in mmol quanta m^{-2} s^{-1}; $[BFH_t]$ is the concentration of protons buffered in thylakoids. $[Q^-]$, [PQH], $[P^-_{700}]$, $[Fd^-]$, [NADPH] are given relative to the fully reduced state, others are in μM. (*a*) Initial state of the model; (*b*) sucrose synthesis rate limited by the decreasing of SPSase; (*c*) light limitation; (*d*) CO_2 limitation.)

	$[CO_2]$	PFD	P	$[Q^-]$	[PQH]	$[BFH_t]$	pH_t	ΔpH	$[P^-_{700}]$	$[Fd^-]$	[NADPH]	$[P_i]$	[adenyl phosphate]	[RuBP]	[PGA]	
(*a*)	50	120	6197	0.326	0.840	64413	5.04	2.72	0.457	0.431	0.685	1618	1119	489	4769	
(*b*)	50	120	4034	0.352	0.903	70143	4.93	2.87	0.548	0.699	0.920	446	310	71	3057	
(*c*)	50	10	974	0.036	0.751	51077	5.28	2.39	0.821	0.038	0.480	886	106	13	3354	
(*d*)	1	120	1249	0.527	0.979	75210	4.82	3.01	0.807	0.971	0.999	716	4915	9020	26	
	[T3P]	[FBP]	[HP]	[E4P]	[SBP]	[S7P]	[P5P]	$[P_{i,c}]$	$[UTP_c]$	$[T3P_c]$	$[PGA_c]$	$[F2,6BP_c]$	$[FBP_c]$	$[HP_c]$	[UDPG]	[SUCP]
(*a*)	4064	2252	12994	140	303	1143	1097	1471	133	2825	1193	5	1719	8535	231	277
(*b*)	2610	1265	20377	163	281	2077	1250	595	41	2632	1024	9	1882	9762	314	145
(*c*)	952	335	22952	124	103	3324	1029	2378	114	2373	2558	27	2426	5633	494	51
(*d*)	612	117	6470	48	22	552	302	3447	746	2728	70	35	3096	4653	1108	77

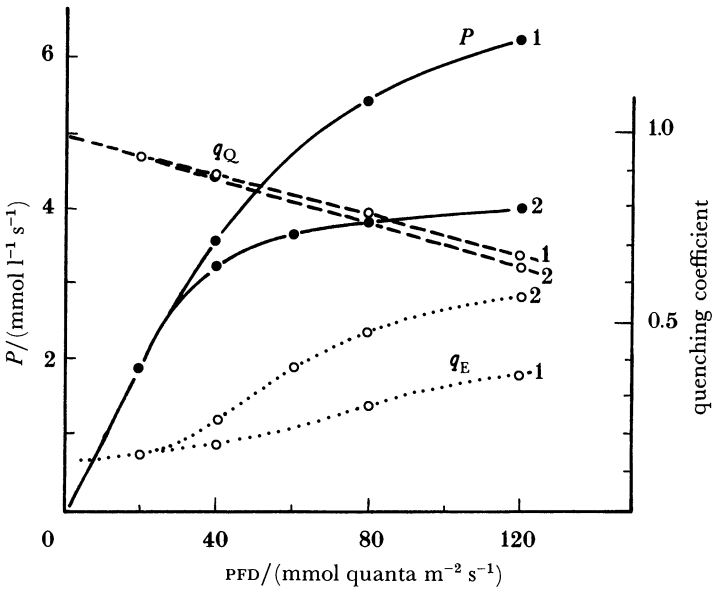

FIGURE 2. Calculated steady-state light curves of CO_2 uptake rate P, photochemical (q_Q) and energy-dependent (q_E) quenching coefficients. Curves 1 correspond to a non-limiting, curves 2 to a limiting sucrose synthesis rate. Quenching coefficients have been defined according to Schreiber (1986).

figure 1 of Weis & Berry (1987), with the exception that at extremely low light intensities, a significant q_E is developing.

We hoped to be able to simulate oscillations in photosynthesis when two controlled reactions, the cytosolic FBPase and PSII activity controlled by thylakoid protonation-reduction, were incorporated into one system. Unfortunately, only a weak overshoot in the CO_2 uptake rate could be seen in the transient from a low to a high CO_2 concentration (not shown). We cannot state that this model is not able to oscillate at all. It is possible that we were unable to find the necessary combination of parameters. Owing to the complexity of the system, standard analytical procedures for studying the stability are not applicable, and only numerical experimentation seems to be a suitable way of studying the behaviour of the system. However, it still seems that oscillations cannot be explained on the basis of these two known regulations of the photosynthetic metabolism and their real cause must be hidden somewhere else.

Studying oscillations in photosynthesis experimentally, we realized that the first minimum always occurs, though following oscillations may sometimes be substituted for a slower but steady rise in the CO_2 uptake rate (figure 3). This means that the first minimum should be considered as a collapse in the stability of the photosynthetic machinery which is then reversed by a regulation. If this regulation is too rapid and operates over a lag, the steady state will be reached via a series of oscillations. In some plants the regulation is slower, and consequently the recovery of photosynthesis is also slower, with no or only rudimentary oscillations superimposed on the basic trend (Laisk & Oja 1976; Laisk 1977).

Proceeding from this observation, we started to study conditions leading to the collapse of the photosynthetic machinery. There are several possible ways of reaching this state, mostly based on disproportions between the rates through sequential irreversible steps of metabolism. However, one of them is different, and its kinetics seems to be close to the observed kinetics of oscillations. This way of generating collapse is based on disproportions between the rates of synthesis of ATP and NADPH during electron transport.

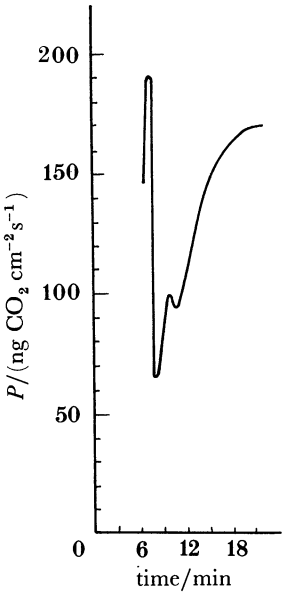

FIGURE 3. Transient in the CO_2 uptake rate P^{-2-1} in an aspen leaf after the CO_2 concentration was increased from zero to 2000 µl l^{-1} (Laisk & Oja 1976).

We have supposed that linear electron flow was insufficient to produce a necessary amount of ATP, and some cyclic electron flow around PSI was necessary. A first-order reaction was assumed to draw electrons back to PQ from reduced ferredoxin (Fd^-). At the transients in the photosynthetic rate, the redox states of PQH/PQ and Fd^-/Fd changed until the new requisite rate of cyclic flow was kinetically obtained. This adjustment was rapid and there was no transient discrepancy between the rates of ATP and NADPH production. As a result, a small overshoot in the CO_2 uptake occurred, but there were no oscillations. One may assume that the flux of electrons from the acceptor of PSI to the cytochrome *b*/*f* complex and further to PQ is under this control. In this case, at low rates of CO_2 uptake (low light, low CO_2) the cyclic flow is turned down to a low value necessary to support the slow CO_2 uptake rate. After a rapid transition to high CO_2 and high light, the CO_2 uptake rate increases rapidly. However, the low rate of cyclic electron flow is insufficient to sustain the necessary ATP concentration, and ATP levels fall rapidly, together with the CO_2 uptake rate. An example of such a numerical experiment is shown in figure 4. As we can see, 20 s after switching on high CO_2 concentration, the CO_2 uptake rate declines to a minimum determined by preset cyclic electron flow rate $V_{m,46} = 1750$ mM s^{-1}. At this stage, ΔpH declines and together with it adenyl phosphate high-energy reaches a minimum. Correspondingly, the whole electron-transport chain, including NADPH and Q^-, is maximally reduced. The q_E quenching ($LHCII_Q$) declines and fluorescence is at a maximum. Up to this moment the calculated situation fits well with the experimental data available, but difficulties arise later on.

To reverse ATP limitation, the $V_{m,46}$ of cyclic electron flow was suddenly increased to 3500 mM s^{-1}. As a result, photosynthesis increased rapidly, proceeded through a maximum, and declined again. In the second minimum NADPH was down, but ΔpH was at an extreme maximum. High-energy adenylates were not very high because of phosphate limitation in this situation. Fluorescence and Q^- rose again in this minimum, because the electron-transport rate was restricted by a very high ΔpH.

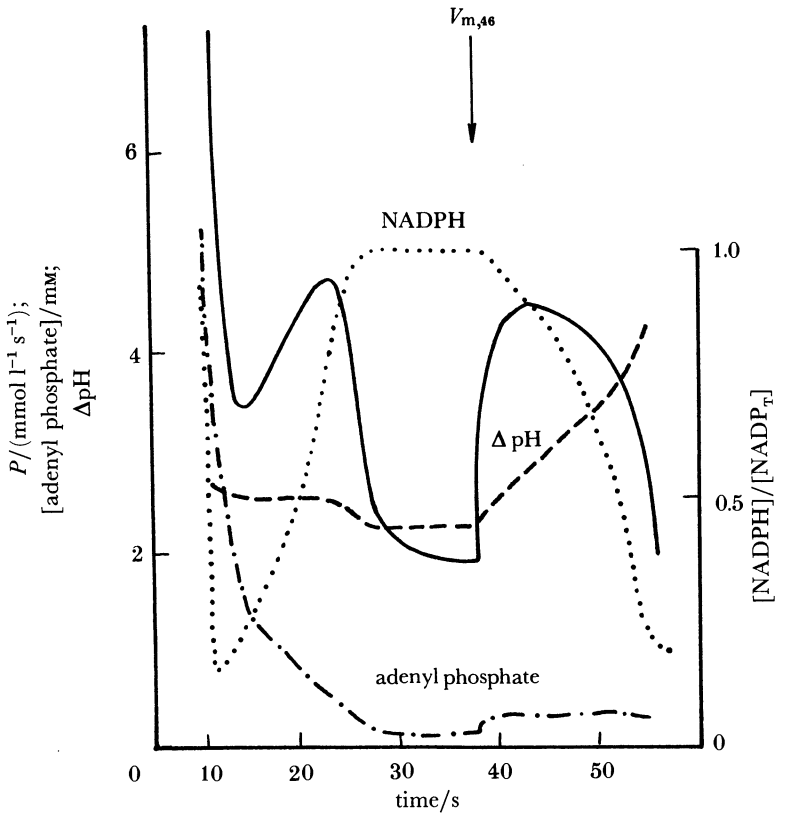

FIGURE 4. Transients in the CO_2 fixation rate P, adenyl phosphate concentration, transmembrane ΔpH, and NADPH reduction state after an increase in CO_2 concentration from 1 to 50 μmol l^{-1} (at $t = 10$). At 1 μmol l^{-1} CO_2, $V_{m,46}$ of the cyclic electron flow was set to 1750 mmol s^{-1}, which was sufficient for stable photosynthesis. Then the concentration of CO_2 was changed to 50 μmol l^{-1} (results plotted from that moment). After 27 s, $V_{m,46}$ was increased to 5000 mmol s^{-1}.

This mechanism of oscillations works well in the first minimum, but in the second minimum ΔpH is in a counterphase; that is, ΔpH oscillates with a frequency half of the frequency of oscillations in photosynthesis. It is known that the light-scattering signal at 550 nm, which is believed to reflect the energization of thylakoid membranes, oscillates with the same frequency as the CO_2 uptake rate (Sivak *et al.* 1984). Therefore this mechanism may be a real cause for oscillations only in this case, if the increase in ΔpH reverses before the energization of thylakoids reaches extremely high values and becomes efficient in the control of the electron-transport rate. In this case, ΔpH will oscillate with the same frequency as the CO_2 uptake rate.

To incorporate these manual control actions into the model, we supposed that the cyclic electron flow was controlled by the level of the energization of thylakoids similarly to the way the efficiency of PSII was controlled (equation (8) without PQH). In this case, the calculated transient from low to high CO_2 (figure 5) has a temporary maximum and a deep minimum, and increases slowly to a steady state. This response is similar to those experimental transients that also have one minimum and a slow rise (figure 3). The reason why the transient is non-oscillatory is the absence of a time lag between the variation of energization of the membranes and the regulation of the cyclic electron flow.

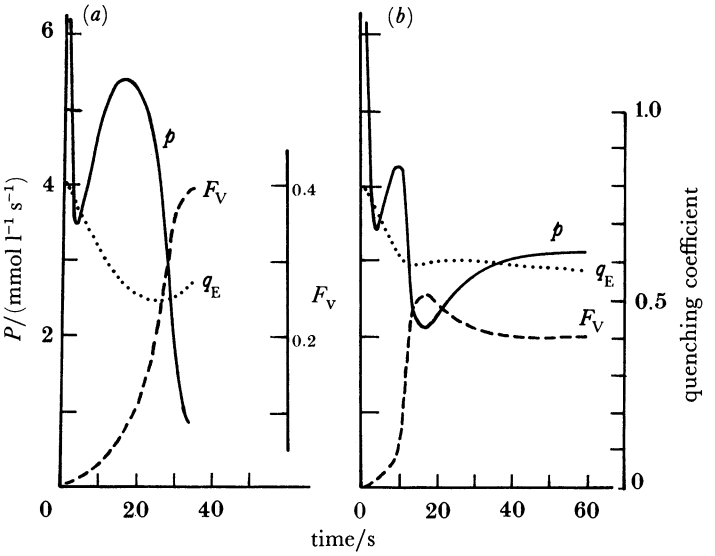

FIGURE 5. Transients in the CO_2 fixation rate, P, variable fluorescence, F_V, and energy-dependent quenching, q_E, after the concentration of CO_2 was changed from 1 to 50 μmol l^{-1}. (*a*) Rate constant for cyclic electron flow set low; (*b*) rate constant for cyclic electron flow made dependent on the energization state of the thylakoid membranes.

DISCUSSION

In spite of not yet being able to simulate mathematically oscillations in photosynthesis, there is still some progress towards understanding their mechanism and, in parallel with this particular task, there has been progress in understanding thermodynamic principles of the control of photosynthesis in general.

Perhaps the most important understanding is that photosynthesis is solely a light-driven process. The Z-scheme, as it is conventionally represented, often fails to emphasize the fact that, although the energy of a 680 nm photon is 1.8 eV, only about 1 eV is used to lift an electron from the level of P^-_{680}/P_{680} to Q^-/Q, and from P^-_{700}/P_{700} to Fd^-/Fd. The remainder of the energy of photons in both photosystems (about 0.8 eV or 18 kcal mol^{-1}) is available as a free energy change driving the reaction. When the pigment P_{680} has absorbed a photon and is in the excited state, the thermodynamic pressure towards charge separation depends on the ratio of rate constants for charge separation and for returning the electron back to the ground state. The problems of energy storage in the photochemical event have been theoretically considered by Ross *et al.* (1976). In general, if even a part of the 0.8 eV excess potential acts as a driving force for the photoreactions, it still means that the primary photoacts are extremely difficult to reverse by the build-up of reaction products. In the steady-state situation, the frequency of the absorbtion of photons by PSII determines the flux rate of electrons through the electron-transport chain, Calvin cycle, sucrose synthesis pathway, phloem transport, etc. ending with the growth processes of the plant. Through these pathways electrons are running energetically downstream. In addition to these two photoreactions, there are several reactions that have relatively high free energy changes in the process of carbon metabolism: RuBP carboxylase, chloroplast FBPase and SBPase, Ru5Pkinase and cytosolic FBPase. These reactions cooperate, generally in sequence, in the transport of electrons from water to CO_2 and further in the formation of sucrose. To keep the pools of intermediates between the successive

irreversible steps of metabolism from overfilling or running down, the enzymes catalysing irreversible steps have to be under control. This control guarantees the stability of the operation of the photosynthetic machinery (figure 6). With poor regulation, some intermediate sugar phosphate pools start to increase without a limit until almost all phosphate available is trapped in this pool, and photophosphorylation will stop. The most logical way to control a sequence of irreversible reactions would be to apply product inhibition, in this way suppressing the maximum concentration of the product accommodating just after an irreversible reaction. Unfortunately, however, there is no experimental evidence of an efficient control of FBPases by hexose monophosphates. Therefore, in the model, hexoses are the most efficient traps of phosphate. Evidently the incorporation of the control of the chloroplast FBPase into the model is one of the most vital tasks.

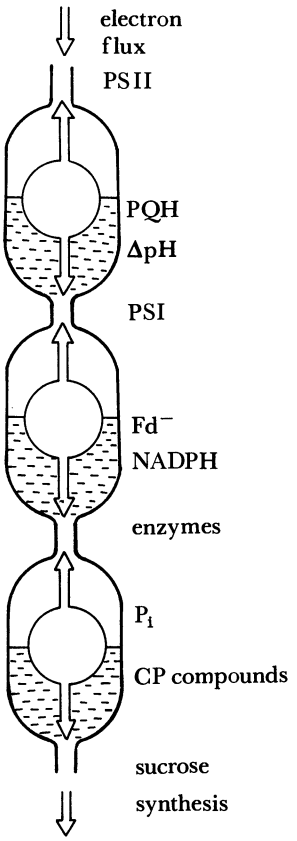

FIGURE 6. Flux control in photosynthesis: a hydraulic model.

When the rate of photosynthesis was limited by the rate of sucrose synthesis (controlled by SPSase, the so called 'phosphate limitation'), most of the phosphate was trapped in hexoses (table 1). Phosphate limitation was stable only in the case when the reactions in the pathways of sucrose and starch synthesis were less sensitive to the decrease of ATP (or UTP) concentration than the photosynthetic rate. This means that the apparent K_m(ATP) of ribulose 5-phosphate kinase has to be greater than K_m(ATP) for ADPglucose pyrophosphorylase and K_m(UTP) for UDPglucose pyrophosphorylase. Otherwise, when a decrease in ATP concentration causes a more rapid decrease in sucrose and starch synthesis than in the CO_2

assimilation rate, the whole system becomes unstable and ends up with all the phosphate trapped in hexose phosphates, with a CO_2 uptake rate approaching zero.

When light intensity exceeds the capacity of the plant to utilize the photosynthetic product, there appears to be an inevitable disproportion between the supply of electrons from PSII and their flow rate through the following metabolic chain. As the reverse reaction at PSII occurs only when the intermediates of the electron transport chain are highly reduced, this way of controlling the electron flow may be dangerous to the plant, causing uncontrolled reductions that may damage the photosynthetic machinery. Photoinhibition seems to be one of the results. The only possible way of controlling the balance of such a system is to do it at the beginning, at PSII. This is the reason why the proposal by Weis & Berry (1987) that the efficiency of the PSII centres were controlled by the energization state of the thylakoids was so attractive. The incorporation of it into the mathematical model showed that it was insufficient to assume that the control was exercised only by the energization state of thylakoids; an additional parallel control by the redox state of PQ was also necessary (Laisk & Walker 1989). Generally speaking, light 'pushes' the photosynthetic metabolism through two parallel springs, ATP and NADPH, and the distribution of forces on them must be carefully controlled.

Our calculations showed that the phosphate limitation of photosynthesis at saturating light and CO_2 is possible. Free orthophosphate in the stroma declines to 446 μM (table 1) and high-energy adenyl phosphates to 310 μM (1119 μM in non-phosphate limitation). According to adenylate kinase equilibrium, this gives a ATP/ADP ratio of 0.13. Contrary to expectations, Δ pH and proton charge in thylakoids did not rise much. There was also a remarkable increase in the trapping of phosphates in hexoses.

Furbank *et al.* (1987) have studied the regulation of photosynthesis in isolated spinach chloroplasts during phosphate limitation. In the absence of added P_i in the medium, the stromal P_i concentration rapidly decreased to a constant steady state between 1.5 and 2.5 mM in the light. As there may be about 1 mM of bound phosphate not available to metabolism (Robinson & Giersch 1987), the P_i concentration calculated by us seems to be realistic. Though a little increase in the transthylakoid ΔpH and in the non-photochemical fluorescence quenching, q_E, was observed during the phosphate-limited photosynthesis, these parameters increased more after P_i was added to phosphate-limited chloroplasts. It was concluded that electron transport was not limited by inability to discharge transthylakoid ΔpH. From table 1 we can see that under the phosphate limitation there is a limited increase in ΔpH, but much more remarkable is the increase in NADPH reduction state. Evidently, electron transport is actually limited by the low levels of 1,3-diphosphoglycerate under low phosphate conditions. Therefore, phosphate limitation in photosynthesis always produces a very high reduction state in the NADPH/NADP system.

During oscillations in photosynthesis, the rate of electron and carbon flow through the whole system is variable. The period of oscillations (*ca.* 60 s at room temperature) is relatively long, and the amount of carbon assimilated during that time is greater than the sum of all intermediate pools in the electron-transport chain and carbon metabolism (Laisk & Oja 1976). It is therefore impossible that the phase shift necessary for generating oscillations is a result of chemical conversions of intermediates. Evidently oscillations occur in the result of delayed interactions of the control systems of photosynthesis.

We tried to generate the delay chemically as the time necessary to synthesize F2,6BP and to inactivate PSII, but unsuccessfully, because both were modelled as inertial links of the feedback

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chain. It is difficult to understand how a delay can be created in PSII unless we involve conformational changes in enzyme proteins. The time lag will be more easily understood if the process involves reorganizations in membrane structures or physical movements of electron carriers in the membranes. Probably, we were too discrete in avoiding assumptions about the presence of time lag and trying to derive it from the kinetics of the system as a whole. Perhaps the regulation of the activity of PSII still occurs with a time lag because it may involve physical reorganizations in the membrane structure?

The hypothesis about phosphate turnover as the main cause of oscillations seems to be inconsistent with the experimental evidence. If the minima in the photosynthetic rate were caused by an insufficient supply of phosphate to ATPase, the membrane energization should increase at that time. In fact, when the photosynthetic rate declines, the q_E quenching decreases and the light-scattering signal also shows that the energization of thylakoids is close to minimum when the CO_2 uptake rate is in the minimum (Sivak *et al.* 1984). The measurements of CO_2 solubility in the leaf also show minimum solubility (minimum energization) in the minimum of photosynthesis (O. Kiirats & U. Gerst, unpublished data). Evidently, the phosphate limitation of photosynthesis is not a simple limitation of ATP synthesis, but involves regulatory events in the electron transport and carbon reduction cycle (Furbank *et al.* 1989). In the light of this evidence, we looked for a formal mechanism that would result in a simultaneous decline in the rate of CO_2 uptake and ΔpH in thylakoid membranes.

The idea based on the insufficient ATP/NADPH ratio gives a good agreement with experimental data in the first minimum of oscillations. Proton gradient declines to minimum and this causes a decrease in q_E quenching. At the same time, the over-reduction of the electron-transport chain occurs with a consequent increase in fluorescence. However, this may not be the only way of inducing oscillations in such a complicated system as photosynthetic metabolism. There may be other mechanisms that would also result in a low energization state in the first minimum of oscillations, when the rate of electron transport between the two photosystems is subject to a regulation, for instance. There is some evidence that the rate of re-reduction of P_{700} after rapid oxidation with a light flash is different and dependent on the preconditioning of the leaf (U. Schreiber, personal communication). Kiirats (1985) showed that the rate of oxygen evolution in 1–2 s light flashes did not much exceed the previous steady rate of photosynthesis of the leaf, and concluded that the ability of the electron-transport system was adjusted to the CO_2 uptake rate limited by a low CO_2 concentration. The ferredoxin–$NADP^+$ oxidoreductase is light-activated (Ruhle *et al.* 1987). If oscillations are caused by the control of the rate of electron transport in the minimum of photosynthesis then both NADPH reduction and pH gradient must be in the minimum. When the ATP/NADPH synthesis ratio is poor, NADPH reduction must be at a maximum during the minimum in photosynthesis. The measurements of the redox state of the ferredoxin–NADPH system during oscillations would provide necessary additional information for understanding the oscillatory control of photosynthesis.

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Discussion

J. Barber (*AFRC Photosynthesis Research Group, Imperial College, London, U.K.*). Without any doubt, the non-photochemical quenching of chlorophyll fluorescence does not involve energy dissipation via PSI as Dr Laisk seems to be suggesting. Can Dr Laisk, in his modelling studies of oscillations, take advantage of the fact that there are differences between shade and sun leaves?

A. Laisk. The analysis of the model will help to understand thermodynamic necessities for the parallel adaptation of the light and dark reactions to different light conditions. For adaptation

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to the sunlight, an ability to control PSII activity, to shade the balanced development of PSII and PSI light-harvesting complexes are important. Björkman and Boardman have shown that in sun plants there are highter protein plastoquinone and cytochrome to chlorophyll ratios, and their carboxylation and electron transport conductances are higher than in shade plants. In model terms it means higher V_ms in the dark reactions and greater rate constants in the light reactions, which result in higher maximum photosynthetic rates in sun leaves.

U. Schreiber (*Department of Botany, Universität Würzburg*, F.R.G.). In my opinion, energization of the thylakoid membrane plays a key role in the oscillations. It is known that there is a clear-cut delay of about 15 s between internal acidification of the thylakoids and the conformational change of the membrane which leads to q_E-quenching and increased light scattering. One may imagine such delay to result from the lateral movement of membrane protein complexes as described by J. Barber. This may be the delay Dr Laisk needs in his model to obtain oscillations. The important aspect is that proton uptake starts a chain of events at the level of pigment–protein complexes that requires a certain time to find its first expression in changed energy distribution, electron transport and proton uptake.

A. Laisk. Thank you; your comment encourages me to introduce a delay between the increase in the proton pool and the decrease in PSII efficiency. At present this step is modelled as an inertial link, i.e. when the signal appears, an immediate response, though slow, follows. Oscillations are more readily generated when the response appears rapidly, but after a time lag.

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