Oscillations in the Photosynthetic Calvin Cycle – Examination of a Mathematical Model

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Oscillations in the photosynthetic reaction system have been extensively studied over recent decades,[1-5] but no convincing mechanistic explanation for their appearance would yet seem to be available. A recent examination of the kinetic structure of the Calvin photosynthesis cycle and ancillary pathway of starch and sucrose formation[6] has led to the detection of a large number of two-reactant subsystems which exhibit such kinetic characteristics that they, in principle, may serve as a source of oscillations. The purpose of this investigation is to examine to what extent some of these potentially oscillating subsystems would actually be expected to trigger oscillations under physiological conditions. This requires analysis of the kinetics predicted by a realistic mathematical model of the photosynthetic reaction system.

Several mathematical models have been designed in order to elucidate the mechanistic origin of the photosynthetic oscillations.[7-10] Most of these models have been put forward to test a postulated hypothetical explanation for the oscillations and have been greatly oversimplified or derived with physiologically unreasonable assumptions. The present analysis, therefore, will be based on a general-purpose model,[11] which utilizes experimentally documented rate equations and realistic values for kinetic constants and other reaction parameters involved. This model has been shown to account satisfactorily for experimentally observed steady-state rates and reactant concentrations in the photosynthetic process of carbohydrate formation[11,12] which makes reasonable the belief that the model should also provide a satisfactory description of the transient-state kinetic properties of the reaction system.

In the present study, the transient-state kinetic predictions of the model are examined and shown to allow for the appearance of oscillatory transients under a variety of experimental conditions. The mathematically predicted oscillations are kinetically characterized and their mechanistic origin discussed.

Results and discussion

Basic characteristics of the mathematical model considered. Pettersson and Ryde-Pettersson have described a realistic mathematical model for photosynthetic carbohydrate formation in isolated chloroplasts of C_3 plants under conditions of saturating light and carbon dioxide.[11] This model considers the Calvin cycle and treats ATP synthesis as a system-dependent input step. Starch production within the chloroplast, and the export of photosynthetic products to the external reaction medium through the action of the phosphate translocator, are included as output processes. The model is based on the assumption that all reaction steps are equilibrating except for those catalyzed by ribulose 1,5-bisphosphate carboxylase, fructose 1,6-bisphosphatase, sedoheptulose 1,7-bisphosphatase, ribulose 5-phosphate kinase, ADP-glucose pyrophosphatase and the phosphate translocator, which means that five independent concentration variables have to be considered. The non-equilibrating reaction rates are described by experimentally supported rate equations including all well-documented activating or inhibitory kinetic interactions. The model may be used to express steady-state reaction rates and reactant concentrations within the chloroplast as a function of a parameter (the concentration $[P_{ex}]$ of orthophosphate in the external medium) that may be readily varied experimentally.

Transient-state kinetics predicted by the model. For previously reported[11] realistic values of parameters (kinetic constants and levels of certain reactant pools) in the above model, the system examined exhibits a single stable steady-state mode of operation which may be attained at external orthophosphate concentrations $[P_{ex}]$ below 1.9 mM. The kinetics of the system near this steady state are governed approximately by five exponential transients with rate parameters λ_k which represents the eigenvalues of the Jacobian matrix characterizing the rate behaviour of the system. Elements of the Jacobian matrix were determined for different values of $[P_{ex}]$ by numerical differentiation of the

kinetic differential equations that define the model and the corresponding eigenvalues were calculated by standard numerical methods.

Typical values of the transient rate parameters λ_k are shown in Table 1. For $[P_{ex}]$ within the ranges 6–20 μM and 0.6–1.85 mM, two of the rate parameters are given by a conjugated pair of complex eigenvalues such that the system will exhibit oscillations when relaxing to the steady state. With certain minor modifications of parameter values in the model (e.g. change of the kinetic constant for inhibition of ribulose 5-phosphate kinase by 3-phosphoglycerate leading to less than 1 % change in the steady state reactant levels), the system may allow for the appearance of two pairs of complex conjugated transient rate parameters. A typical example is given in Table 1, case B.

The period of the predicted oscillations falls typically within the range 2–400 s. In experiments with green algae period times of 4–60 s have been observed.[1] With leaves, protoplasts and chloroplasts from higher plants a period of about 1 min is normal but it may rise to higher values in low light.[2–5] The present model, therefore, accounts satisfactorily for the oscillation periods observed experimentally.

Photosynthetic oscillations recorded experimentally under optimal conditions are relatively weakly damped, 3–10 min being required to decrease the amplitude of the oscillatory transients to 10 % of the initial value.[1–3] The damping factor of the oscillations now modelled exceeds 2 s^{-1}, however, which means that the damping to 90 % is completed within < 5 s. Hence it may be concluded that the modelled oscillations are so strongly damped that they will be concealed by the overlapping slow non-oscillatory transient(s) contributing to the kinetics of the reaction system. This is illustrated by the typical relaxation trajectories of reactant concentrations in Fig. 1, which exhibit only slight resemblance to the photosynthetic oscillations observed experimentally.

Source of the oscillations. The model prediction that one or two oscillatory transients appear under a variety of physiologically relevant conditions indicates that there is a great
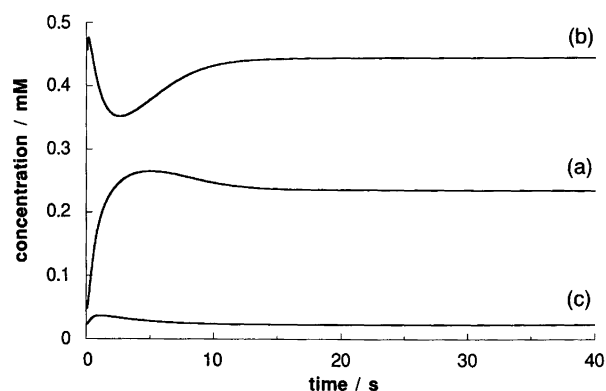

Fig. 1. Trajectories for independent Calvin cycle state variables. The time-course for reattainment of steady-state concentrations of (a) dihydroxyacetone phosphate, (b) ATP and (c) ribulose 5-phosphate, following an initial perturbation of the concentration of dihydroxyacetone phosphate to 20 % of its steady-state value. Qualitatively similar time-courses were obtained for analogous initial perturbations of other concentration variables in the system. Parameter values as in Ref. 11 with $[P_{ex}] = 1.0$ mM.

potential for oscillations in the Calvin cycle. This is consistent with the recent observation that no less than 19 different potential two-reactant sources of oscillations may be analytically identified in the reaction system now considered.[6] The question then arises as to whether the oscillations predicted by the model can be attributed to one of the analytically identified potential two-reactant sources. To answer this question, use was made of the fact that all relevant cases of the latter potential sources involve at least one modifying kinetic interaction (activation or inhibition of a certain enzymic reaction step). The influence of each modifying kinetic interaction on the transient-state kinetics of the reaction system was examined by systematic deletion of the corresponding terms in the rate equations constituting the kinetic model. Somewhat unexpectedly, the oscillatory transient-state rate behaviour of the system was found to persist after elimination of any single modifying kinetic interaction. This means that the oscillations occurring according to the mathematical model do not depend on any particular modifying kinetic coupling. Hence, they cannot be attributed to any one of the analytically identified potential two-reactant sources.

As discussed previously,[6,7,11] all carbohydrate metabolites participating in the Calvin cycle are subjected to a moiety-conservation constraint with phosphate as the conserved moiety and this constraint alone may be sufficient to induce oscillations. Such a mechanism for photosynthetic oscillations has been examined in a simplified form by Giersch[7] and the results were similar to the present ones. This suggests that the phosphate moiety conservation constraint of the Calvin cycle system may be the mechanistic origin also of the oscillations predicted by the model now examined. If so, the triggering 'source' comprises all of the

Table 1. Transient rate parameters of the model examined.[a]

Case	$[P_{ex}]$/mM			λ_k/s^{-1}		
A	1.5	−0.0098	−0.65	−1.2	−6.4±2.0 i	
	1.0	−0.016	−0.57	−0.81	−5.0±1.5 i	
	0.5	−0.021	−0.26	−0.72	−3.8	−4.3
	0.1	−0.0099	−0.032	−0.61	−3.8	−5.8
	0.01	−0.014	−0.040	−2.8±0.58 i		−28
	0.001	−0.0027	−0.00157	−0.85	−2.9	−34
B	1.0	−0.016	−0.67±0.033 i		−5.0±1.3 i	

[a]Rate parameters, λ_k, calculated numerically at different values of the concentration of inorganic orthophosphate in the external medium, $[P_{ex}]$, using previously reported[11] values for (A) all model parameters, (B) all model parameters except K_{i131} which was increased from 2.0 to 3.0 mM. $i = \sqrt{-1}$.

five independent variables in the model and hence the kinetic reaction system as a whole.

Previous investigations of the transient-state rate behaviour of biological systems have usually been based on the assumption that oscillations are generated by a specific source, i.e. by a specific two-reactant subsystem capable of triggering oscillations.[6,7,13–15] From a strictly theoretical point of view, however, such an assumption cannot be generally justified. This is borne out by the present observation that the oscillations predicted by the examined Calvin cycle model cannot be attributed to any one of the analytically identified potential two-reactant sources. Results now reported provide the inference that the mechanistic events and kinetic interrelationships giving rise to metabolic oscillations in realistic biological systems may be so complex that it is not useful to identify a specific source of the oscillations. Unambiguous conclusions regarding the oscillatory behaviour of metabolic networks can be drawn only by characterization of the transient-state kinetic properties of the system as a whole.

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