

## Oscillations in a Chloroplast Suspension System

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**Abstract.** Results of experimental and theoretical study of a photosynthetic system presented with rather complicated temporal behaviour are shown. Experimental results are comparable with those obtained previously by using the electrical method, and thus provide a support to the applicability of the more convenient spectrophotometric method. A possible explanation of a multimode nature of the oscillations observed is presented.

**Key words:** Photosynthetic system — Oscillations — Nonlinear medium — Mode multiplication

### Introduction

Photosynthesis was included in the extensive group of periodic processes quite late. The theory of periodicity of this system was developed many years ago (Benson et al. 1954; Chernavskaya and Chernavskii 1960) but only a few papers dealing with its experimental study have been published so far (e.g. Wilson and Calvin 1955; Butler 1957; Laisk 1977). These studies were based on the elementary knowledge of the photosynthetic assimilation process of carbon dioxide by a plant. If accompanied by the uptake of water and the presence of a photosensitive pigment (usually chlorophyll, located in cell organelles called chloroplasts) as well as external illumination, it results in the production of saccharides and oxygen loss.

The entire mechanism is explained by the ability of plants to absorb the light energy of the sun radiation (absorption of quanta of electromagnetic radiation). The light energy is then transformed to chemical energy. This occurs via electron transfer and combined chemical reactions producing "energy rich" chemical compounds, whereas the yielded chemical energy is used for fixation of carbon dioxide. The weeds and green plants are using light energy of the wavelength range from 400 to 700 nm, and the photosynthesizing bacteria of wider range from 300 up to 950 nm. Photosynthetic assimilation of carbon dioxide takes place in chloroplasts, the cell organelles of green plant tissues. They are oval in shape, 1–10  $\mu\text{m}$  large, arranged in a characteristic lamellar structure (Cerdonio and Noble 1986).

From physical point of view the photosynthetic process consists of two phases (Chernavskaya and Chernavskii 1960):

1. light phase, when absorption and transformation of light energy take place,
2. enzymatic phase requiring energy supply, when reduction of carbon dioxide to saccharides occurs.

It is quite obvious from the facts mentioned above that photosynthesis is sensitive to various external conditions. Studying the temporal behaviour of the system seems to be a convenient possibility how to investigate these effects.

## Materials and Methods

Samples of chloroplast thylakoids isolated from lettuce were used for experiments and the same method as described in our previous paper (Krempaský et al. 1993) with some final modifications:

– the final pellet resuspended in 10 % glycerol solution was stored in a freezer, and “activity” of chloroplasts in the samples was checked after thawing them using a known method, the Hill’s reaction (Hill 1937);

– immediately prior to experiment the suspension was diluted with the damping solution in a 1/18 volume ratio (the composition of the damping solution: 17.115 g of sucrose added to 100 ml of 0.4 mol/l sodium phosphate buffer, pH 7.1).

A glass cuvette (10 × 10 mm) was filled with 1.8 ml of the chloroplast suspension. The absorbance of this sample was measured. White light of a halogen lamp (Narva, 50 W, 12 V) and water filter (optical path 2 cm, to avoid heating of the sample) were used, the cuvette optical path being 1 cm. The outcoming beam was focused on the slit of a colorimeter (Spekol). The detected intensity was amplified and recorded by a computer. A colorimeter emulating the functions of monochromator and photomultiplier was used for our experimental setup, similar to that described elsewhere (Sørensen and Hynne 1989).

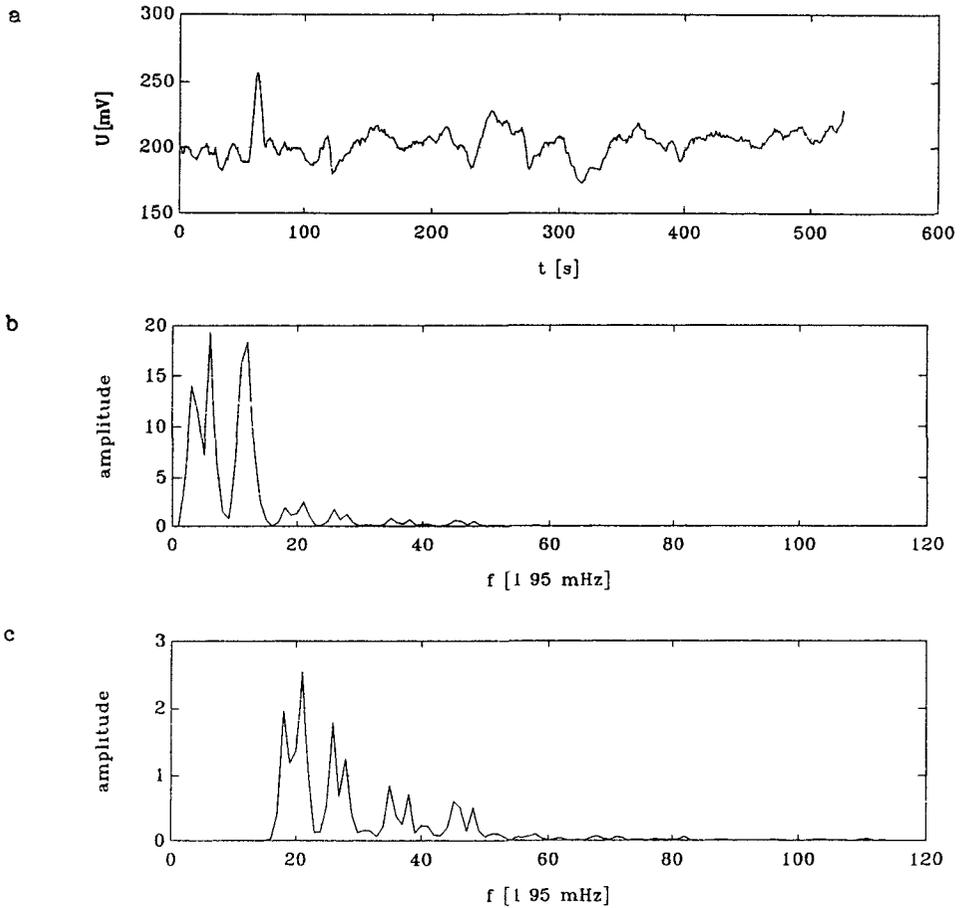
## Results

Figs. 1a and 2a show typical measured time courses of the light-induced absorbance at constant illumination in chloroplast suspension samples at room temperature. Fig. 3a shows the results of the same measurement obtained on the background only. One can see that the background signals are at least one order weaker.

The samples were illuminated by white light of a constant intensity of 140 W/m<sup>2</sup>. The absorbance was measured at 630 nm wavelength.

After mixing all components, the chloroplast suspension was allowed to rest for 2 min prior to starting absorbance measurement. The recordings were carried out within the first 30 min after the suspension preparation as the chloroplast suspension is assumed to keep its properties unchanged during this time (Aon et al. 1988).

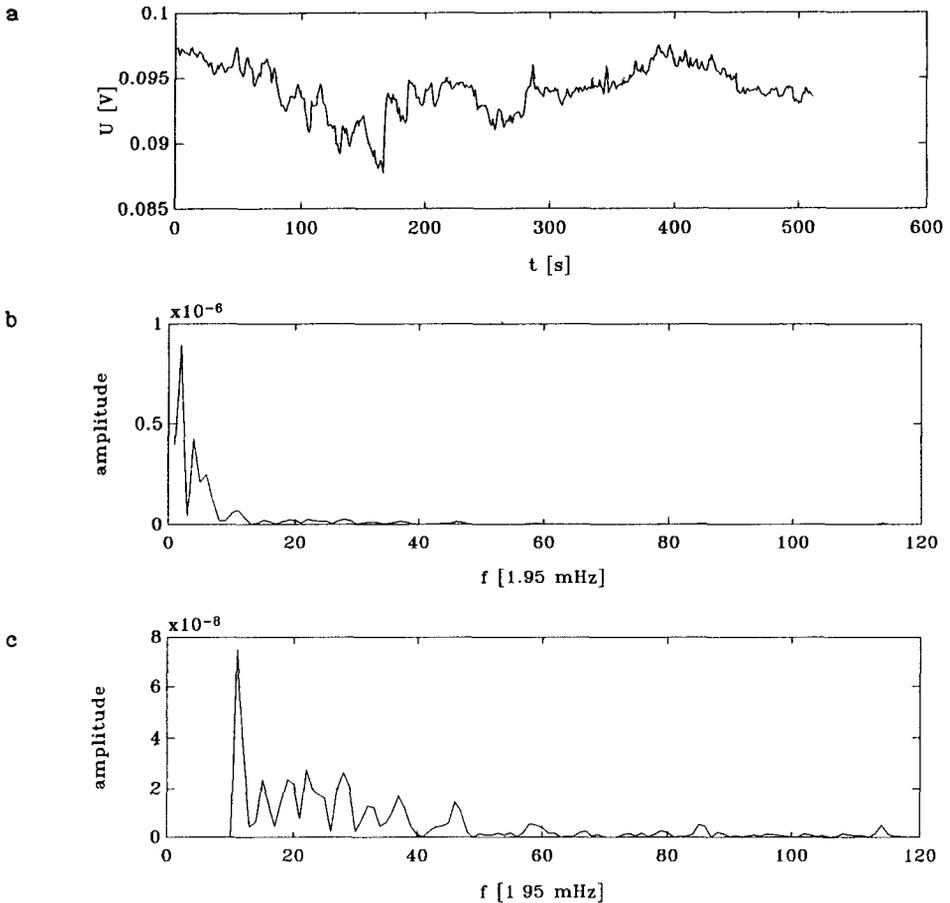
Figs. 1b–3b give the power spectral density obtained by fast Fourier transform.



**Figure 1.** Time course of light-induced absorbance measured on a chloroplast suspension at 630 nm,  $140 \text{ W/m}^2$  (a). Power spectral density (b). Power spectral density with the basic frequency peaks cut off (c).

mation for discrete data (Press et al. 1992). In Fig. 1b, three strong frequency components corresponding to 164 s, 86 s and 43 s appear. In Fig. 2b the peaks corresponding to 234 s, 126 s and 82 s can be seen. Power spectral density of the background measurement is shown in Fig. 3b.

Figs. 1c–3c give the power spectral density with strong frequency components cut off to make the lower frequency peaks visible.

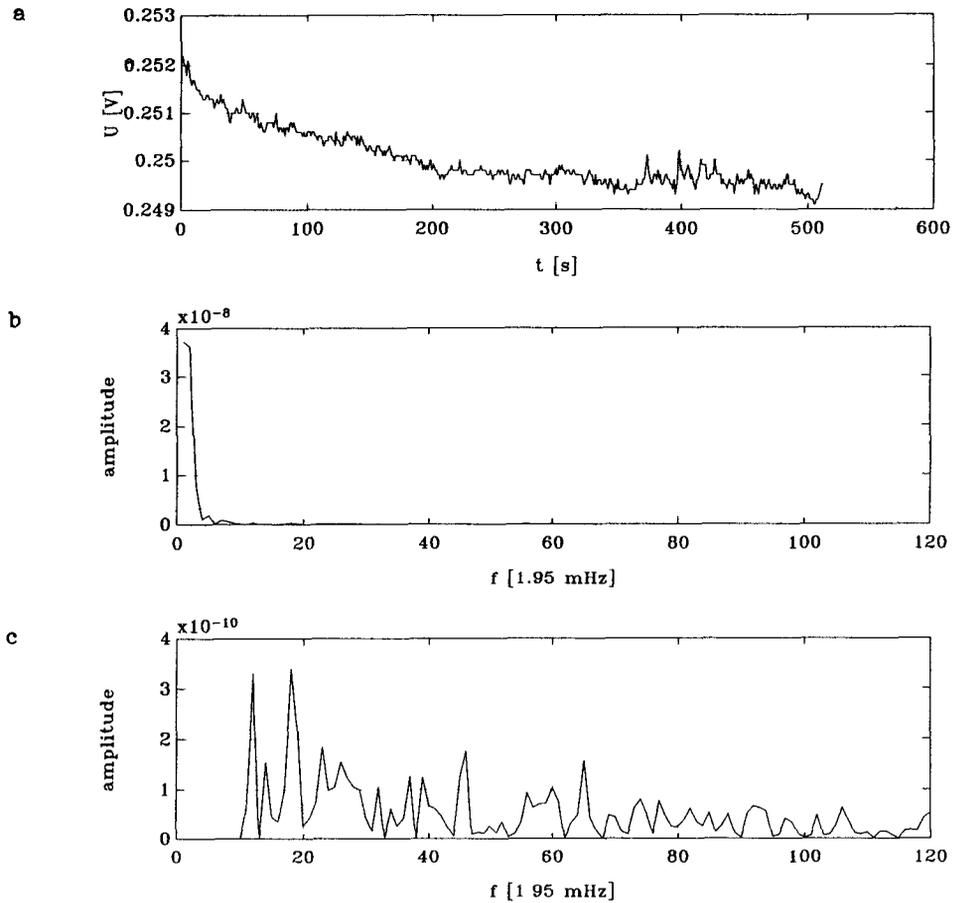


**Figure 2.** Time course of light-induced absorbance measured on a chloroplast suspension in the same conditions as described in Fig. 1 (a), giving different basic frequency peaks in the power spectral density (b). Power spectral density with the basic frequency cut off (c).

## Discussion

The experimental results mentioned above can be summarized as follows:

1. Absorbance variations are detectable in chloroplast suspension samples illuminated by white light.
2. The time course of the light absorbance always shows a complicated oscillatory behaviour.
3. Several discrete frequencies distinctly appear in the Fourier spectrum of the absorbance time course.



**Figure 3.** Time course of light-induced absorbance obtained on the background only in the same conditions as described in Fig 1 (a). Power spectral density (b) Power spectral density with the basic frequency peaks cut off (c).

4. In each spectrum more than 10 different frequency peaks can be distinguished.

In a photosynthetic process light induces the formation of various chemical compounds. Their concentrations can periodically vary over time which is typical of chemical oscillating reactions. The concentration changes were supposed to be detectable by means of absorbance measurements (Pâques and Brouers 1985). We observed "high amplitude" oscillations at the recording wavelength of 630 nm. It falls within the range of 550–680 nm assumed to be a range of high photosynthetic power (Emerson and Lewis 1943). For instance, at  $\lambda < 550$  nm, i.e. blue-green region, incident light is absorbed more by carotenoids than by chlorophyll a.

Chlorophyll a is photosynthetically less efficient in this region, implying that only a smaller portion of the incident light energy is used, thus the total effectivity of the photosynthetic power is decreased. A certain decrease of the photosynthetic power is characteristic for the region of  $\lambda > 680$  nm as well. According to Emerson's hypothesis products of two light-dependent reaction systems (pigment system I and II) characterized by their own production level subsequently react with each other. Here, the difference in production levels is assumed to be the reason of photosynthetic power decrease.

Several periods of photosynthetic oscillations of the order of hundreds of seconds measured at different levels in various photosynthesizing systems have been mentioned in the literature (see, e.g. Wilson and Calvin 1955; Pâques and Brouers 1985; Aon et al. 1988). The basic frequency peaks (Figs. 1*b*–3*b*) obtained in our experiments using a simple spectrophotometric method correspond to the above range ( $10^1$ – $10^2$  s).

## Theory

It seems to be difficult to explain the appearance of more than 10 clearly apparent frequency peaks in each of the Fourier spectra. One possibility is to assume that they are quite reliably reproducible under different circumstances or with using different recording methods; consequently, if this is true it may be stated that photosynthetic oscillations include more than 10 different modes. A question then arises concerning the type of the generating mechanisms they correspond to. A single oscillating system working in deterministic chaos mode has been assumed (Krempaský et al. 1993). However, in such a case the Fourier spectrum shows a typical spread form (Mikhailov et al. 1991), and the spectra in Figs. 1*b*–3*b* do not correspond to it. Another possible explanation, involving an inhomogenous model based on the assumption of a set of more than 10 relatively independent and spatially distributed "biochemical oscillators" running simultaneously or subsequently in the photosynthetic process, each of them characterized by its eigenfrequency, does not seem to be probable either, in particular considering the fact the chemical part of photosynthesis (the so-called Calvin cycle) only involves three relatively independent processes: carboxylation, reduction and regeneration.

Let us consider three autonomous processes in photosynthesis operating in the limit cycle mode thus generating three independent periodical modes. According to this idea the appearance of only three eigenfrequencies would be expected in each time course indeed. In Figs. 1*b*–3*b* we can distinguish several (in some cases exactly three) clean-cut (as if basic) frequencies, however there are additionally approximately ten different modes. The multiplication mechanism of basic modes, due to which essentially more modes (in our case in excess of 10) appear, requires

explanation. We shall try to show that in principle two mechanisms can be operative here:

1. a generating mechanism of higher harmonics in a nonlinear medium,
2. a doubling mode mechanism as a result of the periodic effect on the rate constants of biochemical processes.

Let us assume three systems connected in series oscillating with eigenfrequencies  $\omega_{10}$ ,  $\omega_{20}$ ,  $\omega_{30}$ . The general equation for nonlinear system dynamics is

$$\frac{d^2u}{dt^2} = F(u, u^2, u^3, \dots) \quad (1)$$

where  $u$  is the characteristic state parameter.

Under external periodic influence, the equation for such a system acquires the form

$$\frac{d^2u}{dt^2} = F(u, u^2, u^3, \dots) + A \sin(\omega t) \quad (2)$$

where  $A$  is the amplitude. It is analogical to the problem of interaction of external electromagnetic wave with a nonlinear medium, such as a laser emitting radiation into a nonlinear medium. It is known that in such a case the generation of a new wave of  $m$ -multiple angular frequency corresponds to each term of the integer nonlinearity order ( $m$ ). For instance,

$$y = A \sin(\omega t) + \frac{bA^3}{32a^2}(\sin(\omega t) - \sin(3\omega t)) \quad (3)$$

appears to be the solution to the equation

$$\frac{d^2y}{dt^2} = -ay - by^3 + C \sin(\omega t) \quad (4)$$

where  $a$ ,  $b$  and  $C$  are the constants. The appearance of higher (second and third) harmonics was indeed confirmed. Thus, if a periodic signal is applied to second and third oscillator, higher harmonics of  $2\omega$  and  $3\omega$ , can be generated here in addition to the basic enforced frequency.

The known Kuramoto-Shivashinskii equation (Akhromeieva et al. 1989)

$$\frac{du}{dt} = (a + i\omega)u - b|u|^2u \quad (5)$$

where  $u$  is the characteristic state parameter, serves as a starting point of the mathematical analysis of biochemical oscillators. Assuming a solution

$$u(t) = u_0(t) \exp[i\varphi(t) + i\omega t] \quad (6)$$

it is easy to prove that the system described by equation (5) shows oscillations of the limit cycle mode expressed by

$$u(t) = \left(\frac{a}{b}\right)^{1/2} - \exp[i\varphi(0) + i\omega t]. \quad (7)$$

Using equation (5) it is easy to prove (Haken 1983), that if a mode of angular frequency  $\omega$  is present in the system, a mode of angular frequency  $\omega/2$  can also be generated in certain circumstances. If the concentration of ions varies periodically in time, the corresponding electromagnetic field affecting the values of rate constants of biochemical reactions varies in time as well. Let us vary, for instance, the value of constant  $b$  with angular frequency  $\omega$  in the equation (5), to yield

$$b = b(0) \exp[-i\omega t]. \quad (8)$$

Inserting it into equation (5) we obtain

$$\frac{du}{dt} = (a + i\omega)u - b(0) \exp[-i\omega t]|u|^2 u. \quad (9)$$

Its solution is expressed by the function

$$u(t) = u_0 \exp[i\varphi(0)] \exp\left[i\frac{\omega}{2}t\right]. \quad (10)$$

Thus, a mode of half-value angular frequency appears in the system in these circumstances as well. In theory multiplication mode concerning the oscillators in question is also possible. Several combinations can be found resulting in more than 10 modes at the output.

## Conclusion

The aim of this paper was to present temporal behaviour of a chloroplast suspension system under constant illumination as studied by means of the spectrophotometric method. The behaviour of the system studied corresponds to what has been reported previously about results obtained by a more complicated electric measuring method on the same system (Krempaský et al. 1993). Nevertheless, the chemical intermediates which take part in the oscillation process still remain to be determined. The measured time interval was relatively short because decreasing "activity" of chloroplasts after the first 30 min of measurement presents a limiting factor. Therefore, the Fourier spectra are not sufficiently reliable due to insufficient number of points. The idea is to find a method to prolong the "lifetime" of chloroplasts with the aim to obtain more statistically relevant data. The theory presented herein was aimed at giving possible explanation of the nature of the Fourier spectra obtained. It was shown that the theory of mode multiplication in periodic conditions enables to derive numbers of modes similar to those observed experimentally.

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