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TRANSIENT INDUCED OSCILLATIONS IN THE LEVEL OF ATP IN *CHLORELLA FUSCA*

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SUMMARY

After changing an environmental condition, light intensity or CO_2 concentration, the ATP level in whole cell populations of *Chlorella fusca* shifts rapidly, then gradually returns to the original value in characteristic oscillations. The length of one period is 40 s and seems to be independent of the temperature between 18 and 25 °C. These oscillations may reflect the simultaneous action of the different control systems for ATP producing, consuming and translocating reactions located in different compartments of the eukaryotic cell.

INTRODUCTION

Oscillations and clock phenomena in biological systems have been well known for many years and can be seen in plants, animals and microorganisms. They seem to be a fundamental characteristic of life. The increasing literature on oscillatory phenomena in biochemistry has been summarized recently by Hess and Boiteux[1]. Most of the cited work deals with oscillations in glycolysis. Only a little information is available on rhythmic changes during photosynthesis. ATP has a central position in the metabolism of all organisms. While many reactions are known which are driven by the energy of hydrolysis of ATP, there are only a few known in which ATP is formed from ADP in substantial amounts. In the green cell such reactions are photophosphorylation in the chloroplasts and oxidative phosphorylation in the mitochondria. The level of ATP seems to be in a definite relationship to the cell carbon under various environmental conditions[2,3]. Also it is well known that the ATP concentration shows dramatic changes after altering environmental conditions, *i.e.* the light. After a certain time the original ATP level is reestablished under the new conditions[2-6]. The ATP producing and consuming reactions seem to equilibrate during a transition time of several minutes. Depending on the accuracy and the complexity of the regulatory system, these transitions should show some more or less pronounced oscillations rather than be continuous and asymptotic. Small rhythmic changes are indeed seen in the concentrations of intermediary products of photosynthesis[7] and in the level of free phosphate in algal cells[8] after light to dark transitions. The present paper describes the appearance of quite large oscillations in the ATP level in whole cells of *Chlorella fusca* after sudden environmental changes. Some of these results were described previously[9].

MATERIAL AND METHODS

The organism used in all experiments was *Chlorella fusca*, strain 211-8b (Algensammlung Göttingen). The algae were grown continously with turbidostatic control in a 5-l fermenter (Chemap GF 007) in the medium of Kessler and Czygan[10]. The cell suspension was kept homogeneous by stirring with a triple flat blade turbine at 2000 rev./min. A detailed description of the fermentation apparatus is given by Schneider[11]. The absorbance of the suspension was kept at 0.25 at 570 nm with a light path of 0.1 cm in a Hitachi 101 spectrophotometer. To ensure a fast sampling rate, an excess pressure was produced in the fermentation vessel, forcing the cells out. The time required to transfer the cells from the fermenter to the killing reagent was less than 1 s. To stop the metabolism immediately, the cells (4.5 ml) were injected into precooled perchloric acid (final concn 2 %), mixed rapidly and cooled to 0 °C in liquid N_2. At this temperature ATP was extracted by the perchloric acid for 40 min. The suspension was then neutralized to pH 6.8–7.4 with 2 M K_2CO_3. After cell material and insoluble $KClO_4$ were removed by centrifugation, the ATP was determined in the supernatant liquid by the luciferin–luciferase method[12]. The apparatus used to measure the light produced by luciferase was a gift from Dr. Welsch. High sensitivity and reproducibility were obtained by using an electric syringe (Fisons diluter) for the enzyme injection. Since the extraction of the ATP was not completed even after 2 h, a strict standardisation was absolutely necessary for reproducible results. The time periods for which each sample was kept in liquid nitrogen and in the perchloric acid for ATP extraction and the time interval until the determination of the ATP were held constant for all the samples. The reproducibility of these determinations as seen in multiple assays for calibration was $\pm$ 2 %. This also seemed at least to be the overall reproducibility of the ATP determination in the cells, as judged from the measurements of ATP in the cells before the environmental changes inducing the described oscillations in the level of ATP. CO_2 in the gas phase was determined with an infrared gas analyzer (Beckman 215).

RESULTS

Light to dark transitions

Fig. 1 shows the changes in the ATP content of the cells in two similar but independent experiments during the first 3 min after turning off the light. 20 s after this environmental change the level of ATP reached a minimum, which was about 50 % of the original value in the light. Then the level of ATP in the cells increased again showing strong oscillations having a period of around 40 s. The amplitude seemed either to increase or to remain constant during the first oscillations. Later the ATP level continued to oscillate but the original wave-form disappeared (Fig. 2), possibly because of the successive loss of the synchronisation effect of the light to dark transition. Even 30 min after darkening, irregular fluctuations of $\pm$ 10 % were seen when samples were taken in 8-s intervals. After 24 h under constant conditions the results of the ATP determination were finally within the experimental error of the method $\pm$ 2 %).

Dark to light transitions

When changing from dark to light (Fig. 3), an opposite response of the ATP level in the *Chlorella* cells was observed. Within 20 s the ATP content increased by 50% followed by a rapid decrease occuring in oscillations of 40-s periods as described before. After a few minutes the original value was reached again, but fluctuations of a more or less irregular nature went on as before.

Influence of temperature

Similar experiments were done at 25 and 18 °C. As shown in Fig. 4, lowering the temperature from 25 to 18 °C had little influence on the frequency of the initial oscillations, however, the amplitude seemed to be reduced significantly. The first

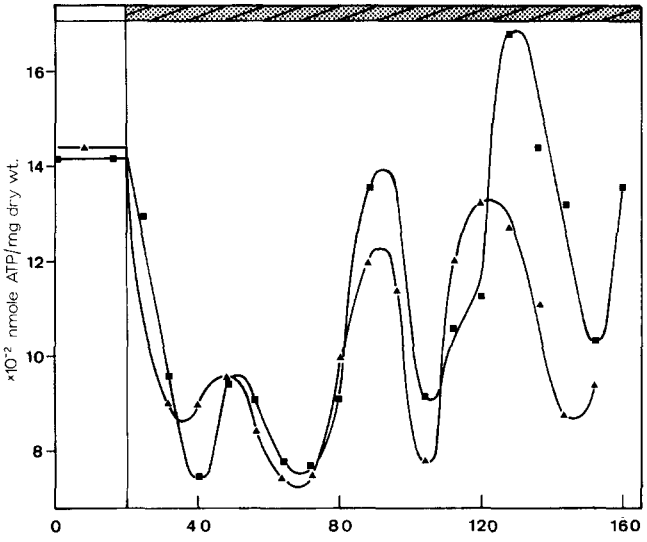

Fig. 1. Changes in the level of ATP during 3 min after a light to dark transition in two independant experiments. Conditions: sampling intervals 6–8 s; stirring, 2000 rev./min; temp., 25 °C; pH 6.0; light, 24 fluorescent lamps 8 W each, resulting in 10^5 ergs·cm^{-2}·s^{-1}; gassing with air (CO_2 content 350 ppm); density of the cell population: absorbance at 566 nm in a 0.1-cm cuvette = 0.25.

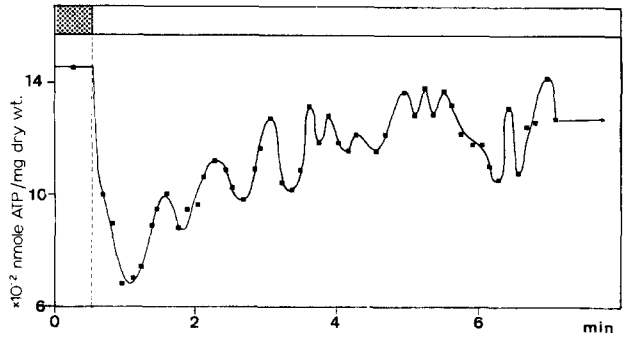

Fig. 2. Changes in the level of ATP during 8 min after a light to dark transition. Sampling intervals 8 s, other conditions as in Fig. 1.

minimum after 20 s was lacking, indicating a possible temperature dependence of the initial regulatory process.

Reaction to changes in the CO_2 concentration

By adding CO_2 absorption flasks to the gas circuit, the CO_2 content of the incoming air was lowered from 375 ppm to zero. As seen in Fig. 5, the CO_2 concentration in the outgoing air dropped from 130 to 10 ppm within 2 min. A rapid increase in the ATP content was measured simultaneous to the change in CO_2.

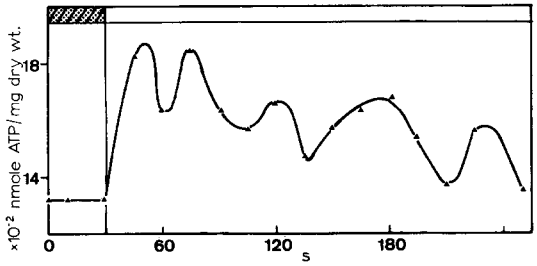

Fig. 3. Changes in the level of ATP during 4 min after a dark to light transition. Sampling intervals 15 s, temp. 18 °C, other conditions as in Fig. 1.

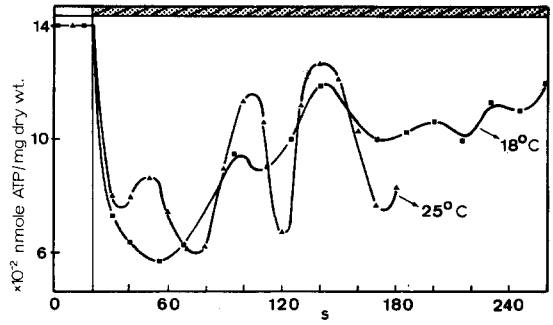

Fig. 4. Changes in the level of ATP during 4 min after a light to dark transition at 25 °C and 18 °C. Sampling intervals 8 s, other conditions as in Fig. 1.

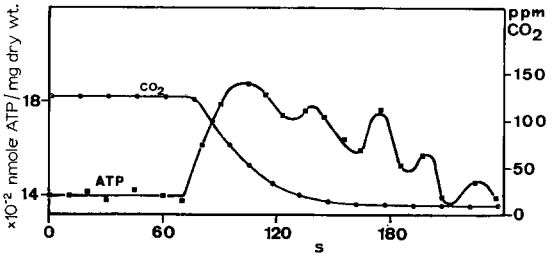

Fig. 5. Changes in the level of ATP during 3 min after lowering the CO_2 concentration in the medium. CO_2 concentration in the outflowing gas indicated on the right-side ordinate. Sampling intervals 6–10 s, other conditions as in Fig. 1.

Again a maximum was reached after 20 s. In several oscillations the ATP level dropped back to the original value in about 3 min. The impressive reaction of the ATP level to a decrease in CO_2 concentration of only 25 % shows how sensitive the response of ATP is to environmental changes that can influence photosynthesis.

DISCUSSION

The results described show clearly that the equilibrium state of the ATP level in the cell is upset after drastically altering the environment and is reestablished not by a continuous change but by large and probably undamped oscillations. This phenomenon suggests a complex regulatory system.

The decrease in the ATP level after turning off the light is certainly due to the sudden interruption of photophosphorylation. For a short time the ATP consuming reactions outweigh the producing reactions, resulting in a rapid decrease in the ATP level. The following increase may reflect an enhanced respiratory activity and ATP production in the mitochondria. Also a strong and simultaneous inhibition of the ATP consuming reactions in the chloroplast may cause an increase in the level of ATP. Since ATP seems to permeate the chloroplast membrane freely[13,14], inhibition of respiration in the light by a high ATP/ADP ratio would be reversed in the dark and the ATP subsequently produced outside the chloroplast could be translocated into the chloroplast and used there in energy-coupled dark reactions. The opposite reaction of the ATP level in the cells after dark to light transitions is certainly due to the immediate activation of the phosphorylation in the light, producing a high ATP concentration in the chloroplast. The decrease to the original value seems to be a result of the rapid activation of ATP consuming reactions in the chloroplast and the inhibition of respiration by ATP leaking out of the chloroplast. Since the ATP level in isolated chloroplasts remains high in the light[15], the drop in the ATP content in whole cells may be due mainly to the inhibition of respiration.

The increase in ATP seen after a sudden withdrawal of CO_2 may be explained by a decrease in the rates of some of the ATP consuming reactions in the chloroplast. The regulation back to the original level could be the result of a further inhibition of the respiratory activity in the light. On the other hand, several recent investigations show that electron transport in chloroplasts is also controlled by the ATP/ADP ratio[14,16]. Therefore, a high ratio would reduce further ATP production by photophosphorylation.

Oscillations are specific indicators of regulation and are well known in biological systems. In a simple open chain of reactions like glycolysis, the observed oscillations can be explained by assuming only one regulatory point, the activity of phosphofructokinase. On the other hand, the described photosynthetic system is a closed one and is complicated greatly by the existence of several ATP producing and numerous ATP consuming reactions. Furthermore, in our system the different producing and consuming reactions are often separated from each other, and the regulation of each reaction occurs in specific compartments of the cell. The regulation at different sites is further complicated by the translocation of ATP, either passive or by carrier, among the different compartments. The sum of the mentioned systems, individually regulated and connected by translocation, may be reflected in the observed oscillations of the ATP level in the cell.

Oscillations of similar frequencies are known in other photosynthetic systems. After light to dark transitions oscillations of the membrane-potential in Hydrodiction[17] and of the oxygen evolution rate in *Chlorella*[18] have been observed. As in our experiments, these different oscillations found in whole cell populations

are seen only after synchronizing the metabolism by suddenly changing an environmental factor.

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