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EFFECT OF COPPER ON PHOTOSYNTHESIS IN SYNCHRONOUS *CHLORELLA* CELLS

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Abstract

The inhibitory effect of copper on photosynthesis in ***Chlorella*** cells was investigated with special observation of the cell stage. During the light period of a synchronous culture treated with light/dark change the senstivity of O_2-evolution to Cu^{2+} fluctuated. The most sensitive stage occurred between 6th and 8th hour after illumination and the most insensitive one near the end of light period. Also the carbohydrate synthesis at these two stages was differently influenced by Cu. The study of the effect of Cu on fluorescence emission at low temperature showed that PS II was more susceptible to Cu than PS I. The results pointed out that both the light and dark reactions of photosynthesis were only partially inhibited by Cu. The possible action of Cu on photosynthesis *in vivo* was discussed.

Introduction

Copper is a well known algicide and has been proved to be able to directly influence the photosynthesis of algae (Greenfield, 1942; McBrien *et al.*, 1967; Gross *et al.*, 1970; Steemann Nielsen *et al.*, 1969, 1971). The site on which Cu might affect has also been studied. In general, Cu has been found to exert its inhibitory effect either on the oxidizing site of photosystem II (Cedeno-Maldonado *et al.*, 1972; Habermann, 1969) or on the reducing site of photosystem I (Shioi *et al.*, 1978a, b). A direct mediation of Cu in the peroxidation of photosynthetic membranes and chlorophyll molecules has been mentioned (Sandmann *et al.*, 1980, 1982). Moreover, Cu has been found to influence the photosynthetic energy conversion (Uribe *et al.*, 1982). However, most of these results were obtained from the study either with isolated chloroplasts or with broken cell fractions. Substantially the effect of Cu in intact cells might be quite different from that observed *in vitro*. In this article the effect of Cu on photosynthesis was revised with studying with intact *Chlorella*

cells. For this purpose of study a synchronous cultures was used. The inhibitory effect of Cu on photosynthesis was investigated with special observation of cell stage.

Materials and Methods

Chlorella fusca var. *vacuolata* (strain 211-11 m from the Collection of Algae in Göttingen (SAG), G. F. R.) was photoautotrophically cultivated as described by Lorenzen (1968). Nutrient solutions were prepared according to Kuhl (1962). The synchronous culture was obtained by cultivating under light/dark change (LD) of 14:10 hours at 32°C with daily diluting the culture to constant cell number of 1.56×10^6 per ml before illumination. The photosynthetic activity was estimated by O_2-evolution that was polarographically measured with a Clark-type oxygen electrode as described by Tischner (1974). Carbohydrate was quantified with the anthrone reaction (Roe, 1955). Chlorophyll was determined according to Mackinney (1941). Copper was added in form of sulfate salt dissolved in water. For measurement of fluorescence emission at 77°K algal suspensions were adsorbed onto a cheese-cloth after concentrating with centrifugation. The samples were then rapidly cooled to 77°K with liquid nitrogen. The fluorescence emission, excited at 430 nm, was measured with the instrument set up by Harnischfeger (1977).

Results

To study the time course of Cu effect on photosynthetic O_2-evolution 100 μM Cu, the critical concentration resulting in the total inhibition of cell growth, was added to algal culture already grown 6 hours in the light. The change of O_2-evolution due to Cu-treatment was then measured with an O_2-electrode. As shown in Fig. 1, the inhibition of O_2-evolution was detectable immediately after the addition of Cu. The inhibition was diphasic in the time course. When algal culture was incubated in the light (L) after Cu-treatment, only about 20% of the total O_2-evolution was inhibited within first 30 min. A pronounced increase in inhibition was detected between 30 and 60 min after Cu-treatment. The maximal inhibition was reached after 60 min. When the Cu-treated culture was incubated in darkness (D) instead of in L, the diphasic course of inhibition was still visible. However, the inhibitory effect of Cu has been lowered to some extent.

Basing on the results shown above the study of the concentration spectrum of Cu for the inhibition of O_2-evolution was undertaken 60 min after Cu-treatment. As shown in Fig. 2, the critical concentration resulting in a marked inhibition of O_2-evolution was 10 μM. Between 20 μm and 100 μM the effect of Cu on inhibiting O_2-evolution was approximately proportional to the added concentrations. It is worth to note

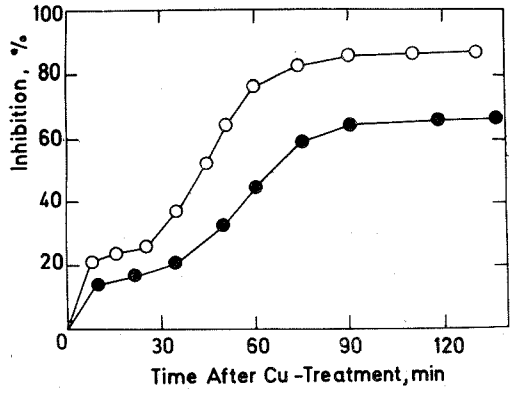

Fig. 1. Time course of the inhibition of photosynthetic O_2-evolution by copper in *Chlorella*. Algal culture which had already grown 6 hours in the light was treated with 100 μM Cu and then incubated in the light (○) or in the dark (●).

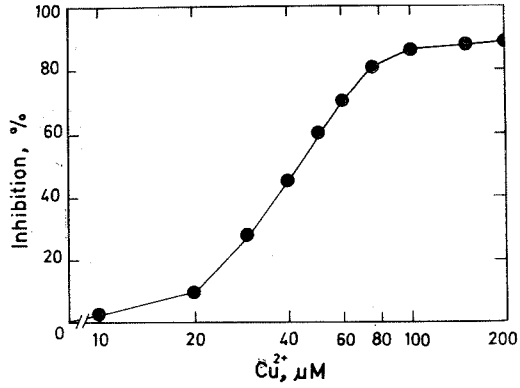

Fig. 2. Inhibition of photosynthetic O_2-evolution in *Chlorella* cells already grown 6 hours in the light due to increasing copper concentrations.

that the addition of lethal dose of Cu, over 100 μM, did not result in a total inhibition of photosynthetic O_2-evolution.

It has been established that the photosynthetic activity of algae fluctuates during the LD-cycle (Pirson *et al.*, 1958). For this studied algal strain the activity of O_2-evolution increased after onset of light and reached its maximum at the fourth hour in L-time (Fig. 3). The sensitivity of O_2-evolution to deleterious Cu also

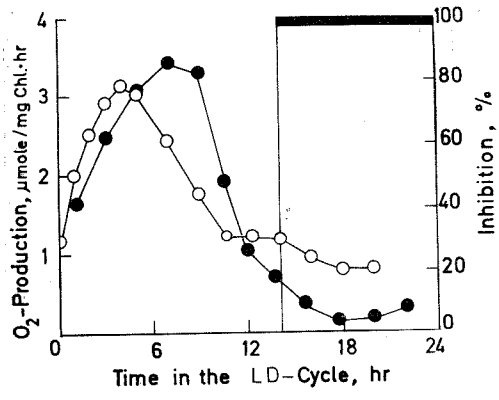

Fig. 3. Photosynthetic activity (○) of *Chlorella* and its sensitivity (●) to toxic copper (100 μM) added at different stages in LD-cycle.

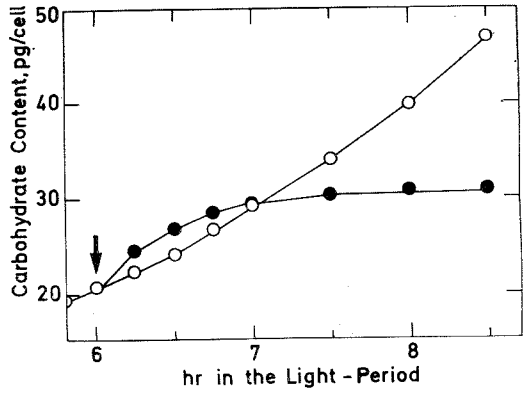

Fig. 4. Course of the change of cellular carbohydrate content in *Chlorella* already grown 6 hours in the light due to the treatment of 100 μM Cu (●) in comparing with the control cells (○). ↓: addition of Cu.

varied throughout the LD-cycle. When 100 μM Cu was added to algal culture at different stages in LD-cycle, the sensitivity of cells to Cu could be estimated by measuring the decrease in O_2-evolution at 60th min after Cu-treatment. As shown in Fig. 3, there was a highest sensitive stage occurring between 6th and 8th hour after onset of light. Thereafter the sensitivity to Cu decreased with increasing the time in L. In the L-time, the cells around the end of illumination had the lowest sensitivity to Cu. The lowest sensitivity of O_2-evolution to Cu throughout the LD-cycle was in the D-time, between 18th and 20th hour from the beginning of LD-cycle.

Both the time courses of fluctuation of photosynthetic activity and its sensitivity to Cu during the LD-cycle did not meet well with each other. The highest sensitive stage to Cu did not fall into the stage with highest photosynthetic activity. The inhibitory effect of Cu on photosynthesis therefore seems not to be correlated with the activity of photosynthesis.

To investigate the effect of Cu on the synthesis of photosynthates the change of carbohydrate content due to Cu-treatment was measured. 100 μM Cu was added to the culture from the most sensitive stage as well as from the less sensitive stage, 6th and 13th hour in L-time, respectively. In the test with cells already grown 6 hours in L the Cu-treated cells had higher carbohydrate content than control cells (Fig. 4). The Cu-treated cells had still increased their carbohydrate content for at least one hour. In cells already grown 13 hours in L the Cu-effect

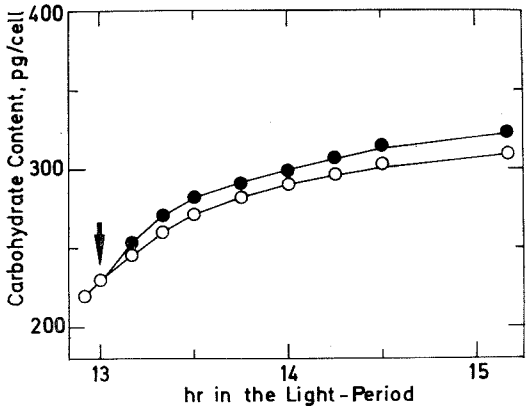

Fig. 5. Course of the change of cellular carbohydrate content in *Chlorella* already grown 13 hours in the light due to the treatment of 100 μM Cu (●) in comparing with the control cells (○). ↓: addition of Cu. Algal cultures were not transfered to darkness after 14th hour.

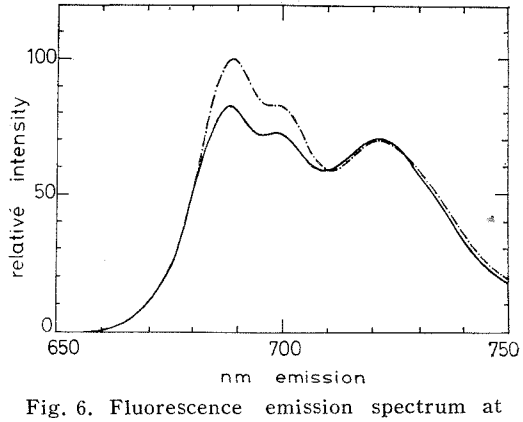

Fig. 6. Fluorescence emission spectrum at 77°K of *Chlorella*. Samples were excited at 430 nm. Dotted line: control cells; solid line: cells treated with 100 μM Cu.

on influencing the synthesis of photosynthates is somewhat different from that observed above. Although the Cu-treated cells had also higher carbohydrate content than control cells, a cease in increase of carbohydrate content due to Cu-treatment was not observed (Fig. 5). Virtually, the Cu-treated cells increased their carbohydrate content with nearly the same rate as the control cells during the test time up to two hours.

An another approach to study the effect of Cu on photosynthesis is to measure the change of fluorescence emission due to Cu-treatment. The fluorescence emission of *Chlorella* at 77°C had three visible characteristic peaks localizing at 686, 698 nm, both belonged to photosystem II (PS II), and 725 nm which could be assigned to PS I (Harnischfeger, 1977). When algal cells were treated with 100 μM, the fluorescence emission after one hour showed a marked decrease in intensity at the both peaks belonging to PS II. The emission peak at 725 nm was little affected by Cu-treatment (Fig. 6). It is evident that the treatment of algal cells with lethal dose of Cu does not result in a decrease in fluorescence intensity to a large extent. No more than 20% of fluorescence intensity was decreased due to the treatment of 100 μM Cu indeed.

Discussion

The results demonstrated above show that there is a delay in the expression of inhibitory effect of Cu on photosynthetic O_2-evolution. To reach the maximal inhibition about one hour treatment was needed. To explain this phenomenon the uptake of Cu by cells was taken into consideration. Foster (1977), Butler *et al.* (1980) and Wu (1980) had showed that a great part of the uptaken Cu were excluded by plasmalemma on cell surface. The delayed expression of Cu-effect on O_2-evolution might probably be correlated with this exclusion system. The rapid increase in inhibition of O_2-evolution might be resulted from the damage of plasmalemma by Cu.

It has been established that the plasma membrane may change their properties such as permeability during the life cycle of cells (Paech, 1940; Fischer, 1950; Biebl *et al.*, 1955). During the LD-cycle of *Chlorella* there is also a physiologically sensitive stage (Tischner, 1974; Nelle, 1976; Wu, 1980) which approximately meets with the stage of the initiation of DNA synthesis and the first cell division (Pirson *et al.*, 1959). It is supposed that the fluctuation of the sensitivity of photosynthesis in *Chlorella* to Cu might be linked with the change of the membrane property, i.e. permeability, during the LD-cycle.

It is interesting that the Cu-treated cells have in general higher carbohydrate content shortly after Cu-treatment than the untreated cells. Probably it is resulted from the accumulation of photosynthates due to the disturbance of the transforma-

tion of photosynthates to other cellular substances by Cu. Comparing the time course of inhibition of photosynthate synthesis with that of O_2-evolution at different stages in LD-cycle it is found that both of the inhibitions follow approximately in similar course. It is therefore likely that the inhibition of photosynthate synthesis is linked with the inhibition of O_2-evolution.

The use of fluorescence emission at low temperature as the analysis of photosynthesis has been well developed and the general considerations for this method has been noted and discussed (Harnischfeger, 1977). Arndt (1974) has developed a method to investigate the toxic effects of Cu and other heavy metals on chloroplasts by means of fluorescence emission, so-called Kautsky-effect. The toxic effect of Cu can clearly be observed from the decreasing intensity of fluorescence emission by his method. The results demonstrated above show however that only the fluorescence emission of PS II is significantly influenced by Cu. This fact meets well with the assumption mentioned by Cedeno-Maldonado *et al.* (1972) that PS II is the site of highest sensitive one to Cu.

It is proposed that magnesium atom in the center of chlorophyll molecule may be displaced by Cu (Gross *et al.*, 1970). Such displacement in atom may result in the change of chlorophyll property (De Filippis, 1979). This displaced molecule, Cu-porphyrin, does not fluoresce (Haurowitz, 1935). A decrease in fluorescence intensity can thus be observed in emission spectrum after Cu-treatment.

Other important actions of Cu on inhibiting photosynthesis might be of the Cu-mediated peroxidation of photosynthetic membranes or the inhibition of energy conversion by Cu. Sandmann and Böger (1980) have shown that Cu may catalyze the formation of hydroxyl radical and Fenton-type reactions that result in the destruction of unsaturated membrane fatty acids. The inhibition of photosynthetic energy conversion due to a Cu-mediated oxidation of sulfhydryl groups on coupling factor 1 was noted by Uribe *et al.* (1982). Both of the Cu-mediated oxidations may all result in the change of the functions of photosynthetic apparatus and therefore also the fluorescence emission.

In conclusion, the inhibitory effect of Cu on photosynthesis *in vivo* is dependent upon the permeability of plasmalemma, which might fluctuate during the LD-cycle of cultivation. The inhibitory effect of Cu is versatile. However, PS II is the highest sensitive site to Cu. Among the light and dark reactions of photosynthesis the inhibition by Cu other than on PS II seems to be of little importance to the inhibitory effect of Cu on photosynthesis.

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銅對綠藻在同步培養下光合作用之影響

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銅對綠藻（*Chlorella*）光合作用之抑制作用與藻細胞發育階段有關。在不同之發育階段，光合作用之氧氣產生能力，對銅有不同之敏感度。約在光期之第六至八小時爲最敏感期，而在光期之末期及暗期爲最不敏感，此敏感性與光合作用之活性無關，而與細胞膜之透析性直接有關。不同敏感時期之合成醣類，受銅之抑制亦有不同。從結果顯示受銅抑制較大的，不是在醣類合成，而在氧氣產生之初期階段。以低溫螢光檢定銅之抑制作用，顯示銅之主要作用確在第二光系統，第一光系統少受其影響。銅抑制光合作用之其他可能途徑，並予討論之。