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Certain aspects of the chemotaxic reaction of chloroplasts in *Funaria hygrometrica*

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Abstract

1. The investigations here described were undertaken in order to establish whether the products of glycine oxidation — a reaction catalysed by the specific glycine oxidase — when applied in the external environment of *Funaria hygrometrica* leaves and penetrating into the leaf cells — have a chemoinductive effect on chloroplast translocation.
2. The experiments were performed both in light and in darkness with glyoxalate and glycine, and with NH_4 and H_2O_2 only in light.
3. Glyoxalate (10^{-4}M) exhibits a marked chemoinductive effect both in light and darkness, whereas glycine only in light, the remaining above mentioned compounds show no activity.
4. Other compounds with a similar chemical structure such as glycollate, glyoxal, oxalic acid and acetic acid were tested. None of them influence chloroplast translocation in *Funaria* leaves.

INTRODUCTION

In the moss *Funaria hygrometrica*, as in many other plants, the chloroplasts can change their position in the cytoplasm layer of the cell. Light has long been known to be a factor exerting an important influence on the change of position of the chloroplasts.

In cells of leaves kept in darkness all chloroplasts are arranged along the walls adjacent to the neighbouring cells. Since the leaves of *Funaria* have one cell layer the walls between them are the side walls. The outer walls of the cells on the edge of the leaf are deprived of chloroplasts.

In cells exposed to light of low intensity the chloroplasts move to the walls perpendicular to the incident light and remain in what is known as flat arrangement. Under strong light, however, they move

to the side walls forming what is called a profile arrangement (Senn 1908).

So far the mechanism of these movements is quite obscure. Zurzycki (1972) advanced a hypothesis which would explain the primary reaction in chloroplast translocations induced by light. This hypothesis is based on the fact that in the chloroplasts glycollate is formed as product of the Calvin cycle, and is further oxidized to glyoxalate in the glyoxysomes. From the latter compound, by way of transmination, glycine is formed (Tolbert, Yamazaki, 1969), which serves as substrate for the enzyme glycine oxidase.

It is postulated that specific glycine oxidase is the key enzyme for reactions associated with chloroplast translocation. This enzyme is present in the gel of the cell membrane complex and it couples with FMN, the molecules of which are oriented towards the cell wall. Glycine oxidase is light-sensitive (Schmid and Schwarze 1969).

When glycine leaves the glyoxysomes it may be transformed via serine to glucose, or it is oxidated by glycine oxidase to glyoxalate which returns to the glyoxysomes. One or several of the products of this enzymatic reaction (glyoxalate, NH_4, or H_2O_2) which are present in higher concentrations near the cell wall may change the local conditions for the chloroplasts.

Thus, photoinduction in the translocation of chloroplasts consists in chemoinduction by an adquate concentration of products of enzymatic reaction controlled by light. Exposure of the cell to light changes the local concentration of these substances and the space in which at the moment optimal concentration of the products prevails becomes a centre of attraction for the chloroplasts. In optimal conditions the chloroplasts become anchored in the gel layer of the cytoplasm so that it is difficult to centrifuge them out (Virgin 1951, 1952, 1954; Seitz 1967; Zurzycki 1972).

The present study was undertaken to find out if, and in what extent, the products of the above described enzymatic reaction stimulate chloroplast translocation. It had to be established whether a certain concentration gradient produced by the external application of adequate NH_4Cl, H_2O_2 or glyoxalate and glycine concentrations will induce a chemotaxic reaction of chloroplasts.

MATERIAL AND METHODS

Funaria hygrometrica leaves collected from natural habitats, and in winter from a light thermostat were used for the experiments.

The material was prepared after Senn (1908). This method consisted in placing the leaves previously exposed to light or kept in darkness on

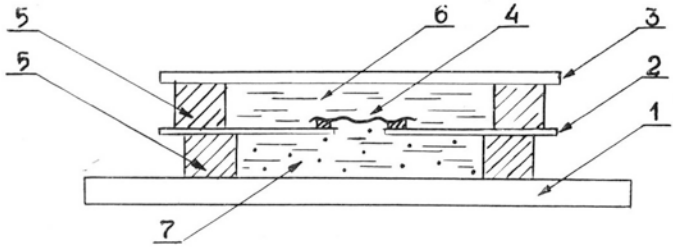

Fig. 1. Diagram of *Funaria hygrometrica* leaf preparation
1. Slide; 2. mica slide with bored hole; 3. cover slip; 4. leaf stuck with gelatine; 5. vaseline rings; 6. water; 7. solution of compound tested

a perforated mica slide over the hole. A suitable solution was put on a slide under the mica plate and water was poured over the leaf. Owing to diffusion of the solution to the leaf cells, an appropriate concentration gradient was achieved stimulating in certain cases chloroplast translocation.

The preparation was as follows: freshly cut leaves were stuck with 10 per cent gelatin to the mica slide over the bored hole 0.8 cm in diametre. On a slide several drops of a suitable solution were placed in a circle of vaseline and the mica slide was put on top with the leaf on its upper side. In another vaseline circle on the mica slide distilled water was placed and all was covered with a cover slip (Fig. 1).

The following chemical compounds were used:
Glycine from 10^{-2} to 10^{-6}M
Glyoxalate from 10^{-2}M to 10^{-6}M
NH_4Cl 10^{-2}, 10^{-4}, 10^{-6}M
H_2O_2 10^{-2}, 10^{-4}, 10^{-6}M
Glycollate 10^{-2}M
Glyoxal 10^{-2}M
Acetic acid 10^{-2}M
Oxalic acid 10^{-2}M
Usually the pH was adjusted to 5.6—5.7 with 1 per cent KOH.

For control of the ability of chloroplasts to chemotactic translocation, 0.25 per cent $MgSO_4$ was used, since, as reported by Senn (1908), this solution has a marked chemotaxic influence on the chloroplasts of *Funaria* leaves, and it is the most active from among the 15 inorganic compounds tested.

The preparations were irradiated 2h with dispersed light and then placed in darkness for 18 h. In some experiments material was pretreated 12 h in darkness. The preparations were processed under red light and then placed for 18 h in darkness.

Observation and evaluation of results were done under a PZO light microscope with 10×5 magnification.

The number of chloroplasts on the lower cell wall (contacting the solution tested) and on the upper wall was counted and their ratio (s) was calculated.

For 0.25 per cent $MgSO_4$ this varied from 1.8 to 2.4. These changes were due to seasonal changes in the plant material. All the experiments were performed in the period October—May in which s = 2. Each final results is a mean for 100 cells (10 preparations).

OBSERVATIONS AND RESULTS

The experiments proved that glyoxalate has a distinct chemotaxic influence on chloroplast translocation (Tabl 1, Fig. 2). In the highest concentrations used (10^{-2}, 10^{-3}M) its influence was somewhat weaker. A concentration of 10^{-4}M (s = 2 and 2.1) was found to be optimal. As the solution was diluted, the inductive effect of glyoxalate decreased.

Glyoxalate was used in two versions of pH: 1) acid, pH 3.1, and 2) adjusted with 1 per cent KOH to pH 5.7 (Table 1, Fig. 2).

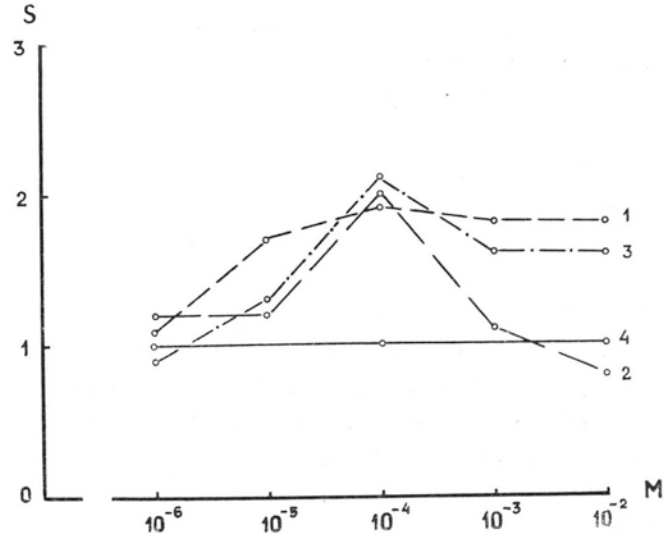

Fig. 2. Influence of glycine and its oxidation products on chloroplast translocation in *Funaria hygrometrica* leaves

Axis x — concentration in moles per litre, axis y — ratio of mean number of chloroplasts in 100 cells on lower cell wall to mean number of chloroplasts on upper cell wall of *Funaria* leaves. Curves: 1. glycine; 2. glyoxalate, pH 3.1; 3. glyoxalate pH 5.7; 4. NH_4

Table 1

Influence of glycine and its oxidation products on chloroplast translocation in *Funaria hygrometrica* laves

Concentration / Solution	10^{-2}M	10^{-3}M	10^{-4}M	10^{-5}M	10^{-6}M	10^{-4} darkness
	g d	g d	g d	g d	g d	g d
glycine pH 5,8	1,5 2,7 s=1,8	1,5 2,7 s=1,8	1,4 2,6 s=1,9	1,3 2,2 s=1,7	2,0 2,2 s=1,1	0 0
glyoxalate pH 3,1	2,1 1,6 s=0,8	2,2 2,4 s=1,1	1,1 2,1 s=2	1,7 2,1 s=1,2	1,2 1,5 s=1,2	—
glyoxalate pH 5,7	0,9 1,5 s=1,6	0,8 1,3 s=1,6	0,8 1,7 s=2,1	0,7 0.9 s=1,3	1,0 0.9 s=0,9	1,3 1,8 s=1,4
NH_4Cl pH 5,7	0,2 0,2 s=1	—	0,1 0,1 s=1	—	0,5 0,5 s=1	—
H_2O_2 pH 5,7	0 0	—	0 0	—	0 0	—

g – mean number of chloroplasts on upper cell wall of *Funaria* leaves (in 100 cells)
d – mean number of chloroplasts on lower cell wall of *Funaria* leaves (in 100 cells)
s – ratio d to g

Glyoxalate of pH 3.1 exerts a weaker action in higher concentrations (10^{-2}, 10^{-3}M) as compared to the solution of pH 5.7. On the other hand, a concentration of 10^{-4}M proved optimal for both the glyoxalate variants (s = 2 and s = 2.1 respectively). Within the concentration range 10^{-4} to 10^{-6}M glyoxalate activity at pH 5.7 falls, and at pH 3.1 at first falls, but within 10^{-5} to 10^{-6} concentrations it remains at a constant level.

When the influence of light is eliminated, glyoxalate also exhibits a chemotaxic influence on chloroplast translocation, but in a lower extent than in the presence of light (s = 1.4 at 10^{-4}M).

The influence of the next compound tested — glycine — has a different course. In *Funaria* leaves exposed to light, the action of this compound is similar to that of glyoxalate. It stimulates chloroplast translocation almost equally in all the three higher concentrations (s = 1.8 to 1.9). As the concentration is lowered this influence decreases.

In darkness, however, glycine has no influence even in the optimal concentration of 10^{-4}M. All chloroplasts are arranged in a pattern characteristic for darkness along the side walls of the cell.

The remaining two products of enzymatic glycine oxidation — NH_4 and H_2O_2 — have no chemoinductive influence on chloroplast translocation.

It had to be further verified whether the chemotaxic influence of glyoxalate and glycine is not associated with some unspecific chemical

structure of these compounds. For this purpose several compounds with a similar structure such as glycollate, glyoxal, oxalic acid and acetic acid were tested. None of these compounds had a chemoinductive effect on chloroplast translocation.

DISCUSSION

The results here reported are in good agreement with the postulates of the hypothesis concerning primary reactions connected with chloroplast translocation (Z u r z y c k i, 1972). Analysis of these results leads to the conclusions that anchoring of the chloroplasts in the gel layer of the cell cytoplasm is caused by the optimal glyoxalate concentration.

Glycine exhibits a chemoinductive activity towards chloroplasts only in light. This substance is a substrate for the light-sensitive enzyme — glycine oxidase. This enzyme oxidizes glycine to glyoxalate. If a glycine solution of appropriate concentration is applied in the outer environment of a leaf, glycine diffuses into the cells where its concentration increases. In the presence of light glycine oxidase oxidizes this additional glycine so that glyoxalate concentration rises, and this at a certain optimal concentration causes chloroplast translocation. In darkness glycine oxidase is not active, and chemoinduction is not observed in the reaction of chloroplast translocation.

Externally applied glyoxalate has a chemoinductive activity both in light and in darkness. Its action is specific. The remaining products of glycine oxidation, NH_4 and H_2O_2 exhibit no activity.

S e n n (1908) investigated a number of organic and inorganic chemical compounds and described their chemotaxic action on the chloroplasts of *Funaria* leaves. Sulphuric acid and some of its acid and neutral salts ($NaHSO_4$, $KHSO_4$, Na_2SO_4, $MgSO_4$) exhibit a chemotaxic action. Among the compounds with a weaker activity S e n n mentions CO_2, HNO_3, malic acid, asparagine, fructose and glucose.

The highest chemotaxic activity was found in $MgSO_4$, therefore it was used in the present investigations as a test compound.

It results from the observations here recorded that the chemoinductive activity of $MgSO_4$ towards *Funaria* leaf chloroplasts is very similar to the action of glyoxalate in darkness and light and to that of glycine in light. The pH value does not seem to play a major role (in the range of pH 3—6). In S e n n's studies acid and neutral salts (e.g. $NaHSO_4$ and Na_2SO_4) had a similar chemotaxic action.

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Pewne aspekty chemotaktycznej reakcji chloroplastów u Funaria hygrometrica

Streszczenie

1. Badania opisane w niniejszej pracy przeprowadzono w celu stwierdzenia czy produkty utlenienia glicyny — reakcji katalizowanej przez specyficzną oksydazę glicynową, zastosowane w środowisku zewnętrznym liścia *Funaria hygrometrica* i przenikające do jego komórek działają chemoindukcyjnie na przemieszczanie się chloroplastów.

2. Badania przeprowadzono na świetle i w ciemności dla glioksalanu i glicyny, natomiast dla NH_4^+ i H_2O_2 tylko na świetle.

3. Wyraźne działanie chemoindukcyjne wykazuje glioksalan (10^{-4}M), zastosowany na świetle i w ciemności, glicyna tylko na świetle, pozostałe z wymienionych związków nie wykazują żadnego działania.

4. Przebadano również związki o podobnej budowie chemicznej jak glikolan, glioksal, kwas szczawiowy i octowy. Żaden z tych związków nie ma wpływu na przemieszczanie się chloroplastów liści *Funaria*.

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