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Grażyna BYSTRZEJEWSKA[1], Aleksy ŁUKOWSKI[2] and Ryszard LIGOWSKI[3]

[1] Isotope Laboratory
Institute of Botany
University of Warsaw
Krakowskie Przedmieście Str. 26/28
00-927 Warszawa, POLAND

[2] Department of Zoology and Ecology
Institute of Zoology
University of Warsaw
Krakowskie Przedmieście Str. 26/28
00-927 Warszawa, POLAND

[3] Laboratory of Polar Biology
Department of Invertebrate Zoology and Hydrobiology
University of Łódź
Banacha Str. 12/16
90-237 Łódź, POLAND

Changes in dynamics of ^{14}C incorporation and release by Antarctic marine diatoms in the presence of Lindane during subsequent light/dark periods

ABSTRACT: The influence of Lindane on net phytoplankton (mainly diatoms) in samples of waters from the Antarctic was investigated for 24 hours from the introduction of γ HCH 0,02 and 2 ppm into the environment. Changes in intensity of ^{14}C incorporation in the light and in rate of release of ^{14}C by the cells in the dark during consecutive light/dark periods were measured. The effect of two different Lindane concentrations in diatoms occurred 16 hours after introduction of the compound into the environment and was independent of concentration. The effect was manifested by delayed induction of photosynthesis following the dark period and also by changes in dynamic equilibrium between carbon assimilation and dissimilation. The presence of Lindane clearly stimulated ^{14}C incorporation in the light and also enhanced the participation of ^{14}C incorporation in overall CO_2 exchange in the dark.

Key words: Antarctica, diatoms, Lindane, photosynthesis.

Introduction

Isomers of HCH are common in the hydrosphere and atmosphere of the Antarctic (Tanabe, Hidaka and Tatsukawa 1983). This is most probably

a consequence of the transport of the compounds via the atmosphere from those regions of our globe where they are widely used in place of DDT. The compounds have long been known to occur in diatoms in Antarctic waters (Łukowski and Ligowski 1987, 1988).

γ HCH (Lindane) is a compound clearly toxic to Antarctic phytoplankton (Łukowski, Bystrzejewska and Ligowski 1989). Twenty four hours after its introduction into the aqueous environment it inhibited phytoplankton photosynthesis.

In considering primary production by organisms as the result od dynamic equilibrium between the processes of carbon assimilation and dissimilation it seems purposeful to study the effect of Lindane on photosynthetic assimilation of carbon and the release of carbon by the cells in the dark. Knowledge of the metabolic reaction of an organism in response to a toxic factor requires the determination of the states of preceding the inhibitory effect caused by the factor in question. This can be achieved by employing increasingly lower concentrations of the toxic compound or by shortening the time of contact between the organism and the compound.

The first of these methods was used in studies on the effect of Aroclor-1254 (Bystrzejewska, Łukowski and Ligowski 1989) which led to the hypothesis that the concentration of the compound effects the relationship between intensity of photosynthesis and intensity of carbon release from the cells in the light as a result of photorespiration as well as during the extracellular release of organic carbon.

However, since even low concentrations of Lindane inhibited photosynthesis by phytoplankton within 24 hours (Łukowski, Bystrzejewska and Ligowski 1989) it seemed more promising to follow the effect of the compound on phytoplankton in time, i.e. for 24 hours from the moment of contact between HCH and the cell. The aim of this study was to follow changes in intensity of ^{14}C incorporation in the light and rate of ^{14}C release by the cells in the dark in consecutive light/dark periods.

Material and methods

Plant material

In the experiment we have used net phytoplankton that had been taken in the South Shetlands (59°43'8"S, 47°59'7"W) area using a Copenhagen-type net with a mesh size of 55 μm from a water column 100-0 m. Phytoplankton contained diatoms dominated by *Chaetoceros criophilum* Castracane. The collected water sample (with appr. 370 diatom cells in 1 cm^3) was distributed in 10 l partions into three bottles which were placed in an artificially illuminated chamber. The intensity of the light inside the chamber was ca. 2500 lx. The bottles in the chamber were cooled to 2°C by constant flow of water.

Treatments, sampling and assays

Two bottles containing phytoplankton were supplemented with γ HCH (Lindane) in acetone to the concentrations of 0.2 and 2 ppm, respectively. The third bottle received 200 µl of acetone (background). At the same time each bottle received 400 µCi of ^{14}C. The bottles in the chamber were exposed to 12 hours light, followed by 4 hours darkness, 4 hours light and another 4 hours of darkness. Every two hours throughout the experiment three 50 ml samples of the cell suspension were removed. The immediate addition of 5 ml of formaline solution to each sample killed all phytoplankton cells. Each sample was then passed through a 1.2 µm Millipore membrane filter and the radioactivity in the cells retained by the filter estimated. The level of incorporated ^{14}C was determined with the use of Geiger-Müller counter. The radioactivity of samples was expressed in Bq.

Results

The experiments demonstrated (Fig. 1) that during the first 12 hours neither of the studied Lindane concentrations (0.02 and 2 ppm) affected the intensity of ^{14}C incorporation by the diatoms in the light. Moreover, Lindane did not visibly affect the intensity of ^{14}C release by the cells during the following 4 hours of

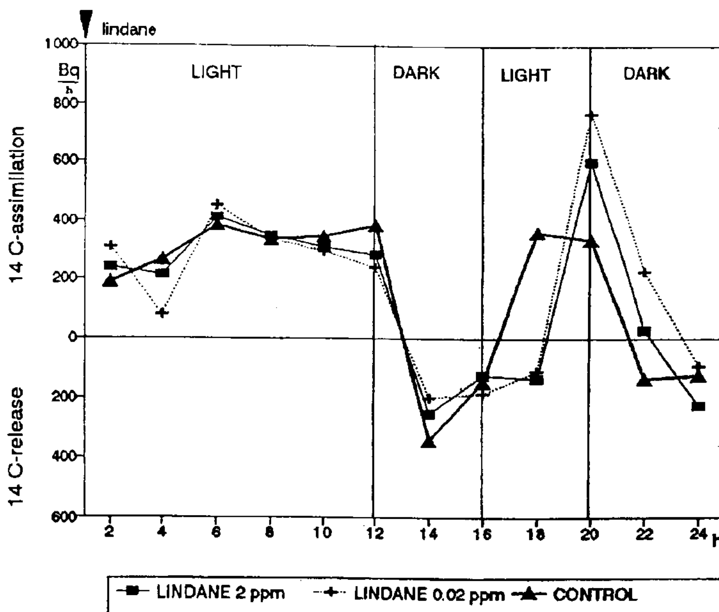

Fig. 1. Changes in intensity of ^{14}C incorporation and release by Antarctic marine diatoms in the presence of Lindane during subsequent light/dark periods

darkness. After this period, which embraced 16 hours from introduction of Lindane into the environment, light did not result in such rapid photosynthetic response by the Lindane-treated cells as observed for the control cells. In the former a rapid increase of intensity of ^{14}C incorporation was observed but the response was delayed in time. Nevertheless, the later incorporation of ^{14}C in the light by the Lindane treated cells reached two-fold higher values than for the control cells. This occurred 20 hours after the introduction of γ HCH, within the last four hours of light.

Both the control and Lindane-treated cells reacted to the second period of darkness with equally strong decreases of ^{14}C incorporation. However, whereas the intensity of ^{14}C release in the control after 2 hours of darkness exceeded that of photosynthesis, a similar state was reached in the presence of Lindane only after 4 hours.

Discussion

The effect of Lindane on the photosynthetic incorporation of ^{14}C observed after 16 hours can be explained by the relatively slow penetration of the compound into the cells, by the fact that the effect is not a direct consequence of the presence of Lindane in the cells, by the further metabolic transformations of the compound or, finally, by its secondary effect on cellular metabolism. In the case of slow penetration of Lindane the effect of the compound on the photosynthetic incorporation of ^{14}C would probably be manifested earlier at the higher of the two concetrations employed. For the same reason the observed photosynthetic effect seems to be not a direct consequence of the presence of Lindane in the cell but rather a secondary effect of its action.

The delayed induction of photosynthesis by light after the period of darkness in the presence of Lindane suggests a reduced pool of metabolic substrates for carboxylation. Replenishing of the pool would occur in the light and involve increased amounts of ATP and $NADPH_2$.

Later compensation of the delayed start of the photosynthetic incorporation of ^{14}C, expressed by two-fold stimulation of the process compared to the control, also supports the notion of a reduced pool of intermediate metabolites of photosynthesis in the presence of Lindane and enhanced metabolic turnover of these compounds.

The domination of ^{14}C incorporation over carbon release observed for about two hours during the second dark period may indicate that the presence of Lindane results in enhanced role of anaplerotic incorporation of CO_2. Anaplerotic CO_2 fixation involves the carboxylation of phosphoenolpyruvate (PEP) and its role in total CO_2 incorporation by phytoplankton has been found to be substantial (Priscu and Goldman 1983). The observed incorporation of CO_2 in the presence of Lindane may indicate that the assimilation of CO_2 in the

light with the participation of RuBP-carboxylase may be accompanied by the incorporation of CO_2 into PEP. Such double carboxylation could help explain the stimulation of CO_2 fixation in the light in the presence of Lindane.

The changes observed in both light and dark point to the pronounced effect of Lindane on CO_2 fixation and lack of such effect on the release of CO_2 in the dark. The results of this study confirm the previously formulated hypothesis (Bystrzejewska, Łukowski and Ligowski 1989) that the relation between intensity of photosynthesis and ^{14}C release by the cells depends on the concentration of CHs (Aroclor-1254). Although the hypothesis related only to processes occurring in the light (photosynthesis, photorespiration and extracellular release of organic carbon) the results of this study, obtained also in the dark, indicate that Lindane affects photosynthesis to a greater degree than respiration and thus affects the dynamic equilibrium between the assimilation and dissimilation of carbon.

The results suggest changes in the biochemical utilization of energy by the cell and the increased participation of β-carboxylation in the photosynthetic and anaplerotic incorporation of CO_2.

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Streszczenie

Badano wpływ lindanu na asymilację i uwalnianie ^{14}C przez fitoplankton sieciowy. Morski fitoplankton, złożony głównie z okrzemek, pobrany był w Antarktyce. Zastosowano trzy warianty prób: próby bez lindanu wprowadzonego z zewnątrz, próby γ HCH w stężeniu 0,02 ppm oraz

w stężeniu 2 ppm. Przez 24 godziny od wprowadzenia lindanu do próbek prowadzono pomiary natężenia włączania ^{14}C na świetle i uwalniania ^{14}C w ciemności. Wyniki porównywano z kontrolą bez lindanu.

Wpływ lindanu na badane procesy przejawiał się po 16 godz. od wprowadzenia γ HCH do środowiska i nie zależał od zastosowanego stężenia lindanu. Efekt wywołany lindanem polegał na opóźnieniu indukcji fotosyntezy po okresie ciemności, a następnie, podczas fotosyntezy, na stymulacji włączania ^{14}C na świetle oraz zwiększeniu udziału włączania ^{14}C podczas wymiany gazowej zachodzącej w ciemności po okresie świetlnym.