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NATURE OF THE AFLATOXIN B_1 - INDUCED INHIBITION OF PHOTOSYNTHESIS IN *CHLORELLA FUSCA* (CHLOROCOCCALES, CHLOROPHYTA)

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طبيعة تثبيط البناء الضوئي بفعل السم الفطري أفلاتوكسين ب ١

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تسببت معاملة طحلب كلوريلا فوسكا بالسم الفطري أفلا توكسين ب١ في تثبيط انطلاق الأكسجين من البناء الضوئي . سببت المعاملات أيضاً إنخفاضا ملحوظا في محتوى الطحلب من الكلوروفيلات والكاروتينويدات و واكب هذا التثبيط للبناء الضوئي حدوث فقد للمكون المتغير لكيناتيكيات إنبعاث لصف كلوروفيل أ .

نوقشت النتائج في ضوء ما سببته المعاملة بالسم الفطري أفلاتوكسين ب١ من تغيرات في التركيب تحت الخلوي الدقيق وبخاصة على مستوى أغشية الثيلاكويد بالبلاستيدات الخضراء .

Key Words: Aflatoxin B_1, *Chlorella,* Chlorophyll fluorescence, Oxygen evolution, Ultrastructure.

ABSTRACT

Treatment with Aflatoxin B_1 (AFB_1) resulted in inhibition of photosynthetic oxygen evolution in *Chlorella fusca* Shih.et Krauss. Treatments also induced a marked reduction of both chlorophyll and carotenoid contents. Inhibition of photosynthesis was manifested by loss of the variable component of Chlorophyll *a* (Chl *a*) fluorescence induction kinetics. Results are discussed in terms of the observed AFB_1 - induced ultrastructural disorders at the level of the chloroplast thylakoid membrane.

INTRODUCTION

AFB_1 has been shown to inhibit growth of *Chlorella pyrinoidosa* Chick [1], and chlorophyll synthesis in mung bean leaves [2]. We have recently reported AFB_1 - induced inhibition of growth and photosynthesis in *Chlorella fusca* Shih. et Krauss [3]. Inhibition of oxygen evolution was observed even at low levels of AFB_1 and in the absence of any appreciable reduction of chlorophyll content. This observation led to the suggestion of a possible inhibitory effect of this hepatocarcinogen at the level of the electron transport chain [3].

Chlorophyll fluorescence was early recognized as a potentially powerful probe for the study of certain aspects of photosynthesis. The versatility of application of Chl *a* fluorescence added further validity to its use in plant physiology [4]. Changes in Chl *a* fluorescence induction kinetics have repeatedly been used to assess damage to the photosynthetic apparatus [5-7]. In this paper Chl *a* fluorescence and transmission electron microscopy techniques were used to further evaluate the nature of the AFB_1 - induced inhibition of photosynthesis.

MATERIALS AND METHODS

Chlorella fusca 211-15 from the Collection of Algal Cultures (Gottingen, Germany) was grown in 12h day/night cycles in a nitrate-rich medium [8] at 25°C and light intensity of 250 umol m^{-2} s^{-1}. AFB_1 in 0.03% dimethylsulfoxide (DMSO) was added to give concentrations of 10 and 100 ug ml^{-1}. DMSO was also added to control cultures. Cell numbers were determined by using a Bright-Line haemocytometer (Reichert-Jung, USA). Chlorophyll and carotenoid contents were determined by the method of Metzner [9]. At designated times, cell numbers were determined and samples containing $1x10^6$ cells ml^{-1} were collected. Photosynthetic oxygen evolution was measured with 5 ml of culture in a Clarke-type

oxygen electrode (Rank Brothers, UK) at 25°C and an irradiance of 800 umol m^{-2} s^{-1} provided by photographic slide-projector. Cells were separated by centrifugation (5,000 g) and dark-adapted on 1 cm diameter filter paper discs at 25°C for 10 min prior to fluorescence measurements. AFB_1 - induced responses were assessed by monitoring changes in F_R, the maximal rate of the induced rise in Chl *a* fluorescence; F_P, the fluorescence peak; and F_Q, the maximal rate of fluorescence quenching (Fig. 1). At the end of the experiment, control and 100 ug ml^{-1} treated cells were separately, fixed in 3% glutaraldehyde for 3h, postfixed in osmium tetroxide for 1 h, dehydrated, and embedded in Spurr's resin [10]. Ultrathin sections were stained with 2% uranyl acetate for 20 min, counter-stained with Reynolds' lead citrate [11] for 2 min, and examined at an accelerating voltage of 100 kV with a Phillips 400 TEM.

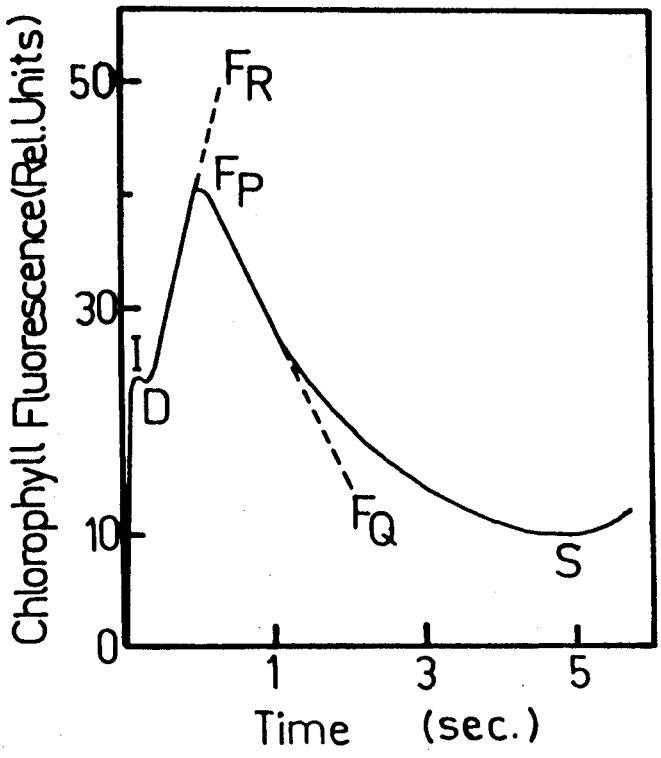

Fig. 1: A generalized diagram of variable Chl *a* fluorescence induction kinetics indicating the I-D dip, the fluorescence peak (F_P), the maximal rate of fluorescence rise (F_R), and the maximal rate of quenching (F_Q).

RESULTS

AFB_1 treatments resulted in marked reduction of total chlorophyll (*a* + *b*) content, Chl *a* : *b* ratio, carotenoid content, and oxygen evolution (Fig. 2). Treatments also induced changes in the pattern of the variable component of Chl *a* fluorescence induction kinetics (Fig. 3). These changes involved reduced values of the variable fluorescence peak, F_P (Fig. 4a), and lower rates of both fluorescence rise, F_R (Fig. 1b), and quenching, F_Q (Fig. 4c). These effects were much more pronounced in cells treated with 100 ug ml^{-1} AFB_1, and were accompanied by major ultrastructural changes of cellular membranes. These changes included separation of cell membrane from cell wall (Plate 1d), breakdown of nuclear membrane (arrows in Plate 1e), and disintegration of the tonoplast (Plate 1f). Ultrastructural changes also occurred in subcellular organelles and included degradation of mitochondrial inner membranes (Plate 1d). Moreover, damage to the photosynthetic apparatus was manifested by disorganization of the arrangement of thylakoid membranes (Plate 1e), and loss of the granular appearance of the stroma (star in Plate 1e).

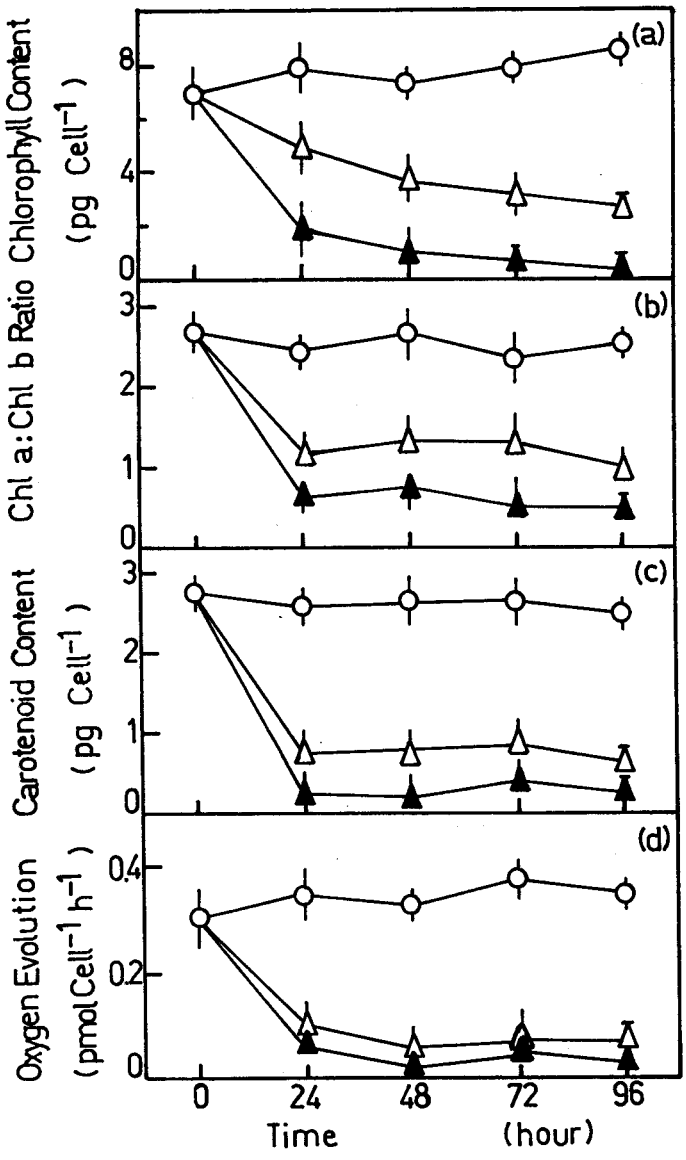

Fig. 2: AFB_1. - induced reduction of total chlorophyll (*a* + *b*) contents *(a)*, Chl *a*: *b* ratio (b), caritenoid content (c), and inhibition of photosynthetic oxygen evolution (d) in *Chlorella fusca*. (O) Control, (Δ) 10 μg ml^{-1}, and (Δ)100 μg ml^{-1} (± se, n = 5).

DISCUSSION

The AFB_1 - induced reduction of total chlorophyll (*a*+*b*) content in *Chlorella fusca* (Fig. 2a) has previously been reported in *Chlorella pyrenoidosa* [1], and in mung bean leaves [2]. Reduced chlorophyll content was attributed to a possible direct inhibitory effect on chlorophyll synthesis [2]. However, treatments also resulted in a marked reduction of carotenoid content (Fig. 2c). This marked reduction of carotenoid content perhaps suggests that the observed loss of

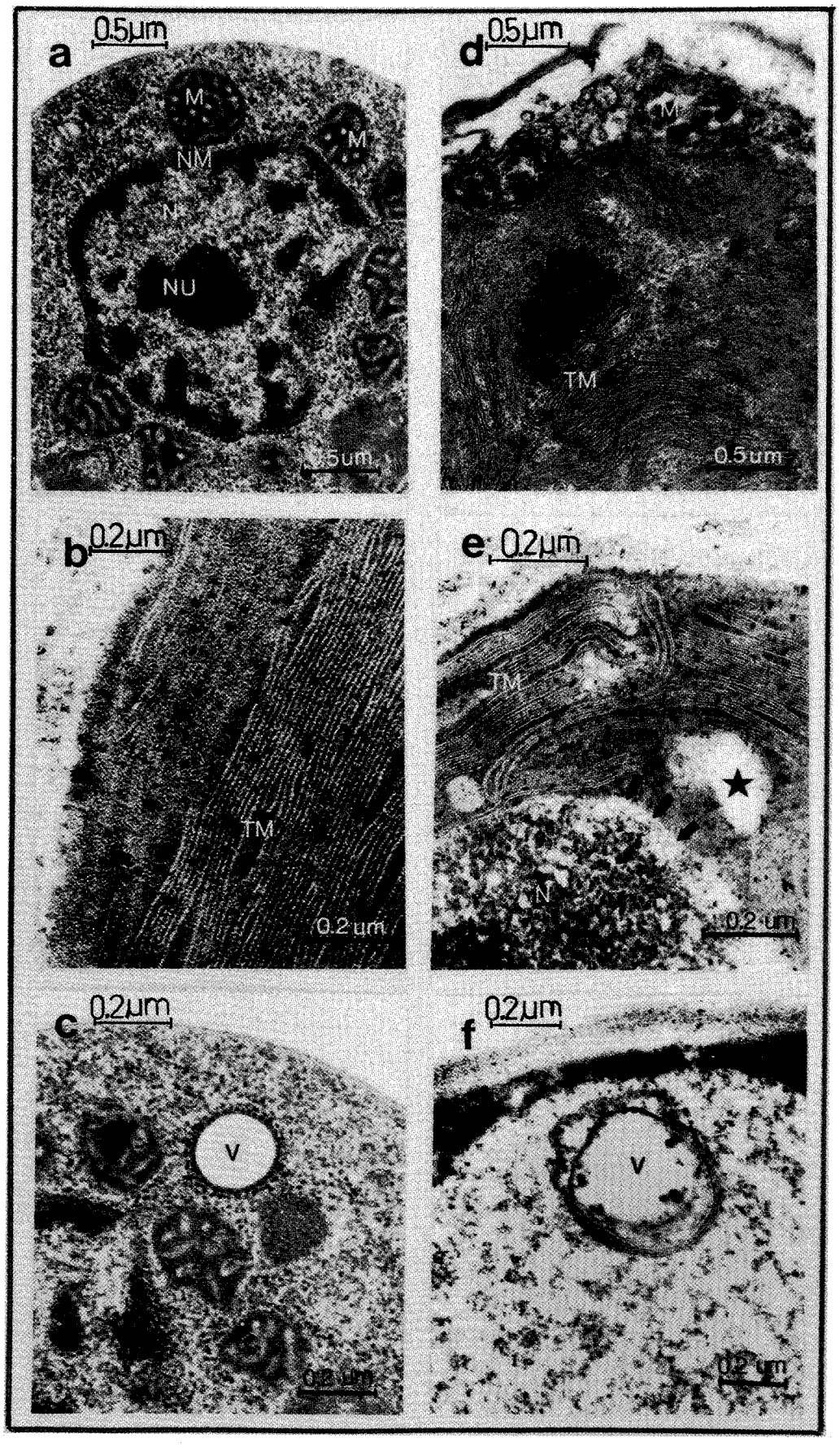

Plate 1: Electron micrographs showing the ultrastructural features of control (a-c) and 100 µg ml^{-1} - treated (d-f) *Chlorella fusca* cells. M = mitochondrion, NM = nuclear membrane, NU = Nucleolus, TM = thylakoid membrane, V = vacoule.

chlorophyll could, at least in part, be due to lack of carotenoid photoprotective role. A similar loss of chlorophyll was reported in *Chlorella fusca* treated with herbicides known to inhibit carotenogenesis [12].

Chl *a* fluorescence originates from the PS II pigment population and explicitly reflects PS II activity [13, 14]. The variable component is sensitive both to the rate of electron

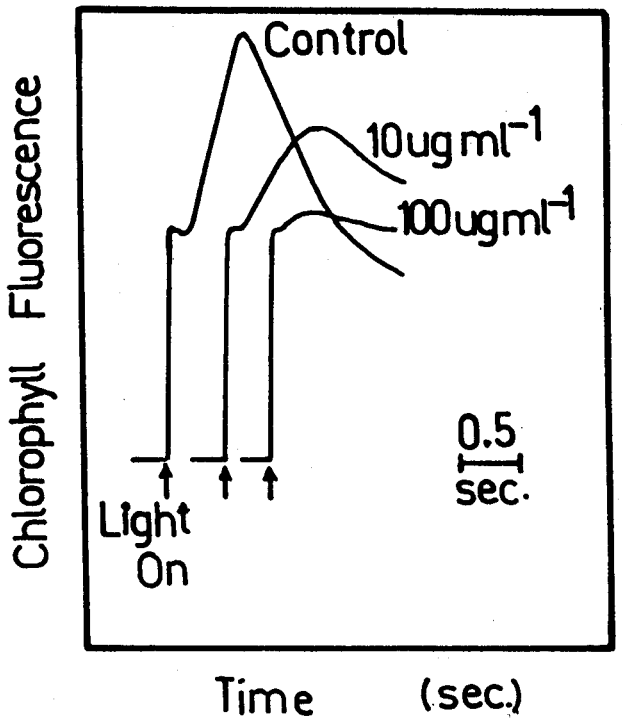

Fig. 3: Patterns of Chl *a* fluorescence induction kinetics in *Chlorella fusca* cells after a 3 h exposure to different levels of AFB_1.

transport through PS II and to changes that accompany phosphorylating electron transport [15]. After the I-D dip (Fig. 1), the rise of fluorescence to F_P indicates reduction of Q_A [16]. The observed low values of F_P and F_R in AFB_1 - treated cells (Fig. 4) thus reflect inhibited electron transport through PS II. Following F_P, fluorescence is quenched to a quasi steady-state level S (Fig. 1), signaling dissipation of available excitation via routes other than fluorescence [13]. Although this includes all processes involved in lowering the fluorescence yield, photochemical quenching denotes the proportion of excitons captured by open traps and being converted to chemical energy in PS II. Hence, oxidation of Q_A causes quenching [16], and the loss of quenching ability found here in AFB_1 - treated cells can possibly be due to damage on the donor side of PS II. The electron micrographs (Plate 1) show general disruption of membrane organization as a major effect of AFB_1 treatment. A similar AFB_1 - induced degradation of liver mitochondria has previously been reported [17]. Visible disorganization of thylakoid membranes is likely to be correlated with disruption of functional integrity of the photosynthetic apparatus, possibly including physical separation of the light harvesting complex (LHC II) from PS II.

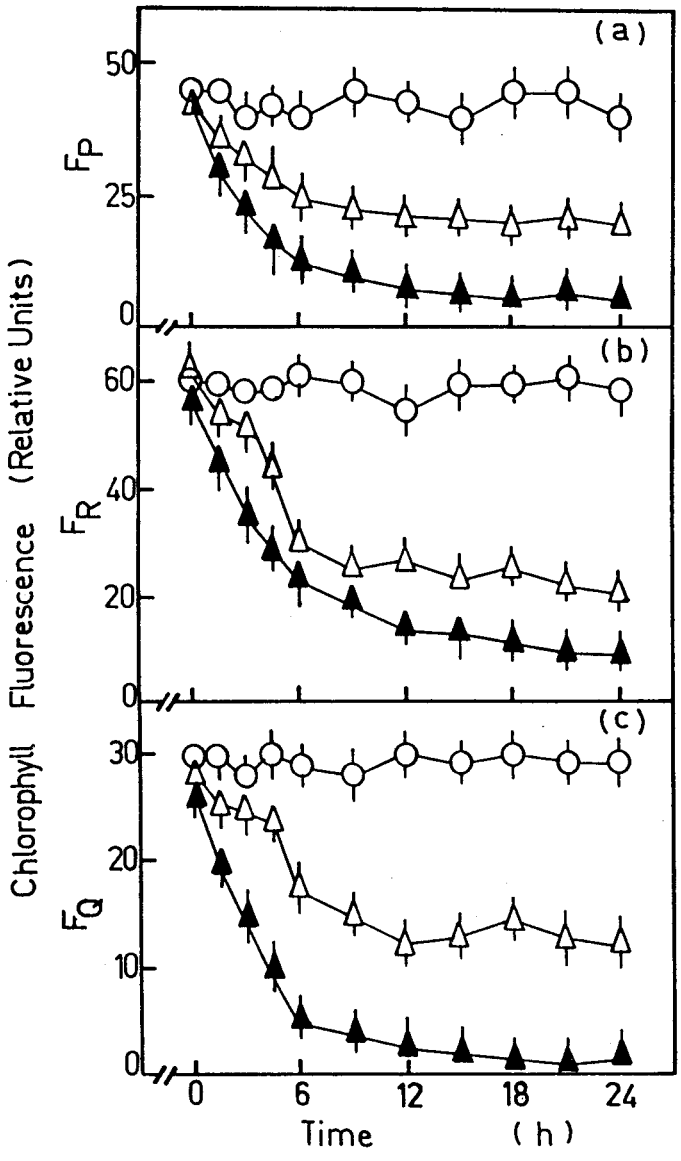

Fig. 4: AFB_1 - induced changes in the parameters F_P, F_R, and F_Q of the variable Chl *a* fluorescence in *Chlorella fusca*. (O) control, (Δ) 10 μg ml^{-1}, and (Δ) 100 μg ml^{-1} (± se, n = 5).

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