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Effects of CO_2 Enrichment on Microstructure and Ultrastructure of Two Species of Freshwater Green Algae

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Abstract: In order to investigate the morphological response of freshwater green algae to elevated CO_2 concentration, *Chlamydomonas reinhardtii* Dang and *Scenedesmus obliquus* Kütz were cultured with enriched CO_2, and their microstructure and ultrastructure were examined by microscopy and electron microscopy. The effect of CO_2 enrichment to 186 μmol/L was insignificant on the shape and size of *C. reinhardtii*, but significant in reducing the volume of *S. obliquus*. High-CO_2 increased the amount of chloroplast. The pyrenoids occurred in low-CO_2-grown cells but not in high-CO_2-grown ones and more starch granules were observed in the former.

Key words: *Chlamydomonas reinhardtii*; CO_2; microstructure; pyrenoid; *Scenedesmus obliquus*; starch granules; ultrastructure

Atmospheric CO_2 concentration has been increasing since the beginning of the past century mainly due to industrial combustion of fossil fuels associated with increased human activities, and it is predicted that the atmospheric CO_2 concentration will be doubled during the 21th century to 700 $\mu L \cdot L^{-1}$ [1]. Such a CO_2 rise is supposed to result in a change of pH and dissolved inorganic carbon (DIC) in aquatic environments. An increase in atmospheric CO_2 from 35 to 50 Pa would increase sea-surface DIC from 2.25 to 2.32 $mol \cdot m^{-3}$, decrease pH from 8.15 to 8.01 at 15 ℃ with a alkalinity of 2.47 $mol \cdot m^{-3}$ [2]. Growth of marine phytoplankton under optimal light and nutrient conditions was shown to be limited by the supply of CO_2 in seawater[3], and doubled CO_2 in oceanic surface water was demonstrated to stimulate marine productivity[4]. However, little is known on this aspect about freshwater algae. When unicellular freshwater green algae were cultured at 5% CO_2, their photosynthetic characteristics were significantly affected. With CO_2 affinity being lowered, CO_2 compensation point increased, carbonic anhydrase (CA) activity decreased[5-8]. Although these findings are fundamental in understanding the photosynthetic physiology of these freshwater algae, they can hardly lead to any ecological implications in terms of the impacts of the atmospheric CO_2 rise, because CO_2 concentrations used in the previous studies are hundreds times higher than the present atmospheric CO_2 concentration.

Freshwater green algae are main primary producers in freshwater ecosystems. Little has been documented with regard to their changes of microstructure and ultrastructure under CO_2-enriched environment. The present study aims to investigate the effects of varied CO_2 concentrations on the micro- and ultrastructure of the common freshwater green algae, *Chlamydomonas reinhardtii* and *Scenedesmus obliquus*.

1 Materials and Methods

Freshwater green algae, *Chlamydomonas reinhardtii* Dang and *Scenedesmus obliquus* Kütz were obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences. Prior to experiments, both species were cultured in Bristol's medium to log phase. All experiments were performed in 1 000 mL batch cultures with modified Bristol's medium containing 20 mmol/L Tris and 20 mg/L $NaHCO_3$ with pH adjusted to 7.2, 8.2 and 9.0 with HCl or NaOH, respectively. Cells were cultured in a plant growth chamber (EF7, Conviron) with a 12∶12 LD cycle. Temperature and illumination were controlled at 25 ℃ and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Half of the medium in each culture was renewed before the start of illumination every day. Dissolved inorganic carbon (DIC) was measured with a total organic carbon analyser (TOC-5000, Shimazdu) during illumination phase, thereafter certain amount of $NaHCO_3$ were added to maintain DIC concentration less than 10% to compensate the amount of CO_2 consumed by photosynthesis. pH was also monitored and maintained constant. CO_2 concentrations in the media were calculated from pH and DIC values according to Stumm and Morgan[2]. After seven days, the cultures were harvested and resuspended in fresh Bristol's medium. Cells were observed and photographed by optical microscopy with a photograph system (BX-50, Olympus). For the ultrastructural examinations, harvested cells were washed three times with phosphate buffer (pH 7.0), and fixed for two hours in 5% (V/V) glutaraldehyde solutions, and then further rinsed twice in the same buffer before post-fixed with 2% osmium tetraoxide for about 1 h, and subsequently embedded in agar and dehydrated in a grade of alcohol-water series. The material was embedded in diallyl phthalate for

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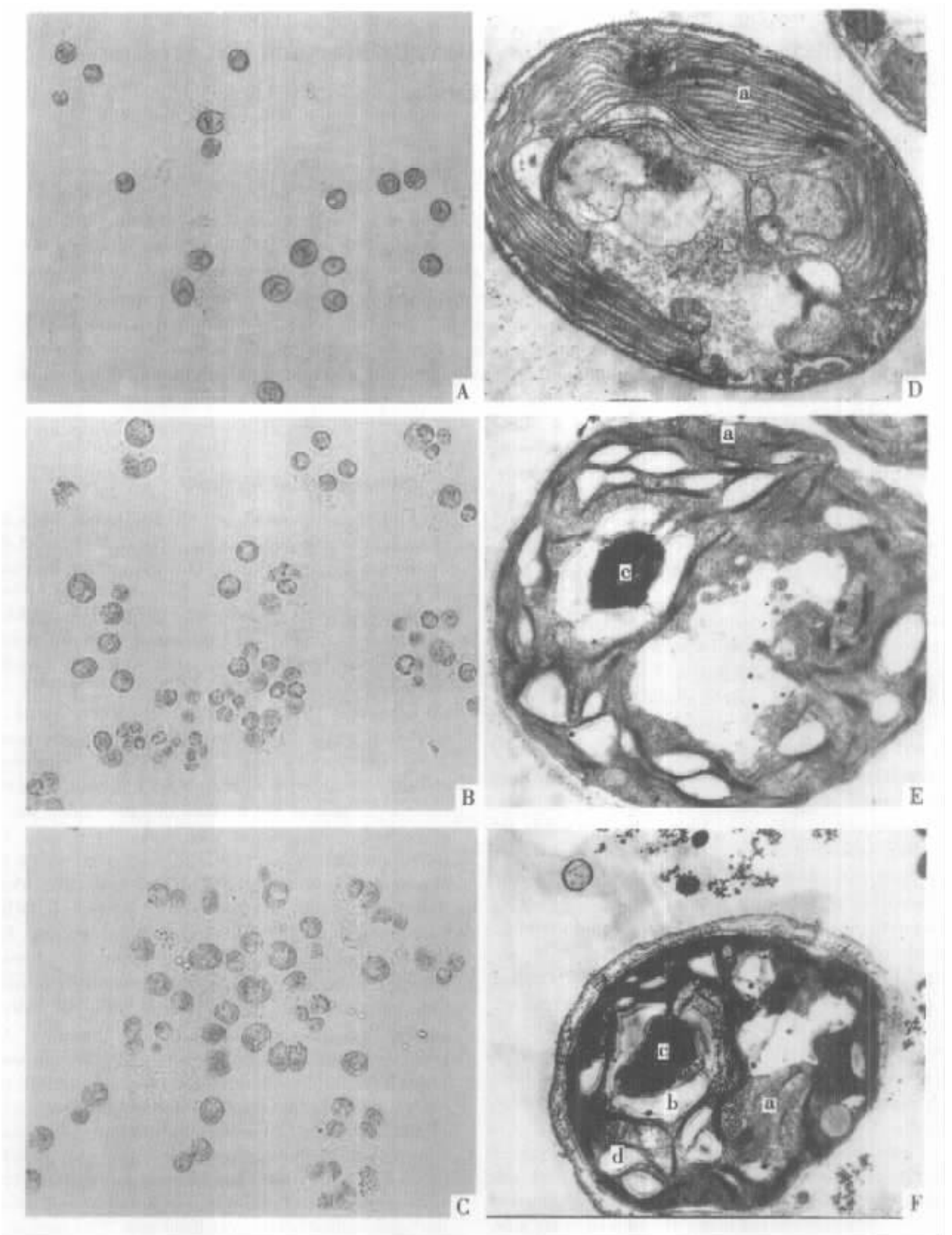

Fig.1. Microstructure and ultrastructure of *Chlamydomonas reinhardtii* grown under different CO_2 levels.
A, B, C. Microstructure, ×400. D, E, F. Ultrastructure, ×10 000. A, D. 186 μmol/L CO_2. B, E. 21 μmol/L CO_2. C, F. 3 μmol/L CO_2. a, chloroplast; b, starch plate; c, pyrenoid; d, starch granule.

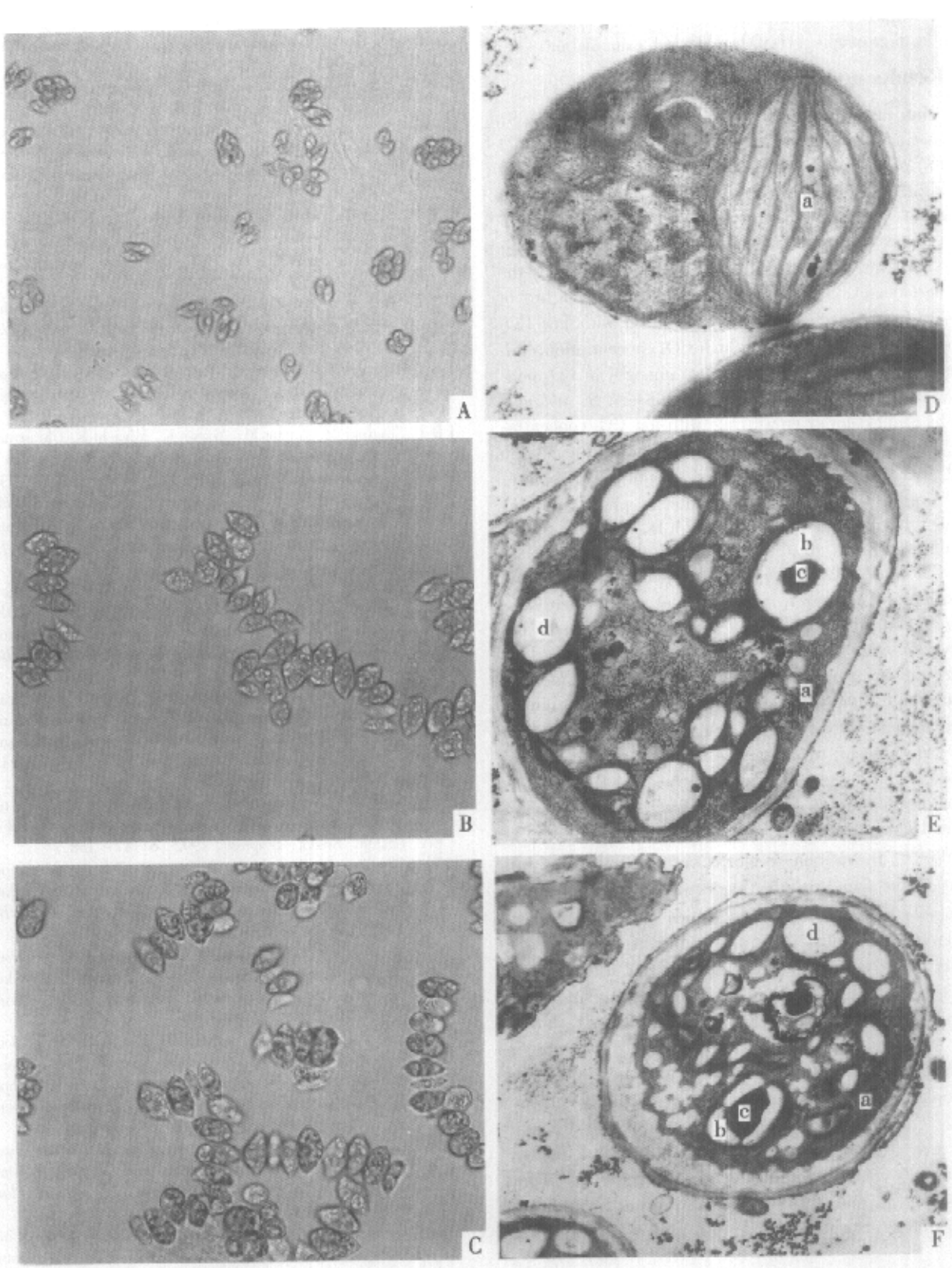

Fig. 2. Microstructure and ultrastructure of *Scenedesmus obliquus* grown under different CO_2 levels.
A, B, C. Microstructure, ×400. D, E, F. Ultrastructure, ×7 000. A, D. 186 μmol/L CO_2. B, E. 21 μmol/L CO_2. C, F. 3 mol/L CO_2. a, chloroplast; b, starch plate; c, pyrenoid; d, starch granule.

three days. Sections were cut with ultramicrotome and double stained with uranyl acetate and lead citrate before electron microscopic (H-300, Hitachi) examination.

2 Results and Discussion

The microstructures of *C. reinhardtii* and *S. obliquus* cultured under varied CO_2 concentrations (3, 21 and 186 μmol/L CO_2) are shown in Fig.1 (A, B and C) and Fig.2 (A, B and C). The size and shape of *C. reinhardtii* grown at 186 μmol/L CO_2 (high-CO_2-grown) were similar to that at 3 and 21 μmol/L CO_2 (low-CO_2-grown). It was obvious that they were not affected by the CO_2 enrichment. However, cell volume enlarged two or three times when *S. obliquus* was grown with low CO_2 concentration in contrast with high CO_2 concentration, implying a species-specific response to changes in CO_2 concentration. The enlargement of the volume in *S. obliquus* may be due to slower growth rate with low CO_2 concentration. In the present study, high CO_2 concentration (186 μmol/L CO_2) resulted in increased amount of chloroplast, indicating an effect on light utilization by photosynthesis. Figure 1 (D, E and F) and Fig. 2 (D, E and F) show the ultrastructures of *C. reinhardtii* and *S. obliquus* grown at varied CO_2 levels. Developed pyrenoids were found in low-CO_2-grown cells, but not in the high-CO_2-grown cells. This implied that pyrenoid formation of *C. reinhardtii* and *S. obliquus* was linked to the low CO_2 concentration. Similar results also occurred in *Chlorella vulgaris* and *Dunaliella tertiolecta* when they were grown under ambient level of CO_2[9-11]. Pyrenoids isolated from both *Eremosphera viridis* and *C. reinhardtii* contained Rubisco[12-15], and about 50% of the total Rubisco was in the pyrenoids of *C. reinhardtii* when grown with 5% CO_2 and more than 90% in the pyrenoids with 0.035% CO_2[16, 17]. In addition, a part of carbonic anhydrase was induced and found in the pyrenoids of low-CO_2-grown *Dunaliella* cells[10], so the pyrenoids developed in low-CO_2-grown cells might play a key role in increasing the affinity for CO_2 during photosynthesis so that transportation of CO_2 to Rubisco is facilitated. In the present study, ultrastructure of *C. reinhardtii* and *S. obliquus* showed more starch granules in low-CO_2-grown cell. Changes in starch granules could alter the structure and function of chloroplast, and result in physiological variation of chloroplasts[18,19]. In the meantime, excessive starch accumulation may lead to slower diffusion of CO_2 in chloroplast, that usually cause inhibition of photosynthesis in higher plants[20-22], and these might also occur in the two species of green algae studied.

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CO_2浓度变化对两种淡水绿藻的显微结构和超微结构的影响

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摘要: 为了探讨淡水绿藻在适应 CO_2 浓度变化过程中细胞形态和结构的变化,通过普通显微镜和电子显微镜观察了在不同 CO_2 浓度培养下的莱因衣藻(*Chlamydomonas reinhardtii* Dang)和斜生栅藻(*Scenedesmus obliquus* Kütz)细胞。结果表明,CO_2 浓度变化对莱因衣藻细胞体积没有明显的影响,但斜生栅藻在低浓度 CO_2 培养下细胞体积明显增大,并可见细胞内含有大量颗粒。两种绿藻细胞的超微结构显示,在低浓度 CO_2 培养下,细胞内叶绿体数目明显减少,并可见明显的淀粉盘包围的蛋白核 细胞内还可见大量的淀粉粒。而在高浓度 CO_2 培养下,这两种绿藻细胞内均未见明显的蛋白核和大量淀粉粒出现。

关键词: 莱因衣藻;CO_2;显微结构;蛋白核;斜生栅藻;淀粉粒;超微结构

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