The Influence of 5-Aminolevulinic Acid on Protochlorophyllide and Protochlorophyll Accumulation in Dark-Grown *Scenedesmus*

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Scenedesmus obliquus, 5-Aminolevulinic Acid, Protochlorophyll, Protochlorophyllide

The intermediate of chlorophyll biosynthesis, 5-aminolevulinic acid (ALA), is a necessary prerequisite for the formation of protochlorophyllide (PChlide) and protochlorophyll (PChl) in the dark. The application of ALA to a dark-grown culture of the pigment mutant C-2A′ of *Scenedesmus obliquus* increased the amount of PChlide 30-fold and the amount of PChl about 10-fold. The rates of ALA-dependent formation of PChlide and PChl reach their maximum values at different concentrations of added ALA. Similarly, the kinetics of PChlide and PChl formation in cells incubated with ALA are different. Cells of *Scenedesmus* mutant C-2A′ incubated with various concentrations of ALA for different periods provide a good tool for future studies differentiating between PChlide and PChl metabolism. – The incorporation of Chl deriving from either PChl or PChlide into different pigment protein complexes is discussed.

Introduction

It is well documented that angiosperms accumulate protochlorophyllide (PChlide) [1–5] and small amounts of protochlorophyll (PChl) [6–11] in darkness. In these plants the conversion of PChlide to chlorophyllide (Chlide) is light-dependent [12, 13] whereas a photoconversion of PChl to Chl could not be observed [14].

We have previously investigated pigment mutant C-2A′ of *Scenedesmus,* which demonstrates similar features of chlorophyll biosynthesis as higher plants. It forms only traces of chlorophyll (Chl) in darkness and accumulates measurable amounts of PChlide [5] and PChl [11] in a certain growth phase. These Chl precursors are completely degraded when cells are kept in darkness for serveral days [5]. Both, PChlide and PChl, are reduced to Chlide and Chl, respectively, in the light [11]. The synthesis of Chl precursors in the dark can be enhanced by the addition of 5-aminolevulinic acid (ALA), the first specific precursor of tetrapyrrol biosynthesis.

Abbreviations: ALA, 5-aminolevulinic acid; Chl, chlorophyll; Chlide, chlorophyllide; PChl, protochlorophyll; PChlide, protochlorophyllide; TLC, thin layer chromatography.

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In this paper we report on a differential effect of ALA on the amount and time course of PChlide and PChl formation, and we discuss its possible implication for PChl metabolism.

Experimental

Organism and growth conditions

Pigment mutant C-2A′ of *Scenedesmus obliquus* [15] was used in this study. This mutant forms only traces of Chl when grown heterotrophically in darkness, but greens in the light [16]. The cells were grown in 250 ml of inorganic medium supplemented with glucose (0.5%) and yeast extract (0.25%) [17], in 500 ml Erlenmeyer flasks in complete darkness in a gyratory incubator (Type G-25, New Brunswick Scientific Inc.) at 32 °C. Every 2 to 3 days 30 ml of the culture were transferred into fresh culture medium.

Pigment extraction and separation

After centrifugation of the mutant Cells (1400 × *g*, 5 min), the pellet was exhaustively extracted with hot methanol in darkness. The various pigments were separated from this methanolic extract on analytical TLC plates (Silica gel 60; 20 × 20 cm; 0.2 mm; Merck # 5553, Darmstadt, F.R.G.). The TLC plates were developed twice in a solvent system consisting of petroleum ether (40–60 °C fraction): 2-propanol: water = 100:10:0.25 (v/v/v). After the separation the bands

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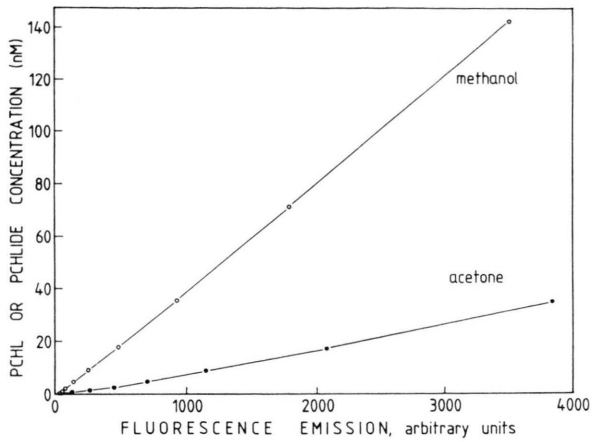

Fig. 1. Calibration of fluorescence emission against the concentrations of PChlide in methanol (—○—) and PChl in acetone (—●—). The solutions of PChlide and PChl were excited at 435 nm or 440 nm, respectively. The concentration of the samples was determined by their absorbance which was measured with a Kontron spectrophotometer (Uvikon 820). Fluorescence emission was monitored at 645 nm (PChlide in methanol) and 630 nm (PChl in acetone). The fluorescence data were obtained with a Shimadzu spectrofluorophotometer RF-540 (for details see [11]).

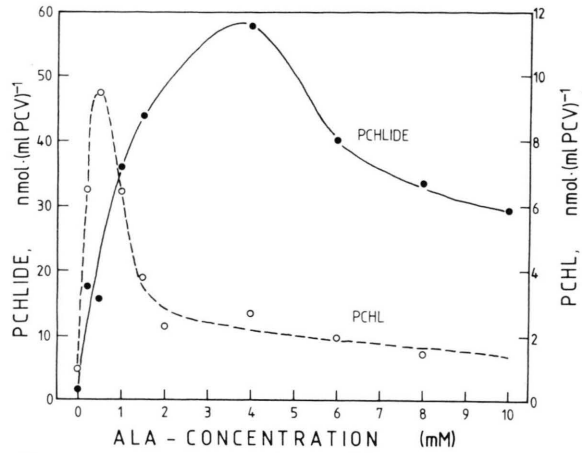

Fig. 2. Concentrations of protochlorophyllide (—●—) and protochlorophyll (– –○– –) in the pigment mutant C-2A′ of *Scenedesmus obliquus* incubated with increasing ALA concentrations in the dark for 20 h.

of PChlide and PChl were scratched off and eluted with methanol and acetone, respectively.

The amounts of PChlide and PChl were determined fluorometrically. PChlide in methanol was excited at 440 nm and its fluorescence measured at 645 nm, whereas PChl in acetone was excited at 435 nm and its fluorescence measured at 630 nm. Fluorescence emission values were calibrated against known concentrations of PChlide and PChl (Fig. 1).

Results

In earlier experiments we demonstrated that the formation of ALA is light-dependent in pigment mutant C-2A′ of the unicellular green alga *Scenedesmus obliquus* [18]. However, addition of ALA did not result in Chl formation in darkness, indicating further light-dependent steps in Chl formation. In this contribution we investigate the influence of ALA on the accumulation of PChlide and PChl. Dark-grown cells of mutant C-2A′ of *Scenedesmus obliquus* were incubated with different concentrations of ALA ranging from 0.2 to 10 mM. After 20 h of growth in the dark PChlide and PChl were extracted and quantitatively determined by their fluorescence. PChl and PChlide formation as a function of ALA concentration demonstrate large differences (Fig. 2). The PChl level reaches a sharp maximum at an ALA concentration of 0.5 mM, representing a tenfold increase over the control level. The level of PChlide shows a broader maximum as a function of the ALA concentration. At an ALA concentration of approximately 4.0 mM, PChlide increases about 30-fold (Fig. 2). It is obvious that the concentration of ALA influences the PChlide in a different way. The ratio of PChlide/PChl is low in darkness and changes from 2 at 0.5 mM ALA to 22 at 4.0 mM ALA added.

To establish the kinetics of PChlide and PChl formation during incubation with ALA, cells of the C-2A′ mutant were incubated with ALA (0.5 or 5.0 mM) and analyzed after different time intervals. At the high concentration of ALA (5.0 mM) PChlide reaches a maximum after about 16 h, and there is a small increase in PChl after 24 h. At the low level of ALA (0.5 mM), PChlide increases steadily whereas the PChl concentration reaches a sharp maximum after 6 h incubation and declines thereafter (Fig. 3). These experiments suggest that the biosyntheses of PChlide and PChl do not take place in a parallel fashion and that the ratio of PChlide to PChl is regulated by the amount of ALA.

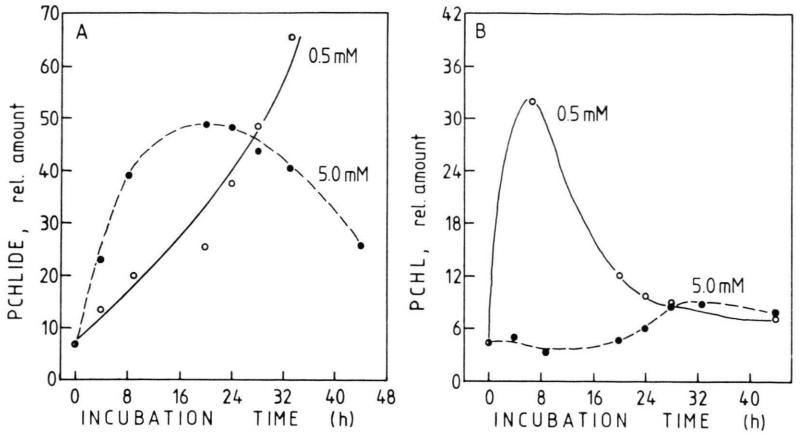

Fig. 3. Time course of protochlorophyllide (A) and protochlorophyll (B) formation in the dark by the pigment mutant C-2A′ of *Scenedesmus obliquus* grown in the presence of 0.5 and 5.0 mM ALA, respectively.

Discussion

Our investigation demonstrates that the addition of ALA causes an increase in the amounts of PChlide and PChl, confirming that ALA is the limiting factor for PChlide and PChl biosynthesis in darkness. Moreover, the results suggest that the concentration of ALA regulates the ratio of PChlide to PChl, and the rate of synthesis of PChlide and PChl in different ways. Overimposed to the biosynthesis is a general degradation of PChl(ide) observed in the dark [5]. PChl is mainly formed in the initial phase of the incubation in the presence of low concentrations of ALA. The amount of ALA in dark-grown cells is very small [3] and the ratio of PChlide/PChl is very low. With the onset of illumination PChl is photoconverted directly and quickly to Chl [11], whereas PChlide is photoconverted to Chlide. Its subsequent phytylation to Chl takes 1–2 h [19].

These different time courses in the formation of Chl from PChl and PChlide might suggest that the Chl of the reaction centers of the photosynthetic apparatus derive from the PChl, whereas the light-harvesting chlorophylls are preferentially formed from the PChlides. This speculation is in agreement with the fact that the reaction centers of mutant C-2A′ are formed immediately after transfer to light, whereas the light-harvesting pigments start to appear after 1–2 h when the biosynthesis of the bulk chlorophylls occurs [19]. This delay in the appearance might be due to the more time consuming phytylation process. – We will investigate the PChl metabolism under this aspect in the near future.

Acknowledgements

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[1] Y. Matsuda, T. Kikuchi, and M. R. Ishida, Plant Cell Physiol. **12,** 127 (1971).
[2] A. Wild, V. Trostmann, I. Kietzmann, and K.-H. Fuldner, Planta **140,** 45 (1978).
[3] T. Oh-hama and E. Hase, Plant Cell Physiol. **21,** 1263 (1980).
[4] Y. Shioi and T. Sasa, Plant Cell Physiol. **25,** 139 (1984).
[5] H. Senger and G. Brinkmann, Physiol. Plant. **68,** 119 (1986).
[6] B. Singh, Phytomorphology **3,** 224 (1953).
[7] O. T. G. Jones, Biochem. J. **101,** 153 (1966).
[8] C. Cohen and J. A. Schiff, Photochem. Photobiol. **24,** 555 (1976).
[9] T. Sasa and K. Sugahara, Plant Cell Physiol. **17,** 273 (1976).
[10] C. Sundqvist, H. Ryberg, B. Böddi, and F. Lang, Physiol. Plant. **48,** 297 (1980).
[11] K. Kotzabasis, M. P. Schüring, and H. Senger, Physiol. Plant. **75,** 221 (1988).
[12] W. T. Griffiths, Biochem. J. **152,** 623 (1975).
[13] W. T. Griffiths, Biochem. J. **174,** 681 (1978).
[14] Y. Shioi and T. Sasa, Plant Cell Physiol. **24,** 835 (1983).
[15] N. I. Bishop, Methods Enzymol. **23,** 130 (1971).
[16] H. Senger and N. I. Bishop, Plant Cell Physiol. **13,** 633 (1972).
[17] N. I. Bishop and J. Wong, Biochim. Biophys. Acta **234,** 433 (1971).
[18] T. Oh-hama and H. Senger, Plant Cell Physiol. **16,** 395 (1975).
[19] K. Humbeck and N. I. Bishop, Plant Cell Physiol. **27,** 1407 (1986).