Photosynthetic Activities in Tobacco Plants I

Cytochrome f from Tobacco Leaves

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Abstract

In order to investigate the photosynthetic activity of tobacco plants with special reference to the relationship between this activity and the content of cytochrome f, the method of extraction and some properties of this compound was studied.

Cytochrome f was extracted from tobacco chloroplasts, using ammoniac ethanol solution containing Triton X-100. Tobacco cytochrome f had properties similar to those of parsley cytochrome f.

The concentration of cytochrome f in the extract was 0.18 to 0.2 μ moles and not affected by the variety of tobacco plants. As the concentration of chlorophyll of tobacco plants varied remarkably among the varieties, the ratio of chlorophyll/cytochrome f differed due to the difference of the starting materials.

Introduction

The presence of cytochrome f in higher plants was first discovered by Hill and Scarisbrick¹⁾, and the substance was obtained in highly purified form from parsley Davenport and Hill²⁾. Recently Forti and his co-workers³⁾ presented a new method for the purification of cytochrome f, modifying the extraction procedures of the former investigators.

In our laboratory, efforts have been done to the investigation of the photosynthetic activity of tobacco plants (e.g. CO₂-uptake and dry matter production) with special reference to the relationship between this activity and the content of the photosynthetically active components, namely chlorophylls, cytochromes and some quinones. During the quantitative estimation of cytochrome f, it was found that the extraction procedure of Forti and his co-workers³⁾ was inadequate in the case of tobacco leaves, because of high content of gummy substances, which prevented further purification of cytochrome f. To avoid this difficulty, the chloroplasts were prepared from tobacco leaves and cytochrome f was extracted from the isolated chloroplasts, since cytochrome f is known to be localized in chloroplasts^{1,2)}.

Material and Methods

Chloroplasts were prepared from the laminae of tobacco leaves (*Nicotiana tabacum*, sp.) obtained from Utsunomiya Tobacco Experiment Station. Six hundred grams of laminae were homogenized in a Waring blender for 30 sec. with 1200 ml of ice-cold

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0.5 M NaCl. The homogenate was then squeezed through cheese cloth and centrifuged at 200 *g* for 4 min. The sedimented residue was removed and the supernatant was recentrifuged at 1,000 *g* for 10 min. The sedimented chloroplasts were resuspended in 0.5 M NaCl and recentrifuged at 1,000 *g* for 10 min. The washing procedure was repeated twice.

To the chloroplast suspension, cold (-10°) ethanol containing 1% (v/v) Triton x-100 and 1.5% (v/v) ammonia solution (sp. gr. 0.880)³⁾ was added with vigorous mechanical stirring, to give a final concentration of 56% ethanol on feeding the stirring, care was taken to maintain as constant possible a proportion of ethanol: chloroplasts. After 15 min stirring, this mixture was centrifuged at 19,000 *g* for 20 min (at -5°). The supernatant obtained was clear and yellowish-green. Cytochrome pigment was then precipitated by the addition of 1.1 vol of cold (-10°) acetone with stirring and allowed to stand for 1 hr. The precipitate was collected by centrifugation (3,000 *g*, 10 min.), and then washed in a centrifuge tube, with a cold (-10°) mixture of acetone-ethanol-water (2:1:1, v/v) made alkaline with ammonia (0.5% ammonia solution of 0.880 specific gravity). During the washing, the temperature of the room was kept at -5° . After centrifugation (3,000 *g*, 10 min.), the precipitate was suspended in 0.5 saturated ammonium sulfate of pH 8.0. The suspension was again centrifuged at 3,000 *g*, for 10 min., and the supernatant was discarded. Cytochrome was extracted from the precipitate with 0.05 M Tris buffer (pH 8.0)

containing 0.5% Triton x-100. Extraction was continued overnight with stirring in a cold room (-5°). The volume of the buffer solution which was required for an efficient extraction of cytochrome f was rather small (20-50 ml).

The further steps of the purification of cytochrome f were quite identical to the procedure of Forti *et al.*, including further ammonium sulfate fractionation and column chromatography on Sephadex G-100 and G-200³⁾.

Fig. 1. The absorption spectra of cytochrome f extracted from tobacco chloroplasts. Continuous line, reduced form. Broken line, oxidized form. The cytochrome f is dissolved in 0.05 M Tris buffer (pH 8.0) containing 0.5% Triton X-100.

Result and Discussion

The absorption spectrum of the purified tobacco cytochrome f in the reduced form, is shown in Fig. 1. The spectrum is substantially identical to that of cytochrome f published by Forti and his co-workers³⁾, except that the peak at 330 mμ is almost negligible in our preparation. The spectrum of the oxidized form obtained by oxidation with potassium ferricyanide²⁾ is also shown Fig. 1. The tobacco cytochrome f was not autooxidizable in the air, and the oxidized form was obtained only

by addition of oxidizing agents. Keilin and Hartree⁴⁾ showed that cytochrome c is readily oxidized by potassium ferricyanide, copper sulfate and hydrogen peroxide. Davenport and Hill²⁾ found that potassium ferricyanide is the only common oxidizing agent which oxidizes a solution of ferrocytochrome f to ferricytochrome f. The tobacco cytochrome was not readily oxidized by these reagents. Potassium ferricyanide ($E'_0 = +0.43$ V) oxidized the cytochrome but a relatively high concentration of the oxidant was required. The resistance of the tobacco cytochrome against the reagents which readily oxidize cytochrome c indicates that the tobacco cytochrome has an oxidizing potential higher than that of cytochrome c ($E'_0 = +0.254$ V). When ascorbate was added to the oxidized preparation of cytochrome f, the band at $555\text{ m}\mu$ reappeared. As far as the shape of the absorption spectrum shows, this band was identical in position and intensity to the band which had originally been observed before the addition of the oxidizing reagent. Ascorbate has a redox potential of $+0.065$ V and can reduce ferricytochrome f ($E'_0 = +0.365$ V), but is incapable of reducing ferricytochrome b_6 ($E'_0 = -0.06$ V)⁵⁾. These findings indicate that the cytochrome pigment extracted from tobacco chloroplasts is, in fact, cytochrome f.

On the extraction step from the chloroplasts, as Davenport and Hill²⁾ have indicated, the final concentration of ethanol in the chloroplast-ethanol suspension should be maintained strictly at 55-56%. When more concentrated ethanol was used, a compound having an absorption peak at $542\text{ m}\mu$ was extracted, which prevented the quantitative estimation and the purification of cytochrome f. This compound was considered to be a carotenoid-protein complex. If more dilute ethanol was used, the extraction of cytochrome f from chloroplasts was incomplete.

The concentration of cytochrome f in the crude extract was calculated to be from 0.18 to $0.20 \times 10^{-6}\text{M}$ by using a value of $\epsilon_{555}^{\text{red}} = 2.6 \times 10^4$. This means that about one half of the cytochrome f initially present in the chloroplast was extracted by this procedure. If the efficiency of this extracting procedure was not affected by the variety or the age of tobacco plants, the similarity in concentration of cytochrome f in each extract may indicate that the concentration of this compound in leaves did not materially differ from variety of tobacco. On the other hand, there was a remarkable variation in pigmentation of leaves among various varieties of tobacco plants (Table 1). Actu-

Table 1. Chlorophyll contents of young tobacco leaves. The tobacco plants were grown in the green house of Utsunomiya Tobacco Experiment Station. Leaves at 11th stalk position from the top were sampled from 60 day-old plants. Each value was estimated according to Shimizu *et al.*⁶⁾

	Chlorophyll* (a + b)	Pheophytin*	Total Chlorophyll*
Domestic : Matsukawa	1064.6	56.2	1120.8
Burley : Burley 21	835.0	29.6	864.6
: Ky 57	539.0	28.6	567.6
Flue Cured : Bright Yellow	824.6	52.7	877.3
: Hicks	607.9	38.8	246.7
Bright Consolation	355.2	67.9	423.1

(* μg per 1 gm fresh weight)

ally, the ratio of chlorophyll/cytochrome f in each variety was remarkably affected by the concentration of chlorophyll in the leaf. In the Burley variety Kentucky 57 and the virescent variety Bright Consolation, the chlorophyll concentrations were found to be appreciably less than in other varieties. However, these two varieties show

normal growth and the total amounts of the harvested leaves were not inferior to the yields in other more highly pigmented varieties. These findings may suggest that the photosynthetic activity per chlorophyll unit in the less pigmented varieties is higher than that in the much highly pigmented ones. The relationship between the photosynthetic activity and the molar ratio chlorophyll/cytochrome *f* in tobacco plants will be investigated in detail.

References

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摘 要

堀田ルリ・清水 碩・玉置英之助：タバコ植物における光合成活性 I
タバコのチトクローム *f*

タバコ植物の光合成活性を調べるために、チトクローム *f* の定量をこころみた。タバコの葉には、いろいろな物質が多量に含有されているために、従来おこなわれていた方法でチトクローム *f* を定量することは不可能であることがわかった。そこで葉緑体を取り出し、この葉緑体についてチトクローム *f* の定量をおこなった。

Triton x-100 を含むアンモニア性エタノールで抽出し、さらに、アセトン、硫酸等で不純物を取除いた。この方法によると、葉緑体に存在するチトクローム *f* のおよそ半量が抽出される。抽出されるチトクロームの量は、タバコの種類によってほとんど影響をうけないがクロロフィル含量は、種類によって大きく変わる。したがって、チトクローム *f* 1 分子に対するクロロフィル分子数の比は、種類によって大きく変わるものと思われる。(専売公社中央研究所)