Studies on the Phosphorylase Action in Plants

I. Histochemical Investigation of Phosphorylase in Growing Leaf Epidermis

by Isao KATO*

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Yin *et al.*^{1,2)}, using a histochemical method, have found strong action of phosphorylase in stomatal guard cells of tobacco and broad bean leaves, and suggested that the enzyme plays a part in stomatal movements by changing osmotic value of the cells.

Although in leaves of a variety of plants, phosphorylase action likely appears solely in guard cells, in *Phytolacca americana* and *Datura tatula*, it is detectable also in other epidermal cells other than guard cells (Kato and Fukuda³⁾). On the other hand, Konagamitsu and Ono⁴⁾ have observed the phosphorylase action in epidermal and mesophyll cells of young leaves, but only in guard cells of mature leaves of liliaceous plants.

The purpose of the present study is to disclose more definitely the change in distribution of phosphorylase in growing leaf cells so as to gain some clue to the role which may be played by the enzyme in plant cells.

Material and Methods

The leaf epidermal tissues of *Tradescantia reflexa*, *Datura tatula* and *Vigna catiang* were used as the experimental materials. Most experiments were made with *Tradescantia*, because of the ease of stripping off epidermal tissues from the leaf blade.

The slices of stripped epidermis were incubated for about 24 hrs. in a reaction mixture which was kept at 25° in a thermostat. Then the materials were stained with iodine solution, and were examined under a microscope to see whether starch is formed or not. The control samples were incubated in the reaction mixture without G-1-P (α -D-glucose-1-phosphate).

The reaction mixture consisted of 1% G-1-P solution, adjusted to pH 6.0 with acetate buffer, and a few drops of toluene which was known to increase the permeability of cells to G-1-P (Stocking⁵⁾).

Results

Experiment in *Tradescantia reflexa* Along the axis of a leaf blade (3~5 cm. in length) of *Tradescantia*, a zonal distribution of the age is present. Thus the blade was arbitrarily separated into four parts, namely a, b, c and d (Fig. 1). Epidermal tissues excised from b, c and d contained stomatal cells at different maturation

Fig. 1. Schematic diagram of a young leaf (3~5 cm. in length) of *Tradescantia reflexa* showing four maturation stages (a, b, c and d) examined.

* Biological Institute, College of General Education, Hiroshima University, Hiroshima, Japan.

tion stages and those from part a were consisted solely of meristematic cells, as shown in Fig. 2.

(A)~(D) in Fig. 2 show starch formation in epidermal tissues treated with G-1-P solution. Each protodermal cell in the basal part (part a in Fig. 1) containing starch

Fig. 2. Starch formation in parts a, b, c and d of leaf epidermis of *Tradescantia reflexa* treated with G-1-P solution. Stained with iodine ($\times$ ca.300). A~D, treated with G-1-P. A'~D', untreated with G-1-P.

of amylose type turned blue with iodine solution (Fig. 2A). This starch is the product of phosphorylase action in the tissue, because it was not detectable in the control (Fig. 2A').

In the stage of stomatal mother cells in part b of Fig. 1, phosphorylase action was detectable in every cell of the tissue, but a strong action was found in the mother cells and a fairly strong action was also seen in the subsidiary cells surrounding the mother cells (Fig. 2B). In the control tissue, however, there was no starch formation (Fig. 2B').

In part c, in which guard cells and stomata are formed by the division of stomatal mother cells, starch formation, i.e., phosphorylase action was remarkable in guard cells and still remained in subsidiary cells though weaker than that in the former stages (Fig. 2C). In the control tissues no starch formation was observed (Fig. 2C').

In the epidermis which was at perfect maturation (part d in Fig. 1) phosphorylase action was strong only in guard cells and not detectable in subsidiary and other epidermal cells. By the way, some guard cells at stage d contained starch grains a priori, so epidermal tissues containing no starch as shown in Fig. 2D' were selected for the present use.

The localization of phosphorylase was also examined in transections of young and grown parts of a leaf blade. In the young part (part a in Fig. 1) strong action of phosphorylase was found in every epidermal cell rather than in mesophyll cells (Fig. 3E), but in the older part (part d in Fig. 1) the enzyme action was found only in mesophyll and guard cells, and not in epidermal cells (Fig. 3F).

Fig. 3. Transections of a leaf blade of *Tradescantia reflexa* treated with G-1-P solution. Stained with iodine. ($\times$ ca. 300). E, young epidermis. F, mature epidermis.

Fig. 4. Starch formation in shoot apex of *Tradescantia reflexa*. Stained with iodine. ($\times$ ca. 300). G, longisection of vegetative shoot treated with G-1-P. G', control.

The strong action of the enzyme was also found in vegetative shoot apex of *Tradescantia*: in tunica, corpus and leaf primordia (Fig. 4 G).

Fig. 5. Starch formation in leaf epidermis treated and untreated with G-1-P solution. Stained with iodine. ($\times$ ca. 300). *Datura tatula*....H, H', young epidermis; I, I', mature epidermis. *Vigna catianga*....J, J', young epidermis, K, K' mature epidermis.

Experiment in *Datura tatula* and *Vigna cati*ang Phosphorylase action in the leaf epidermis of *Datura tatula* and *Vigna cati*ang was also investigated (Fig. 5). In *Tradescantia* rather regularly zonal distribution of stomatal cells with respect to maturity was found along the blade axis, while in *Datura* and *Vigna*, as is the case for most dicotyledons, no such regularity was found and different developmental stages of stomata were mixed in a mosaic fashion, and fully mature guard cells often appeared adjacent to stomatal mother cells.

Starch formation was observed in all young epidermal cells (Fig. 5 H, J), but only in guard cells in mature epidermal cells (Fig. 5 I, K).

Discussion

The location of phosphorylase in epidermal cells other than guard cells has little been reported (Yin and Tung^{2,6}), Alvin⁷), Maruo and Nakamura⁸), Ono^{9,10}), Fukuda and Kato¹¹)).

In this experiment the phosphorylase action was examined in the leaf epidermis of various developmental stages of *Tradescantia reflexa*, *Datura tatula* and *Vigna cati*ang. In the early stage of development a strong action of the enzyme was found in every epidermal cell. With the maturation of the tissues the enzyme action became stronger in stomatal cells and weaker in the other epidermal cells until in fully mature epidermis it was found exclusively in guard cells.

According to Kato and Kato³), in the leaf epidermis of *Phytolacca americana* and *Datura tatula* sometimes a remarkable phosphorylase action was detectable not only in guard cells but also in other epidermal ones. In view of the present findings it seems probable that the leaf epidermis tested by these authors was not enough mature.

Yin and Sun¹) studied the distribution of phosphorylase in germinating seeds, e.g. those of soybean and found that the enzyme was present in root tip, young leaf and stem tip. Aimi and Kodera¹²) reported that the enzyme action was rather strong in young roots, and weak in older roots and leaves of germinating wheat plants.

In *Tradescantia*, as shown above, the strong action of the enzyme was found in various parts of vegetative shoot apex, i.e., tunica, corps, and protoderm and mesophyll of primordial leaves, whereas the epidermis of grown-up leaf contained it only in its guard cells.

These results are consistent with an idea that the phosphorylase action may take a part in the regulation of osmotic values of the plant cells, which would associate with stomatal movements in mature guard cells or with growth in young cells.

Summary

1) Phosphorylase action in the epidermal tissues isolated from growing leaves of *Tradescantia reflexa*, *Datura tatula* and *Vigna cati*ang was investigated. The tissues were incubated with G-1-P, and starch formed was detected with iodine reaction under a microscope.

2) In protodermal tissue a remarkable phosphorylase action was detectable in every cell.

3) With the maturation of epidermal tissue, the phosphorylase action in differentiating guard cells or stomatal mother cells became stronger, while in other epidermal cells it became weaker until it completely disappeared.

4) Meristematic shoot apex cells which contained no starch showed positive phosphorylase action.

5) It was presumed that phosphorylase action took an important role in the regulation of osmotic values of plant cells, which might be related to the growth of the young tissue as well as to the movement of stomata in leaf epidermis.

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摘 要

加藤勇夫： 植物における phosphorylase 作用の研究 I. 気孔発生過程の表皮組織における phosphorylase 作用

1) 多くの植物で気孔の孔辺細胞には顕著な phosphorylase 作用が検出されるが、孔辺細胞と同じく原表皮組織から発達した一般表皮細胞では通常この酵素作用を認めない。本実験では、発育過程における葉の表皮組織で、これらの兩種細胞における phosphorylase 作用の消長をムラサキツユクサ、ヨウシュチョウセンアサガオおよび、ササゲを材料として顕微化学的に検討した。

2) 原表皮組織には自然状態ではでんぷんの存在を認めないが、G-1-P 溶液に浸しておくと、組織のすべての細胞が顕著なよう素反応（でんぷん形成）を示す。

3) 組織の生長につれて、そこに分化した孔辺細胞では phosphorylase の作用はしだいに強まるが、その他の表皮細胞では逆に弱まってゆき、生長の完成した表皮では孔辺細胞にのみ phosphorylase の作用が認められる。

4) 茎の生長点の付近にある若い組織には、自然状態ではでんぷんの存在を認めないが、組織の G-1-P 液処理によって茎の先端部および葉原基の組織全体に顕著な phosphorylase 作用が検出される。

5) 気孔の孔辺細胞における phosphorylase 作用はこの細胞の滲透圧変化に関与することによって気孔開閉に寄与するといわれるが、幼細胞の含有する phosphorylase は、同じく滲透圧変化を介して生長に寄与するのではないかと考えられる。(広島大学教養部生物学教室)