Studies on "Bluing Effect" in the Petals of Red Rose II. Observation on the development of the tannin body in the upper epidermal cells of bluing petals

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Received July 6, 1972

In a previous paper (Yasuda 1970), it was indicated that the bluing effect of red rose petals is mainly due to the blue spherule presented in the upper epidermal cells of fresh petals, and it was also suggested that some tannic substance is a basic component of this spherule. At the same time, a possibility was foreseen that the bluish color of this spherule may be exhibited essentially through a combination of the following three component: anthocyanin, tannic substance and iron. A question arises how the spherule is formed. As a first step in resolving this question, the present study was designed to make observations on the development of the blue spherule which will be called "tannin body" in this paper.

Material and methods

The plant used in the present investigation was the same as the previous one (Yasuda 1970), a red rose cultivar, cl-Crimson Glory, grown outdoors. Two types of the microscopic specimens were prepared from petals exhibiting the bluing effect in various intensities, one being fixed material and the other fresh epidermis.

The small pieces of petal (5 × 5 mm square) were fixed with Kaiser's fluid¹ for 18 hours. After completion of fixation they were thoroughly washed in running water for 4 hours to remove traces of the fixing reagents. The dehydration was performed through a series of butylalcohol, and at last embedded in paraffin as usual. Paraffin blocks were cut 20 micra in thickness. The sections were stained with toluidine blue, fuchsin, fast green and the combination of toluidine blue and fuchsin. The fresh epidermis was floated onto distilled water subsequently to being peeled off from the petals, and used for observation without any fixation and staining, being mounted only in water.

Observation

The structure of upper epidermal cells of rose petals

Since general views of the cross section of the rose petals and the contours of

¹ Kaiser's fluid is composed of 10 g of a mercuric chloride, glacial acetic acid (3 ml) and distilled water (300 ml).

upper epidermal cells were previously described (Yasuda 1964, 1965), only the internal structure of upper epidermal cells is briefly explained here.

Since the upper epidermal cells of rose petals does not differ fundamentally from general plant cells with reference to the cellular constituents, detailed descriptions about each cellular elements will be omitted. However, it is desirable to give illustrations of the features of the cytoplasm, because it seems to be important in considering the initial appearance of the tannin body.

In the less mature cells it is recognized, in addition to the parietal cytoplasm surrounding the central vacuole, that some cytoplasmic strands bridge the central

Fig. 1. Schematic figure showing the upper epidermal cells of rose petals. a: less mature cell b: mature cell. Abbreviations: n, nucleus; cw, cell wall; cv, central vacuole; c, cytoplasm; cs, cytoplasmic strands. Cytoplasm includes plastids and granules, while they are not shown in the figure. Arrow marks the region at which several cytoplasmic strands fuse to acquire a radiated shape.

vacuole, especially in the upper region of the cell (Fig. 1 a). Several strands fuse with one another at a certain place to acquire a radiated shape (arrow, Fig. 1 a). On the other hand, in the mature cells only a narrow layer of parietal cytoplasm is encountered (Fig. 1 b), and the cytoplasmic strands are not found probably because they have been attracted to the parietal cytoplasmic layer as the cell matured.

Observation of the development of the tannin body

The present observations were made by comparing two kinds of specimens, fresh and fixed materials.

For convenience of explanation the developmental sequence is divided into the following three stages according to the shapes of the tannin body.

A. The early stage: In the fresh epidermis of the petals showing the bluing effect in a slight degree, several filamentous bodies appear in the cell, being aggregated into a radiated forms and being tinged with blue color (Fig. 2). In the fixed material of the petals in the same degree of bluing there is found a radiated structure sensitive to toluidine blue, fuchsin and fast green in the position and the form corresponding to the filamentous body in the fresh materials (Fig. 3 a). Each strand of this radiated structure grows thicker as the bluing progresses in degree, and the radiated appearance becomes somewhat star-like in both specimens (Fig. 3a').

B. The middle stage: A tannin body is encountered in every cell of both specimens prepared from the petals producing the bluing effect in a more advanced degree than that of the preceding stage. In the fixed material the typical tannin body comprises at least the three following layers as investigated below (Fig. 3 b). A central layer: this occupies the bulk of the tannin body, and is stained well with toluidine blue, and slightly with fuchsin and fast green. A layer

enveloping the central layer: this layer is densely stained with fuchsin and fast green. The thickness of this layer differs among the different tannin bodies. A membrane-like layer: this layer is situated on the surface of the tannin body, being

Fig. 2. The fresh epidermis peeled off from the petal showing the bluing effect in a slight degree. Arrows indicate the blue filamentous bodies aggregated into radiated form. $\times 800$.

Fig. 3. Upper epidermal cells of the petals exhibiting the bluing effect in various degree. The petals were fixed with Kaiser's solution and stained with the combination of toluidine blue and fuchsin. $\times 800$. a and a': early stage, b: middle stage, c: latter stage.

sensitive to toluidine blue. In the typical examples this layer is distinguishable very clearly, but in some cases it is found to be slight and occasionally it was not

observed.

A part of the tannin body is partially associated with the parietal cytoplasmic layer. In the fresh epidermis, these three layers are not distinguishable.

C. The latter stage: The upper epidermal cells of the petals fully exhibiting the bluing effect, include a tannin body which has a massive appearance. The feature varies according to the specimens, being ellipsoid-, gourd-, or ovoid-shaped and the like in appearances. In the fixed material, typical examples show the three layers having the same pattern as those of the middle stage. A flank of the tannin body also is in contact with the parietal cytoplasm (Fig. 3 c). Tonoplast is not discernible through the growing stages.

Discussion

From a comparison between two kinds of specimens, it is clear that the structures which are stained with the dyes used in the fixed materials correspond to the blue spherule in the fresh epidermises, throughout all the growing stages. As discussed previously (Yasuda 1970), in the rose petals the regions positive for Kaiser's solution-toluidine blue method can account for the abundant inclusion in tannic substance. Hence, the present author would like to interpret the blue staining with toluidine blue as an identification of tannic substance, giving the name of tannin body to the blue spherule.

As for the reason why the tannin body indicates the radiated feature in the early stage, the explanation may be given that the tannin body originates at the region where the cytoplasmic strands crossed radiately. That the regions sensitive to toluidine blue also stained well with fuchsin and fast green, may suggest that the cytoplasm adjacent to the origin of the tannin body has a special property, for instance, some colloidal state.

From the staining of the tannin bodies in the fixed materials in the middle and latter stages, it seems reasonable to assume that the central layer is abundant in tannic substances, that the layer enveloping the central layer is composed of cytoplasm acquiring some special properties and that the membrane-like layer has a cytoplasmic character accumulating tannic substances. If this assumption is true, then it may be more likely that the tannin body has its origin in the interior of the cytoplasmic strands.

From the fact that the part of tannin body in the middle and latter stages is associated with the parietal cytoplasmic layer and nevertheless is initiated in a part of the cytoplasmic strands, the explanation can be that the tannin body may be attracted to the parietal cytoplasmic layer as the cytoplasmic strands are drawn to the layer.

The relationship between the tannin body and tonoplast is also a question to be determined. If the tannin body has its origin in the interior of the cytoplasm, the tonoplast would be expected to exist on the exterior of the tannin body (Fig. 4 a), unless it tears the tonoplast in the course of development. When the tannin body which has originated in the interior of the cytoplasm gets partially out into the central vacuole by tearing the tonoplast in the course of development, the tono-

plast must then be illustrated as shown in Fig. 4 b. Fig. 4 c would be available for these cases in which the origin of the tannin body occurs on the surface of the cytoplasmic strands.

There is literature concerned with tannin cells,* but information correlating to the developmental process of tannin bodies is sparse. Tokugawa *et al.* (1936) made an observation on the formation of tannin cells in the fruit of persimmon, and Toriyama (1955) studied the development of the tannin vacuole in the motor cells of *Mimosa pulvinus*. In the fruits of persimmon, a tannic substance begins to accumulate in some small vacuoles, in near association with the formation of the vacuole in the cytoplasm. In the motor cells of the *Mimosa pulvinus*, a tannic substance appears in a particular vacuole termed the "tannin vacuole" in the cytoplasm. These examples would make it more likely that, in the upper epidermal cells of rose petals, the origin of the tannin body is in the interior of the cytoplasm with an accumulation of the tannic substance in a special vacuolar system.

The tannin body of the rose petals, looked at in the middle stage of development, is superficially similar to that of the oriental persimmon (Kitagawa 1968) and of the *Mimosa* motor cell (Toriyama 1954, 1966), and especially bears more resemblance to the latter material. Hence, it seems desirable to make further observations on the blue spherule, that is the tannin body in the rose petals, in conjunction with the results on the motor cell of *Mimosa*.

Though some additional work is needed, the present observations can provide a possible explanation, for the bluing development in the red rose petals, that the tannin body (having certain points of likeness to the tannin vacuole) grows in an upper epidermal cell and anthocyanin (previously contained in the cell sap) reacts to it with iron (probably accumulated by the transpiration stream).

There are two discussions on the cause of the bluing effect in the rose petals reported to date, one cause being attributed to the lack of tannic substance in the petal tissue (Currey 1927), and the other cause ascribed to the rise of the pH value in the cell sap denying Currey's theory (Weinstein 1957). In both experiments,

Fig. 4. Schematic figure illustrating the relationship between tannin body and tonoplast. a) indicates that the tannin body originating in the inside of cytoplasm develops without tearing the tonoplast, b) that the tannin body originated in the inside of cytoplasm gets out partially into the central vacuole by tearing the tonoplast in the course of development and c) that the origin of the tannin body occurs at the surface of the cytoplasmic strand. Abbreviations: tb, tannin body; cv, central vacuole; to, tonoplast.

* The literatures concerning these subjects were cited by Toriyama (1953).

the tannin contents were estimated from extracts from whole flower. However, as shown in this paper there can be a sample that the tannic substance is localized under a massive state in a cell. Hence, it seems quite inadvisable to consider the cause of the bluing effect in the rose petals to be due to the difference in the total contents of the tannic substance in a flower.

Summary

1. The tannin body begins to appear as a radiated structure in the early stage of development. Such a structure of the tannin body seems to be resulted from that the origin occurs at the region where the several cytoplasmic strands running across the central vacuole fuse each other to form a radiated shape.

2. Each thread of the radiated structure grows thicker, and the radiated shape of tannin body becomes somewhat star-like.

3. The star-like structure develops into a spherical body in the middle stage of the development. Following three layers are distinguished in the typical body: a) a central layer which seems to be composed principally of tannic substance, b) a layer enveloping the central layer, perhaps having special property of cytoplasm, c) a membrane-like layer accumulating tannic substance and covering the body.

4. The spherical tannin body becomes a massive appearance at the latter stage of development.

5. A part of the tannin body is in contact with parietal cytoplasmic layer.

6. It is suggested that the tannin body in the rose petals superficially resembles the tannin vacuole in the motor cell of *Mimosa pulvinus*.

Acknowledgement

The author expresses his gratitude to Professor Dr. H. Toriyama of Tokyo Woman's Christian College for his valuable advice and criticism in the preparation of this paper. Also, the present author wishes to thank Professor D. McCoy, Science English Center, Faculty of Science and Engineering, Sophia University, Tokyo, for his generous help in the preparation of this manuscript.

Literature cited

- Currey, G. S. 1927. J. Proc. Roy. Soc. N. S. Wales **61**: 307-314.
Kitagawa, H. 1968. Jour. Japan. Soc. Hort. Sci. **37**: 89-94.
Tokugawa, Y. and Yuasa, A. 1936. Bot. Mag. Tokyo **50**: 277-283.
Toriyama, H. 1953. Cytologia **18**: 283-293.
— 1954. Cytologia **19**: 29-40.
— 1955. Bot. Mag. Tokyo **68**: 203-208.
— 1966. Protein Nucleic Acid Enzyme **11**: 599-602.
Weinstein, L. H. 1957. Contris. Boyce Thompson Inst. **19**: 33-48.
Yasuda, H. 1964. Jour. Fac. Lib. Art. Sci. Shinshu Univ. No. 14 (II): 31-37.
— 1965. Jour. Fac. Lib. Art. Sci. Shinshu Univ. No. 15 (II): 15-21.
— 1970. Bot. Mag. Tokyo **83**: 233-236.
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