Accumulation and Conversion of 3-O-Methyl-D-Glucose in Guard Cells of *Commelina communis* L.

Peter Dittrich and Klaus Fischer

Botanisches Institut der Universität München, Menzingerstraße 67, D-8000 München 19

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Young plants of *Commelina communis* L. were allowed to take up [U-^{14}C]-3-O-methyl-D-glucose through the cut stem via the transpiration stream. The distribution of this tracer carbohydrate in the plant was analyzed and its accumulation investigated in the epidermal tissue by microautoradiography. Over feeding periods from 1 to 24 h 3-O-methyl-D-glucose accumulated predominantly in the guard cells of the stomata, whereas the other epidermal cells contained only minor amounts. Thus, guard cells, despite of the lack of plasmodesmata and the photosynthetic carbon reduction cycle, can easily obtain the carbohydrates from photosynthetic tissues necessary to drive the turgor mechanism of opening and closing. Depending upon the part of the plant, 3-O-methyl-D-glucose was converted during a period of 4 h up to 30% to the corresponding 6-phosphate despite the alledged inert nature in metabolism of this artificial carbohydrate.

Introduction

Guard cells of stomata were shown to contain chloroplasts but also to be devoid of the Calvin Cycle [1], and therefore to depend upon the import of carbohydrates from photosynthetically active tissues. Hence, isolated strips of epidermal tissue can be incubated on solutions of labelled carbohydrates, which are taken up and converted to starch by the guard cells [2]. This short-distance transport does not proceed via plasmodesmata, but has to pass the control of the plasmalemma, since fully differentiated guard cells do not possess plasmatic connections to neighbouring cells. In order to test the feasibility of a long-distance transport from source leaves or the stem via the vascular bundles to the sink of the epidermis we used a non-physiological carbohydrate, 3-O-methyl-D-glucose. This compound is considered to be transported similar to naturally occurring sugars, but supposedly is not subject to conversion reactions, which might take place on the way between source and sink. The plant with which the experiments were carried out was *Commelina communis* L., whose leaves can be easily stripped of its epidermis.

Abbreviations: 3-O-MG, 3-O-methyl-D-glucose.

Reprint requests to P. Dittrich.

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Materials and Methods

Plants of *Commelina communis* L. were cultivated in a greenhouse at 23–24 °C during the day and at 16–18 °C during the night. Daylength was extended to 16 h by illumination with mercury vapour lamps. Germination of *Commelina* seeds was accelerated and standardized by incubation for 24 h in a solution of 2×10^{-5} M gibberellic acid and kinetin in dry acetone prior to planting in wet soil.

The plants were used in this study when they had fully developed two leaves beside the cotyledon. The stem was cut close to the surface of the soil and subsequently shortened with a razor blade under water to the appropriate length. After being transferred to a 1 ml conical Eppendorff vessel, containing 0.4 ml of the [U-^{14}C]-3-O-methyl-D-glucose solution (12.5 µCi/ml; 74.2 Ci/mol), the plants were allowed to take up to tracer solution, which was replaced by refills with distilled water for periods up to 24 h. The feeding experiments were carried out at 24 °C and about 45% relative humidity under constant illumination of 15 W/cm² by an incandescent lamp. The air was circulated slowly to promote transpiration.

For the determination of 3-O-MG taken up and converted, the plant tissues were homogenized and extracted three times in boiling 70% ethanol. The radioactivity of the extracts was determined in a scintillation counter, and after evaporation of the extraction solvent the mixture of metabolites was subjected to descending paper chromatography according to Willmer and Dittrich [3].

In order to localize the radioactivity within the epidermis, detached epidermal strips were quickly rinsed with distilled water, instantly frozen in liquid nitrogen and freeze-dried. The samples of approximately 10 mm² were subsequently subjected to microautoradiography according to Dittrich and Raschke [4].

The conversion product of 3-O-MG detected on paperchromatograms after autoradiography on Kodak X-ray film was eluted with water and incubated with alkaline phosphatase. This treatment resulted

in the formation of 3-O-MG. Since the untreated derivative exhibited a charge in high-voltage paper electrophoresis comparable to a monophosphate ester, the compound was oxidized with J_2 in methanolic KOH according to Floss and Simon [5] in order to discriminate between a phosphate ester at carbon 1 or carbon 6 of 3-O-MG. The reaction product turned out to be the phosphate ester of 3-O-methyl-gluconic acid. The identity of the latter was corroborated by cochromatography with the authentic compound.

Results

The young *Commelina* plants took up the solution of labelled 3-O-MG within 30 min. One hour thereafter the first tissue samples were cut out of the lower and upper epidermis of the secondary leaf (second leaf after the cotyledon). The localization by microautoradiography of the radioactivity in the epidermal strips revealed that the tracer transported via the transpiration stream had already reached the epidermis; the label was predominantly located in the guard cells. However, a slight staining with silvergrains of the area between the stomata indicated that some 3-O-MG had also been taken up by normal epidermal cells. This pattern of guard cells being heavily labelled and the epidermal cells containing significantly less 3-O-MG prevailed up to 24 h of incubation time.

Since after short periods more than 80% of total label resided in the tissue of the stem and the cotyledon, a 4 h period was chosen to investigate metabolites and distribution of label. The data compiled in Table I show a rather uniform distribution of the tracer over the entire plant. In the secondary leaf, which was dissected into upper and lower epidermis and the mesophyll tissue in between, more than 30% of the radioactivity resided in the epidermal tissue and there mainly in the guard cells. These metabolically very active cells apparently constitute a sink for compounds – in this case a carbohydrate – transported in the vascular system.

Table I. Distribution of label in *Commelina communis* L. after 4 h of [U-^{14}C]-3-O-methyl-D-glucose uptake. Percent of total activity in the plant.

Tissue	Fresh weight [mg]	3-O-MG	3-O-MG-phosphate
stem	81.6	28.1	1.8
cotyledon	28.9	26.8	3.1
primary leaf	60.2	14.2	0.5
secondary leaf	87.4	22.2	3.2
upper epidermis		2.6	0.8
mesophyll		16.0	1.4
lower epidermis		3.6	1.0

It was interestingly to note that the alledgedly inert methylated glucose was converted by the plant tissue into a monophosphate. By means of alkaline oxidation with J_2 this derivative was turned into the corresponding gluconic acid. Since this treatment excluded the phosphate group being bound to carbon 1 of 3-O-MG, it appears to be very likely that carbon atom 6 represents the site of the phosphate ester bond. This assumption is drawn mainly from a biochemical standpoint; an enzyme of the hexokinase-type could be responsible for this reaction. From a pure chemical standpoint one would have to take also into consideration a phosphate ester in position 2 or 4. Minor conversion rates of non-physiological compounds infiltrated into plants would not be remarkable; however, in *Commelina communis* L., depending upon the organ of the plant, up to 30% of the radioactivity recovered were found in 3-O-MG-phosphate. The possibility that the phosphorylation was an artefact due to bacterial contamination in the feeding vessel could be ruled out, since the feeding solution monitored during uptake did not develop any contamination. The fact that epidermal tissues contained the highest levels of 3-O-MG-phosphate could give rise to the speculation of a selective uptake mechanism that favours the import of the phosphate which was prior phosphorylated in the vascular tissue or a source leaf. Incubation of isolated strips of epidermis on a solution of 3-O-MG led to the uptake of this compound and its subsequent conversion to its phosphorylated derivative. Concluding from this observation all parts of *Commelina communis* L. are capable of phosphorylating 3-O-MG. The parameters which regulate this conversion, however, cannot be found solely in age or metabolic activity, since as described in Table I the single cotyledon contains much more of the phosphate than the subsequent primary leaf, but somewhat less than the secondary leaf.

Discussion

The provision of energy in guard cells for the operation of the opening and closing mechanism is

not a matter of photosynthetic activity in these cells; they are devoid of the Calvin Cycle and consequently depend upon the import of energy, presumably in form of carbohydrates. The lack of cytoplasmic connections between guard and epidermal cells facilitates the independent operation of the turgor mechanism on the one hand, but simultaneously aggravates the mode of transport and provision of required metabolites. Though guard cells had been shown to take up carbohydrates when floating on incubation solutions [2], we demonstrated in this study that a carbohydrate, 3-O-MG, fed via the transpiration stream is distributed into all parts of the plant, but is predominantly transported into the epidermis. There 3-O-MG accumulates in the guard cells. Thus the supply of stomata with carbohydrates even from distant photosynthetically active tissue does not appear to pose any problem. Since sucrose is the major carbohydrate transported *in vivo* in the vascular system we can assume according to the results with the model compound 3-O-MG that guard cells are mainly supplied and refueled with sucrose carried by the transpiration flow. The uptake of sucrose from the cell-free space into the guard cell, however, is apparently not catalyzed by a proton cotransport in comparison to the uptake of glucose or even 3-O-MG, since no interference with the potassium uptake during opening was observed by Dittrich and Mayer [6]. During a related study on environmental pollution, Hampp and Ziegler [7] investigated the uptake of lead by plants. Feeding lead nitrate to spinach plants via the cut stem, the transpiration flow carried the lead into the epidermal tissue where it accumulated in the guard cells. The epidermal cells in between contained only traces of lead.

The conversion of 3-O-MG into its monophosphate at carbon 6 is probably catalyzed by an enzyme of the hexokinase-type. Usually this carbohydrate is considered not to be subjected to metabolic reactions and consequently sugar transport and uptake studies frequently employ this compound. From 3-O-MG feeding to *Neocosmospora* [8], however, it was reported that some conversion to an unidentified compound occurred, while a study of Kaback [9] with the phosphotransferase-system of *E. coli* emphasized that the uptake is mediated by a phosphorylation at carbon 6. These cases are rather rare; however, our findings with 3-O-MG and those by Zemek *et al.* [10] with 2-deoxyglucose recommend strongly that the use of compounds alledgedly not subject to turnover reactions be closely controlled.

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