Some Observations on the Saponin Accumulation in Oat Seedlings and on the Transformation of the Avenacosides to the Antibiotic 26-Desgluco-avenacosides

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The accumulation of the steroidal saponins avenacoside A and B in oat was investigated in green and etiolated seedlings and was found to be strictly bound to the growth of the seedlings. No significant differences were detected between green and etiolated seedlings, showing that there is no relation between saponin accumulation and plastid development. As the role of the oat saponins has to be seen as a protective device against fungi and bacteria, the transformation of the inactive avenacosides to their corresponding antibiotic 26-desgluco-avenacosides was tested in dependence of pH and temperature. During disruption of leaves, transformation occurs almost completely over a wide pH-range (pH 4–pH 10); the degree of transformation exhibits no distinct temperature optimum but a drastic decrease at 70 °C.

Introduction

Recently published experiments [1] have led to the conclusion that the prolamellar body (PLB) of oat is not built up by steroidal saponins. The "PLB-saponins" are artificial transformation products of the avenacosides A and B, which are mainly localized in the vacuoles [2]. Contradictory earlier conclusions may be due to an adsorption of desglucoavenacosides, originating from the vacuolar avenacosides during disruption of the cell, to PLB-tubules; such an attachment can also be demonstrated with PLBs from saponin-free plants, *i.e.* rye or wheat [3].

The function of saponins in primary leaves and coleoptiles must be regarded to as a protective device against fungi and bacteria [3–6]. If intact oat leaves are wounded, the avenacosides are converted within a very short time to the active 26-desgluco-avenacosides. This conversion is most likely catalysed by a very specific β-glucosidase, which splits off only the glucose molecule attached to C-26 but does not affect the glucose molecules of the side chain at the C-3 position [*cf.* 7].

In order to get more information about the capacity of this "saponin-system" we investigated the accumulation of saponins in oat seedlings and the pH- and temperature dependence of the transformation process.

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Materials and Methods

Growing and extraction conditions

Green and etiolated seedlings of *Avena sativa* L. (var. Flämingskrone) were grown as described elsewhere [1]. For investigations on the saponin accumulation the overground parts of the seedlings (leaves + coleoptiles) were harvested and extracted with boiling methanol [1, 10].

Determination of saponins

Saponins were determined by HPLC on RP-8-columns using a water-acetonitrile gradient [10]. All extracts were diluted to 40% methanol in water prior to injection.

Transformation experiments

The avenacosides of oat are quickly transformed to their corresponding 26-desgluco-avenacosides if the plants are homogenized in water. The influence of the pH was investigated by homogenizing the plants in the following buffer systems: citrate buffer 0.1 M (pH 1–6), phosphate buffer 0.15 M (pH 6–8), borate buffer 0.2 M (pH 8–9) and glycocoll buffer 0.1 M (pH 9–12). The resulting homogenates were extracted after an incubation of 5 minutes by adding methanol to 80%. Temperature dependence was tested by homogenizing the plants in water of different temperatures: intact plants were incubated in the water for 5 minutes and after homogenization extracted as described above.

Results and Discussion

As shown recently [1] the concentration of the steroidal saponins and the composition of the saponin mixture in oat leaves is similar in green and etiolated primary leaves and does not change significantly on a dry weight basis during growth or greening of the plants. These results lead to the assumption that the amount of steroidal saponins per seedling is strictly bound to growth. To proof this assumption more thoroughly we determined the saponin concentration in the overground parts of the seedlings (leaves + coleoptiles) during growth.

As shown in Figs. 1 and 2 saponins accumulate during growth in green as well as in etiolated seedlings. The concentration of the 26-desgluco-avenacosides is neglectible small and may be due to a transformation during extraction [1]. If the growth is reduced or stopped during ageing also the saponin production decreases or comes to a complete stop. This decrease in saponin production was observed at the 9–11 day of growth; within this period a decline in growth was obvious from a decrease in dry weight and pigment accumulation. Saponin accumulation was found to be similar in green and in etiolated seedlings. The only difference found was in the ratio avenacoside A/avenacoside B. In green seedlings the accumulation curve of avenacoside B intersects the curve of avenacoside A at day 4, whereas this intersection occurs at day 7 in etiolated seedlings. This difference can be explained by the different growth rate of coleoptiles in green and etiolated seedlings. As coleoptiles almost exclusively contain avenacoside A [3], the late intersection in etiolated seedlings may be caused by a more intensive growth of coleoptiles during etiolation.

As shown by the present results the saponin accumulation in green and etiolated seedlings depends on the growth only. Earlier assumptions that the prolamellar body (PLB) in etioplasts is built up by these steroidal saponins [8, 9] could be rejected; these earlier results may be explained by an adsorption of transformed saponins to PLB-tubules [1, 3]. The transformed saponins 26-desgluco-avenacoside A and B originate from the avenacosides A and B if the cell is wounded. This transformation process seems to be a protective device against fungi and bacteria as the 26-desgluco-avenacosides, but not the avenacosides, possess antibiotic activity [5–7]. Lüning and Schlösser [5] presented evidence for a β-glucosidase to be responsible for the transformation. They tested a crude enzyme preparation, using *p*-nitro-phenyl-β-D-glucopyranoside as substrate for the *in vitro* assay and found a pH-

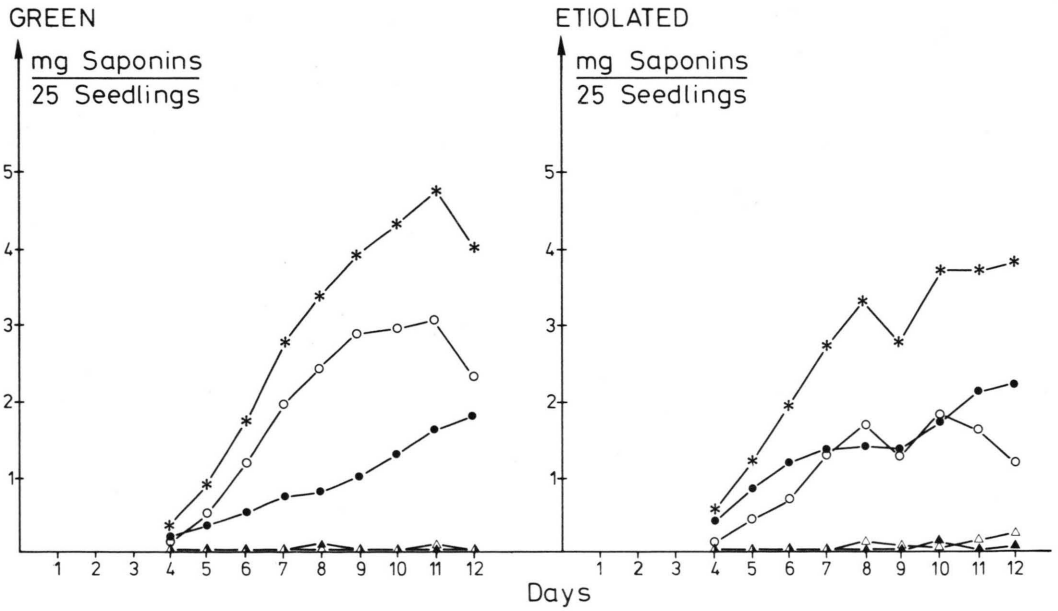

Fig. 1. Accumulation of steroidal saponins in the overground parts of green and etiolated seedlings of oat during growth from day 4 after germination till day 12. ●, Avenacoside A; ○, avenacoside B; ▲, 26-desgluco-avenacoside A; △, 26-desgluco-avenacoside B; *, total saponins.

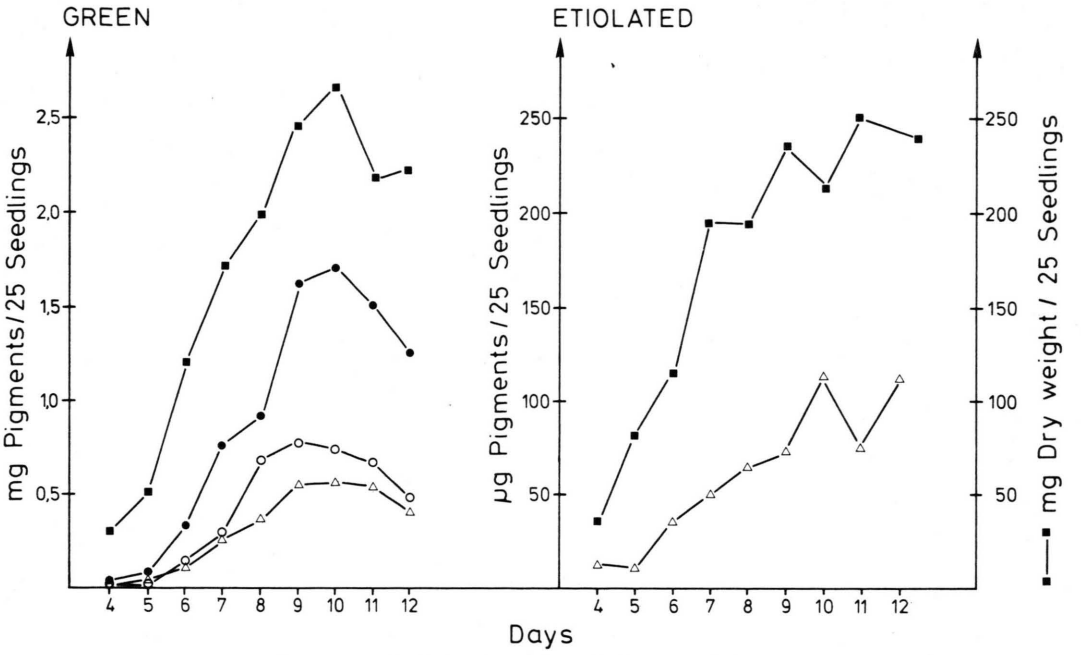

Fig. 2. Growth parameters in green and etiolated seedlings during growth from day 4 after germination till day 12. ■, dry weight; ●, chlorophyll a; ○, chlorophyll b; △, carotenoids.

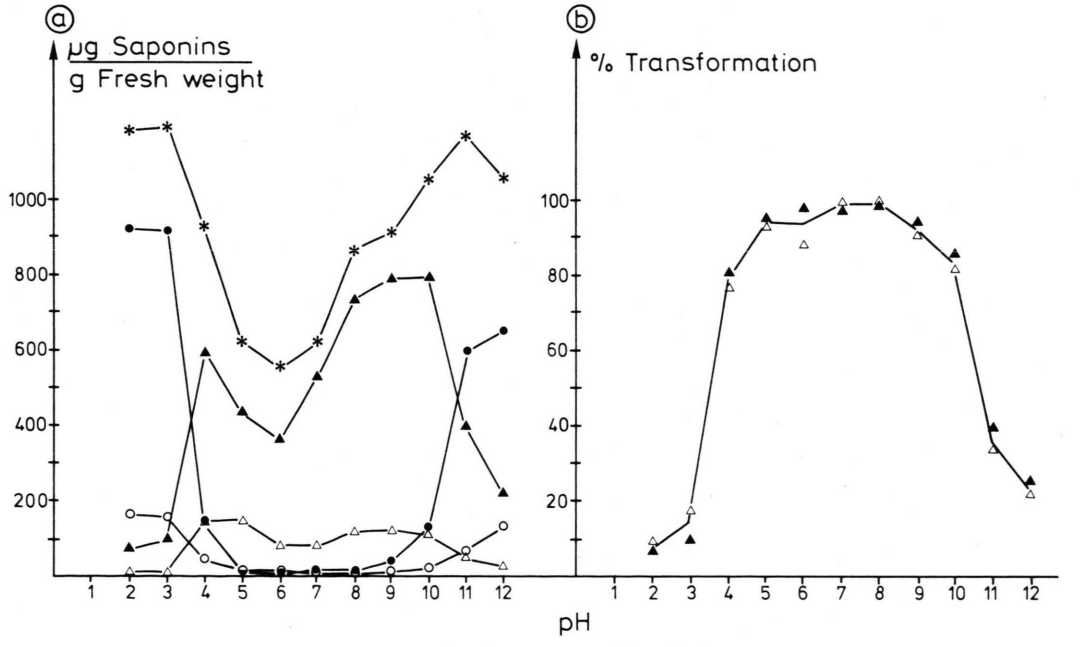

Fig. 3. Transformation of avenacosides to 26-desgluco-avenacosides during homogenisation of leaves in buffers depending on the pH. a) Saponin concentrations in extracts of homogenates. ○, avenacoside A; ●, avenacoside B; △, desgluco-avenacoside A; ▲, desgluco-avenacoside B; *, total saponins. b) Percentage of transformation expressed as portion 26-desgluco-avenacosides per total saponins. △, transformation of avenacoside A to 26-desgluco-avenacoside A; ▲, transformation of avenacoside B to 26-desgluco-avenacoside B.

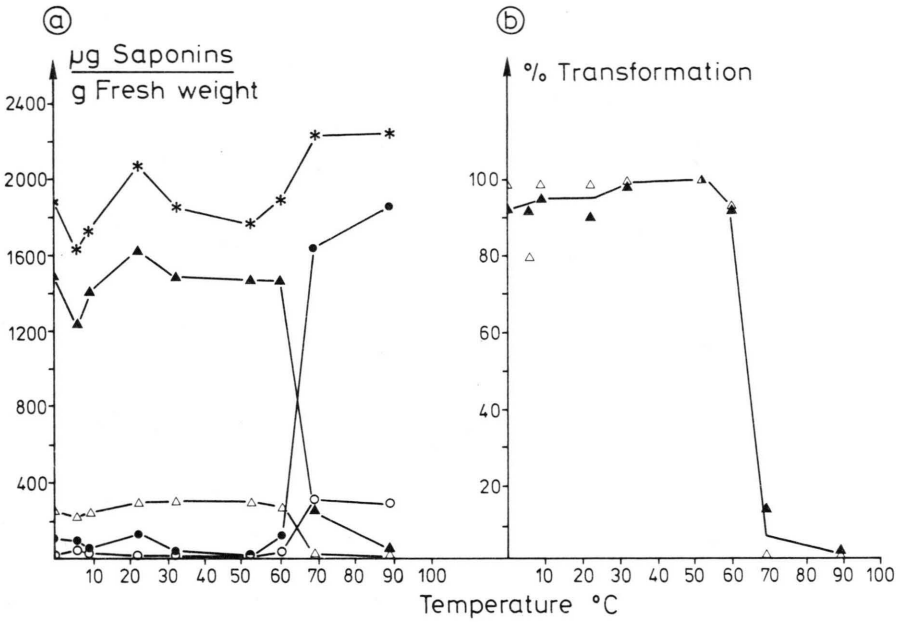

Fig. 4. Transformation of avenacosides to 26-desgluco-avenacosides during homogenisation of leaves in water depending on the temperature. a), b) for explanation see Fig. 3.

optimum of about pH 6 which is typically found for β-glucosidases. However, the enzymatic cleavage of this artificial substrate may show a pH-optimum which is not identical with the pH-optimum of the natural substrate. In first experiments we tried to characterize the transformation of the avenacosides in crude leaf homogenates by blending the leaves in buffers of different pH and at different temperatures. By investigating the transformed saponins we thus used the natural substrate.

As shown in Fig. 3 the transformation during disruption of leaves in buffers occurs almost completely over a wide pH-range from pH 4–pH 10. This broad pH-optimum is contradictory to the findings of other authors [5] and may indicate that the transformation is not catalysed by a "typical" β-glucosidase. "Typical" β-glucosidases cleave all glucose moieties of avenacosides as they all are bound by β-bonds. However, during the very fast transformation of the avenacosides to the desgluco-avenacosides only the glucose moiety at the C-26 is cleaved. Nevertheless, the influence of a "typical" β-glucosidase on the saponin composition in extracts of homogenized leaves can be observed as shown in Fig. 3a. Around pH 6 a decrease in the saponin concentration is visible which may be due to a destruction of the avenacosides by cleavage of the other glucose moieties to products with a more reduced sugar content. These products are not measured with the HPLC-method used. Therefore the presence of at least two different β-glucosidases has to be assumed: one, responsible for the specific transformation (= 26-desgluco-avenacosidase), and the other, for a general cleavage of β-glycosides. However, it can not be excluded that a chemical or physical process is responsible for the specific transformation. One attempt to solve this question might be the investigation of the temperature dependence of the transformation process.

Therefore leaves were homogenized at different temperatures in water. As shown in Fig. 4 the degree of transformation exhibits no distinct temperature optimum as it would be expected for an enzymatically catalyzed reaction. The degree of transformation reaches 100% in the range of 0 °C–60 °C for both avenacosides. However, it is drastically decreased by temperatures higher than 65 °C–70 °C. This "denaturation" might be seen as evidence for an enzyme system, though it so far can not be excluded that the avenacoside-transformation in oat is a physical or chemical process, initiated by disruption of the cells. This question can only be

answered by a purification of the transforming system.

A purified enzyme fraction should show a high affinity to the natural substrates avenacoside A and B and a high transformation velocity. Nevertheless, uncommon properties of the transformation system may be expected because of the function of these oat saponins; the protective device against fungi and bacteria should also operate under unfavourable conditions. This transformation system is under investigation now.

Acknowledgements

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