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CHANGES IN UDPG:STEROL GLUCOSYLTRANSFERASE ACTIVITY IN *CALENDULA OFFICINALIS*

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Key Word Index—*Calendula officinalis*; Compositae; biosynthesis; steryl glucosides; acylated steryl glucosides.

Abstract—UDPG:sterol glucosyltransferase and acyltransferase which catalyse acylation of steryl glucosides are active in leaves, roots and flowers during the whole vegetative period of *Calendula officinalis*. The high activity of glucosyltransferase in young, developing tissues and its subsequent rapid decrease in activity in mature organs suggests that steryl glucosides are involved in the formation of some cell structures rather than in sterol transport as such within the plant.

INTRODUCTION

ENZYMATIC synthesis of steryl 3β-D-glucosides (SG) using UDPG as the sugar donor, as well as enzymatic transformation of steryl glucosides into monoacyl derivatives (ASG) using certain complex lipids as the acyl donors, has been demonstrated in various subcellular particulate fractions from a number of higher plants.[1–7]

Steryl glucosides and their acylated forms occur commonly in higher plants together with free sterols and steryl esters.[8–10] However, their physiological functions are not clear. It seems that studies of the distribution of the various enzymatic activities participating in the formation of sterols and their complex forms within the plant during development would contribute to a better understanding of the role of individual sterols in plants. This paper describes such a study using *Calendula officinalis*.

RESULTS AND DISCUSSION

In the course of incubation of homogenates prepared from various organs of *C. officinalis* with UDP-glucose-[U-^{14}C] a parallel synthesis of steryl glucosides (SG) and acylated steryl glucosides (ASG) was observed. The radioactivity of these two classes of compounds always amounted to 75–95% of all the radioactive glycolipids soluble in *n*-butanol. Previously,[7] we observed that in subcellular preparations from mature leaves synthesis of SG occurred mainly with endogenous sterols and was only slightly stimulated by addition of exogenous

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sterols. The present experiments, however, show that addition of sitosterol emulsion to homogenates of certain tissues (e.g. embryo axes of young seedlings) causes up to a tenfold enhancement of SG synthesis, indicating that endogenous levels of sterols available for glucosylation can be different in various tissues. For this reason emulsified sitosterol was always added to the incubation mixture. Since it is known[5–7] that ASG are formed by enzymatic acylation of SG, total incorporation of ^{14}C into both these compounds represents a measure of UDPG:sterol glucosyltransferase activity. Radioactivity incorporation into ASG, however, can be considered as only an approximate measure of acyltransferase activity since the levels of endogenous acyl donors in various tissues are not known.

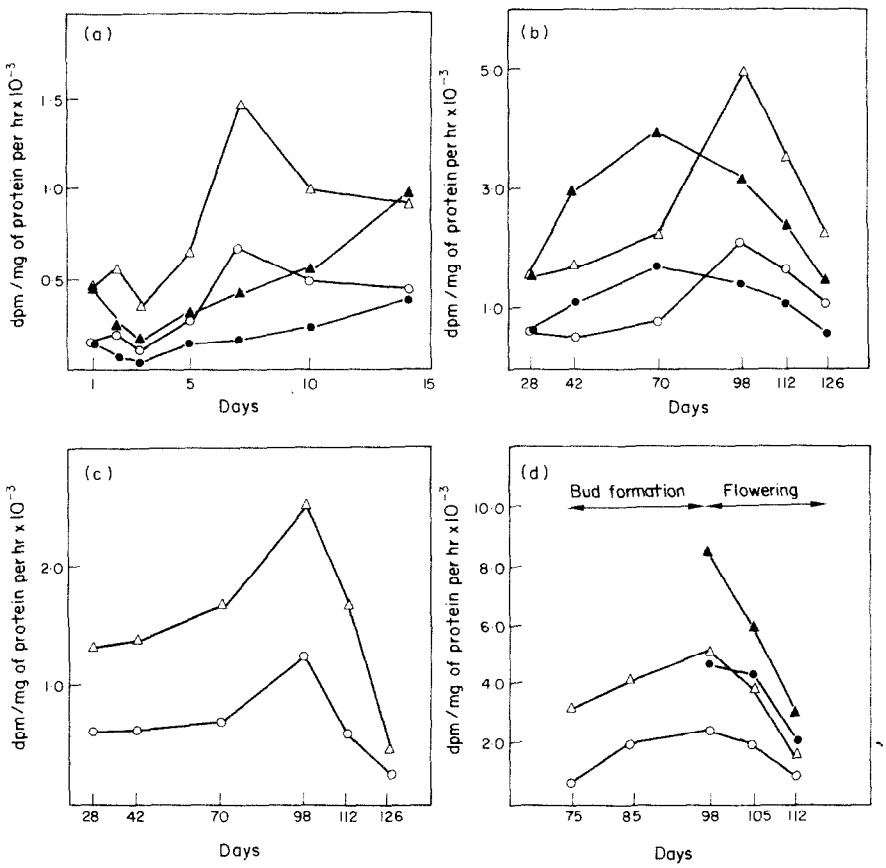

FIG. 1. BIOSYNTHESIS OF STERYL GLUCOSIDES (SG) AND ACYLATED STERYL GLUCOSIDES (ASG) IN HOMOGENATES FROM VARIOUS ORGANS OF DEVELOPING *Calendula officinalis* PLANTS.
(a) Embryo axes: SG + ASG(△), ASG(○); cotyledons: SG + ASG(▲), ASG(●). (b) tip leaves: SG + ASG(△), ASG(○); basal leaves: SG + ASG(▲), ASG(●). (c) roots: SG + ASG(△), ASG(○). (d) whole influorescence: SG + ASG(△), ASG(○); ligulate flowers: SG + ASG(▲), ASG(●).

Figure 1a shows changes in activity of UDPG:sterol glucotransferase in young seedlings up to the end of cotyledon growth (14-day-old plants). The ability to synthesize SG and ASG was established even in the embryos of 1-day-old seeds. Earlier stages of germination were not investigated because of difficulties in separation of the embryos from

the seed coat. From the second day the activity was determined separately in embryo axes and in cotyledons. An initial small decrease of glucosyltransferase activity (per mg of protein) was observed in both parts. On the third day a small increase of activity was found in cotyledons. On the other hand in embryo axes a rapid increase of activity was observed with a maximum on the 7th day followed by a decrease to the activity level of cotyledons. Figures 1b and 1c show changes of SG and ASG synthesis in leaves and roots of the older plants (after 28 days of development). After 42 days, the activity was determined separately for tip and basal leaves. A rapid increase of SG and ASG synthesis was observed in young, developing organs followed by a decrease in mature tissues. In the basal leaves maximal glucosyltransferase activity was found at the beginning of flower bud formation (70 days), that is, at the end of growth of these leaves. In the tip (younger) leaves and in the roots maximal activity was observed later during the full flowering period (98 days). Figure 1d shows SG and ASG synthesis in homogenates from the whole inflorescence and from the separated ligulate flowers. Maximal glucosyltransferase activity was found immediately after opening of the flower bud, followed by a rapid decrease. The capacity of flowers for SG and ASG synthesis markedly exceeds that of the whole inflorescence and all other organs of the plant. Under the incubation conditions 30–50% of total radioactivity of both types of steryl glucosides was usually associated with ASG. Only in homogenates from old flowers and roots did acylation occur at higher levels. This may be explained by similarities in the changes of acyltransferase activity with those of UDPG:sterol glucosyltransferase in green parts as well as in young roots and flowers.

The results show that all organs of *C. officinalis* plants are capable of SG and ASG synthesis during the whole vegetative period. This observation contradicts the suggestion that SG represents the intercellular transportation form of sterols from areas of sterol synthesis (e.g. mature leaves) to areas of intensive growth (e.g. immature leaves, flower buds).[11] Our observations indicating that young, rapidly developing tissues show the highest activity of glucosyltransferase suggests that SG are required for membrane expansion, a similar role to that postulated for free sterols and steryl esters.[12,13] This conclusion is also in agreement with some recent studies on changes of SG and ASG content in developing plants[10] as well as with studies on the subcellular distribution of these compounds.[14,15] In germinating *Raphanus sativus* the accumulation of SG and ASG proceeds in parallel with the accumulation of free sterols and is closely correlated with the increase of plant dry weight.[10] It has also been found that in *Lactuca sativa* leaves[14] and *Solanum tuberosum*[15] tubers both types of steryl glycosides are localized exclusively in subcellular particles such as chloroplasts, mitochondria and microsomes, their distribution pattern being similar to that of free sterols.[15]

EXPERIMENTAL

Calendula officinalis L. cv. Radio plants were grown at 3000 lx for 16 hr per day and at 24° during the day and at 16° during the night. Seedlings (up to 2 weeks) were cultivated on filter paper moistened with H_2O. Older plants were cultivated on sand enriched with mineral medium.[16]

Homogenates. Plant tissues were homogenized in a Potter homogenizer with cold 0·1 M Tris–HCl buffer, pH 7·5 containing 1 mM EDTA (3 ml per g of tissue). The homogenate was centrifuged at 3000 g.

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Enzyme activity. Samples containing 1 ml supernatant; 0·025 ml UDP-D-glucose-[U-^{14}C] (0·9 nM, 5·5 × 10^5 dpm) and 0·2 ml (200 μg) sitosterol emulsified in 12·5% aq. Tween 80 were incubated at 30°. Linear incorporation of ^{14}C into steryl glucosides was observed for periods of 0·5 to 2 hr depending on the preparation activity. The enzymatic reaction was terminated by addition of MeOH (3 ml) and boiling for 3 min. BuOH (15 ml) was added to each sample and precipitated proteins removed by centrifugation. The supernatant was then washed with H_2O (5 × 5 ml) and evaporated to dryness. Radioactive SG and ASG were separated by TLC on silica gel with $CHCl_3$–MeOH–H_2O (62:7·5:2) as solvent (R_f values 0·27 and 0·40 respectively) and located on the chromatograms by autoradiography. Radioactivity was measured after elution from silica gel with hot MeOH as described previously.[7] Protein was estimated according to Lowry *et al.*[17] All results are mean values from at least 3 independent determinations.

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