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# BIOSYNTHESIS AND METABOLISM OF STERYL GLYCOSIDES IN *SINAPIS ALBA* SEEDLINGS

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**Key Word Index**—*Sinapis alba*; Cruciferae; steryl glucosides; biosynthesis; metabolism.

**Abstract**—With $^{14}CO_2$, D-glucose-[U-$^{14}$C] and DL-mevalonate-[4*R*-4-$^3H_1$] used as precursors, a study was made of the labelling dynamics of the steryl glucosides (SG) and steryl acylglucosides (ASG) in *Sinapis alba* seedlings. The radioactivity of the sterol and sugar moieties, as well as of the fatty acid moieties in the case of ASG, was analysed separately. The course of incorporation of $^{14}$C from $^{14}CO_2$ and glucose-[U-$^{14}$C] into the sugar part of SG and ASG indicated that about 2/3 of the whole pool of the newly synthesized sterol glycosides of both types underwent rapid deglucosylation. Likewise, fatty acids in the ASG pool were rapidly exchanged. The present results point to a high metabolic activity of the sterol glycoside derivatives in plant cells.

## INTRODUCTION

Despite the widespread occurrence of steryl 3$\beta$-D-monoglucosides (SG) and of their 6′-*O*-acyl derivatives (ASG) in higher plants, the biological role of these compounds remains obscure. The existing hypotheses concerning their possible function in the cells can be divided into two views. The first considers glycosides to be substances of relatively low metabolic activity, such as final products of sterol metabolism [1], reserve forms of sterols [2] or inactive components of certain cellular structures [3]. The second group of hypotheses regards steryl glycosides as metabolically active substances. For example, their participation in intercellular transport of sterols from the sites of intense synthesis to sites of intense growth has been suggested [4]. Processes of glucosylation–deglucosylation of sterols incorporated into membranes have been attributed a role in the regulation of the properties of these membranes [5]. Moreover, the possible function of steryl glycosides as sugar residue carriers in some transglucosylation processes has been considered [6].

Several authors [7–9] have studied the labelling rate of the sterol moiety in steryl glycosides following incubation of the plant with radioactive precursors. These experiments have confirmed that the biosynthesis of the compounds discussed proceeds according to the sequence: free sterols → SG → ASG. Recent enzymic studies also afford strong evidence for this sequence [10–12]. However, these studies do not unequivocally demonstrate, whether and to what an extent SG and ASG are further metabolized. More extensive information on the metabolism of steryl glycosides can be obtained from a comparison of the dynamics of labelling of the different moieties making up the SG and ASG molecules, e.g. of sterols, glucose and fatty acids. Such experiments are described in the present paper.

## RESULTS AND DISCUSSION

White mustard seedlings (7-day-old) were incubated with $^{14}CO_2$ for 1 hr. After a lapse of 0–75 hr lipids were extracted with chloroform–methanol, whereupon free sterols (FS), steryl 3$\beta$-D-glucosides (SG) and steryl 6′-*O*-acyl-3$\beta$-D-glucosides (ASG) were separated by TLC. The glycosidic forms of sterols were hydrolyzed and the radioactivity was determined separately in the glucose, sterols, and fatty acids (in the case of AGS). It can be seen in Fig. 1a that during the experimental period the radioactivity gradually increased both in free sterols and sterols obtained from SG and ASG. A certain delay in labelling of SG and ASG sterols is consistent with the reaction sequence FS → SG → ASG, for which strong evidence was adduced by enzymic studies [10–12]. The course of glucose labelling in SG and ASG is entirely different (Fig. 1b). After completion of the incubation with $^{14}CO_2$, the radioactivity of the sugar moiety of SG attained a maximum in 2 hr; at this time the radioactivity of this moiety was about 24 times higher than that of the sterol moiety. However, the radioactivity in the glucose in SG and ASG rapidly decreased, to attain after about 24 hr a constant level corresponding to less than 1/2 of the maximum radioactivity. The course of labelling of the sugar in ASG was, in principle, similar. The shift of the maximum labelling of glucose in ASG (about 6 hr after completion of the incubation), as compared with SG, can be explained in the light of the above-mentioned reaction sequence for the biosynthesis of ASG. In this experiment the labelling of fatty acids from ASG was even more rapid than labelling of the sugar. It attained a maximum after 2 hr whereupon it dropped abruptly and after 75 hr it amounted to only 1/10 of the maximum value.

$^{14}CO_2$ is a relatively distant precursor for both the sugar moiety and the aglycone of SG and ASG. Figures 2a and 2b present the results of a similar experiment in which the plants were incubated with more specific precursors of the sugar and sterol moieties, i.e. with a mixture of D-glucose-[U-$^{14}$C] and DL-mevalonate-[4*R*-4-$^3H_1$]. It is seen that the results resembled those obtained in the experiment with $^{14}CO_2$. Incorporation of $^3$H into the sterols, both free as well as in SG and

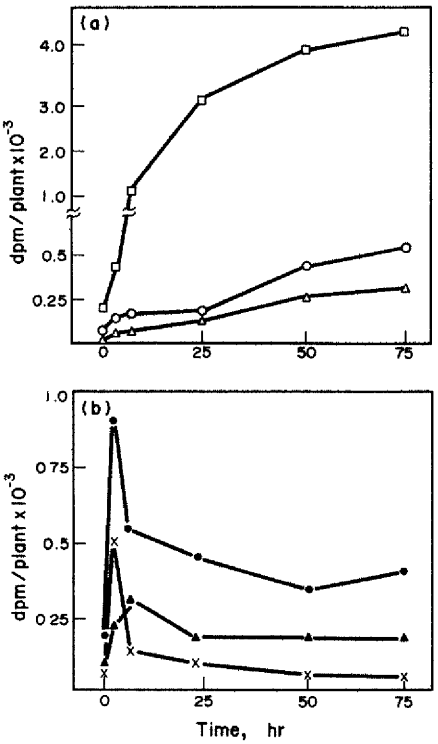


Fig. 1. Time-course of labelling of free sterols, SG and ASG after incubation of *S. alba* seedlings with $^{14}CO_2$. (a) $^{14}C$-incorporation into free sterols (□), sterols of SG (○) and sterols of ASG (△); (b) $^{14}C$-incorporation into glucose of SG (●), glucose of ASG (▲) and fatty acids of ASG (X).

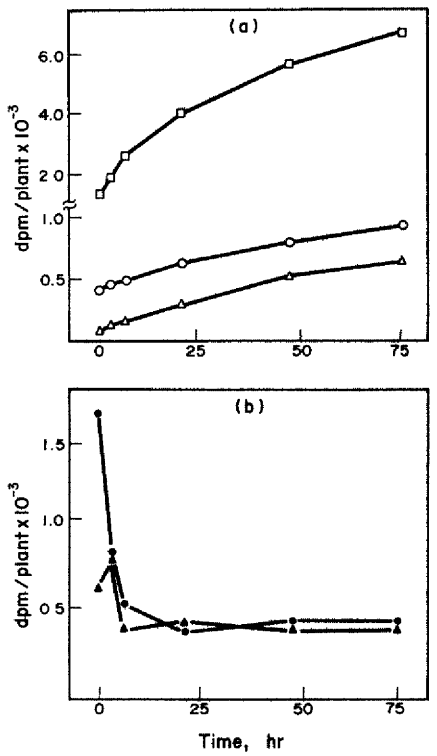


Fig. 2. Time-course of labelling of free sterols, SG and ASG after incubation *S. alba* seedlings with D-glucose-[U-$^{14}C$] and DL-mevalonate-[4*R*-4-$^3H_1$]. (a) $^3H$-incorporation into free sterols (□), sterols of SG (○) and sterols of ASG (△); (b) $^{14}C$-incorporation into glucose of SG (●) and glucose of ASG (▲).

ASG, gradually increased during the whole experimental period. Labelling of the sugar moiety, as compared with the experiment using $^{14}CO_2$, was more rapid, attaining a maximum in the case of SG immediately after completion of the incubation, and in the case of ASG 2 hr later. After longer incubation periods the glucose labelling in both SG and ASG dropped and subsequently became constant as found in the $^{14}CO_2$ experiment. This stabilization of the radioactivity possibly proves that a part of the SG and ASG pool (according to the data from Figures 1b and 2b, not more than 1/2–1/3) is characterized by a relatively low metabolic activity and shows no rapid turnover.

It is stressed that, according to control experiments, the contents of FS, SG and ASG exhibit a gradual slight increase. Seven-day-old seedlings contain (per 100 plants) 820 μg FS, 205 μg SG and 80 μg ASG, whereas after further incubation during 120 hr under conditions described in the Experimental these contents increase to 1120, 260 and 92 μg, respectively.

The present results uniquivocally prove that in *S. alba* seedlings at least a part of the pool of newly synthesized SG and ASG is rapidly metabolized. This is clearly indicated by the labelling rate of the sugar moieties in these compounds, and in particular by the rapid drop in this labelling upon prolonged incubation, with a steady—though slow—increase in labelling of the sterol residues and a slight rise of the total SG and ASG contents in the plants. Moreover, in the case of the ASG fraction, the results point to rapid exchange of the fatty acid residues. This indicates that in *S. alba* seedlings, rapid processes of FS glucosylation and SG acylation are accompanied by rapid de-esterification and de-glucosylation. The present observations are clearly incompatible with hypotheses which consider steryl glycosides to be metabolically inactive substances.

According to a number of authors [10–12], different subcellular fractions of various tissues of higher plants, including *S. alba* [12], are characterized by an unexpectedly high activity of UDPG: sterol glucosyltransferase and complex lipids: SG transacylase, when compared with the low SG and ASG contents of the plants. As previously shown[13], in *C. officinalis*, both these enzymes exhibit high activity even during the vegetative periods characterized by a marked drop in the SG content [14]. In the light of the present results it seems that the above observations should be interpreted in terms of an equilibrium between the processes of SG and ASG synthesis and degradation.

Kauss [6] has investigated the *in vitro* synthesis of SG and ASG by the subcellular fractions of bean leaves and found that the newly synthesized steryl glycosides are not metabolized any further. The discrepancy between these results and ours can probably be explained by the strict regulation of the processes of metabolism of SG and ASG in an intact plant or by the inactivation of the enzymes responsible for these processes during cell distruption.

On the basis of similar experiments Benson [15] showed that chloroplast galactosylglycerides are rapidly metabolized, and suggested that they may act as sugar carriers through the membrane. A similar function for steryl glycosides cannot be ruled out. It has been

shown [10–12] that UDPG: sterol glucosyltransferase is localized in specific fractions of the cell membranes.

## EXPERIMENTAL

*Administration of precursors.* $^{14}CO_2$ (15.4 mCi/mmol, 250 $\mu$Ci per 150 7-day-old seedlings) was administered during 1 hr with 20000 lux illumination. Under these conditions 98% of $^{14}C$ was assimilated. D-glucose-[U-$^{14}C$] (180 mCi/mmol, 110 $\mu$Ci per 110 seedlings and mevalonate-[4*R*-4-$^{3}H_1$] (250 mCi/mmol, 20 $\mu$Ci per 110 seedlings) were administered by immersion of the plant roots in an aq. sol (2.8 ml) of both precursors during 1 hr under illumination conditions as above; non-incorporated precursors were removed by repeated washing. After precursor administration the seedlings were incubated, with 3000 lx illumination, on wet filter paper for 0–75 hr.

*Isolation of sterols and their derivatives.* Lipids were extracted with a mixture of $CHCl_3$–MeOH (2:1). To the extract sitosterol as well as plant SG and ASG fractions were added as carriers (about 2 mg each). Free and bound sterols were isolated and subsequently purified by repeated TLC on Si gel using the previously described systems [16]. Samples of SG and ASG were hydrolyzed with boiling 6% HCl in 50% MeOH (2 hr). Sterols and fatty acids were isolated from the hydrolyzate, as previously reported [16]. The radiochemical purity of sterols, SG, ASG, glucose and fatty acids was checked by TLC in various systems [16] as well as by autoradiography. Control experiments, in which a known amount of steryl glucoside-[$^{14}C$] (obtained by the enzymic method [12]) was added to a seedling homogenate, showed that the recovery of $^{14}C$ after extraction, TLC purification and hydrolysis was 80–85%.

*Radioactivity measurement.* Radioactivity was measured by scintillation counting. The scintillator was PPO (3 g/l) and POPOP (0.3 g/l) in toluene for free sterols and fatty acids, and Insta-Gel (Packard) for aq. solns of glucose.

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