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OCCURRENCE OF UDPG:STEROL GLUCOSYLTRANSFERASE ACTIVITY IN SOME LOWER PLANTS

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Abstract—A study was made of the sterol glucosylating ability of cell-free homogenates obtained from 16 species of photosynthesizing and nonphotosynthesizing lower plants (2 species of Chlorophyceae, 2 species of Cyanophyceae, 1 species of Phycomycetes, 3 species of Ascomycetes, 3 species of Basidomycetes, 1 species of Myxomycetes, 3 species of Musci and 1 species of Sphenopsida). Except for the blue-green and green algae, all the remaining species showed distinct *in vitro* synthesis of steryl monoglucosides from UDPG and cholesterol or sitosterol. Preliminary studies on the specificity of the relevant enzymes pointed to a correlation between the sterol composition of the plant and the specificity of its glucosylating enzyme. The enzyme from Ascomycetes and Basidomycetes utilized ergosterol better than cholesterol or sitosterol. Enzymic preparations from mosses utilized sitosterol the best.

INTRODUCTION

The ability to synthesize steryl β-D-monoglucosides, with utilization of UDPG as the sugar donor, has been demonstrated in cell-free preparations obtained from many higher plants [1–7]. The enzyme has been found in seeds [2], etiolated [5] and non-etiolated [3, 4] seedlings, flowers [3], roots [1–3] and leaves [4]. Studies of partly purified enzymic preparations [2, 3] suggest that the synthesis of steryl glucosides is catalysed by specific UDPG: sterol glucosyltransferase strongly bound with cell membranes [8, 9]. Since recent studies indicate that steryl glucosides, in addition to free sterols, are common components of cell membranes [10, 11], it seems possible that in higher plants UDPG: sterol glucosyltransferase plays an important role in the biogenesis or regulation of membrane structures. Much less is known about the occurrence and possible function of steryl glucosides in lower plants; so far they have been detected in only a few representatives of these plants, e.g. in some yeast species [12] and Mycoplasma [13]. Recently, in studies on UDPG metabolism in the myxomycete *Physarum polycephalum* we found an unexpectedly high activity of specific UDPG: sterol glucosyltransferase, which we partly purified and characterized [14]. This led us to undertake a more systematic search for a similar enzyme in representatives of various groups of photosynthesizing and non-photosynthesizing lower plants.

RESULTS AND DISCUSSION

To make certain of the sterol glucosylation ability of cell-free homogenates, we applied in parallel two enzymic tests: (i) incubation with UDP-glucose-[6-^{3}H] and non-labelled sitosterol and (ii) incubation with cholesterol-[4-^{14}C] and non-labelled UDPG. In both cases we performed parallel incubations without an addition of non-labelled sitosterol or UDPG, in order to determine the rate of steryl glucoside formation with utilization of endogenous acceptors or donors of sugar residues. The resulting radioactive steryl glucosides were isolated by co-chromatography with non-radioactive synthetic cholesterol β-D-glucoside. In the case of the incubation with cholesterol-[4-^{14}C], the chromatographic resolution was additionally verified by autoradiography.

The results obtained with homogenates from various lower plants are presented in Table 1. Results obtained under identical incubation conditions of the homogenate from 14-day-old *Calendula officinalis* seedlings (Compositae) are also given for comparative purposes. This higher plant exhibits an UDPG: sterol glucosyltransferase activity similar to that observed in many other higher plants belonging to various families [3]. It is seen from Table 1 that except for representatives of blue-green and green algae, homogenates from all the remaining lower plants studied synthesized steryl monoglucosides. Distinct incorporation of radioactive cholesterol or UDPG into the substance co-chromatographing with cholesteryl β-D-monoglucoside was observed in both incubations. With homogenates from the fungi *Fusarium oxysporum* (Ascomycetes) and *Phytophtora infestans* (Phycomycetes) the synthesis rate was similar to that found in *C. officinalis*, especially when the differences in protein content in the incubated samples were taken into account. The remaining fungi exhibited several-fold lower activity. Mosses and the horsetail species showed an even lower, though distinctly perceptible *in vitro* synthesis of steryl glucosides. The activity of the homogenate from the myxomycete *Physarum polycephalum* was particularly high, thus confirming the suitability of this organism for enzyme isolation.

Incubation with radioactive UDPG with the addition of non-labelled sitosterol enhanced the steryl glucoside synthesis 1.5–4 times. This stimulation was different for

Table 1. Formation of steryl glucosides by cell-free preparations from various lower plants and *Calendula officinalis*

Plant	Protein content in the incubation mixture (mg)	Steryl glucoside formation (dpm × 10^{-3}) from UDPG-glucose-[6-^{3}H]		from cholesterol-[4-^{14}C]	
		−sitosterol	+sitosterol	−UDPG	+UDPG
Cyanophyceae					
Anabaena cylindrica	0.08	0	0	0	0
Cylindrospermum sp.	0.20	0	0	0	0
Chlorophyceae					
Chlorella vulgaris	0.42	0	0	0	0
Scenedesmus obliquus	0.39	0	0	0	0
Phycomycetes					
Phytophora infestans	0.09	0	3.66	0	2.87
Ascomycetes					
Fusarium oxysporum	0.13	9.37	13.43	0	7.08
Verticillium albo-atrum	0.11	0.88	1.60	0.29	8.20
Aspergillus nidulans	0.13	1.35	1.86	0.74	2.40
Basidomycetes					
Agaricus campestris	0.64	1.07	2.14	0.47	3.44
Boletus edulis	0.10	0.89	2.17	0.13	1.28
Leccinium scabrum	0.08	0.85	2.22	0.13	0.94
Myxomycetes					
Physarum polycephalum	0.13	34.83	60.06	0.16	63.87
Musci					
Leucobryum glaucum	0.18	0.67	1.86	0	1.83
Polytrichum sp.	0.32	0.43	1.42	0	1.12
Rhytiadiadephus sp.	0.60	0.61	1.63	0	1.58
Sphenopsida					
Equisetum arvense	0.32	0.18	0.57	0.08	0.28
Higher plant					
Calendula officinalis	0.26	3.82	8.04	0.15	14.08

various species, thus testifying to differences in the concentration or availability of endogenous sterols: however, the results seemed to indicate preferential utilization of endogenous sterols.

It was of interest to find substantial synthesis of steryl monoglucosides in the homogenate from the parasitic fungus *Phytophtora infestans* (Phycomycetes). While not synthesizing its own sterols, this organism can grow on sterol-free media. However, sterols are indispensable for its sexual reproduction [15]. When growing on media containing sterols this fungus incorporates them into membrane structures [15]. In accordance with the above facts, homogenates from *P. infestans* cultured on mineral medium with an addition of sucrose only, exhibit no steryl glucoside synthesis in the absence of exogenous sterol. Elliott and Knights [16] reported that cholesterol supplied in the medium to the related species *Phytophtora cactorum* was converted *in vivo* partly to cholesteryl esters and partly to an unidentified polar metabolite. On the basis of our results it seems possible that cholesteryl glucoside was this metabolite.

Under conditions of incubation with cholesterol-[4-^{14}C] in the absence of UDPG glucoside synthesis was as a rule slight or imperceptible. Only in some fungi was fairly intense synthesis observed; this was probably due to a high concentration of endogenous sugar donors in homogenates from these fungi.

The incubations summarized in Table 1 were carried out under standard time conditions (4 or 14 hr for incubation with UDPG-[6-^{3}H] or cholesterol-[4-^{14}C], respectively). More detailed studies of the synthesis rate were only performed on some selected species belonging to different systematic groups (Fig. 1). Usually the content of synthesized glucoside continuously increased between 0.5 and 14 hr. With *Aspergillus nidulans* a prolongation of the incubation time caused a drop indicating that the homogenate contained an active glucosidase which removed part of the glucoside formed.

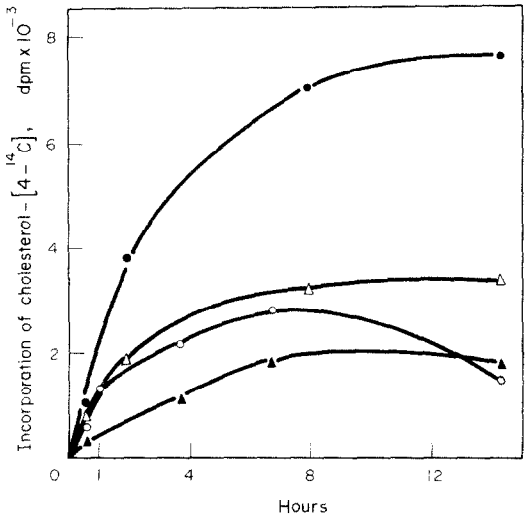

Fig. 1. Time-course of steryl glucoside formation in cell-free homogenates from *Aspergillus nidulans* (—○—), *Verticillium albo-atrum* (—●—), *Agaricus campestris* (—△—) and *Leucobryum glaucum* (—▲—) incubated with cholesterol-[4-^{14}C] and UDPG.

Table 2. Glucosylation of different sterols by acetone precipitated enzyme preparations from various plants

Plant	Relative activity (%) +cholesterol	+sitosterol	+ergosterol
Verticillium albo-atrum	100	92	212
Agaricus campestris	100	80	170
Physarum polycephalum	100	435	48
Polytrichum sp.	100	199	56
Leucobryum glaucum	100	175	94
Calendula officinalis	100	166	115

Enzyme preparations (1 mg/ml) were incubated with 0.1 μCi of UDP-glucose-[6-^{3}H] and 10 μg of unlabelled sterol for 4 hr.

It is known that the various systematic groups of lower plants exhibit differences in their sterol compositions [17]. Ascomycetes and Basidomycetes in general contain ergosterol as the main component. Mosses and horsetails are similar to higher plants, and contain mainly C_{29} sterols (sitosterol and stigmasterol). It has been reported that the myxomycete *Physarum polycephalum* contains also sitosterol and stigmasterol as the main components [18]; however, recently evidence has been presented [19] that sterols of this organism have the opposite configuration of the ethyl group at C-24. Thus, it seemed of interest to compare in selected species the specificity of the sterol glucosylating systems towards some sterols. These experiments (Table 2) were performed with acetone-precipitated enzyme preparations, partly deprived of endogenous sterols. Marked differences in enzyme specificity between various plants were observed. *Verticillium albo-atrum* and *Agaricus campestris*, an Ascomycete and a Basidomycete respectively, utilized ergosterol about twice as effectively as cholesterol or sitosterol. In the remaining species tested, sitosterol was definitely the best acceptor of the glucosyl residue from UDPG. The enzyme preparation from *Ph. polycephalum* (Myxomycetes) utilized sitosterol 9 times more rapidly than ergosterol, and the preparation from *Polytrichum* sp. (Musci) used sitosterol *ca* 3.5 times more rapidly. Likewise, in the case of *Leucobryum glaucum* (Musci) and *Calendula officinalis* (a higher plant) sitosterol was utilized more rapidly than ergosterol but at a rate similar to that of cholesterol utilization. Thus, the present results suggest a correlation between the sterol composition of the species investigated and the specificity of the enzyme participating in steryl glucoside synthesis. This can be regarded as indirect evidence indicating that the physiological function of the investigated enzyme in fact is sterol glucosylation, i.e. the enzyme is not a low-specific glucosyltransferase.

EXPERIMENTAL

Plant material. Green algae *Chlorella vulgaris* (Greifswald A-23), *Scenedesmus obliquus* (Prinsheim A-125) as well as blue-green algae *Cylindrospermum* sp. (isolate obtained from the Dept. of Microbiology, Warsaw University) and *Anabaena cylindrica* (Greifswald A-19) were cultured under illumination on Bold's mineral medium containing 1% peptone and 2% glucose. About 2-week-old cultures were used. Parasitic fungi *Phytophtora infestans*, strain R-3, *Verticillium albo-atrum* and *Fusarium oxysporum* were isolates obtained from Dept. of Genetics, Warsaw Agricultural Academy. *Aspergillus nidulans*, strain bi-1, originated from the Dept. of Genetics, Glasgow University. All these fungi were cultured for 10 days on Copek-Dox mineral medium containing 0.3% sucrose. Young frutifications of higher fungi were collected in a forest near Warsaw in October. Myxomycete *Physarum polycephalum*, strain M_3C IV, was grown as described previously [14]. The remaining plants (Musci and Sphenopsida) were obtained from the Botanical Garden of Warsaw University. Young leaves were used.

Enzymic preparations. Fresh plants were homogenized in 0.1 M Tris–HCl, pH 7.3, using 10 ml buffer per 1 g material. Frozen (−20°) algal cells were homogenized in a mortar by grinding with glass beads (100 mesh, BDH Chemical Ltd., 2 g of beads/1 g of fresh cells). The remaining plants were homogenized in a Potter-Elvehjem homogenizer for 3–5 min. Homogenates were centrifuged at 300 *g* (10 min) and supernatants used for incubations. For prepn of Me_2CO precipitated enzyme, the homogenate was added drop-wise to a 20-fold amount of cold (−18°) Me_2CO. The protein ppt. was collected by centrifugation, washed 2× with cold Me_2CO and dried *in vacuo*.

Measurement of sterol glucosylation. Variant A: The incubation mixture contained in a total vol. of 0.7 ml: 0.5 ml cell-free homogenate; 0.035 ml EtOH soln of cholesterol-[4-^{14}C] (0.1 μCi, sp. act. 47 mCi/mmol) and non-labelled UDPG, diNa salt (2.7 μmol). Variant B: The incubation mixture contained in a total vol. of 0.7 ml: 0.5 ml cell-free homogenate or of a suspension (2 mg/ml) of the Me_2CO-precipitated prepn in the buffer as above; UDP-glucose-[6-^{3}H] (0.1 μCi, sp. act. 6.1 Ci/mmol); egg lecithin (120 μg) and non-labelled sterol (0.3 μmol). Sterol and lecithin were added in EtOH soln (0.035 ml). Incubations were carried out at 30° for either 4 hr (with UDPG-[6-^{3}H]) or for up to 14 hr (with cholesterol-[4-^{14}C]). The reaction was stopped by boiling with 1 ml MeOH. Radioactive products were extracted with *n*-BuOH, purified by TLC on Si gel in $CHCl_3$–MeOH–H_2O (128:15:4) and the radioactivity measured as previously described [14]. Protein was determined by the Lowry method [20].

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