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Enzymic Glucosylation of Gibberellins*

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Abstract. Starting from the well-known conversion of exogenously applied free gibberellic acid (GA_3) to its 3(O)-glucoside by intact immature fruits of runner beans (*Phaseolus coccineus* L.), a protein fraction has been prepared from this plant material possessing glucosylating activity towards GAs. This glucosyltransferase is located in the pericarp only and utilizes preferably UDP-glucose as a sugar donor. The product formed enzymically from GA_3 and UDP-glucose could be identified by derivatization and comparison with the authentic compound to be GA_3-3(O)-glucoside. Among 15 native or chemically modified GAs, the enzyme glucosylates only GA_3 and to a lower extent GA_7 and GA_{30}, indicating a high enzyme specificity with regard to the A ring of gibberellins. The physiological significance of the enzymic GA_3-3(O)-glucoside formation in *Phaseolus coccineus* is not clear, since this glucoside is not known to be endogenous in this plant. The enzyme preparation did not glucosylate substances of phenolic structure, such as hydroquinone, aesculetin, and quercetin. Glucosylation of GA_3 was achieved also by enzyme preparations from *Vigna sinensis* and from cell suspension cultures of *Digitalis purpurea*. A number of other plant materials showed no activity.

Gibberellin (GA) conjugation with glucose is considered to be one main process of GA metabolism in regulating the biologically active level of GAs and in

* Gibberellins 100. For part 99 see Liebisch et al. 1984a.

Abbreviations: GA_1, GA_4 = gibberellin A_1, gibberellin A_4, etc; ME = 2-mercaptoethanol; DTE = dithioerythritol; TLC = thin-layer chromatography; HPLC = high performance liquid chromatography; PVP = polyvinylpyrrolidone (Polyclar AT).

forming transport and storage forms (Sembdner 1974, Sembdner et al. 1976, Dathe et al. 1978). In the endogenous GA conjugates known, glucose is linked either to a hydroxyl group or to the carboxyl group forming GA-O-glucosides or GA-glucosyl esters, respectively. Exogenous application of free GAs to intact plants or isolated plant organs results in formation of GA conjugates of different kinds and amounts, depending on the plant species and organs used as well as their developmental stage (for review, cf. Sembdner et al. 1980, Schneider 1983). If GA_3, e.g., is fed to maturing fruits of runner beans (*Phaseolus coccineus* L.), its transformation to GA_3-3(O)-β-D-glucopyranoside can be observed (Sembdner et al. 1968, Sembdner et al. 1972, Liebisch et al. 1984a). A similar conversion of GA_3 is known to occur in young plants of *Phaseolus vulgaris* L. (Asakawa et al. 1974) and in both seedlings and maturing seeds of *Pharbitis nil* Choisy (Barendse and de Klerk 1975).

Despite the well-characterized GA conjugate formation based on labeling experiments as well as structural elucidation of the GA conjugates formed, nothing is known about the enzymes catalyzing this conversion of free GAs. Cytokinins are known to be transformed in radish (*Raphanus sativus* L.) cotyledons by cytokinin glucosyltransferases using UDP-glucose as the donor of glucose (Entsch and Letham 1979, Entsch et al. 1979). A glucosyltransferase catalyzing the transfer of glucose from UDP-glucose to abscisic acid with the formation of abscisic acid glucosyl ester has been prepared from cell suspension cultures of *Macleaya microcarpa* (Maxim.) Fedde (Lehmann and Schütte 1980). This paper deals with the glucosylation of GA_3 and structurally related GAs *in vitro* and describes some results concerning the characterization of the enzyme. Preliminary results were presented by Müller et al. (1974).

Materials and Methods

Chemicals

GA_3 was purchased from Phylaxia, Budapest, Hungary, and further purified by chromatography and recrystallization. Other GAs and the phenolic substances were from the Institute's collection. Dithioerythritol was obtained from Reanal, Budapest, Hungary; polyvinylpyrrolidone (Polyclar AT) was from Serva, Heidelberg, FRG; p-chloromercuribenzoate, UDP-glucose, TDP-glucose, CDP-glucose, and GDP-glucose were from Calbiochem, Los Angeles, USA; ADP-glucose and UDP-galactose were from Sigma Chemical Corp., St. Louis, USA. [6α-^{3}H]-GA_3 (3.7×10^9 Bq/mmole) was synthesized at the Institute (Lischewski et al. 1982).

Plant Material

Seeds of *Phaseolus coccineus* L. cv. "Preisgewinner," of *Triticum aestivum* L. cv. "Carola," and of *Calendula officinalis* L. were purchased from VEB Saat- und Pflanzgut, Quedlinburg, GDR. Seed material from all other legumes (cf. Table 1) and *Pharbitis purpurea* (L.) Voigt were from the collection of the

Zentralinstitut für Genetik und Kulturpflanzenforschung der Akademie der Wissenschaften der DDR, Gatersleben, GDR.

Phaseolus coccineus was grown under field conditions from June to October or in greenhouses from March to July. Immature fruits (characterized by green to slightly yellow pericarp, seeds completely developed with black spots, and length 22–35 mm) were obtained in September–October or June–July, respectively. The other species of *Phaseolus* and *Vigna* were cultivated in greenhouses from April to September and immature fruits collected in August–September. Seeds of *Pharbitis purpurea* were germinated according to the method of Barendse and de Klerk (1975). Immature fruits were harvested from greenhouse-grown plants in October. Seeds of *Triticum aestivum* were germinated on wet filter paper at 20°C in the dark for 4 d (length of the coleoptiles about 0.5–1.5 cm). *Calendula officinalis* was cultivated outdoors and leaves of flowering plants were used. The cell suspension culture of *Digitalis purpurea* L. is described by Tewes et al. (1982). Cells were harvested after 11 d of culture.

Screening Experiments

1. Seedlings, leaves, and immature fruits of plants mentioned in Table 1 were processed at 4°C as follows: 50 g fresh material was ground in a mortar with quartz sand and 75 ml 0.05 M Tris-HCl buffer, pH 7.4 (10^{-2} M ME). The homogenate was squeezed through eight layers of cheesecloth and centrifuged for 15 min at 1000 × *g*. The supernatant was then centrifuged for 30 min at 20,000 × *g*. Five ml of buffer was added to the sediment and the suspension obtained was used immediately for incubation (sediment fraction). The supernatant was filtered through a column of Sephadex G-25 (1.8 × 50 cm) and the protein fraction lyophilized and redissolved in the same buffer to give the supernatant fraction with a protein concentration of about 10 mg/ml. Two hundred twenty-five g cells of *Digitalis purpurea* were homogenized with 120 ml 0.1 M Tris-HCl buffer, pH 7.4 (10^{-2} M ME) at 0°C in the presence of 10 g PVP and the homogenate treated as described above to prepare sediment and supernatant fractions.

2. For checking the GA_3 glucosylating activity, an incubation mixture was prepared containing 1 μmol GA_3, 0.5 μmol UDP-glucose, 0.2 ml 0.05 M Tris-HCl buffer pH 7.4 (10^{-2} M ME) in a total volume of 0.5 ml. The reaction was started by addition of 0.5 ml enzyme preparation (sediment or supernatant fraction) and terminated after an incubation period of 3 h at 37°C by the addition of 2 ml methanol. The precipitate was removed by centrifugation and the supernatant dried under vacuum. The residue was dissolved in 1 ml methanol, and aliquots were tested by TLC using solvent systems A and B.

Enzyme Preparation from Pericarp of Phaseolus coccineus

All operations were performed at 0–4°C. Two hundred g fresh or 25 g lyophilized and powdered material was homogenized with quartz sand in a mortar

or extracted by stirring for 2 h with 300 ml 0.12 M Tris-HCl buffer, pH 8.2 (10^{-2} M ME). The homogenate or crude extract was squeezed through eight layers of cheesecloth and centrifuged for 15 min at 1000 × *g*. The sediment was discarded and the supernatant centrifuged again for 30 min at 20,000 × *g*. At times, the 20,000 × *g* supernatant derived from fresh material was ultracentrifuged for 1 h at 100,000 × *g* to obtain the microsomal fraction. Usually the 20,000 × *g* supernatant was brought to 50% saturation by addition of solid $(NH_4)_2SO_4$ over a period of 10 min with continuous addition of 5 M NH_4OH to maintain the pH at 8.2. The mixture was allowed to stand for a further 15 min and then centrifuged at 3,600 × *g* for 20 min. The supernatant was brought to 80% saturation, and the precipitate was collected in the same way and redissolved in a minimum volume of 0.05 M Tris-HCl buffer, pH 8.2 (10^{-2} M ME). Residual $(NH_4)_2SO_4$ was removed from this fraction by dialysis against the same buffer. After the addition of solid DTE to give a concentration of 10^{-2} M, the protein preparation was used immediately for assay of glucosyltransferase activity or stored at −25°C.

Enzyme Assay

The standard assay mixture for glucosyltransferase activity contained 50 nmole [6α-^{3}H]-GA_3 (about 3.5 × 10^5 dpm), 500 nmole UDP-glucose, 50 µl 0.05 M Tris-HCl buffer, pH 8.2 (10^{-2} M ME), and 50 µl enzyme solution in a total volume of 110 µl. In control experiments, UDP-glucose was omitted or enzyme solution added after heat denaturation. The reaction was started by the addition of protein and terminated, after incubation at 25°C for 2 h, by adding 1 ml methanol. The solvent was removed under vacuum at 45°C, the residue dissolved in 100 µl ethanol, and 5 µl aliquots applied to Silufol sheets and developed in solvent system A. Areas corresponding to spots of GA_3-3(O)-glucoside run on reference strips next to the enzymic reaction product were cut out and counted (without spraying) in a liquid scintillation spectrometer (Tricarb 2660, Packard Instruments).

For testing acceptor specificity (Fig. 1) the assay system contained 1 µmol GA (nonradioactive), 1.5 µmol UDP-glucose, 0.2 ml 0.05 M Tris-HCl buffer, pH 8.2 (10^{-2} M ME), and 0.5 ml enzyme solution in a total volume of 1 ml. Incubation conditions and processing were the same as for standard assay with the exception of the use of silica gel plates for TLC (solvent systems A, B). Reference compounds (3(O)-β-D-glucopyranosides of GA_1, GA_3, GA_4, GA_7; GA_8-2(O)-β-D-glucopyranoside, GA_5-13(O)-β-D-glucopyranoside, or GA_3-(O)-β-D-glucopyranosyl ester; cf. Schneider 1981) were chromatographed on the same plate. When reference compounds were not available (e.g., GA_{30}-3(O)-glucoside), the plates were checked for the appearance of GA conjugates more polar than the free GAs applied.

Product Identification of GA_3 Glucosylation

The residues of enzyme incubations were purified on a DEAE-Sephadex A-25 column (1 × 23 cm, acetate form in 80% methanol, v/v) according to Gräbner

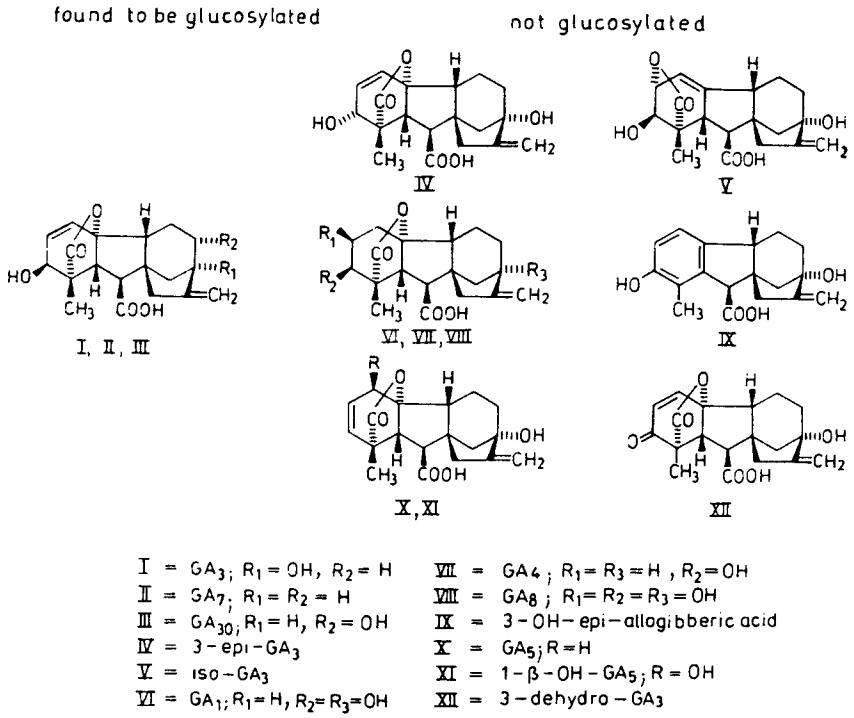


Fig. 1. Gibberellins checked as acceptors for glucosyltransferase.

et al. (1976). Fractions containing the GA_3 conjugate were collected and dried. The residue was dissolved in 100 µl dry pyridine and mixed with 100 µl acetic anhydride. The acetylation was allowed to proceed for 30 min and stopped by evaporation under vacuum. Aliquots were chromatographed together with GA_3 - 3(O) - β - D - tetra - acetyl - glucopyranoside and GA_3 - 3(O) - acetyl - 13(O) -β-D-tetra-acetyl-glucopyranoside (Schneider et al. 1974) in solvent systems C and D.

For enzymatic hydrolysis, part of the GA_3 conjugate derived from the DEAE-Sephadex A-25 column was incubated either with cellulase (from *Aspergillus niger;* Serva, Heidelberg, FRG) in 0.1 M acetate buffer, pH 4.6, or with α-glucosidase (from yeast; Boehringer, Mannheim, FRG) in 0.067 M phosphate buffer, pH 6.8, as described by Müller et al. (1978). The incubation mixture was checked by TLC for GA_3 conjugate and free GA_3 in solvent systems A and B.

Thin-Layer Chromatography

GA compounds were chromatographed by TLC on Silufol sheets (Kavalier, Czechoslovakia) or plates with silica gel G (Merck, Darmstadt, FRG), respectively, using the following solvent systems:

A. chloroform/methanol/acetic acid/water (40:15:3:2).

B. n-propanol/5 M ammonia (5:1).

C. chloroform/ethyl acetate/acetic acid (14:6:1).

D. benzene/n-butanol/acetic acid (14:5:1).

Plates were sprayed with 85% H_2SO_4 and checked under UV (360 nm) before and after heating at 110°C for 20 min.

Protein Estimation

The protein content was determined by the Folin method (Lowry et al. 1951); crystalline bovine serum albumin was used as a standard.

Results

Screening of Plant Material

Seed maturation in different plant species is often accompanied by a conversion of free GAs to glucose derivatives (Hiraga et al. 1974). For this reason, ripening fruits should be a suitable material in which to look for enzymes catalyzing the transfer of β-D-glucose to GAs. According to N-glucosylation of cytokinins and O-glucosylation of phenolic compounds (Ibrahim and Grisebach 1976, Köster and Barz 1981), sterols (Wojciechowski et al. 1979), and cardiac glycosides (Franz and Meier 1969), these enzymes are expected to be O-glucosyltransferases using UDP-glucose as the sugar donor.

Therefore, in a screening experiment, various plant species have been checked for GA_3-glucosylating activity using protein fractions from homogenates and UDP-glucose. Homogenates were separated at 20,000 × *g*. The sediments were immediately checked, whereas supernatants were at first purified by gel filtration on Sephadex G-25. The results are summarized in Table 1a. Enzyme activity glucosylating GA_3 has been detected mainly in soluble fractions prepared from immature fruits of some leguminous plants. The highest transferase activity was found in *P. coccineus*. The ability of glucosylating GAs other than GA_3, however, cannot be excluded, as other GAs have not been applied in these incubations. In addition to species listed in Table 1a, other plant materials have also been tested (Table 1b). Wheat seedlings (*Triticum aestivum* L.), leaves of *Calendula officinalis* L., and cell suspension cultures of *Digitalis purpurea* L. are known to contain enzymes forming hydroquinone-β-D-glucoside (arbutin) (Goncalves 1963), and sterol-β-D-glucosides (Wojciechowski 1972, Yoshikawa and Furuya 1979). Enzyme preparations from *Triticum* and *Calendula* did not transform GA_3 at all. Very low activity has been demonstrated using *Digitalis* preparations. Fruits of *P. coccineus* have been selected for further experiments on enzymic GA_3 glucosylation.

Table 1. Glucosylation of GA_3 *in vitro* using crude enzyme preparations from several plant sources (the number of crosses characterizes relative amounts of GA_3 conjugate formed on the basis of TLC separation).

Plants	Relative activity 20,000 × *g*	
a. Immature fruits	Supernatant	Sediment
Phaseolus angularis (Willd.) W.F. Wight	+ +	–
Phaseolus aureus Roxb.	+	–
Phaseolus calcaratus Roxb.	–	–
Phaseolus coccineus L.	+ + +	–
Phaseolus semierectus L.	–	–
Phaseolus lunatus L.	–	–
Phaseolus vulgaris L.	–	–
Vigna sinensis (Stickm.) Savi ex Hassk. ssp. *sesquipedalis* (L.) Van Es.	–	+
Vigna sinensis (Stickm.) Savi ex Hassk. ssp. *sinensis*	–	–
Pharbitis purpurea L.) Voigt	–	–
b. Other plant material		
Triticum aestivum L. (seedlings, 4 d)	–	–
Calendula officinalis L. (leaves of flowering plants)	–	–
Digitalis purpurea L. (cell suspension cultures, 11 d)	+	–

Estimation and Localization of Enzyme Activity in P. coccineus

With regard to the low levels of biologically active GAs in plants, only trace amounts of enzyme(s) may be expected. Therefore, the method for quantification of the enzyme reaction has to be a highly efficient one concerning product separation and sensitivity. In the case of cytokinin glucosylation, the glucosides formed have been determined by reversed phase HPLC (Entsch et al. 1979). Unfortunately, this method became available only very recently in our laboratory. Thus, a radiotracer has been employed in combination with TLC on precoated silica plates. Attempts to use UDP-[^{14}C]-glucose failed because of difficulties in the separation of cleavage products of the nucleotide sugar and the GA_3 conjugate. Therefore, [6α-^{3}H]-GA_3 (Lischewski et al. 1982) with a specific radioactivity of about 3.7×10^9 Bq mmole^{-1} has been applied. A sufficient separation of [^{3}H]-labeled free and conjugated GA_3 has been achieved by TLC using solvent system A. This method is reproducible and permits the quantification of the conjugated GA_3 in amounts as low as 10 pmole.

Enzyme activity was found to vary during fruit development in *P. coccineus*. The most active stage appears when the pericarp is still green and the seeds are developing. In the natural vegetation period, this stage normally is reached at the end of September when there are short-day conditions.

The transferase was found to be localized only in the pericarp; it seems to

Table 2. Partial purification of GA_3 glucosyltransferase.

Fraction	Total protein (mg)	nmole GA_3 glucoside × mg protein × 2 h^{-1} [c]
Crude extract	446[a]	—[b]
Crude extract after Sephadex G-25 filtration	397	0.58
0 to 50% satur. $(NH_4)_2SO_4$	209	0.6
50 to 80% satur. $(NH_4)_2SO_4$	101	1.3
80 to 100% satur. $(NH_4)_2SO_4$	7	0.36

Assay: 5 nmole [6α-^{3}H]-GA_3, 500 nmole UDP-glucose, 50 µl 0.05 M Tris-HCl buffer, pH 8.2 (10^{-2} M ME), 50 µl enzyme preparation (80–435 µg protein)

[a] From 25 g lyophilized pericarp of *Phaseolus coccineus*.

[b] No enzyme activity could be detected at this stage.

[c] The formation of GA_3 glucoside is linear over a period of at least 3 h.

be a soluble enzyme not bound to membranes or other subcellular structures according to ultra-centrifugal experiments. No activity glucosylating GAs has been found in protein preparations from seeds.

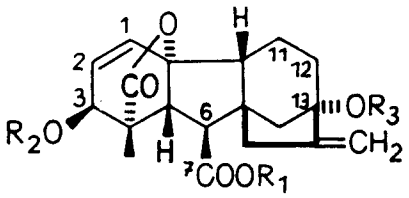
Enzyme Preparation

The pericarp of freshly harvested fruits was immediately frozen, lyophilized, and powdered. The enzymic activity remains stable for at least 2 years during storage of the dry material at temperatures between +2° and +6°C. No enzyme activity is detectable in homogenates or crude extracts. This is due to structurally unknown inhibitors of low-molecular weight. Only if crude extracts are filtered through Sephadex G-25 can enzyme activity be detected in the protein fraction eluted within the void volume. The low-molecular weight fraction inhibits the enzyme activity when aliquots are added to the protein fraction. These inhibitory substances can also be separated from the enzyme by ammonium sulfate precipitation.

The addition of ME or DTE to the extraction buffer enhances the enzyme activity, whereas p-chloromercuribenzoate (5×10^{-4} M) inhibits the reaction completely. DTE is the preferred additive for storage of enzyme preparations. The steps of enzyme enrichment are summarized in Table 2. The 50 to 80% saturated ammonium sulfate fraction contains the enzyme activity enriched to about 2-fold. This protein redissolved in Tris-HCl buffer of pH 8.2 and dialyzed against the buffer provides an enzyme preparation stable for about 8 weeks when stored at −25°C in the presence of 10^{-2} M DTE. After a storage of 8 months under these conditions, about one third of the activity disappears. All further experiments have been conducted using this active protein fraction.

Product Analysis and Stoichiometry

Glucosylation of GA_3 is possible at positions 3, 13, and 7 of the GA_3 molecule forming glucosides and the glucosyl ester, respectively (Fig. 2). With respect



$R_1 = R_2 = R_3 = H$	GA_3
R_1 = β-D-glucose $R_2 = R_3 = H$	GA_3 glucosyl ester
$R_1 = H$, R_2 = β-D-glucose $R_3 = H$	GA_3-3(O)-glucoside
$R_1 = R_2 = H$ R_3 = β-D-glucose	GA_3-13(O)-glucoside

Fig. 2. Monoglucosyl derivatives of GA_3.

to monoglucosylation, three products may be formed: GA_3-3(O)-glucoside, GA_3-13(O)-glucoside, and GA_3-7-(O)-glucosyl ester. The product formed by the transferase differs in its R_f values in different solvent systems from the glucosyl ester and shows the same chromatographic behavior as the (O)-glucosides. Ion exchange chromatography on DEAE-Sephadex-A-25 (Gräbner et al. 1976) excludes the formation of the glucosyl ester because the GA_3 conjugate formed is strongly bound to the column and eluted by acetic acid like authentic GA_3-3(O)-glucoside. In order to distinguish between 3(O)- and 13(O)-glucosylation, the product eluted from DEAE-Sephadex was short-time-acetylated (Schneider et al. 1974). The resulting derivative was found to be identical by TLC with authentic synthetic GA_3-3(O)-β-D-tetra-acetyl-glucopyranoside. Under these conditions, acetylation of the tertiary 13-hydroxyl group does not take place and thus, GA_3-13(O)-glucoside having a free secondary hydroxyl group at position 3 would have been acetylated to the corresponding penta-acetate. Therefore, the identification of a tetra-acetyl derivative demonstrates that the transferase catalyzes 3(O)-glucosylation of GA_3.

The β-glucosidic linkage of the sugar moiety could not yet be confirmed by NMR data because of minute amounts available. However, the behavior toward enzymic hydrolysis accords with that of authentic GA_3-3(O)-β-D-glucopyranoside (Müller et al. 1978). β-Glucosidase (cellulase from *Aspergillus*) is able to split the glucoside partially with the formation of GA_3 and glucose, whereas α-glucosidase (from yeast) is ineffective. The stoichiometry of UDP-glucose utilization by the transferase reaction could not be studied in detail as some cleavage of UDP-glucose occurs spontaneously.

Specificity toward Nucleotide Sugars

In parallel experiments, different donors were added to the same enzyme preparation at saturating concentrations. The data presented in Table 3 show that UDP-glucose is used preferentially, and TDP-glucose is incorporated to 16% of the amount of UDP-glucose. The three further activated glucose derivatives applied were found to be inactive. It is noteworthy that UDP-galactose is accepted as a donor substrate.

Specificity toward Gibberellins

In order to study structural prerequisites of gibberellin glucosylation by the transferase, 15 natural or chemically modified gibberellins (Fig. 1, not all pre-

Table 3. Donor specificity of the GA_3 glucosyltransferase.

Donor	dpm GA_3 glucoside formed
Experiment 1	
Uridine-5'-diphosphoglucose	6.2×10^4
Thymidine-5'-diphosphoglucose	1.0×10^4
Cytidine-5'-diphosphoglucose	0
Adenosine-5'-diphosphoglucose	0
Guanosine-5'-diphosphoglucose	0
Experiment 2	
Uridine-5'-diphosphoglucose	4.8×10^4
Uridine-5'-diphosphogalactose	3.1×10^4

Assay: 5 nmole [6α-^{3}H]-GA_3; 500 nmole nucleotide sugar, 50 µl 0.05 M Tris-HCl buffer, pH 8.2, 10^{-2} M ME; 50 µl enzyme (710 µg protein).

sented) were checked as acceptors using TLC techniques. Only GA_3 (I) and to much smaller extents GA_7 (II) and GA_{30} (III) were found to be glucosylated.

The modification of ring A of the GAs either by epimerization of the 3-β-hydroxyl group (IV), or hydrogenation of the 1,2-double bond (VI, VII), by rearrangement of the lactone ring (V), by aromatization (IX), or by an additional hydroxylation at position 2 (VIII) results in loss of acceptor properties. Thus, structural features necessary for glucosylation of a gibberellin seem to be the 3-β-hydroxyl group and the 1,2-double bond. The ring A structure with the hydroxyl group shifted to 1β-position and the double bond to $\Delta^{2,3}$ (XI) is not accepted by the enzyme, and gibberellins with a hydroxyl group only in position 13 (X, XII) are not glucosylated.

Additional attempts to glucosylate simple phenolics, such as hydroquinone, aesculetin and quercetin, by the enzyme preparation were unsuccessful, indicating the glucosylating activity to be probably specific for gibberellins. Furthermore, no formation of glucosyl esters of GA_3 and ferulic acid could be observed with this *Phaseolus* enzyme or with preparations from immature fruits of *Pharbitis purpurea,* including modified conditions of incubation (Corner and Swain 1965).

In summary the enzyme is proposed to be UDP-glucose: gibberellin A_3-3(O)-glucosyltransferase (trivial name: GA_3-3(O)-glucosyltransferase); the enzymic reaction is shown in Fig. 3.

Discussion

The results presented demonstrate the detection of a GA_3 glucosylating enzyme in plants and describe the partial characterization of the enzyme from the pericarp of *Phaseolus coccineus*. It glucosylates GA_3 to the GA_3-3(O)-glucoside by transfer of glucose from UDP-glucose. This is the first report on gibberellin-O-glucoside formation *in vitro* supporting the studies on the formation of GA_3-3(O)-glucoside by intact fruits of *P. coccineus* after application of free GA_3 (Liebisch et al. 1984a). A glucosyltransferase catalyzing the formation of GA

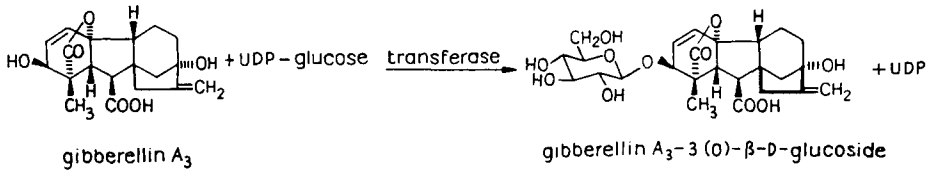


Fig. 3. Reaction catalyzed by UDP-glucose: gibberellin A_3-3(O)-glucosyltransferase.

glucosyl esters has been recently found in cell suspension cultures of *Lycopersicon* (Liebisch et al. 1984b).

The enzyme could not be detected in homogenates or crude extract without the removal of low-molecular-weight substances inhibiting the enzyme activity. Similar results have been published concerning the glucosylation of cytokinins (Entsch et al. 1979). The GA_3 glucosyltransferase was shown to prefer UDP-glucose over TDP-glucose, contrary to the enzyme catalyzing cytokinin glucosylation, which reacts to UDP-glucose and TDP-glucose equally. The formation of a GA_3 conjugate by applying UDP-galactose as a donor substrate showed the transferase to be specific with regard to the uridine ring system independent of the sugar moiety. This is supported by the fact that other nucleotide sugars are utilized little or not at all. GA_3-3(O)-glucoside and the product formed from UDP-galactose are not separable by TLC. The similarity in the rate of UDP-galactose and UDP-glucose consumption indicates the formation of GA_3 galactoside; nevertheless, a preceding epimerase reaction cannot be excluded. In the case of cytokinin glycosylation, a much lower UDP-galactose utilization led to the formation of a galactoside identified by HPLC (Entsch et al. 1979).

The GA glucosyltransferase was found to catalyze glucosylation of the 3β-OH group of GA_3, GA_7, and GA_{30}, though the product of GA_{30} glucosylation was identified only tentatively without direct comparison with authentic GA_{30}-3(O)-glucoside. Glucosylation of 1β-hydroxy-GA_5 with a modified structure of ring A did not occur.

To summarize the acceptor studies: the enzyme is thought to be highly specific to GAs with a 3β-hydroxyl group, a $\Delta^{1,2}$-double bond, and a 19,10-lactone in ring A. This conclusion, of course, contains some speculation. Studies have to be continued to improve analytical techniques and to determine whether under varied conditions the enzyme might be able to catalyze also the formation of other GA glucosides.

GA_8-2(O)-β-D-glucopyranoside has been isolated as an endogenous GA conjugate from maturing fruits of *P. coccineus* (Schreiber et al. 1970). Nevertheless, the enzyme seems not to glucosylate GA_8, although a formation of GA_8-2(O)-glucoside below the detection limit on TLC (0.45 μg) cannot be excluded. In field-grown plants of *P. coccineus*, the GA_3 glucosyltransferase is present in pericarp only, whereas GA_8 glucoside predominates in the seed. However, in greenhouse-grown *P. coccineus*, both GA_8 and GA_8-2(O)-glucoside were identified by GC-MS in the pericarp (Rivier et al. 1981). Physiological and biochemical differences in plants grown under natural conditions or in the greenhouse are well known. In our investigations, transferase activity was not al-

ways detectable in plants cultivated in the greenhouse, and attempts to detect a GA_8 glucosylating enzyme in ripening seeds have not yet been successful.

The physiological significance of GA_3 glucosylation is not clear. Although the application of GA_3 to fruits of *P. coccineus* unequivocally results in the formation of GA_3-3(O)-glucoside *in vivo* (Liebisch et al. 1984a), possibly as a deactivation process, there are no data in the literature on the endogenous occurrence of this glucoside in runner bean fruits. The aglycone substrate, GA_3, was identified by GC-MS only in an early immature stage of developing seeds of *P. coccineus* (Durley et al. 1971). The glucosyltransferase described in this paper has been isolated from the pericarp at a late stage of development, just before ripening, but low enzyme activity could also be detected in the early immature stages. In *Phaseolus vulgaris*, glucosylating enzymes are apparently absent in early immature stages of seed development; they appear during maturation (Yamane et al. 1977).

Further studies are necessary on the enzyme(s) involved in the formation of GA glucosides in *P. coccineus* considering also the role of conditions of growth (field/greenhouse, day length) and developmental stages of the fruits.

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