Enzymatic Studies on the Reversible Synthesis of Nicotinic Acid-N-glucoside in Heterotrophic Parsley Cell Suspension Cultures

Barbara Upmeier, Jürgen E. Thomzik*, and Wolfgang Barz

Lehrstuhl für Biochemie der Pflanzen, Westfälische Wilhelms-Universität Münster, Hindenburgplatz 55, D-4400 Münster, Bundesrepublik Deutschland

Z. Naturforsch. **43c,** 835–842 (1988); received August 1/September 30, 1988

Petroselinum hortense, Umbelliferae, Plant Cell Cultures, Nicotinic Acid, Nicotinic Acid-N-glucoside

A soluble enzyme catalyzing the transfer of the glucose moiety from UDP-glucose to the nitrogen atom of nicotinic acid was detected in protein preparations from heterotrophic cell suspension cultures of parsley (*Petroselinum hortense* Hoffm.). Enzyme activity was enriched 22-fold by ammonium sulfate precipitation, gel filtration and ion exchange chromatography. The UDP-glucose : nicotinic acid-N-glucosyltransferase showed a pH-optimum at pH 7.8–8.2 and a temperature optimum at 30 °C. The apparent K_M values were determined to be 170 μM for nicotinic acid and 1.2 mM for the cosubstrate. The native enzyme had a molecular mass of about 46 kDa. The glucosyltransferase reaction was shown to be reversible. The transfer of the glucose molecule from nicotinic acid-N-glucoside to uridinediphosphate yielding uridinediphosphoglucose and nicotinic acid could be demonstrated indicating that nicotinic acid-N-glucoside has a high group-transfer potential.

Introduction

Nicotinic acid can be regarded as an important connecting link between primary and secondary metabolism in higher plants. Being a constituent of the pyridine nucleotide cycle, nicotinate is a precursor as well as a degradation product of the pyridine nucleotide coenzymes NAD and NADP which play a decisive role in oxidation-reduction reactions [1]. Nicotinic acid also functions as a building moiety for pyridine alkaloids [2]. Finally, plant cells are able to completely degrade nicotinic acid [3] or to form conjugates [4–6].

Conjugation reactions play a pivotal role in plant metabolism as they allow vacuolar storage, detoxification and metabolic regulation of primary and secondary constituents in plant cells [7, 8]. Glucose is one of the most important conjugation moieties [7]. In suspension-cultured parsley cells *(Petroselinum hortense)* the rapid conversion of exogenously applied and endogenously synthesized nicotinic acid to the N-glucosyl conjugate has been observed [9, 10]. This reaction when measured in cell suspension cultures seems to be restricted to members of the subclass Asteridae and some higher orders of the subclass Rosidae [11]. Nicotinic acid-N-glucoside has been shown to rapidly turnover in parsley cells. This reaction possibly assigns to the conjugate the function of a storage form for nicotinic acid, because it can provide free nicotinate for the synthesis of NAD and NADP in the pyridine nucleotide cycle [12]. In this report we describe the isolation and characterization of uridinediphosphoglucose : nicotinic acid-N-glucosyltransferase with the intention to further elucidate the physiological role of nicotinic acid-N-glucoside in plant cell metabolism.

Material and Methods

Plant material

Heterotrophic parsley (*Petroselinum hortense* Hoffm.) cell suspension cultures were cultivated on Gamborg B_5-medium [13] under conditions previously described [14]. For enzyme purification cell cultures were harvested 7–10 days after inoculation.

Chemicals

Nicotinic acid, nicotinamide and nicotinic acid methylester were purchased from MERCK (Darmstadt, F.R.G.). Picolinic acid, iso-nicotinic acid, uridinediphosphate and uridinediphosphoglucose were obtained from SIGMA (Munich, F.R.G.), 6-hydroxynicotinic acid was from SCHUCHARDT

Abbreviation: EDTA, ethylenediamine tetraacetic acid.

* Present address: BAYER AG, Pflanzenschutzzentrum Monheim, D-5090 Leverkusen, Bundesrepublik Deutschland.

Reprint requests to Prof. Dr. W. Barz.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/88/1100–0835 $ 01.30/0

(Munich, F.R.G.). 3-Hydroxypyridine and quinolinic acid were supplied by EGA-CHEMIE (Steinheim, F.R.G.), 2-hydroxypyridine came from ALDRICH (Beerse, Belgium). Pyridine-3-aldehyde, *p*-nitrophenyl-β-D-glucoside and tetrabutylammonium hydrogensulfate were purchased from SERVA (Heidelberg, F.R.G.) and trigonelline from ROTH (Karlsruhe, F.R.G.). Nicotinic acid-N-glucoside was from our previous studies [9]. PHARMACIA (Uppsala, Sweden) supplied all materials for protein purification.

The radiochemicals [7-^{14}C]nicotinic acid (2.07 GBq/mmol) and uridinediphospho-[U-^{14}C]glucose (7.4 GBq/mmol) were obtained from THE RADIOCHEMICAL CENTER AMERSHAM (Amersham, U.K.).

Buffer systems

The buffers were buffer A, 200 mM Tris/HCl, 10 mM EDTA, 100 mM sucrose, pH 8.0; buffer B, 20 mM Tris/HCl, pH 8.0; buffer C, 100 mM Tris/HCl, pH 8.0; buffer D, 10 mM potassium phosphate, pH 7.8.

For the determination of the pH-optimum the following buffer systems were used at 200 mM concentration: pH-range 2.5–5.5 citrate/phosphate; pH-range 5.5–8.5 potassium phosphate; pH-range 7.5–11.0 Tris/HCl.

Protein determination

Protein concentrations were determined according to Bradford [15] with bovine serum albumin as reference.

Enzyme purification

The cell cultures were separated from the growth medium by filtration and homogenized in a chilled mortar with buffer A (1 ml/g fr. wt.) and quartz sand. All procedures were carried out at 4 °C. The homogenate was centrifuged at $1000 \times g$ for 10 min and subsequently at $20{,}000 \times g$ for 20 min. The supernatant was fractionated by addition of ammonium sulfate in two steps between 0–40% and 40–70% saturation. After each step the precipitated protein was recovered by centrifugation. The resulting sediment of the second precipitation was dissolved in a small volume of buffer B and desalted on Sephadex G-25 (PD-10 columns, Pharmacia, Uppsala, Sweden) using buffer B. The protein material was applied to a DEAE-cellulose column (2.0 × 12 cm) previously equilibrated with buffer B. After elution of unbound protein with 60 ml of buffer B a linear gradient of NaCl in buffer B with a slope of 0–0.5 M NaCl in a total volume of 180 ml was applied. Protein was eluted with a flow rate of 25 ml/h and fractions of 2.5 ml each were collected. The fractions with pronounced glucosyltransferase activity were pooled and concentrated by ultrafiltration (PM 10, Amicon, Osterhout, Netherlands) to a volume of 5 ml. This sample was applied to a Sephadex G-100 column (2.5 × 70 cm) and chromatographed using buffer C (flow rate 25 ml/h) and a fraction volume of 3 ml. The fractions containing glucosyltransferase activity were combined, reduced to 2.5 ml by ultrafiltration and transferred into buffer D using a prepacked Sephadex G-25 (PD-10) column. The resulting protein preparation was charged onto a column of hydroxylapatite (1.7 × 3.0 cm) previously equilibrated and then washed with 10 ml of buffer D. The enzyme was eluted with a linear gradient of 80 ml buffer D ranging from 10–250 mM potassium phosphate at pH 7.8 (flow rate 35 ml/h; fraction size 3 ml). Active fractions were pooled, concentrated and immediately used for studies on enzyme properties.

Enzyme assays

Standard glucosyltransferase assays contained 100 µl enzyme preparation, 50 nmol nicotinic acid containing 3.7 kBq [7-^{14}C]nicotinic acid and 100 nmol UDPG in a total volume of 150 µl buffer B. The enzyme reaction was started by adding the cosubstrate, incubated for 1 h at 30 °C and stopped by transferring the assay tubes to a boiling water bath. Denaturated protein was removed by centrifugation, and 100 µl of the supernatant were subjected to ion exchange chromatography on Dowex 1X8 (mesh 100–200, formate form). The Dowex-resin (2 ml) had been equilibrated with distilled water and packed into a 5 ml syringe. After application of the enzyme assay solution the reaction product nicotinic acid-N-glucoside could be removed by washing the resin with 5 ml distilled water. Radioactivity in this fraction was measured by liquid scintillation counting. Nicotinic acid could subsequently be eluted from the resin with 15 ml 8 M formic acid.

The glucohydrolase assay was performed in sodium citrate–phosphate buffer (50 mM, pH 5.5) with 2 mM *p*-nitrophenol-β-D-glucoside as substrate

and 100 µl protein preparation. After incubation for 10–30 min at 30 °C the reaction was stopped by adding 2 ml of 1 M Na_2CO_3. The product of the enzymatic reaction was determined photometrically at 400 nm.

Product identification

The identity of the enzymatically formed glucoside was verified using thin layer chromatography on silica gel plates (20 × 20 cm, Si GF_{254}, MERCK, Darmstadt, F.R.G.) with synthetic nicotinic acid-N-glucoside [9] as reference. Chromatographic solvents were acetone : water (7:3), *n*-butanol : acetic acid : water (6:3:1), chloroform : methanol (4:1) and methylethylketone : methanol : formic acid : water (11:6:1:2). In addition, the product of the enzymic reaction was subjected to high performance liquid chromatography, and its retention time was compared with that of authentic nicotinic acid-N-glucoside.

High performance liquid chromatography

Separation of nicotinic acid and nicotinic acid-N-glucoside was achieved on a Si 60 LiChrosorb column (250 × 4 mm, 5 µm, MERCK, Darmstadt, F.R.G.) monitoring the UV absorption at 261 nm. Chromatograms were developed using a linear gradient of 100% acetonitrile to 50% acetonitrile in 1.5% (w/v) phosphoric acid within 30 min at a flow of 0.8 ml/min. Chromatography of UDP-glucose was performed on a reversed phase (RP-18) LiChrosorb column (250 × 4 mm, 5 µm, MERCK, Darmstadt, F.R.G.) monitoring the UV absorption at 260 nm. The chromatogram was developed under isocratic conditions using ion pair chromatography with 0.1 M potassium hydrogenphosphate, 0.1 M ammonium acetate and 25 mM tetrabutylammonium hydrogensulfate, pH 5.45.

Determination of molecular mass

The molecular mass of the native enzyme was determined by gel filtration according to Andrews [16] using phosphorylase (M_r 99,000), bovine serum albumin (M_r 67,000), ovalbumin (M_r 45,000) and chymotrypsinogen A (M_r 25,000) as marker proteins.

The void volume and the pore volume of the column (Sephadex G 100, 2.5 × 70 cm) were determined with Blue Dextran 2000 (PHARMACIA, Freiburg, F.R.G.) and DNP-alanine (SERVA, Heidelberg, F.R.G.), respectively. Analytical SDS-PAGE was performed after each purification step under denaturating conditions according to Weber and Osborn [17] and to Shapiro *et al.* [18]. Gels (80 × 80 mm) were stained with SERVA Blue G. Bovine serum albumin, ovalbumin, chymotrypsinogen and myoglobin were used as markers. The mixture contained 0.2% bromphenol blue as tracking dye.

Substrate specificity

The reaction mixtures contained 100 µl enzyme preparation, 50 or 100 nmol of the substrate and 100 nmol UDPG with 1.85 kBq uridinediphospho-[U-^{14}C]glucose in a total volume of 150 µl. After incubating the assays for 30–100 min at 28 °C the reaction was stopped by addition of 150 µl methanol. Chromatographic separation was performed on silica or cellulose thin layer plates in methylethylketone : methanol : formic acid : water (11:6:1:2) and ethyl acetate : methanol : formic acid : water (5:3:1:1).

Enzymatic synthesis of ^{14}C-labelled nicotinic acid-N-glucoside

For the enzymatic syntheses of labelled samples of nicotinic acid-N-glucoside a protein extract from parsley cells was used which had been desalted on Sephadex G-25 and transferred to buffer B. An aliquot (1 ml) of the protein preparation was incubated with 500 nmol nicotinic acid and 1000 nmol UDP-glucose for 2 h at 30 °C in a total volume of 1.5 ml. The assays contained 9.25 kBq [7-^{14}C]nicotinic acid (2.07 GBq/mmol) or 9.25 kBq UDP-[U-^{14}C]glucose (7.4 GBq/mmol), respectively, depending on whether the label was to be incorporated into the pyridine or into the glucose moiety of the product. After addition of 1 ml of methanol to stop the enzyme reaction the nicotinic acid-N-glucoside was purified by ion exchange and high performance liquid chromatography.

Reversibility of the nicotinic acid-N-glucosyltransferase reaction

Enzyme assays consisted of 300 µl protein preparation and 300 nmol nicotinic acid-N-glucoside containing 133 kBq [7-^{14}C]nicotinic acid-N-glucoside or 150 nmol nicotinic acid-N-glucoside containing 133 kBq nicotinic acid-N-[U-^{14}C]glucoside in a final volume of 400 µl buffer B. After 20 min of incubation at 30 °C 300 nmol UDP (30 µl) were added and

the assays were incubated for another 90 min. Samples of 30 µl each were withdrawn from the assays every 10 or 15 min. These aliquots were mixed with 20 µl of methanol, the precipitated protein was removed by centrifugation and the samples were subjected to high performance liquid chromatography to detect nicotinic acid or UDP-glucose. The eluate was fractionated and radioactivity was measured by liquid scintillation counting.

Results

Enzyme reaction

Enzyme activity for the conversion of nicotinic acid to the N-glucosyl conjugate (Fig. 1) was detected in crude protein preparations from heterotrophic cell suspension cultures of *Petroselinum hortense* Hoffm. The addition of EDTA and sucrose to the extraction buffer led to a nearly 3-fold increase in enzyme activity.

The product of the enzymatic reaction was identified by comparing its chromatographic behaviour with that of synthetic nicotinic acid-N-glucoside [9] during HPLC analysis and thin layer chromatography in different solvent systems. Radioactive nicotinic acid-N-glucoside could likewise be detected when uridinediphospho-[U-^{14}C]glucose was added to the incubation assay instead of [7-^{14}C]nicotinic acid, thus proving that UDPG is a suitable glucosyl donor for the transfer reaction.

The UDP-glucose : nicotinic acid-N-glucosyltransferase activity could be detected in the suspension cultured parsley cells during the whole growth cycle (Fig. 2). The specific activity reached a transient maximum during the early lag-phase of growth, whereas during the logarithmic, linear and stationary phases it remained rather constant at 40–50 pkat/mg protein. At every stage of cell growth a glucohydrolase activity towards the artificial substrate *p*-nitrophenol-β-D-glucoside could also be detected.

Enzyme purification

The results of the purification procedure are depicted in Table I. The nicotinic acid-N-glucosyltransferase was purified by precipitation with ammonium sulfate (40–70% saturation) which already eliminated 70% of the protein while retaining 53% of the enzyme activity. The enzyme preparation was further subjected to ion exchange chromatography on DEAE-cellulose. The enzyme activity was eluted at approximately 0.25–0.3 M salt concentration. Gel filtration on Sephadex G-100 resulted in a 19-fold increase in specific activity as compared with the crude extract.

In spite of the removal of 99% of the protein after another adsorption chromatography step on hy-

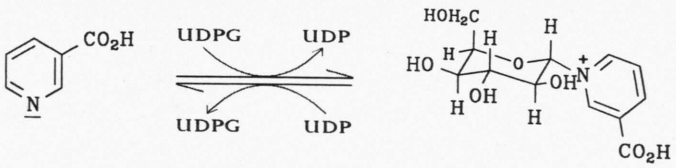

Fig. 1. Reversible conjugation reaction catalyzed by UDP-glucose : nicotinic acid-N-glucosyltransferase.

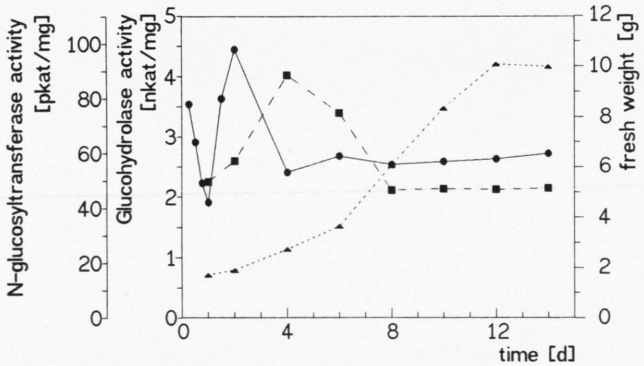

Fig. 2. UDP-glucose : nicotinic acid-N-glucosyltransferase (●——●) and glucohydrolase (■——■) activities during a growth cycle of parsley cell suspension cultures (▲——▲).

Table I. Purification of UDP-glucose:nicotinic acid-N-glucosyltransferase from heterotrophic cell suspension cultures of *Petroselinum hortense* Hoffm.

Purification step	Protein [mg]	Total act. [nkat]	Specific act. [pkat/mg]	Recovery [%]	Purification [-fold]
Crude extract	282.2	7.5	26.6	100	1
$(NH_4)_2SO_4$ fractionation 40–70% saturation	85.4	4.0	46.8	53	2
DEAE-cellulose	25.7	4.7	182.9	63	7
Sephadex G-100	4.6	2.3	500.0	30	19
Hydroxylapatite	1.4	0.82	585.7	11	22

droxylapatite the nicotinic acid-N-glucosyltransferase preparation presumably still contained O-glucosyltransferase activities as demonstrated by the conversion of various phenolic compounds to the corresponding glucosides (Table II).

Kinetic properties

The purification procedure described in Table I yielded an about 22-fold enriched preparation of nicotinic acid-N-glucosyltransferase which was used for kinetic studies of the enzyme.

Table II. Substrate specificity of UDP-glucose:nicotinic acid-N-glucosyltransferase from parsley cell cultures.

Substrate	Rel. activity [%]
Nicotinic acid	100
iso-Nicotinic acid	0
Picolinic acid	0
Nicotinic acid methyl ester	30
Nicotinamide	0
Trigonelline	0
Quinolinic acid	0
6-Hydroxy nicotinic acid	0
2-Hydroxypyridine	0
3-Hydroxypyridine	2
2,6-Dihydroxypyridine	0
3-Methylpyridine	0
Pyridine-3-aldehyde	0
Histidine	0
Proline	0
Adenine	0
Aniline	0
Catechol	59
Vanillin	28
Vanillinic acid	40
Anthranilic acid	0
Benzoic acid	0
p-Hydroxybenzoic acid	2
2,3-Dihydroxybenzoic acid	0
Protocatechuic acid	16

The glucosylation reaction was linear for up to 40 min of incubation at 30 °C. The enzyme exhibited a pH-optimum at pH 7.8–8.2. At pH 11.0 the glucosyltransferase still revealed 50% of the maximum activity, whereas at pH 5.0 it was nearly inactive. The temperature optimum was determined to be between 25 and 30 °C, though at 15 °C glucosyltransferase activity still could be detected.

The apparent K_M values for nicotinic acid and UDP-glucose were found to be 1.7×10^{-4} M and 1.2×10^{-3} M, respectively, with a maximum velocity of 50 pkat/mg protein. The data quoted were obtained using the Lineweaver-Burk method of linear transformation.

Stability of the enzyme and inhibitory effects

Purification of the nicotinic acid-N-glucosyltransferase was hampered by the instability of the enzyme during the extraction from the cells. The addition of EDTA and sucrose to the extraction buffer allowed partial stabilization of the enzyme activity in such crude protein preparations. The glucosyltransferase did not require divalent cations for enzymatic activity, because Ca^{2+}, Mg^{2+} and Mn^{2+} ions did not significantly affect the enzyme reaction. However, 10 mM Zn^{2+} caused a 90% inhibition of the glucosyltransferase activity. *p*-Chloromercuribenzoate (0.5 mM) exercised a strong inhibitory effect on the enzyme reaction, although the glucosyltransferase was not dependent on sulfhydryl protecting agents.

Molecular mass of the glucosyltransferase

Chromatographic behaviour during five separate runs on a gel filtration column (Sephadex G-100) indicated a molecular mass of the native enzyme in the range of $46(\pm 2) \times 10^3$. SDS-PAGE with the partially purified enzyme revealed one major protein

band with an approximate molecular mass of 45–46 kDa. Although another minor protein band with a molecular weight of about 56–58 kDa could be detected with Coomassie Blue staining, the results suggest a monomeric structure of the nicotinic acid-N-glucosyltransferase with a molecular weight of 46 kDa.

Substrate specificity

The nicotinic acid-N-glucosyltransferase exhibited a remarkable specificity for nicotinic acid. A substantial number of other pyridine derivatives were also tested (Table II), but in addition to nicotinic acid itself a glucosyltransfer reaction could only be observed with nicotinic acid methylester. The conversion of several phenolic compounds to the corresponding O-glucosides can best be attributed to an O-glucosyltransferase activity in the enzyme preparation due to the limited extent of protein purification.

Among different nucleotide sugars tested, only UDP-glucose served as a glucosyl donor, whereas CDP-glucose, GDP-glucose, ADP-glucose and TDP-glucose failed to show any activity.

Reversibility of the glucosyltransferase reaction

When [7-^{14}C]nicotinic acid-N-glucoside was incubated with the partially purified glucosyltransferase, or with crude protein preparations from the parsley cells, free [7-^{14}C]nicotinic acid could only be detected, when UDP had been added to the enzyme assays. Under the same conditions the application of nicotinic acid-N-[U-^{14}C]glucoside as substrate yielded labelled UDP-glucose as could be judged from scintillation counting of relevant samples after high performance liquid chromatography of the enzyme assay. In the absence of UDP, a cleavage of the N-glycosidic bond of nicotinic acid-N-glucoside could not be observed, although an enzyme activity hydrolyzing *p*-nitrophenylglucoside, an accepted artificial substrate for glucohydrolases, was still present in the glucosyltransferase preparation (Fig. 2).

Kinetic studies on the formation of free nicotinate and UDP-glucose from nicotinic acid-N-glucoside and UDP are shown in Fig. 3. After the addition of UDP to the enzyme assays a very rapid conversion of the glucoside was detected reaching a steady state after 15 min of incubation when about 7% of the nicotinic acid-N-glucoside had been converted to

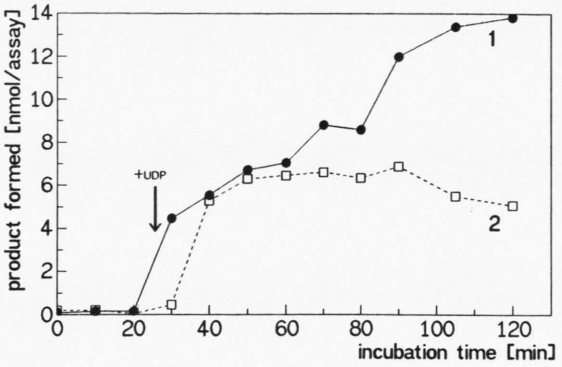

Fig. 3. Reversibility of UDP-glucose:nicotinic acid-N-glucosyltransferase. 1: Formation of [7-^{14}C]nicotinic acid from [7-^{14}C]nicotinic acid-N-glucoside (300 nmol/assay). 2: Formation of UDP-[U-^{14}C]glucose from nicotinic acid-N-[U-^{14}C]glucoside (150 nmol/assay). The arrow indicates the time of the addition of UDP (300 nmol/assay).

UDP-glucose and nicotinic acid. After longer incubation periods degradation of UDP-glucose was observed probably due to hydrolyzing enzyme activities still present in the protein preparation. The removal of UDP-glucose from the equilibrium results in further cleavage of nicotinic acid-N-glucoside to re-establish the steady state conditions. This leads to a further increase in the amount of free nicotinic acid in the reaction mixture.

Discussion

In previous studies it could be demonstrated that nicotinic acid is converted to the N-glucosyl conjugate by heterotrophic cell suspension cultures of *Petroselinum hortense* Hoffm. [9]. This formation of a N-glucoside occurs alternatively to N-methylation [11]; the nicotinic acid N-methyltransferase has recently been characterized in our laboratory using soybean cell cultures [19].

The glucosyltransferase reaction depicted in Fig. 1 has now been studied at the enzymatic level. The UDP-glucose:nicotinic acid-N-glucosyltransferase was partially purified and shown to exhibit a very narrow substrate specificity (Table II), which has also been shown for a variety of other plant glucosyltransferases (for references see [20]). The nicotinic acid-N-glucosyltransferase is specific for the tertiary nitrogen atom of the pyridine ring and requires another functional group in position 3. Among various pyridine derivatives tested, only nicotinic acid,

nicotinic acid methylester and, to a very small extent, 3-hydroxypyridine are converted to the respective N-glucoside by the purified enzyme. Product formation from several phenolic compounds also tested must be attributed to O-glucosyltransferase activities still present in the partial purified protein preparation. In this as in other cases [20] the complete separation of glucosyltransferases appears to be difficult. The conversion of nicotinic acid to the corresponding glucose ester could not be observed, although this compound has previously been shown to occur in parsley cell cultures after feeding of nicotinate [3]. There is evidence that an O-glucosyltransferase from tomato fruits is able to synthesize both the glucoside and the glucose ester from hydroxycinnamic acids depending on the reaction conditions [21], whereas in anthers of tulips the formation of those compounds is due to different separable enzyme activities [22].

With regard to the coenzyme, only UDP-glucose serves as a glucosyl donor among several nucleotide sugars tested. Similar results were obtained for many other plant glucosyltransferases (*e.g.* [23–25]).

The reversibility of the nicotinic acid-N-glucosyltransferase clearly demonstrates that nicotinic acid-N-glucoside has a high group transfer potential and furthermore it may point to a regulatory function of this enzyme in nicotinate metabolism. Among a great variety of plant glucosyltransferases characterized so far, only the UDP-glucose : flavonol-3-O-glucosyltransferase from parsley cell cultures was shown to catalyze a freely reversible reaction [26]. In seedlings of *Raphanus sativus* an enzyme activity for the formation of 1-sinapoylglucose from sinapic acid and UDP-glucose could be detected. The observation that cleavage of the ester bond was stimulated by the addition of UDP may also point to a reversible glucosyltransferase reaction in this system [27].

The ability of plant cells to convert exogenously applied as well as endogenously synthesized compounds to the corresponding glucosyl conjugates has extensively been studied [18, 28]. Although a variety of O-glucosyltransferases has thoroughly been characterized, very little is known about N-glucosyltransferases. Foliar tissues of tomato (*Lycopersicon esculentum* Mill.) are able to metabolize the pesticide metribuzine to the corresponding N-glucoside [29]. As glucosylation alters the polarity and chemical reactivity of aglycones such glucosylation reactions are considered to be important detoxification reactions [30].

With regard to nicotinic acid-N-glucoside, there are several criteria indicating that this compound fulfills the function of a storage form for nicotinic acid; the role of a detoxification product must be attributed to the nicotinate glucose ester which can only be found in parsley cells when exposed to nicotinic acid in concentrations higher than 10^{-5} M [3]. Firstly, the capacity of forming the N-glucosyl conjugate from the primary metabolite nicotinic acid can be detected in suspension cultured parsley cells during the entire growth cycle. Secondly, nicotinic acid-N-glucoside which is formed from exogenously applied as well as endogenously synthesized nicotinic acid is very rapidly metabolized with a half-life of 24 h [9]. Since we were able to observe the enzymatic formation of free nicotinic acid and UDP-glucose from nicotinic acid-N-glucoside and UDP in protein preparations from the parsley cells, the turnover of this N-glucoside can be explained by the reversibility of the UDP-glucose : nicotinic acid-N-glucosyltransferase. Even in crude protein extracts there is no enzyme activity detectable cleaving the N-glycosidic bond of nicotinic acid-N-glucoside by hydrolysis. We postulate that it is the reversible glucosyltransferase reaction that provides free nicotinate for the synthesis of NAD and NADP in the pyridine nucleotide cycle with preservation of the energy of the glycoside in form of UDP-glucose. Further experiments are necessary to demonstrate in more detail this close linkage of nicotinic acid-N-glucoside to the pyridine nucleotide cycle.

Acknowledgements

Our studies were generously supported by the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie.

[1] R. Wagner and K. G. Wagner, Planta **165,** 532–537 (1985).
[2] G. R. Waller and E. K. Nowacki, Alkaloid Biology and Metabolism in Plants, Plenum Press, New York, London 1978.
[3] L. Schwenen, D. Komoßa, and W. Barz, Z. Naturforsch. **41c,** 148–157 (1986).
[4] S. Mizusaki, Y. Tanabe, T. Kisaki, and E. Tamaki, Phytochemistry **9,** 549–554 (1970).
[5] T. A. Scott and A. L. Devonshire, Biochem. J. **124,** 949–950 (1971).
[6] K.-W. Leienbach, V. Heeger, and W. Barz, Hoppe-Seyler's Z. Physiol. Chem. **357,** 1089–1095 (1976).
[7] W. Barz and J. Köster, in: The Biochemistry of Plants, **Vol. 7** (P. K. Stumpf and E. E. Conn, eds.), pp. 35–84, Academic Press, New York, London, Toronto, Sydney, San Francisco 1981.
[8] W. Barz, J. Köster, K.-M. Weltring, and D. Strack, in: Annual Proceedings of the Phytochemical Society of Europe, **Vol. 25** (C. F. Van Sumere, P. J. Lea, eds.), pp. 307–347, Clarendon Press, Oxford 1985.
[9] B. Upmeier, J. E. Thomzik, and W. Barz, Phytochemistry, in press (1988).
[10] K.-W. Leienbach and W. Barz, Hoppe-Seyler's Z. Physiol. Chem. **357,** 1069–1080 (1976).
[11] U. Willeke, V. Heeger, M. Meise, H. Neuhann, I. Schindelmeiser, K. Vordemfelde, and W. Barz, Phytochemistry **18,** 105–110 (1979).
[12] W. Barz, in: Primary and Secondary Metabolism of Plant Cell Cultures (K. H. Neumann, W. Barz, and E. Reinhard, eds.), pp. 186–195, Springer Verlag, Berlin, Heidelberg 1985.
[13] O. L. Gamborg, Can. J. Biochem. **44,** 791–799 (1966).
[14] V. Heeger, K.-W. Leienbach, and W. Barz, Hoppe-Seyler's Z. Physiol. Chem. **357,** 1081–1087 (1976).
[15] M. M. Bradford, Anal. Biochem. **72,** 248–254 (1966).
[16] P. Andrews, Biochem. J. **91,** 222–232 (1964).
[17] K. Weber and N. Osborn, J. Biol. Chem. **244,** 4406–4412 (1969).
[18] A. L. Shapiro, E. Vinuela, J. V. Maizel, Biochem. Biophys. Res. Comm. **5,** 815–820 (1967).
[19] B. Upmeier, W. Groß, S. Köster, and W. Barz, Arch. Biochem. Biophys. **262** (2), 445–454 (1988).
[20] W. Hösel, in: The Biochemistry of Plants, **Vol. 7** (P. K. Stumpf and E. E. Conn, eds.), pp. 725–753, Academic Press, New York, London, Toronto, Sydney, San Francisco 1981.
[21] A. Fleuriet and J. J. Macheix, Z. Naturforsch. **35c,** 967–972 (1980).
[22] P. A. Bäumker, M. Jütte, and R. Wiermann, Z. Naturforsch. **42c,** 1223–1230 (1987).
[23] R. K. Ibrahim and B. Boulay, Plant Sci. Lett. **18,** 177–184 (1980).
[24] T. Shimizu and M. Kojima, J. Biochem. **95,** 205–212 (1984).
[25] W. Kreis, U. May, and E. Reinhard, Plant Cell Reports **5,** 442–445 (1986).
[26] A. Sutter and H. Grisebach, Arch. Biochem. Biophys. **167,** 444–447 (1975).
[27] D. Strack, Z. Naturforsch. **35c,** 204–208 (1980).
[28] M. Tabata, Y. Umetani, M. Ooya, and S. Tanaka, Phytochemistry **27** (3), 809–813 (1988).
[29] D. S. Frear, E. R. Mansager, H. R. Swanson, and F. S. Tanaka, Pestic. Biochem. Physiol. **19,** 270–281 (1983).
[30] E. E. Conn, in: Cellular and Molecular Biology of Plant Stress, pp. 351–365, Alan R. Liss, Inc., New York 1985.