1-*O*-*trans*-Cinnamoyl-β-D-glucopyranose: Alcohol Cinnamoyltransferase Activity in Fruits of Cape Gooseberry (*Physalis peruviana* L.)

Stefan Latza and Ralf G. Berger

Institut für Lebensmittelchemie, Universität Hannover, Wunstorfer Straße 14, D-30453 Hannover, Germany

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Phenylpropanoid Metabolism, Glycoconjugate, Flavour Precursor, Aroma Ester, Methyl Cinnamate

Methyl and ethyl cinnamate are aroma volatiles frequently occurring in fruits. Evidence was obtained that the enzymatic transfer of cinnamic acid to endogenous alcohols present in fruits (methanol, ethanol, 1-propanol) depended on energy-rich 1-*O*-glycosyl esters of cinnamic acid which served as acyl donor molecules. A putative 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose: alcohol cinnamoyltransferase from cape gooseberry (*Physalis peruviana* L.) was active towards 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose and 1-*O*-*trans*-cinnamoyl-β-D-gentiobiose. The enzyme was purified 290-fold by a protocol including ammonium sulphate precipitation, solubilization by Triton X-100, gel permeation and affinity chromatography on concanavalin A. The acidic glycoprotein (pI = 4.8) most probably is membrane bound. The distribution of alcohol cinnamoyltransferase activity in gel chromatography fractions suggests a native M_r of 75,000. For 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose, an apparent K_m of 69 μM was determined. At pH > 6.0, non-enzymatic transesterification superposes the enzymatic transformation.

Introduction

The flavour of many fruits is determined by volatile carboxylic acid esters. Methyl and ethyl cinnamate stand for widely occurring aroma esters (Maarse *et al.*, 1994) possessing pleasant and balsamic, tropical-fruit-notes. Whereas in fruits methyl cinnamate generally is present at low concentrations, in some *Eucalyptus* species *trans*-methyl cinnamate amounts to 94–99% of the oil yield, superseding *Ocimum canum* (basil, methyl cinnamate type), in which methyl cinnamate amounts to 87% of the total oil (Curtis *et al.*, 1990). Apart from its flavour, methyl cinnamate is assumed to possess several bioactivities. The cinnamic acid ester was held responsible for an ovipositional deterrence of the onion fly (Cowles *et al.*, 1990), repellence to blackbirds (Avery and Decker, 1992), and larvicidal activity in *Kaempferiae rhizoma* (Kiuchi *et al.*, 1988). Despite its frequent occurrence in the plant kingdom and its assumed range of bioactivity, the generation of methyl cinnamate in plant tissues remained unclear.

The objective of the present study was to reveal the enzymology associated with methyl and ethyl cinnamate formation in fruits. The biosynthesis of *O*-esters, such as the related hydroxycinnamic acid (HCA) conjugates in plants, in the majority of cases, proceeds via the HCA-coenzyme A thioesters or via the 1-*O*-HCA-β-glucosides (Mock and Strack, 1993). In the latter case, the formation of an *O*-ester by transfer of an HCA-moiety to an HO-bearing acceptor molecule is catalyzed by 1-*O*-acylglucose-dependent *O*-acyltransferases (Strack and Mock, 1993).

In line with these results, 1-*O*-cinnamoylglycosides, as possible non-volatile progenitors, have been identified in several fruits (Latza *et al.*, 1996a, b). In cape gooseberry (*Physalis peruviana* L.), 1-*O*-*trans*-cinnamoyl-β-D-gentiobiose was detected.

Abbreviations: AAT, alcohol acyltransferase; ACT, alcohol cinnamoyltransferase; Con A, concanavalin A; FPLC, fast protein liquid chromatography; GC, gas chromatography; HCA, hydroxycinnamic acid; HPLC, high performance liquid chromatography; IEF, isoelectric focusing; PAA, polyacrylamide; PME, pectin methylesterase; PMSF, phenylmethylsulfonyl fluoride; PVDF, polyvinylidene difluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SPME, solid phase microextraction; TEMED, N,N,N′,N′-tetramethylethylene diamine.

Reprint requests to Prof. Dr. R. G. Berger.
Telefax: (0049) 511–7624547.

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A previous study suggested a remarkable dependency of methyl cinnamate formation from treatment of cape gooseberries with methanol used as a general enzyme inhibitor (Berger *et al.*, 1989). Therefore, fruits of *P. peruviana* were chosen for devising a purification protocol for a putative 1-*O*-cinnamoylglycoside-dependent alcohol cinnamoyltransferase.

Materials and Methods

Plant material

Fruits of Colombian cape gooseberry (*Physalis peruviana* L.) were purchased from the local market. The surrounding dehydrated calycles were removed. Undamaged fruits, free from infection (visually inspected), were selected and stored at 10 °C until used for extraction.

Chemicals

1-*O*-*trans*-Cinnamoyl-β-D-glucopyranose and 1-*O*-*trans*-cinnamoyl-β-D-gentiobiose were synthesized as described (Latza *et al.*, 1996a; Plusquellec *et al.*, 1986). 1-*O*-*trans*-Cinnamoyl-6-*O*-α-L-arabinofuranosyl-β-D-glucopyranose was isolated from fruits of *Psidium guajava* as reported previously (Latza *et al.*, 1996a). The *trans*-cinnamoyl coenzyme A thioester was prepared by a mixed anhydride procedure (Fong and Schulz, 1981).

Isolation and partial purification of alcohol cinnamoyltransferase

All buffers contained 1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma, Germany) as a protease inhibitor. Fruits of cape gooseberry (100 g) were homogenized with 120 ml distilled water, 68 g dry ammonium sulfate and 20 mg PMSF in a Rotor blender (Rotor AG, Switzerland) for 5 min at 14,000 rpm. The seeds were not broken up. After adjustment to pH 5.0 with 2.0 M NaOH, the homogenate was centrifuged at 35,000 × *g* for 20 min. The pellet was resuspended in 40 ml buffer A (50 mM sodium phosphate, pH 5.0, containing 0.1% Triton X-100) and the suspension was centrifuged at 35,000 × *g* for 20 min. To the supernatant, which is termed the crude extract, dry ammonium sulfate (14 g) was added and dissolved by stirring. After centrifugation at 39,000 × *g* for 20 min, the precipitate was redissolved in 2.0 ml buffer A. Insoluble remainders were removed by centrifugation at 10,000 × *g* for 10 min. The enzyme extract was clarified by membrane filtration using a Chromafil 0.45 µm polyvinylidene difluoride filter (PVDF, Macherey-Nagel, Germany).

For gel filtration chromatography, 0.5 ml of enzyme extract was loaded onto a column of Superdex 200 HR 10/30 (29.5 × 1 cm, Pharmacia, Sweden), which was pre-equilibrated with 50 mM sodium phosphate buffer, pH 5.0. Elution was carried out with the same buffer at a flow rate of 0.75 ml/min (Glass HPLC). Active fractions (1.5 ml) were pooled, diluted with the threefold amount of buffer B (20 mM Tris-HCl, pH 7.4, 0.5 M NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$), and adjusted to pH 7.4. For affinity column chromatography, pooled samples of four repeated runs were applied onto a column of Con A Sepharose (FPLC, 4 × 1 cm, Pharmacia), which had been equilibrated with buffer B, at a flow rate of 1 ml/min. The bound activity was eluted with a linear gradient of methyl α-D-glucopyranoside (0.0–0.2 M) in buffer B for 30 min. Active fractions (2 ml) were pooled and dialyzed against 10 mM sodium phosphate buffer, pH 5.0.

Enzyme assays

Enzyme activity of alcohol cinnamoyltransferase was determined by SPME/GC analysis of the methyl cinnamate formed (Yang and Peppard, 1994). The standard assay mixture contained 0.66 M methanol, 0.16 mM 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose and 0.1 M sodium phosphate buffer (buffer C), pH 5.0, in a final volume of 3.0 ml. The reaction was initiated by adding 1.0 ml of enzyme solution (pH 5.0). After incubation for 3 h at 40 °C, the reaction was terminated by instant freezing at -20 °C. Prior to SPME liquid sampling, the rethawed mixture was diluted 1:1 with buffer C. Two ml of the solution were mixed with 0.8 g of dry NaCl and the SPME fiber, consisting of fused silica coated with 85 µm polyacrylate (Supelco Inc., Bellefonte, PA), was inserted. Under stirring, the fiber remained in the liquid for 35 min. For thermal desorption, it was subsequently introduced into the GC injector in splitless mode.

GC was carried out on a HRGC Fractovap 4160 chromatograph (Carlo Erba, Italy) equipped with

a regular split/splitless inlet, a flame-ionization detector, a deactivated FS-Phenyl-Sil pre-column (3 m × 0.32 mm i.d., CS, Germany), and a BC SE 54 fused silica column (25 m × 0.32 mm i.d., film thickness 0.4 μm, Leupold, Germany). Operating conditions were as follows: Carrier gas H_2 at 3.25 ml/min, injector 250 °C, detector 270 °C. The column was held at 60 °C for 5 min and temperature was increased to 250 °C at a rate of 3 °C/min. Dilutions of methyl cinnamate (Fluka, Germany) in 0.1 M buffer C were used to fit an external standard curve in the range of 0.1 μM to 1.0 μM.

Pectin methylesterase (PME) activity in chromatographic fractions was monitored by a dye-based assay (Wojciechowsky and Fall, 1996), using highly esterified Pomosin pectin type B (Hercules/Pomosin, Germany) as a substrate.

Isoelectric focusing and gel activity detection of alcohol cinnamoyltransferase

Isoelectric focusing was performed in polyacrylamide gels (12 × 12 × 0.05 cm, 10% PAA), polymerized from a mixture of 2.3 ml double-distilled water, 7.6 ml acrylamide/ bisacrylamide (32.5/1), 5.3 ml 60% (v/v) glycerol, 0.8 ml ampholytes (pH-range from 3–10), 16 μl 50% (w/v) ammonium persulfate and 7 μl TEMED. Prior to electrophoresis, active Con A fractions were dialysed against double-distilled water and concentrated by evaporation under reduced pressure at 30 °C. The cathode solution was 0.5 M NaOH and the anode solution 0.5 M phosphoric acid. After prefocusing, gels were run at 200 V for 0.5 h, then at 1000 V for additional 2.5 h. Sample proteins and IEF standards (p*I*-range from 4.45–9.6, Bio-Rad, Germany) were stained with Coomassie blue. To positively detect alcohol cinnamoyltransferase activity, half of the gel was left unstained. Sections of the sample lane were cut out and incubated in the standard assay mixture as described above.

Other analytical methods

Standard procedures were used for measuring protein concentrations (Bio-Rad protein assay, based on Bradford, 1976) and for the determination of molecular weights (SDS-PAGE, 12% PAA, Laemmli, 1970). Protein was visualized by staining with Coomassie brilliant blue. In addition, M_r of sample proteins was verified by rechromatography of active Con A fractions on Superdex 200 HR 10/30, as described above (fractions of 0.75 ml).

Studies on the substrate specificity of alcohol cinnamoyltransferase

Substrate specificity was determined in the standard assay mixture, containing 0.16 mM of 1-*O*-*trans*-cinnamoyl-β-D-gentiobiose, 1-*O*-*trans*-cinnamoyl-6-*O*-α-L-arabinofuranosyl-β-D-glucopyranose, *trans*-cinnamoyl CoA and cinnamic acid, respectively, instead of the standard substrate. The alcohol substrate was varied by using 0.66 M ethanol and 1-propanol, respectively, instead of methanol.

Determination of the pH- und temperature-optimum, kinetics

Enzyme assays were carried out as described above, using 0.1 M sodium phosphate buffer with pH between 2.0 and 12.0. Control experiments were carried out by using 1 ml of the respective buffer instead of enzyme solution. The temperature-dependency of alcohol cinnamoyltransferase was analysed by incubation of the standard assay mixture at different temperatures between 10 °C and 70 °C. Determination of the apparent Michaelis-constant for 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose (varied between 6.25 μM and 1.25 mM) was performed with fixed methanol concentration (0.66 M).

Results

Enzyme preparation

An enzyme mixture which showed alcohol cinnamoyltransferase activity was purified about 290-fold from fruits of cape gooseberry (*Physalis peruviana* L.) by a procedure including ammonium sulphate precipitation, solubilization effected by Triton X-100, gel filtration and affinity chromatography. The overall recovery was 20% (Table I).

At about 60% ammonium sulphate saturation, the precipitable fraction of the initial homogenate was sedimented along with cell fragments and seeds. Out of the sediment, solubilization of alcohol cinnamoyltransferase activity could only be obtained after 0.1% Triton X-100 was included. This points to a membrane bound transferase. From the resulting crude extract, a precipitate was

Table I. Purification of alcohol cinnamoyltransferase from cape gooseberry. Activity is expressed as nmol methyl cinnamate formed/min. The temperature was 40 °C. All other conditions are described under Materials and Methods.

Purification step	Total protein [mg]	Total activity [nmol/min]	Specific activity [nmol/mg protein per min]	Yield [%]	Purification -fold
Homogenate	187	5.70	0.030	100	1
Crude extract	31.4	4.46	0.142	78	4.7
Redissolved precipitate	19.0	4.32	0.227	76	7.6
Superdex 200 HR 10/30	1.3	2.76	2.12	48	71
Con A Sepharose	0.13	1.13	8.69	20	290

obtained after re-adjustment to 60% ammonium sulphate saturation. The precipitate was redissolved and used for subsequent gel permeation chromatography on Superdex 200 HR 10/30 (Fig. 1). Fractions corresponding to the major peak of alcohol cinnamoyltransferase activity (fractions 8 to 10, maximum activity in fraction 9) were subjected to affinity chromatography on a Con A Sepharose column (Fig. 2). After non-bound contaminants (flow-through) were washed out, the alcohol cinnamoyltransferase activity was eluted in a linear gradient of methyl α-D-glucopyranoside, indicating that the enzyme is a glycoprotein. Although a single activity peak was eluted from the Con A column at a concentration of about 80 mM methyl α-D-glucopyranoside (Fig. 2), subsequent reanalysis by gel permeation chromatography on Superdex 200 HR 10/30 revealed the presence of apparently three major proteins (Fig. 3). The distribution of alcohol cinnamoyltransferase activity in the chromatographic fractions suggested that the enzyme was the protein eluting first, thus having the highest molecular mass. Small activities of alcohol cinnamoyltransferase in the subsequent fractions were interpreted as a result of chromatographic tailing.

As pectin methylesterases (PME) from fruits frequently are described as glycoproteins (Glover and Brady, 1994), the presence of a PME in the final enzyme extract was expected. Indeed, the protein eluting last was found to exhibit pectin methylesterase activity (Fig. 3). In view of the low

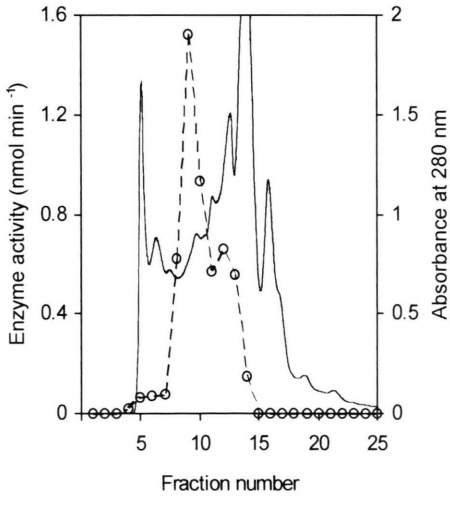

Fig. 1. Fractionation of the redissolved precipitate by gel permeation chromatography on Superdex 200 HR. Fractions (1.5 ml) were assayed for alcohol cinnamoyltransferase activity (○). Maximum activity was detected in fraction 9. Elution was monitored by recording absorbance at 280 nm (—).

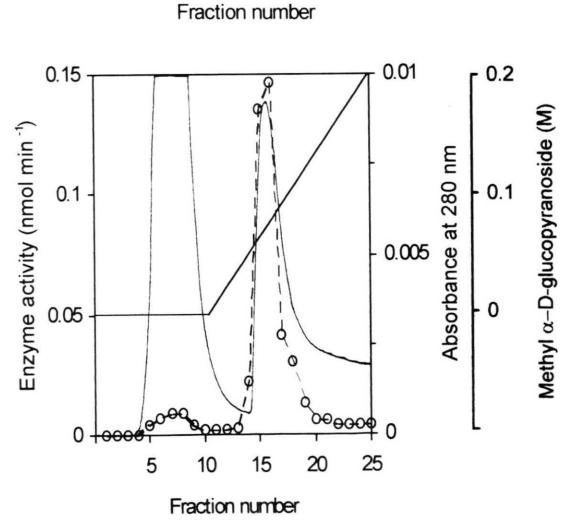

Fig. 2. Fractionation of active fractions from gel filtration by affinity chromatography on Concanavalin A. Fractions (2 ml) were assayed for alcohol cinnamoyltransferase activity (○). Gradient elution was started between fractions 10 and 11. The active protein eluted at a concentration of about 80 mM methyl α-D-glucopyranoside. Absorbance was recorded at 280 nm (—).

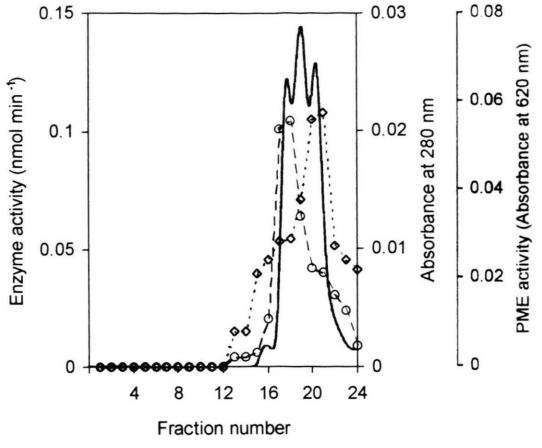

Fig. 3. Rechromatography of active fractions from Con A chromatography on Superdex 200 HR. Fractions (0.75 ml) were collected and assayed for alcohol cinnamoyltransferase (○) and pectin methylesterase activities (◊). Protein elution was monitored at 280 nm (—).

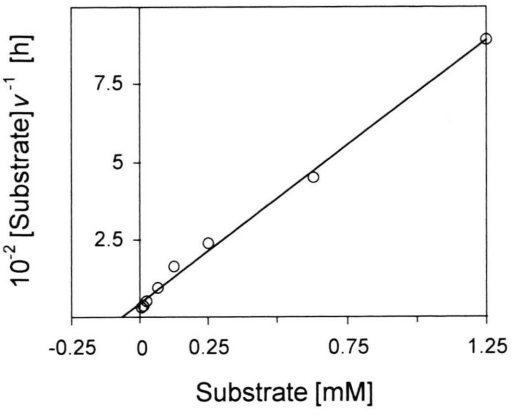

Fig. 4. Single-reciprocal Hanes kinetic plot of alcohol cinnamoyltransferase activity from cape gooseberry at a constant level of methanol (0.66 M). 1-*O-trans*-cinnamoyl-β-D-glucopyranose served as a substrate.

total protein content about 0.1 mg absolute mass and minute specific activity, further attempts to fully separate the alcohol cinnamoyltransferase activity from the interfering polypeptides by FPLC or HPLC-based methods, or by preparative electrophoresis, failed. However, despite the incomplete purification, studies on kinetics and substrate specificity of alcohol cinnamoyltransferase resulted in coherent sets of data, as they were to be expected from a pure activity.

Kinetic properties and substrate specificity

Alcohol cinnamoyltransferase activity conformed to hyperbolic Michaelis-Menten kinetics. Under standard assay conditions, the apparent K_m value for 1-*O-trans*-cinnamoyl-β-D-glucopyranose, obtained from a single-reciprocal analysis (Fig. 4), was 69 μM. Substrate concentrations of 1-*O-trans*-cinnamoyl-β-D-glucopyranose were varied in the range of 6.25 μM to 1.25 mM, whereas the methanol content (second substrate) was kept constant at 0.66 M. Under these conditions no substrate inhibition could be observed, and apparent V_{max} was determined as 24.6 nmol min^{-1} for [Substrate] = 1.25 mM.

At a constant level of 0.16 mM 1-*O-trans*-cinnamoyl-β-D-glucopyranose, concentrations of methanol were varied in the range of 49.3 mM to 7.40 M (0.2- 30.0%, v/v). A linear relationship of product formation was observed up to 3.7 M of methanol (15.0%, v/v), then rates of formation decreased (data not shown). At a methanol content of 7.40 M, still 67% of maximum activity could be observed.

The alcohols methanol, ethanol and 1-propanol all served as a substrate for alcohol cinnamoyltransferase. 1-*O-trans*-Cinnamoyl-β-D-gentiobiose, which is naturally occurring in cape gooseberry (Latza *et al.*, 1996a), was accepted as acyl donor substrate, but the enzyme was inactive towards 1-*O-trans*-cinnamoyl-6-*O*-α-L-arabinofuranosyl-β-D-glucopyranose from guava and towards *trans*-cinnamoyl CoA and free cinnamic acid.

Temperature and pH dependency

The temperature optimum was at 45 °C. As pH had a profound effect on product formation (Fig. 5), its value was kept constant at pH 5.0 during performance of activity assays. In the range between pH 4.0 and 6.0 formation of methyl cinnamate solely was affected by alcohol cinnamoyltransferase activity. At higher pH-values, that do not occur in a physiological environment, non-enzymatic transesterification readily can occur and overrides the enzymatic transformation. Above pH 10, 1-*O-trans*-cinnamoyl-β-D-glucopyranose is subjected to alkaline hydrolysis. As enzymatic and non-enzymatic transesterification competed in the range between pH 6.0 and 10.0, a pH optimum between pH 7.0 and 8.0 was assessed by subtraction

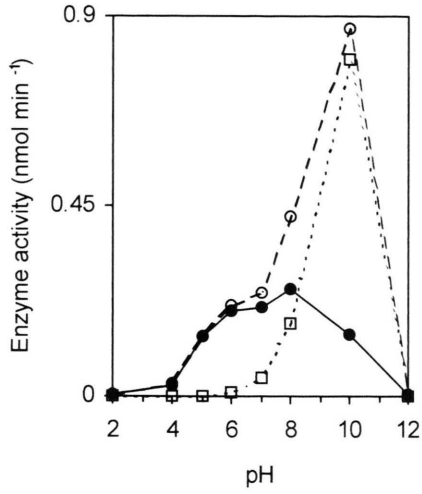

Fig. 5. Alcohol cinnamoyltransferase activity and incubation pH-value. The enzymatic transformation (●) results from subtraction of non-enzymatic transesterification (control, □) from the combined reaction (total, ○).

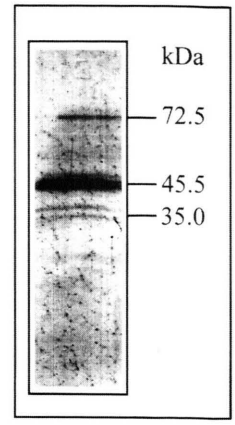

Fig. 6. SDS-polyacrylamide gel electrophoresis of the enzyme extract obtained by Con A chromatography. 0.13 mg of sample protein was applied. M_r of protein bands was calculated from calibration with protein standards on a nearby lane of the gel. By comparison to the results of gel filtration, the upper band is expected to represent the alcohol cinnamoyltransferase, the lower, split band is supposed to reflect a pectin methylesterase.

of non-enzymatic rates (control) from the combined transformation (total).

Isoelectric point and molecular mass

The p*I*-value of alcohol cinnamoyltransferase was determined by native IEF-PAGE based on the mobilities of IEF marker proteins. In accordance with the results of gel filtration chromatography, three bands of sample protein were detected at p*I*-values of 8.4; 7.6 and 4.8. In order to locate the alcohol cinnamoyltransferase activity, half of the slab gel was left unstained, allowing the enzymes to maintain their activity. Respective sections were sliced and assayed for alcohol cinnamoyltransferase activity by incubation in the standard assay mixture. Activity was detected in the section covering p*I* = 4.8, indicating a rather acidic protein. No other protein band contained any transferase activity for the substrates used.

By gel permeation, native M_r-values of 75,000; 48,500 and 31,000 were assigned to the three major bands (Fig. 3). In agreement with the IEF pattern, denaturing SDS-polyacrylamide gel electrophoresis also showed three major bands (Fig. 6). Considering the distribution of alcohol cinnamoyltransferase activity in gel chromatography fractions (Fig. 3), the transferase should exhibit the highest molecular weight of 75.0 kDa, corresponding to 72.5 kDa determined by SDS-PAGE. M_r-values of 45,500 and 35,000 referred to the accompanying proteins. By analogy with the above considerations, the pectin methylesterase of cape gooseberry showed a native molecular weight of 31.0 kDa (35.0 kDa by SDS-PAGE, respectively).

Discussion

The present study describes the enzymatic 1-*O*-acylglucose-dependent transfer of cinnamic acid to methanol, ethanol or 1-propanol by an activity isolated from cape gooseberry (Fig. 7). As acyl donor molecules, 1-*O*-cinnamoylglycosides recently have been identified in some fruits: 1-*O-trans*-Cinnamoyl-β-D-glucopyranose was isolated from strawberry, cowberry, guava (Latza *et al.*, 1996 a,b) and blood orange (Mouly *et al.*, 1997), 1-*O-trans*-cinnamoyl-β-D-gentiobiose was detected in cape gooseberry, and 1-*O-trans*-cinnamoyl-6-*O*-α-L-arabinofuranosyl-β-D-glucopyranose was obtained from fruits of guava (Latza *et al.*, 1996a). For the closely related 1-*O*-sinapoyl-β-D-glucopyranose, it has been shown that 1-*O*-acyl-β-glucosides have a high group-transfer potential, permitting subsequent transacylation reactions (Mock and Strack, 1993). 1-*O*-acylglycosides are regarded as possible pathway alternatives to CoA-dependent activation of

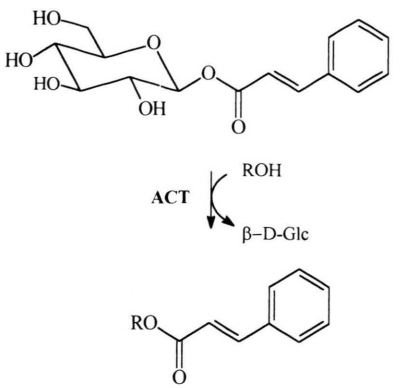

Fig. 7. Alcohol cinnamoyltransferase (ACT) catalyzed transfer of cinnamic acid from 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose to methanol, ethanol or 1-propanol (ROH). The β-D-gentiobiose ester of cinnamic acid also serves as a substrate for ACT.

acyl-moieties, in particular regarding hydroxycinnamic acids (Strack and Mock, 1993). In accordance with this concept, there was no cofactor requirement of alcohol cinnamoyltransferase from cape gooseberry. As the acyl donor molecule, 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose routinely was employed in the standard assay mixture. As a natural substrate occurring in cape gooseberry, 1-*O*-*trans*-cinnamoyl-β-D-gentiobiose was also accepted as an acyl donor, but with much less affinity. From kinetic studies (data not shown), apparent K_m was extrapolated to 3.5 M, compared to 69 μM for 1-*O*-*trans*-Cinnamoyl-β-D-glucopyranose (Fig. 4). 1-*O*-*trans*-cinnamoyl-6-*O*-α-L-arabinofuranosyl-β-D-glucopyranose from guava did not serve as a substrate. Free cinnamic acid and *trans*-cinnamoyl CoA were not transformed to alkyl cinnamates. Additional studies will be necessary to show if *p*-coumaroyl-, caffeoyl-, feruloyl- or sinapoylglucose may serve as acyl-donor substrates. As our main interest referred to aroma biogenesis in fruits, no effort was made to synthesize and apply the ubiquitous 1-*O*-glucose esters of hydroxycinnamic acids.

As the second substrate, methanol, ethanol and 1-propanol were employed as HO-acceptor molecules. These alcohols are the most abundant short chain alkanols of strawberry fruit. In the juice of ripe strawberries, 1.2 mM methanol, 0.2 mM ethanol and 0.1 mM 1-propanol, together amounting to 89% of the free alcohol content, were detected (Ueda *et al.*, 1992).

Respective data on cape gooseberry were not available. The alcohols served directly in methyl, ethyl, and propyl cinnamate formation and were acylated with similar affinity. Since only methyl and ethyl cinnamate are of significance to the aroma of fruits, other alcohols were not tested.

As a result of the performed experiments, proof was given for 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose: alcohol cinnamoyltransferase activity in enzyme extracts of cape gooseberry at pH < 6.0. An issue of particular importance is that the non-enzymatic transesterification reaction can readily result at pH > 6.0 (Fig. 5). To avoid the danger of obtaining artifactual results, assays for measuring alcohol cinnamoyltransferase activity were conducted at pH 5.0. The enzyme activity was monitored by analysis of formed alkyl cinnamates. As this demanded for a method of trace analysis, a recently developed and highly-sensitive variation of the adsorption techniques, solid phase microextraction (SPME, Yang and Peppard, 1994) was applied in combination with high-resolution gas chromatography.

The purification of alcohol cinnamoyltransferase ended up with a protein fraction containing three major proteins. After the last purification step, total protein mass was very low (Table I) and amounted to 0.13 mg. In view of the low specific activity, further attempts to complete the purification, failed. However, alcohol cinnamoyltransferase activity partially could be separated from an accompanying pectin methylesterase activity by gel filtration rechromatography (Fig. 3). Both, SDS-PAGE and IEF-PAGE of the final extract exhibited three major proteins. From IEF-PAGE, a major band at pI = 4.8 unequivocally was assigned to alcohol cinnamoyltransferase activity, whereas a native M_r of 75.0 kDa was concluded from the distribution of transferase activity during gel filtration (Fig. 3).

In view of the low specific activity of 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose: alcohol cinnamoyltransferase, the transferase reaction might reflect a side-activity of a known enzyme. The distinctive feature of the enzyme to yield a carboxylic acid methyl ester in the presence of methanol under aqueous conditions resembles the characteristics of non-specific lipid acyl hydrolases from dif-

ferent plants and microorganisms (Mukherjee, 1994, 1990). Some of these lipolytic enzymes catalyze the transfer of acyl groups from a wide range of glycerolipids to methanol to yield methyl esters of fatty acids. So far, these enzymes seemingly are not dependent on activated acyl moieties. On the contrary, biosynthesis of aroma esters in fruits of strawberry, banana, melon, and apple repeatedly has been reported to be an acyl-CoA dependent reaction, catalyzed by acyl-CoA: alcohol acyltransferases (Fellmann *et al.*, 1993, Pérez *et* al., 1996, Ueda *et al.*, 1992, Wyllie *et al.*, 1996). However, alcohol cinnamoyltransferase from cape gooseberry did not exhibit activity towards *trans*-cinnamoyl CoA. As alcohol cinnamoyltransferase activity also was detected in fruits of strawberry (data not shown), the enzyme seems to coexist with acyl-CoA-dependent alcohol acyltransferases. On account of its high content of pectins (0,68%, Lower, 1992) and polyphenols (2,1 g × kg^{-1}, Perkins-Veazie, 1995), the above described method of enzyme purification turned out to be inapplicable to strawberry fruit. Therefore, strawberry alcohol cinnamoyltransferase was not further analyzed.

A glucose ester-dependent transferase reaction was first discovered in the biosynthesis of indolylacetic acid-*myo*-inositol ester in kernels of *Zea mays* (Michalczuk and Bandurski, 1980); meanwhile, there is increasing evidence that in particular the formation of hydroxycinnamic acid esters via 1-*O*-hydroxycinnamoyl glucosides plays an important role as an alternative to the CoA-dependent transferase reactions (Strack and Mock, 1993). In this report, evidence was provided for a related 1-*O*-cinnamoylglycoside-dependent transferase reaction which could be involved in aroma ester biogenesis.

Acknowledgements

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