Phytochemistry, Vol. 24, No. 3, pp. 407–410, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

DISTRIBUTION OF 1-SINAPOYLGLUCOSE:CHOLINE SINAPOYLTRANSFERASE ACTIVITY IN THE BRASSICACEAE

JOSEF REGENBRECHT and DIETER STRACK*

Botanisches Institut der Universität zu Köln, Gyrhofstrasse 15, D-5000 Köln 41, West Germany

(*Received* 21 *June* 1984)

Key Word Index—*Brassica*; *Raphanus*; *Sinapis*; Brassicaceae; phenylpropanoid metabolism; hydroxycinnamic acid ester; glucose ester; sinapine; 1-sinapoylglucose:choline sinapoyltransferase; transacylase.

Abstract—The occurrence of 1-sinapoylglucose:choline sinapoyltransferase (SCT) in seeds of various members of the Brassicaceae is reported. Within the species and cultivars investigated, a positive correlation was found between extractable levels of enzyme activity and the degree of sinapine accumulation. High enzymatic activities were found in seeds from *Brassica*, *Raphanus* and *Sinapis*, known for their high sinapine content.

INTRODUCTION

The synthesis of cinnamic acid esters is an endergonic reaction which requires activation of the carboxyl group of the phenylpropanoid acid. There are many examples of this being accomplished in higher plants by transferases which are dependent on cinnamoyl-CoA thiolesters [1, 2]. However, we have recently shown that the formation of cinnamic acid esters is also catalysed by transferases which use energy-rich 1-*O*-acyl glucosides as the acyl donor in the synthesis of, for example, *O*-sinapoylcholine (sinapine) [3], *O*-sinapoyl-L-malate [4, 5] and 1,2-di-*O*-sinapoylglucose [6] in seeds and seedlings of red radish, the glucose-activated sinapic acid being synthesized via UDP-activated glucose [7, 8]. The same mechanism of ester formation also occurs in the metabolism of galloyl esters in oak leaves [9, 10]. The first example of this type of transacylation was discovered in plant hormone metabolism in the biosynthesis of IAA-*myo*-inositol in kernels of sweet corn [11].

Among a large number of cinnamic acid esters occurring in higher plants (cf. ref. [12]), members of the Brassicaceae exhibit a characteristic accumulation of sinapine [13] in the seeds [14–16]. The enzyme catalysing the synthesis of sinapine in seeds of red radish can be classified as 1-*O*-sinapoyl-β-D-glucose:choline sinapoyltransferase (SCT) [3].

The extractable transacylase activities from different stages of seed development are closely correlated with the *in vivo* accumulation kinetics of sinapine during the process of seed ripening [3]. The dry, dark brown, mature seeds have about 20% of the maximal activity reached in dark-green immature seed exhibiting a rapid sinapine accumulation. This observation was of interest since it indicated that mature seeds could be used for the easy screening of this enzyme in members of the Brassicaceae.

In this paper we report the presence of SCT activities in seeds of various members of the Brassicaceae. Our results indicate that the new enzyme is widely distributed within this plant family and a correlation between the enzyme levels and the degree of sinapine formation is indicated.

RESULTS AND DISCUSSION

Protein extracts from seeds of 44 members of the Brassicaceae catalysed the formation of sinapine, using 1-

*To whom correspondence should be addressed.

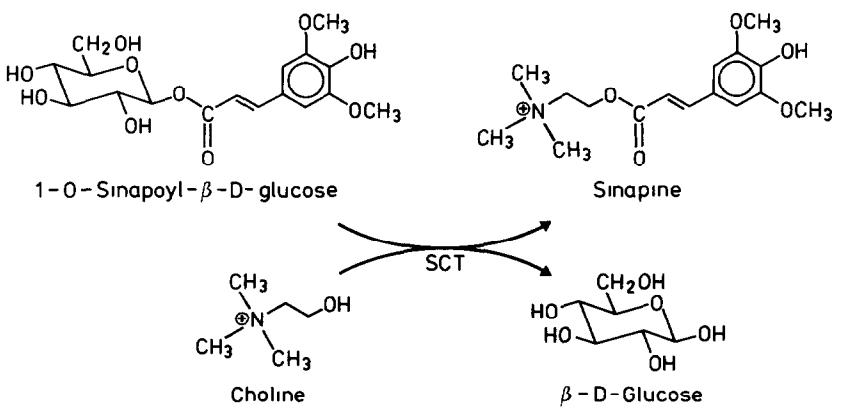

Fig. 1. SCT-catalysed sinapine formation.

O-sinapoyl-β-D-glucose as the acyl donor (Fig. 1; Table 1). The SCT activity was measured by using isocratic HPLC (Fig. 2). This procedure allowed rapid determination of the activity, combined with selective identification of the product. The same system was also used for the quantitative determination of the seed sinapine content. For all plants investigated, the identification of sinapine (a) as a product of the enzymatic reactions and (b) in the seed extracts was checked by TLC (microcrystalline cellulose [3]). The sinapine spots were located under UV (366 nm,

Table 1. Sinapine (nmol) and SCT activity (pkat) per individual seed and per mg seed of various members of the Brassicaceae (values are the means from three or four independent determinations)

Plant	Sinapine		SCT	
	Seed	mg seed	Seed	mg seed
Berteroa incana (L.) DC.	tr*	tr	0.2	0.5
Biscutella lyrata L.	12.8	9.5	2.2	1.5
Brassica juncea (L.) Czern.	27.9	20.2	7.9	5.6
B. napus L. var. *annua* Metzger	121.6	26.2	6.1	1.3
B. n. subsp. *napus* (L.) DC.	110.6	28.6	6.2	1.6
B. nigra (L.) Koch	20.2	21.0	1.4	1.3
B. oleracea L. convar. *acephala* (DC.) Alef. var. *gongylodes* L.	146.3	28.6	36.7	7.4
B. o. a. var. *sabellica* L.	104.7	27.2	13.8	3.9
B. o. convar. *botrytis* (DC.) Alef. var. *botrytis* L.	157.7	38.4	36.9	9.3
B. o. b. var. *italica* Plenck	130.3	36.8	14.5	3.7
B. o. convar. *capitata* (L.) Alef. var. *capitata* L. f. *alba*	100.8	31.1	13.7	4.4
B. o. c. c. f. rubra	78.1	24.2	12.5	3.7
B. o. c. var. *sabauda* L.	88.2	28.3	7.6	2.4
B. o. convar. *oleracea* var. *gemmifera* DC.	64.7	23.3	3.4	1.2
B. rapa L. var. *rapa* (L.) Thell.	49.7	26.1	13.9	6.8
B. r. var. *sylvestris* (LAM.) Briggs	18.8	15.9	3.7	2.0
Camelina microcarpa Andrz.	2.1	6.2	1.2	3.7
C. sativa (L.) Crantz	5.7	7.3	1.8	2.5
Capsella bursa-pastoris (L.) Med.	1.1	12.5	0.2	2.3
Cheiranthus alpinus L.	16.0	12.7	1.3	1.1
C. cheiri L.	35.8	20.5	2.0	1.2
Descurainia sophia (L.) Webb	2.1	18.6	0.1	0.8
Eruca sativa Mill.	1.2	0.5	0.4	0.2
Erysimum L. × *allionii*†	16.1	13 7	tr	tr
Isatis tinctoria L.	60.1	31 0	0.8	0.4
Lepidium densiflorum Schrad.	tr	tr	6.9	2.4
L. latifolium L.	3.0	15.2	tr	tr
L. perfoliatum L.	8.1	10.3	0.7	0.8
L. sativum L.	72.8	35.6	1.7	0.8
Malcomia africana (L.) R. Br.	8.3	25.1	2.1	7.5
Matthiola incana (L.) R. Br.	1.3	1.3	5.3	5.2
Neslia paniculata (L.) Desv.	3.0	8.1	1.8	4.7
Raphanus raphanistrum L.	177.4	16.7	10.2	0.9
R. sativus L. var. *sativus* L.	76.6	14.8	10.3	2.1
Rapistrum rugosum (L.) All.	24.3	3.6	6.9	1.0
Rorippa palustris (L.) Bess.	0.3	5.0	0.3	5.3
R. sylvestris (L.) Bess.	0.8	11.5	0.2	2.7
Sinapis alba L.	176.1	32.1	8.6	1.6
S. arvensis L.	281.3	39.6	36.2	5.1
Sisymbrium altissimum L.	3.5	15.8	0.5	2.8
S. austriacum Jacq.	2.4	11.2	0.3	1 4
S. officinale (L.) Scop.‡	3.1	16.1	3.8	19.4
S. strictissimum L.	5.6	13.5	0.2	0.5
Turritis glabra L.	1.1	17.0	0.3	4.4

*Trace.

†Inhibition of reaction velocity.

‡High deviation from the average K_m value.

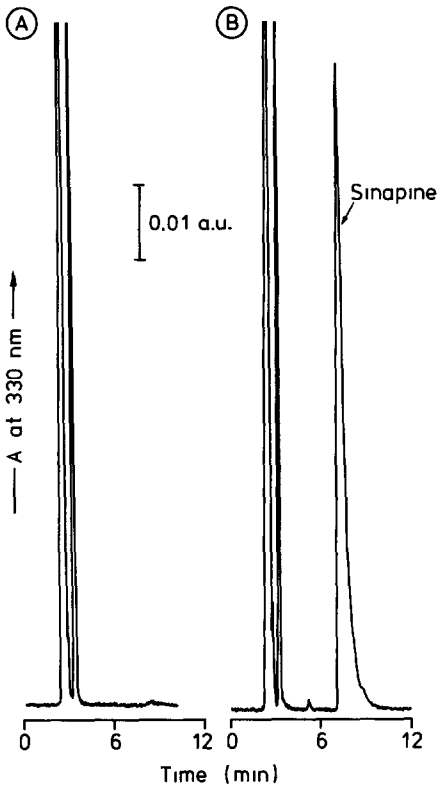

Fig. 2. HPLC analysis of SCT assays after 2 hr of incubation (A without and B with 25 mM choline chloride). Twenty μl of a standard assay was injected onto the column (RP-8) developed isocratically using 30% acetonitrile, 15% glacial acetic acid, 1% *ortho*-phosphoric acid and 0.05% sodium dodecyl sulphate in water at a flow rate of 1 ml/min. Elution was in the order 1-sinapoylglucose, sinapic acid, sinapine. The appearance of free sinapic acid is due to contaminating esterase activities. Enzyme came from seeds of *Brassica oleracea* convar. *capitata* var. *capitata* f. *alba* and was incubated with 0.35 mM 1-sinapoylglucose.

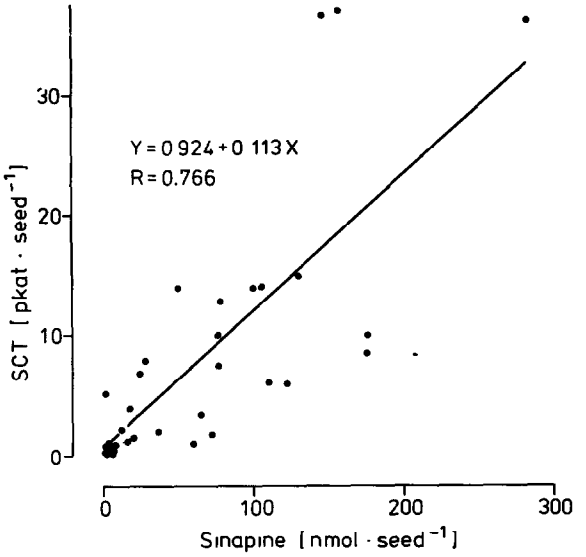

Fig. 3. Relationship between seed sinapine content and extractable SCT activity. Data from Table 1. Seed sinapine content was regressed on SCT activity (n = 39; *Berteroa, L. densiflorum, L. latifolium, Erysimum* and *S. officinale* were excluded).

changing from a dark-blue to a bright-green fluorescence when treated with ammonia vapour) and in daylight by the orange colour obtained with Dragendorff's spray reagent [17].

We investigated 77 species and cultivars of members of the Brassicaceae and from various other taxonomic groups of Angiospermae families. Table 1 lists the 44 plants which showed extractable SCT activity and occurrence of sinapine in the seeds from members of the Brassicaceae. No activity was detected in seeds from plants (not listed) which do not accumulate sinapine.

The overall highest enzymatic activities were found in seeds from *Brassica, Raphanus* and *Sinapis*, i.e. genera which accumulate large amounts of sinapine. In contrast, there were low activities in seeds which contain small amounts of sinapine (Table 1). Despite the fact that the seeds came from different sources and did not ripen under controlled growing conditions of the plants, the relationship of extractable level of SCT activities and the *in situ* amounts of sinapine were found to be positively correlated (Fig. 3). Also the SCT activities in members of the Brassicaceae obviously show similar kinetics, as found for *R. sativus*. Thus a rough correlation between seed sinapine content and SCT activity was possible. Linear least-squares fitting gave a coefficient of correlation of $R = 0.766$ (Fig. 3).

Concerning the correlation between SCT activities and sinapine levels, the specific activities (per mg seed protein) obtained in this screening are of limited value, because the Brassicaceae show such a wide range in the amount of protein stored in aleurone bodies [18]. For example, a relatively high specific SCT activity (301 pkat/mg protein) was found in seeds of *Matthiola incana* which exhibit a rather low sinapine content. On the other hand, we found a relatively low specific activity (31 pkat/mg protein) in seeds of *B. oleracea acephala gongylodes* which show a high sinapine content, but on the basis of the individual seed and seed weight also a high SCT activity. Comparing the data obtained from the latter plant with those from *B. oleracea botrytis botrytis* which showed 208 pkat SCT activity per mg protein, this becomes even more evident.

All enzyme preparations were tested for the presence of possible inhibitors by mixing experiments with the *R. sativus* enzyme preparation. Inhibiting interference with enzyme activities were only found with *Erysimum* × *allionii*, which showed strong enzyme inhibition, and *Sisymbrium officinale*, which exhibited an extremely high K_m value for 1-sinapoylglucose, compared to the other plants investigated. K_m values (41 plants) for 1-sinapoylglucose at 25 mM choline gave an average of 0.79 ± 0.56 mM.

Besides the typical seed constituent sinapine, there are large amounts of choline esters of other phenolic acids found in the Brassicaceae, e.g. feruloyl- and isoferuloylcholine [19, 20] and hydroxybenzoic acid choline esters [21, 22]. It would be of interest to study their biosynthesis to see if, as in sinapine formation, 1-*O*-acyl glucosides are used as activated substrates.

In summary, the transacylase activity described in this paper seems to be widespread among members of the

Brassicaceae. We suggest that the enzyme is specifically involved in the synthesis of sinapine. This report documents the significance of this new type of transferase which uses an energy-rich 1-*O*-acyl glucoside as the acyl donor in the synthesis of a cinnamic acid ester.

EXPERIMENTAL

Plant sources and biochemicals. Seeds from the plants investigated were provided by the botanic gardens of our institute, Sektion Biowissenschaften der Karl-Marx-Universität, Leipzig, and Martin-Luther-Universität, Halle, G.D.R. The seeds from our institute and most of those from the other two institutes were identified by H. Zimmer. Sinapoylcholine (sinapine) and 1-*O*-sinapoyl-β-D-glucose were isolated from seeds and cotyledons, respectively, of red radish (*Raphanus sativus* L. var. *sativus*) [23]. Choline chloride was obtained from Fluka, Neu-Ulm, F.R.G. Other chemicals were from Merck, Darmstadt, F.R.G.; Serva, Heidelberg, F.R.G.; or Pharmacia, Uppsala, Sweden.

Extraction and quantification of sinapine. Twenty-five seeds (or at least 100 mg) were homogenized (Ultra-Turrax homogenizer) in 7 ml MeOH for *ca* 4 min. Then the homogenizer was rinsed with another 3 ml MeOH which was added to the extract. The homogenate was allowed to stand for *ca* 1 hr and was then centrifuged at 3000 *g* for 15 min. The supernatant was analysed by HPLC as described in Fig. 2. The identity of sinapine was proved by co-chromatography [3] and by the colour reaction obtained with Dragendorff's spray reagent [17].

Enzyme preparation. Thirty seeds (or at least 200 mg) were frozen with liquid N_2 and immediately ground in a mortar together with 0.5 g quartz sand, 0.2 g dry insoluble Polyclar AT, and 7 ml 0.1 M KPi buffer, pH 7.0. The homogenate was transferred to a glass tube and allowed to stand with continuous stirring for 1 hr at 4° and subsequently filtered through Miracloth into a centrifuge tube. After centrifugation at 48 000 *g* for 15 min and filtration of the supernatant through Miracloth, the vol. of the crude extract was measured. The enzymatic activity was prepared from the 30–70% $(NH_4)_2SO_4$ precipitable fraction followed by chromatography on Sephadex G-25 (Pharmacia PD-10 columns). Protein was determined according to Bradford [24], using bovine serum albumin as the standard.

Enzyme assay and activity determination. The standard reaction mixture contained, in a total vol. of 100 μl: 30 μl 0.2 M KPi buffer, pH 7.0; 10 μl protein extract; 10 μl 250 mM choline chloride; and 5–50 μl 1-sinapoylglucose (0.2–2.0 mM in H_2O). The reaction was started by the introduction of choline chloride. After incubation at 30° for 2 hr, the reaction was stopped by transferring the mixture to a freezer (−20°). The enzymatic activity was analysed by HPLC (Fig. 2), using sinapine as the standard. The V_{max} and K_m values were calculated from Lineweaver–Burk plots ($n = 6$).

Acknowledgement—This work was financially supported by the Deutsche Forschungsgemeinschaft (Str 170/5-2).

REFERENCES

1. Zenk, M. H. (1979) in *Recent Advances in Phytochemistry, Volume* 12; *Biochemistry of Plant Phenolics* (Swain, T., Harborne, J. B. and Van Sumere, C. F., eds.), pp. 139–176. Plenum, New York.
2. Gross, G. G. (1981) in *The Biochemistry of Plants, Volume* 7; *Secondary Plant Products* (Conn, E. E., ed.), pp. 301–316. Academic Press, New York.
3. Strack, D., Knogge, W. and Dahlbender, B. (1983) *Z. Naturforsch. Teil C* **38**, 21.
4. Tkotz, N. and Strack, D. (1980) *Z. Naturforsch. Teil C* **35**, 835.
5. Strack, D. (1982) *Planta* **155**, 31.
6. Dahlbender, B. and Strack, D. (1984) *J. Plant Physiol.* **116**, 375.
7. Strack, D. (1980) *Z. Naturforsch. Teil C* **35**, 204.
8. Nurmann, G. and Strack, D. (1981) *Z. Pflanzenphysiol.* **102**, 11.
9. Gross, G. G. (1982) *FEBS Letters* **148**, 67.
10. Gross, G. G. (1983) *Z. Naturforsch. Teil C* **38**, 519.
11. Michalczuk, L. and Bandurski, R. S. (1980) *Biochem. Biophys. Res. Commun.* **93**, 588.
12. Herrmann, K. (1978) *Fortschr. Chem. Org. Naturst.* **35**, 73.
13. Gadamer, J. (1896) *Arch. Pharm.* **235**, 44.
14. Schultz, O. E. and Gmelin, R. (1952) *Z. Naturforsch. Teil B* **7**, 500.
15. Schultz, O. E. and Gmelin, R. (1953) *Z. Naturforsch. Teil B* **8**, 151.
16. Kerber, E. and Buchloh, G. (1980) *Angew. Bot.* **54**, 47.
17. Munier, R. and Macheboeuf, R. S. (1980) *Biochem. Biophys. Res. Commun.* **93**, 588.
18. Werker, E. and Vaughn, J. G. (1974) *Planta* **116**, 243.
19. Pagani, F. and Romussi, G. (1979) *Farm. Ed. Sci.* **24**, 257.
20. Gmelin, R. and Kjaer, A. (1970) *Phytochemistry* **9**, 667.
21. Clausen, S., Olsen, O. and Sørensen, H. (1982) *Phytochemistry* **21**, 917.
22. Gmelin, R. and Möhrle, H. (1967) *Arch. Pharm.* **300**, 176.
23. Linscheid, M., Wendisch, D. and Strack, D. (1980) *Z. Naturforsch. Teil C* **35**, 907.
24. Bradford, M. M. (1976) *Analyt Biochem.* **72**, 248.