Pergamon

0031-9422(95)00274

Phytochemistry, Vol. 40, No. 3, pp. 657–660, 1995

0031-9422/95 $9.50 + 0.00

REGULATION OF ACYL–CoA OXIDASES IN MAIZE SEEDLINGS

MARK A. HOOKS,* KORNÉLIA BODE and IVAN COUÉE

Station de Physiologie Végétale, Centre de Recherches de Bordeaux, Institut National de la Recherche Agronomique, BP 81, 33883 Villenave d'Ornon Cedex, France

(*Received in revised form* 9 *February* 1995)

Key Word Index—*Zea mays*; Gramineae; maize; peroxisomes; β-oxidation; regulation; acyl-CoA-oxidase; carbohydrate.

Abstract—Because of the importance of acyl-CoA oxidases in controlling peroxisomal β-oxidation in higher plants, it is necessary to determine their activities and the factors regulating their expression. Acyl-CoA oxidase activities were determined in extracts from different tissues of dark-grown maize plantlets, and in peroxisomes isolated from normal, carbohydrate-starved and carbohydrate-fed maize root tips. Acyl-CoA oxidases exhibited differential and coordinated expression depending on tissue type, tissue development and cellular carbohydrate status.

INTRODUCTION

The natural development and subsistence of a higher plant probably requires catabolism of fatty acids, since β-oxidation is a constitutive property of higher plant tissues [1]. This catabolism proceeds primarily by peroxisomal β-oxidation to produce acetyl-CoA and ultimately organic acids to feed the tricarboxylic acid cycle [2]. β-Oxidation has been best characterized in cotyledon glyoxysomes during seed germination and early growth [3, 4], where enzyme activities of this pathway increase dramatically to provide carbon and energy to the growing seedling [5].

In plant peroxisomes, the first step of peroxisomal β-oxidation is catalysed by a family of acyl-CoA oxidases (ACOX, EC 1.3.3.6) comprised of three enzymes that show different specificities for acyl-CoA chain-length [6]. The long-chain ACOX (LCOX) was first purified from cucumber cotyledon glyoxysomes [7]. The short- (SCOX) and medium-chain (MCOX) ACOXs have been recently purified from maize [6]. A distinct maize LCOX was also identified and shown to have similar substrate specificity to that from cucumber [6].

ACOX has been postulated to be the enzymatic step primarily controlling the flux through peroxisomal β-oxidation in animals [8, 9] and plants [2, 10]. The flux through β-oxidation could be controlled in a global manner by a coordinate expression or repression of ACOXs. Additionally, the presence of three different ACOXs provides a mechanism for differential regulation, whereby the flux through β-oxidation would be dictated by the relationship between acyl-CoA oxidase expression and availability of a particular class of acyl-CoA ester (i.e. short-, medium- or long-chain). By analysing their activities in extracts of different tissues of germinating maize, and in isolated peroxisomes from carbohydrate-fed and carbohydrate-starved maize root tips, we determined that ACOXs are subjected to both types of regulation. This implies that the regulation of peroxisomal β-oxidation during plant development is of general importance in controlling the concentrations of the substrates and products of β-oxidation.

RESULTS AND DISCUSSION

In order to determine whether ACOXs are expressed in plants differentially, coordinately or both, we determined the activities of the three forms in plant tissues of differing developmental state and carbohydrate status. The data presented in Table 1 shows ACOX activity in three different tissues of dark-grown maize plantlets. The substrates, C_6-, C_{12}- and C_{16}-CoA, represent the activities of SCOX, MCOX, and LCOX, respectively [6]. In each tissue, ACOX activities was greatest with C_6-CoA, followed in order by that with C_{12}- and C_{16}-CoA, although ACOX activity with C_{12}-CoA and C_{16}-CoA were essentially similar in roots. It is apparent that the relative ACOX activities with the three substrates are not similar in all tissue types, indicating a dependence of flux on the relative concentrations of each ACOX and the available class of acyl-CoAs. For example, the flux of enoyl-CoAs, the reaction products of ACOXs, into the subsequent steps of β-oxidation appears to be directed to a more equal use of medium- and long-chain acyl-CoAs in roots

*Author to whom correspondence should be addressed.

Table 1. ACOX activities (nkat g fr. wt $^{-1}$) in protein extracts of different tissues of dark-grown maize plantlets*

Tissue	Substrate		
	C_6-CoA	C_{12}-CoA	C_{16}-CoA
Root (2 cm)	2.10 ± 0.58	1.00 ± 0.33	1.15 ± 0.37
Coleoptile	1.89 ± 0.11	0.89 ± 0.33	0.48 ± 0.00
Scutellum	3.27 ± 0.02	2.39 ± 0.03	1.51 ± 0.33

*Mean ± s.d. of two measurements performed on two or more independent tissue extracts.

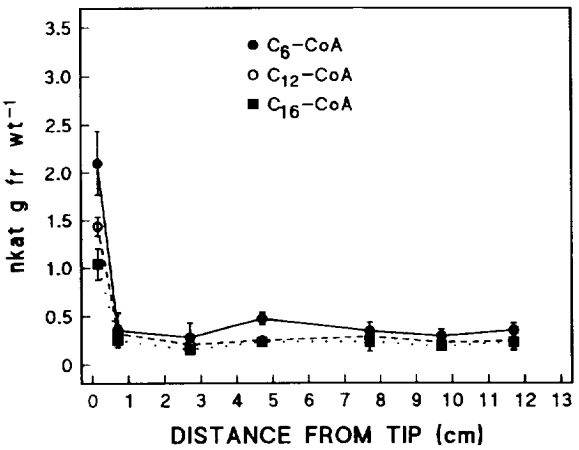

Fig. 1. ACOX activities in protein extracts of maize root segments. Each point represents the mean ± s.d. of single measurements performed on protein extracts of three independent groups of segments.

when compared to coleoptiles, and a greater use of short- than long-chain acyl-CoAs in coleoptiles, when compared to roots and scutella. Our results on ACOX activity in scutella differ from those of Olsen and Huang [11], who observed a higher activity for C_{16}-CoA than for C_{12}-CoA in glyoxysomes of maize scutella. This difference may result from a difference in developmental and metabolic states between scutella from 10 hr-imbibed seeds and those from seeds they first allowed to germinate for 3 days. This does not appear to be due to a technical difference in measuring activities in protein extracts compared to peroxisomes, since peroxisomes isolated from scutella of 10 hr-imbibed seeds showed the same relative ACOX activities with the three substrates as those presented in Table 1.

The seminal root itself exhibits differential development throughout its length, where the tip is composed of actively dividing meristematic cells; the more mature portion contains differentiated cells of lower respiratory metabolism [12]. A relationship between ACOX expression and tissue development, and possibly metabolic state, becomes apparent from the differences in ACOX activities among the root meristem and mature portions (Fig. 1). ACOX activity with all three acyl-CoAs was greater in the tip than in the other segments, with that for C_6-CoA being more than four-fold higher, thus indicating a global change in SCOX, MCOX, and LCOX activity levels from the tip to the mature portions. The difference in activities between the tip and the mature segments may be slightly exaggerated due to the relative facility of extracting protein from the tip compared to the more fibrous mature segments. In addition, the differences in global and relative ACOX activities between roots shown in Fig. 1 and Table 1 may reflect the differences in metabolic state of corresponding regions of roots of different length [13].

In certain tissues of maize plantlets, having SCOX and MCOX activities greater than that of LCOX would be advantageous. High SCOX and MCOX activities would ensure that any short- or medium-chain acyl-CoA esters formed would be completely degraded to acetyl-CoA. This would serve the purposes of providing an additional source of energy and carbon and removing potentially toxic fatty acids. A high production of fatty acids or acyl-CoAs is likely to arise from the rapid turnover of membrane lipids, similarly to that characterized for rapidly dividing animal cells [14]. Fatty acids, particularly short-chain fatty acids, have been shown to have a number of detrimental effects on cells, such as halting proliferation [15] and decreasing the mitotic index [16] of meristem cells. It would not be unreasonable to expect more mature, less metabolically active tissues, to have similar activity levels for the three ACOXs. This may be the case for long maize roots (Fig. 1). The necessity for having a relatively high level of β-oxidation in the more mature tissues, for example in the root, is not immediately clear, though a certain basal level could be expected to support membrane lipid turnover.

Analysis of ACOX activities in peroxisomes isolated from maize root tips demonstrated that ACOX expression was also sensitive to the cellular carbohydrate status in both a coordinate and differential manner (Table 2). Peroxisomes, isolated from excised maize root tips incubated 24 hr without an exogenous carbohydrate supply, exhibited a five- to ten-fold increase in ACOX activity with C_6-, C_{12}- and C_{16}-CoA, relative to that determined in peroxisomes isolated from freshly excised root tips. This higher global level relates to a general increase in β-oxidation to degrade cellular lipids as a source of respiratory carbon for cell survival during carbohydrate starvation of root tips [17, 18]. In contrast, peroxisomes isolated from root tips incubated with exogenous glucose showed a decrease in ACOX activity with C_6-CoA, while that with C_{12}-, and C_{16}-CoA did not change. Though some root growth (*ca* 3 mm) occurred in root tips incubated with glucose, our calculations indicate that the decrease in activity with C_6-CoA, resulting from tissue maturation and differentiation, could account for only a minor portion of this decrease (see Fig. 1). The activities in peroxisomes from carbohydrate-fed root tips probably represent the basal levels necessary to support lipid turnover and avoid fatty acid toxification of cells.

In conclusion, we have shown that maize ACOX levels are tissue-specific, and developmentally and metaboli-

Table 2. Carbohydrate-dependent ACOX activities (nkat g fr. wt^{-1}) in peroxisomes from maize root tips*

Treatment	Substrate		
	C_6-CoA	C_{12}-CoA	C_{16}-CoA
Normal	0.90 ± 0.20	0.35 ± 0.09	0.33 ± 0.06
Starved	4.80 ± 0.30	3.50 ± 0.40	2.30 ± 0.20
Glucose	0.56 ± 0.06	0.41 ± 0.03	0.35 ± 0.00

* Mean ± s.d. of two measurements performed on two or more independent peroxisome isolations.

cally regulated. This regulation can involve either a coordinated or differential expression of the different ACOXs. These results, in combination with the current understanding of the regulatory properties of ACOXs [2, 8, 9, 10], indicate peroxisomal β-oxidation itself to be highly regulated in order to control the levels of substrates and products of this pathway. Such substrates and products requiring strict regulation of cellular concentrations are free fatty acids as previously mentioned, acetyl-CoA, which is proposed to regulate the transcription of photosynthetic genes [19], and fatty acyl-CoA esters themselves, which serve to regulate enzyme activity via covalent modification [20].

EXPERIMENTAL

Preparation of plant material. Maize seeds (*Zea mays* L., cv. DEA; Pioneer France Maïs, France) were soaked in flowing tap H_2O for 3 hr, and layered between sheets of wet Whatman 3 MM filter paper satd with a mineral nutrient soln [21]. Seeds were germinated at 25° in the dark. Groups of 10–15 roots of at least 15 cm in length were obtained according to the method of Ref. [22]. Root segments, corresponding to the first 3 mm and every 10 mm thereafter, were excised, and the corresponding segments were combined and extracted. Seeds used for the scutellum extracts were imbibed for 10 hr in flowing tap H_2O. Maize root tips (3 mm) used for the incubation treatments were excised from 3-day-old germinated seedlings (root length, 3–5 cm) and incubated for 24 hr in a buffered antibiotic–nutrient soln, supplemented or not with 200 mM glucose, and continuously bubbled with O_2–N_2 (1:1) [17].

Protein extraction. Tissue samples were homogenized by Polytron in either 1 ml (expts of Fig. 1) or 2 ml (expts of Table 1) of 150 mM Tris-HCl, pH 7.5, 10% (v/v) glycerol, 10 μM FAD, 1 mM β-mercaptoethanol, 1 mM EDTA, 0.1 mM phenylmethylsulphonyl fluoride and 1% insol. PVPP. The extracts were clarified by centrifugation at 27 000 *g* for 15 min and subsequently by filtration on one layer of Miracloth (Calbiochem). The extracts were placed on ice and then fractionated sequentially between 0–40 and 40–50% satd $(NH_4)_2SO_4$. Underestimation of C_{16}-CoA oxidase activity in extracts was minimized by selective sepn of LCOX and long-chain acyl-CoA thioesterase activities, which pptd, respectively, below and above 40% satd $(NH_4)_2SO_4$. No ACOX activity was found to ppt. above 50% satd $(NH_4)_2SO_4$. For each fractionation step, $(NH_4)_2SO_4$ was added over a period of 30 min and stirred for another 30 min. The ppts were resuspended in 20 mM phosphate buffer, pH 7.5, containing 10% (v/v) glycerol and 10 μM FAD, and assayed directly for ACOX activity. ACOX activities determined for each tissue and for seminal root segments represent the sum of activities of the $(NH_4)_2SO_4$ frs.

Peroxisome isolation. Frs enriched in peroxisomes were isolated by centrifugation on one-step sucrose gradients of 35% and 60% [17].

Enzyme assays. ACOX was assayed by the peroxidase-coupled reaction using *p*-hydroxybenzoic acid as the chromophoric peroxidase substrate [23]. All assays were performed at 50 μM acyl-CoA, which was sufficient for maximal ACOX activity in our assay (Hooks, M.A. and Couée, I., unpublished results). Triton X-100 was included at 0.01% (w/v) in the ACOX assays of peroxisomal frs.

Protein. Protein content was determined by the method of Ref. [24].

REFERENCES

1. Gerhardt, B. (1985) *Phytochemistry* **24**, 351.
2. Kindl, H. (1987) in *The Biochemistry of Plants* (Vol. 9) (Stumpf, P. K. ed.), p. 31.
3. Cooper, T. G. and Beevers, H. (1969a) *J. Biol. Chem.* **244**, 3507.
4. Cooper, T. G. and Beevers, H. (1969b) *J. Biol. Chem.* **244**, 3514.
5. Miernyk, J. A. and Trelease, R. N. (1981) *Plant Physiol.* **67**, 341.
6. Hooks, M. A., Couée, I., Bode, K. and Raymond, P. (1994) *Plant Physiol.* **105**, s710.
7. Kirsch, T., Löffler, H.-G. and Kindl, H. (1986) *J. Biol. Chem.* **261**, 8570.
8. Reubsaet, F. A. G., Veerkamp, J. H., Bukkens, S. G. F., Trijbels, J. M. F. and Monnens, L. A. H. (1988) *Biochim. Biophys. Acta* **958**, 434.
9. Aoyama, T., Souri, M., Kamijo, T., Ushikubo, S. and Hashimoto, T. (1994) *Biochem. Biophys. Res. Commun.* **201**, 1541.
10. Holtman, W. L., Heister, J. C., Mattern, K. A., Bakhuizen, R. and Douma, A. C. (1994) *Plant Sci.* **99**, 43.
11. Olsen, J. A. and Huang, A. H. C. (1988) *Phytochemistry* **27**, 1601.
12. Bingham, I. J. and Stevenson, E. A. (1993) *Physiol. Plant.* **88**, 149.
13. Rost, T. L. and Baum, S. (1988) *Am. J. Bot.* **75**, 414.
14. Van den Bosch, H. (1980) *Biochim. Biophys. Acta* **604**, 191.
15. Lanzagorta, A., de la Torre, J. M. and Aller, P. (1988) *Physiol. Plant.* **72**, 775.
16. Tramontano, W. H., Gelardi, P. and Jouve, D. (1994) *Phytochemistry* **36**, 19.

17. Dieuaide, M., Brouquisse, R., Pradet, A. and Raymond, P. (1992) *Plant Physiol.* **99**, 595.
18. Dieuaide, M., Couée, I., Pradet, A. and Raymond, P. (1993) *Biochem. J.* **296**, 199.
19. Sheen, J. (1990) *Plant Cell* **2**, 1027.
20. Towler, D. A., Gordon, J. I., Adams, S. P. and Glaser, L. (1988) *Ann. Rev. Biochem.* **57**, 69.
21. Saglio, P. and Pradet, A. (1980) *Plant Physiol.* **66**, 516.
22. Roberts, J. K. M. and Testa, P. (1988) *Plant Physiol.* **86**, 1127.
23. Hryb, D. J. and Hogg, J. F. (1979) *Biochem. Biophys. Res. Commun.* **87**, 1200.
24. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248.