Phytochemistry, Vol. 27, No. 6, pp. 1601–1603, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00

GLYOXYSOMAL ACYL-CoA SYNTHETASE AND OXIDASE FROM GERMINATING ELM, RAPE AND MAIZE SEED

JULIE A. OLSEN and ANTHONY H. C. HUANG*

Chemistry Department, Wabash College, Crawfordsville, Indiana, U.S.A.; *Biology Department, University of South Carolina, Columbia, South Carolina, U.S.A.

(*Revised received* 5 *October* 1987)

Key Word Index—*Ulmus Americana*; elm; *Brassica napus*; rape; *Zea Mays*; maize glyoxysome; β-oxidation; acyl-CoA synthetase; acyl-CoA oxidase.

Abstract—The activities of acyl-CoA synthetase (EC 6.2.1.3; 3) and acyl-CoA oxidase (EC 1.3.99.3; 4) in the glyoxysomes from germinating elm, maize and rape seeds were determined. Apparent K_m and V_{max} values for C_{10}–C_{22} fatty acids were calculated. Unlike the seed lipases reported earlier, there is no correlation between the chain length of stored fatty acids and of the optimal substrates for the two glyoxysomal enzymes.

INTRODUCTION

The utilization of stored oils in germinating seeds begins in the lipid bodies with the degradation of triacylglycerols by lipases. Seed lipases have been shown to be specific for the types of fatty acids stored in the triacylglycerols of each particular plant species [1]. Further degradation of the free fatty acids to acetate occurs in the glyoxysomes [2, 3]. The initial enzymatic steps in this degradative pathway involve the formation of an acyl-CoA using an acyl-CoA synthetase [4] followed by an acyl-CoA oxidase catalysed oxidation to produce an enoyl-CoA [2]. The substrate specificity of these two enzymes with reference to the fatty acid chain length has only been determined for a few plant species. Castor bean glyoxysomes contain both an acetate thiokinase and an acyl-CoA synthetase. The latter synthetase is capable of producing CoA-fatty acids from palmitic, oleic and ricinoleic acids, but is relatively inactive for fatty acids of less than ten carbons [4]. The acyl-CoA oxidase in castor bean glyoxysomes is able to oxidize acyl-CoA's of C_4–C_{18}, and in cucumber cotyledons it also oxidizes C_{20} and C_{22} acyl-CoA's [5]. The oxidase has also been found at low activities in peroxisomes of spinach leaves and mung bean hypocotyl, where, based on V_{max} values, activity occurs with C_{10}–C_{18} acyl-CoA's, optimizing at C_{14} acyl-CoA's in both systems [6].

The purpose of this research was to answer the following question: Are the glyoxysomal acyl-CoA synthetase and β-oxidation pathways of individual plant species tailored to handle the fatty acids stored by these species, as is the lipase? If these pathways are selective for the stored fatty acids, then the free fatty acids, generated by lipase activity associated with the lipid bodies, can be moved directly into the glyoxysomes for degradation.

RESULTS AND DISCUSSION

Three plant species with distinctly different storage triacylglycerols were selected. Elm seed stores predominantly very short chain fatty acids, maize kernel primarily C_{16} and C_{18} fatty acids, and rape seed C_{18}, C_{20} and C_{22} fatty acids in the triacylglycerols (Table 1).

We have examined the ability of glyoxysomes isolated from germinating seed of each of the three species to metabolize fatty acids of varying chain length. Fig. 1 and Table 2 show the apparent V_{max} and K_m values respectively for glyoxysomal acyl-CoA synthetase. This enzyme activates fatty acids to form acyl-CoA's for subsequent degradation via the glyoxysomal β-oxidation sequence. The assay used couples the reaction to the β-oxidation sequence and monitors the resulting reduction of NAD. The assumption was made that the synthetase reaction is

Table 1. Percentage composition of seed oils

	Fatty acid										
Source	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:1	22:1
Elm	61.3	5.9	4.6	2.9			11.0	9.0			
Rape (American)							17.6	13.7	9.0	3.0	50.8
Maize kernel					11.5	2.0	26.6	59.0	0.8		

Data compiled from ref. [7].

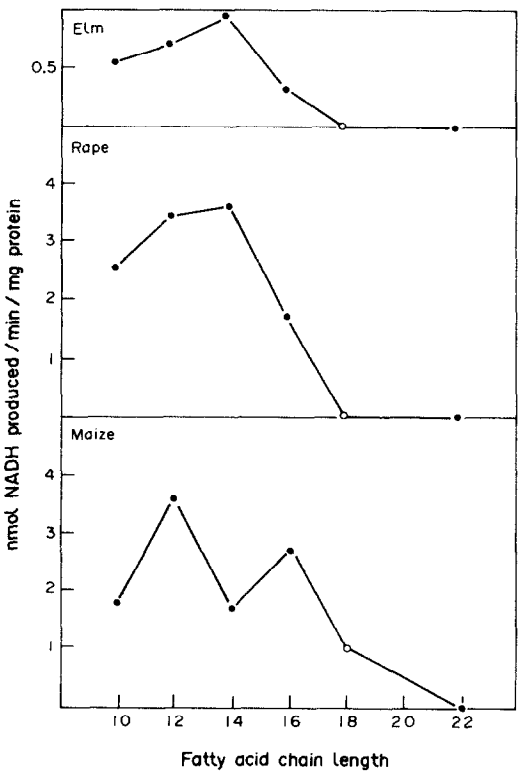

Fig. 1. V_{max} values for glyoxysomal acyl-CoA synthetase from elm, rape and maize seeds, calculated from activities detected at increasing fatty acid-concentrations. The fatty acids used were $C_{10:0}$(decanoic), $C_{12:0}$(lauric), $C_{14:0}$(myristic), $C_{16:0}$(palmitic), $C_{18:0}$(stearic, S), $C_{18:1}$(oleic, 0), $C_{22:1}$(erucic).

Table 2. Apparent K_m values for glyoxysomal acyl-CoA synthetase and oxidase

Fatty acid	K_m(μM) Acyl-CoA synthetase			Acyl-CoA oxidase		
	Elm	Rape	Maize	Elm	Rape	Maize
10:0	7.0	10.1	15.8	9.4	21.4	28.6
12:0	21.0	113.8	100.1	5.4	98.6	29.4
14:0	20.3	78.9	380.3	4.6	111.0	252.1
16:0	2.5	22.1	699.2	3.7	4.8	136.6
18:0	—	—	—	6.9	7.8	73.7
18:1	NA	NA	71.4	7.9	11.4	27.4
22:1	NA	NA	NA	3.8	7.1	22.4

NA, no activity.

the slowest step in the enzymatic series. The validity of the assumption was verified using acyl-CoA's as substrates for the assay and observing that the intial rates of NAD reduction were *ca* twice those of the measured synthetase activities.

By comparing the types of fatty acid stored by each species (Table 1) with the apparent $V_{max}$and K_m values of the synthetase for those fatty acids (Fig. 1 and Table 2), one can see that the enzyme from elm, which stores predominantly C_{10} fatty acid, shows preferential use of the C_{10} through C_{14} substrates for glyoxysomal acyl-CoA synthetase, and is able to react with the C_{16} fatty acid. Oleic acid, $C_{18:1}$, which is stored in appreciable amounts, supports no detectable activity.

Even more striking is the case of rape seed glyoxysomes. Rape seed contains high percentages of C_{18} and C_{22} fatty acids in the stored triacylglycerol (Table 1). However, its acyl-CoA synthetase shows no measurable activity in the *in vitro* assay with these fatty acids, acting most effectively on the smaller C_{10}–C_{16} fatty acids.

Maize kernel contains an acyl-CoA synthetase with preference for fatty acids smaller than the various C_{18} fatty acids it stores. The apparent jog in the V_{max} plot going from C_{12} to C_{16} is reproducible, the C_{14} substrate demonstrates inhibition whereas the C_{16} fatty acid was not saturated under the assay conditions. The enzyme is able to accommodate the $C_{18:1}$ fatty acid; however, it too shows no apparent activity with $C_{22:1}$.

The observed synthetase activities toward different fatty acids do not appear to be the direct consequence of their solubilities for the following reasons: (i) at working concentrations of the fatty acids, there were no appreciable solubility problems. (ii) The observed enzymatic activities do not mirror the relative solubility of the various fatty acids tested, but show classic kinetic behaviour. (iii) The enzymes from the three different species gave different patterns of activities toward the fatty acids.

The activity of the first enzyme in the β-oxidation sequence, acyl-CoA oxidase, towards C_{10}–C_{22} acyl-CoA's was also evaluated, to see if there was a similar specificity for the shorter chain fatty acids or whether the longer acyl-CoA's could enter into the β-oxidation sequence at this point. The oxidase activity was assayed by coupling the enzymatic product, hydrogen peroxide, to a fast peroxidase colour reaction. Fig. 2 and Table 2 show the apparent V_{max} and K_m values for each acyl-CoA tested. The oxidase reactions were in all cases one to two orders of magnitude faster than the synthetase reaction.

In the case of the elm seed enzyme, there is preference for the C_{10} substrate, with low constant activity for the remaining series of substrates, including the $C_{22:1}$, erucic acid. The rape seed glyoxysomal acyl-CoA oxidase shows a strong preference for C_{12} and C_{14} fatty acids, with a residual activity for C_{16}, C_{18} and C_{22} fatty acids. The maize enzyme is best able to act on C_{14} and C_{16} fatty acids, but it can utilize both the longer and smaller chains to a minimal degree.

The observed oxidase activities do not appear to mirror the critical micellular concentrations for the acyl-CoA's tested, and there is considerable variation in the behaviour of the acyl-CoA oxidases with substrates of varying acyl-chain length for the three species.

In general, if the glyoxysomes of the three plant species were supplied with long chain acyl-CoA's, they could metabolize them via β-oxidation. However, these longer chain fatty acids appear to be poor substrates for the glyoxysomal acyl-CoA synthetase in the *in vitro* assay.

This study shows that in contrast to the lipases found in the plant species, the initial enzymes of fatty acid metabolism in the glyoxysomes, with the exception of the elm synthetase, do not show a preference for the respective major storage fatty acids, neither do these enzymes show the same substrate specificity in the three species tested.

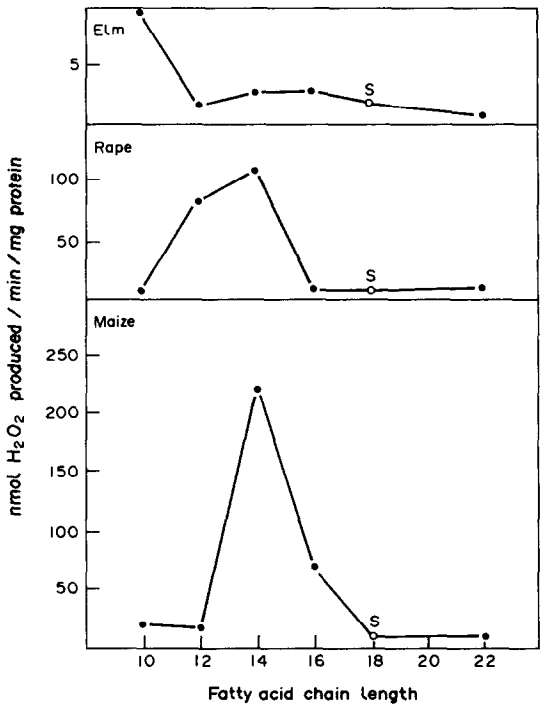

Fig. 2. V_{max} values for glyoxysomal acyl-CoA oxidase from elm, rape and maize seeds, calculated from activities detected at increasing acyl-CoA concentrations. See Fig. 1 for fatty acids used.

EXPERIMENTAL

Fatty acids and acyl-CoA's were purchased from Sigma. Elm seed (*Ulmus Americana*) was collected on the campus of the University of South Carolina. Rape seed (*Brassica napus* L. cv Dwarf Essex) was purchased from a local source. Inbred maize kernel (*Zea mays* MO-17) was obtained from the Illinois Foundation Seed Corporation, Champaign, IL.

The seeds, after soaking for 24 hr, were allowed to germinate in the dark at 26° on moist paper. The glyoxysomes were isolated from 3- to 4-day-old maize scutella, and elm and rape cotyledons at 0–4° as follows. Isolated scutella or cotyledons were rinsed twice in dist. H_2O, and placed in a grinding buffer containing 0.6 M sucrose, 1 mM EDTA, 10 mM KCl, 1 mM $MgCl_2$ 0.15 M Tricine, 2 mM DTT adjusted to pH 7.5 with KOH. After incubation for 10–15 min, the sample was chopped with a new razor blade to form a fairly thick slurry. This slurry was transferred to a mortar and ground very gently. After filtration through Nitrex cloth (Retko, Elmsford, NY) 20 $\times$ 20 μm^2 pore size, the filtrate was subjected to centrifugation of 1500 g for 10 min. The supernate was transferred onto linear sucrose gradients of 26 ml 60% (w/w) to 30%, and the gradients contained 1 mM NaEDTA, pH 7.5. The gradients were centrifuged for 4 hr at 21 000 rpm in a Beckman Rotor SW 28. The glyoxysomal fraction was identified both visually and by the presence of catalase activity. Protein was assayed by the Bradford technique [8].

Acyl-CoA synthetase was assayed spectrophotometrically by coupling into the β-oxidation sequence present in the glyoxysomes and observing the resulting reduction of NAD [2, 4]. The reaction mixture contained 5.5 mM $MgCl_2$, 2.8 mM DTT, 2.8 $\times 10^{-4}$ M CoA-SH, 4.1×10^{-4} M NAD, 3.5 mg/ml BSA, 2.10^{-3} M ATP, in 20 mM KPO_4, pH 7.5. Free fatty acids were dissolved in 20 μl of 95% EtOH, and then diluted with 1 ml of buffer to give stock solns of 2 mM fatty acids. Reactions were initiated by the addition of the glyoxysomal preparations containing about 100 μg of protein, and were monitored at 340 nm. In calculating enzymatic activity an extinction coefficient of 6.2 cm^2/μmol of NADH was used.

Acyl-CoA oxidase was assayed spectrophotometrically by coupling the H_2O_2 product to a peroxidase colour reaction [9]. The reaction mixture contained 13 mM *p*-hydroxybenzoic acid, 50 μM FAD, 1 mM 4-aminoantipyrine, 1 mM NaN_3, 6 units horseradish peroxidase (Sigma), and 175 mM Tris–HCl, pH 8.5. Acyl-CoA concentrations were varied through the micromolar range. The reaction was initiated by addition of the glyoxysomal preparation containing approximately 100 μg of protein and was monitored at 500 nm. An extinction coefficient of 5.1 cm^2/μmol was used to calculate product formation.

Apparent V_{max} and K_m values were taken from computer generated Lineweaver–Burk plots, except in case of the elm synthetase where, due to substrate inhibition, values were determined directly from the plots of rate vs substrate concentration. The V_{max} values for the acyl-CoA synthetase gave an average standard deviation (expressed as a percent of the mean) of 31% for the rape and corn enzymes and 15% for the elm enzyme. The V_{max} values for the acyl-CoA oxidase showed an average standard deviation of 30% for the rape enzyme and 20% for both the corn and elm enzymes.

Acknowledgement—The work was supported by a grant from the National Science Foundation to AHCH and by the assistance of Wabash College to JAO.

REFERENCES

1. Lin, Y. H., Yu, C. and Huang, A. H. C. (1986) *Arch. Biochem. Biophys.* **244**, 346.
2. Cooper, T. G. and Beevers, H. (1969) *J. Biol. Chem.* **244**, 3514.
3. Hutton, D. and Stumpf, P. K. (1969) *Arch. Biochem. Biophys.* **142**, 48.
4. Cooper, T. G. (1971) *J. Biol. Chem.* **246**, 3451.
5. Kirsch, T., Loffler, H. G., and Kindl, H. (1986) *J. Biol. Chem.* **261**, 8570.
6. Gerhardt, B. (1985) *Phytochemistry* **24**, 351.
7. Hilditch, T. P., and Williams, P. N. (1964) *Chemical Constitution of Natural Fats* 4th Edn. Wiley, New York.
8. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248.
9. Hyrb, D. J. and Hogg, J. F. (1979) *Biochem. Biophys. Res. Commun.* **87**, 1200.