SUBSTRATE SPECIFICITY OF COTTON GLYOXYSOMAL ENOYL-CoA HYDRATASE

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1. Introduction

The conversion of fatty acids to hexoses during postgerminative growth of oil seeds involves the coordinated activities of β-oxidation, glyoxylate cycle, and glycolytic enzymes [1]. The least-studied segment of this gluconeogenic pathway has been β-oxidation. We have described the development and subcellular localization of β-oxidation activity in cotyledons of cotton seeds [2]. A discrepancy noted between the in vitro enzyme activities and the in vivo rate of lipid mobilization [2] led us to examine the effects of acyl-chain length and pH on in vitro activities of the β-oxidation enzymes. This report describes the substrate specificity of cotton glyoxysomal enoyl-CoA hydratase (EC 4.2.1.17).

2. Materials and methods

Germination of cotton (*Gossypium hirsutum* L.) seeds and isolation of glyoxysomes from 48-h-old seedlings by zonal rotor, sucrose-density centrifugation have been described [2–4]. Glyoxysomes were subjected to osmotic shock [4], the membranous material sedimented, and the supernatant passed through Sephadex G-25 before assaying enzyme activity. Enoyl-CoA hydratase activity was measured as in [2]. A correction was made for acyl-CoA thioesterase activity [5] with each of the substrates tested. All kinetic data were analyzed according to [6], and lines were generated by linear regression of the data points.

Coenzyme A was purchased from PL Biochemicals (Milwaukee WI). The preparation of *trans*-2-butenoyl-CoA from crotonic anhydride and free CoA has been described [2]. *trans*-2-Hexenoic acid was purchased from Fluka AG, *trans*-2-octenoic acid from Aldrich Chemical Co (Milwaukee WI), *trans*-2-decenoic acid from Fairfield Chemical Co. (Blythewood SC) and *trans*-2-hexadecenoic acid from ICN Pharmaceuticals (Plainview NY). Synthesis of *trans*-2-dodecenoic and *trans*-2-tetradecenoic acids was as in [7]. All acids were purified by recrystallization from light petroleum (60–90°C b.p.); additionally, C_{12} and longer acids were precipitated with perchorate. Alkenoyl-CoAs were prepared by the mixed anhydride method in [8]. Substrate concentrations were determined from A_{263} [9].

3. Results

Maximum in vitro enoyl-CoA hydratase activity was at pH 8.8; 50% of peak activity was at pH 8.2 and 9.5 (fig.1). These values were identical with C_4, C_{10} or C_{16} substrates. The rather sharp pH optimum was considerably more alkaline than that of other β-oxidation enzymes from cotton (acyl-CoA oxidase 7.6, 3-hydroxyacyl-CoA dehydrogenase 7.2, and 3-oxoacyl-CoA thiolase 8.1, unpublished).

K_m-values were relatively low with C_4 and C_6 substrates, but were >10-fold greater with the longer acyl-chains through C_{16} (table 1). Maximum velocities decreased nearly 100-fold as acyl-chain length was increased from C_4–C_{16}. With any of the substrates examined, in vitro activity of enoyl hydratase was in excess of activity required for storage lipid mobilization [2], suggesting that the enoyl hydratase hydration is not the limiting step in fatty acid β-oxidation.

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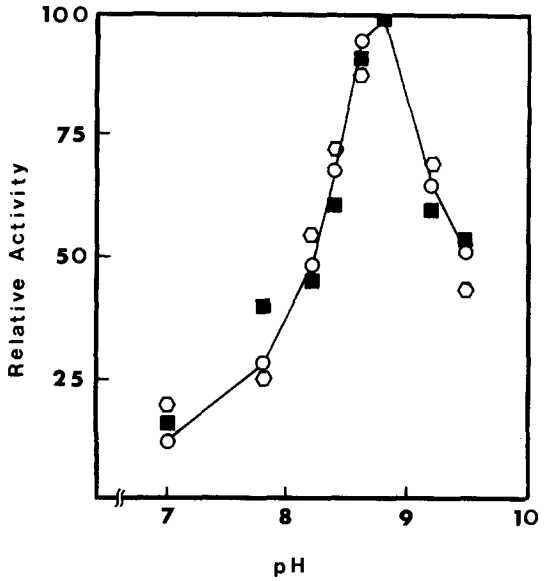

Fig.1. Enoyl-CoA hydratase activity as a function of pH in a wide-range phosphate–citrate–borate buffer. Substrates are: (■) *trans*-2-butenoyl-CoA; (○) *trans*-2-decenoyl-CoA; (○) *trans*-2-hexadecenoyl-CoA.

Table 1
Substrate specificity of cotton glyoxysomal enoyl-CoA hydratase

trans-2-Enoyl-CoA substrate	K_m (μM)	V_{max} (μkat/mg protein)	Relative V_{max} (%)
Butenoyl-CoA	0.16	9.86	100
Hexenoyl-CoA	0.21	1.80	18
Octenoyl-CoA	0.87	1.83	19
Decenoyl-CoA	1.55	1.22	12
Dodecenoyl-CoA	1.97	0.71	7
Tetradecenoyl-CoA	2.38	0.32	3
Hexadecenoyl-CoA	2.80	0.11	1

Kinetic parameters for each substrate were obtained by linear regression of S/v vs S

4. Discussion

This is the first report on substrate specificity for any of the β-oxidation enzymes from a plant source. Enoyl-CoA hydratases from mammalian sources have been studied extensively and substrate specificities examined [10–12]. The reports often refer to short-chain (crotonase) [10] and long-chain hydratases, which prior to the discovery of the β-oxidation sequence in peroxisomes [14], were presumed to operate in the mitochondria. Peroxisomal β-oxidation was suggested to be involved with catabolism of long-chain fatty acids, with the resulting short-chain fatty acids being transported to the mitochondria for further oxidation [14]. This dual compartmentation of β-oxidation sequences may explain the presence of enoyl hydratases with different substrate specificities in mammalian cells. Bacteria also have long- and short-chain enoyl-CoA hydratases [15,16] which apparently operate separately in either the cytosol or plasma membrane [15]. In contrast, β-oxidation in cotton cotyledons is located exclusively in peroxisomes (glyoxysomes) [2], possibly reflecting the ability of cotton peroxisomal enzyme(s) to utilize a wide range of acyl chain lengths.

The results in table 1 clearly illustrate the need to measure β-oxidation enzyme activity with variable chain length substrates (rather than with C_4 only as is often done) before comparing enzyme activities among species, or relating in vitro activities to in vivo rates of fatty acid catabolism. Other examples include rat liver peroxisomal enoyl-CoA hydratase which has much lower K_m-values for C_6 and C_8 substrates than for C_4 [12], and pig heart enoyl-CoA hydratase which is maximally active with a C_8 substrate [13]. It was suggested [17] that 3-oxoacyl-CoA thiolase from cucumber glyoxysomes was rate limiting in β-oxidation, based upon comparisons of in vitro activities of 3-hydroxyacyl-CoA dehydrogenase, enoyl-CoA hydratase and 3-oxoacyl-CoA thiolase, all with C_4 substrates. Such suggestions are premature without knowledge of the substrate specificities of the enzyme involved. In cotton cotyledons, acyl-CoA oxidase activity is more likely rate limiting [2]. Additionally, the high proportion of unsaturated fatty acids in storage lipids of oil seeds requires consideration of the activities of enoyl-CoA isomerase and 3-hydroxyacyl-CoA epimerase when examining rates of the overall process.

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