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β-Hydroxydecanoyl Thio Ester Dehydrase Does Not Catalyze a Rate-Limiting Step in *Escherichia coli* Unsaturated Fatty Acid Synthesis†

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ABSTRACT: The intracellular level of β-hydroxydecanoyl thio ester dehydrase, the product of the *fabA* gene of *Escherichia coli*, was increased by isolation of a putative promotor mutant (termed *fabAup*) or by molecular cloning of the wild-type *fabA* gene into plasmid pBR322. The *fabAup* and plasmid-carrying strains overproduced dehydrase by about 15- and 10-fold, respectively. The phospholipids of all strains that overproduced the dehydrase contained significantly higher levels of saturated fatty acids than isogenic strains producing a normal level of dehydrase. No increased levels of unsaturated fatty acids were observed. This result indicates that, although the dehydrase is required for unsaturated fatty acid synthesis, the level of dehydrase activity in wild-type cells does not limit the rate of unsaturated fatty acid synthesis. The introduction of a plasmid carrying the structural gene for β-ketoacyl acyl carrier protein synthase I into a *fabAup* strain overcame the effect of dehydrase overproduction on fatty acid composition.

Escherichia coli adjusts the fatty acid composition of its phospholipids in response to growth temperature [for review, see DeMendoza & Cronan (1983)]. This regulatory mechanism functions to lower the temperature of the order–disorder lipid phase transition and thus optimizes membrane function at lower temperatures (DeMendoza & Cronan, 1983). Thermal regulation is now known to be an intrinsic property of the fatty acid biosynthetic enzyme β-ketoacyl-ACP[1] synthase II (DeMendoza & Cronan, 1983). However, mutants lacking this enzyme, although completely defective in thermal regulation, synthesize a characteristic mix of saturated and unsaturated fatty acids. This result indicates the existence of an underlying mechanism regulating fatty acid composition that is independent of temperature. This mechanism is the subject of this paper.

The site of regulation of fatty acid composition in the absence of regulation by temperature has often been proposed to be exerted at the level of the acylation of *sn*-glycerol 3-phosphate to form phosphatidic acid, a key intermediate in phospholipid synthesis [for review, see Rock & Cronan (1982)]. However, acylation specificity is not absolute in vivo (Silbert, 1970), and thus, a major site of control must be at the level of fatty acid synthesis. The enzyme, β-hydroxydecanoyl thio ester dehydrase is responsible for the introduction of a cis double bond into the growing acyl chain to give the

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[1] Abbreviations: ACP, acyl carrier protein; NAC, *N*-acetylcysteamine; DNAC, 3-decynoyl-*N*-acetylcysteamine, Tet^R, tetracycline resistant.

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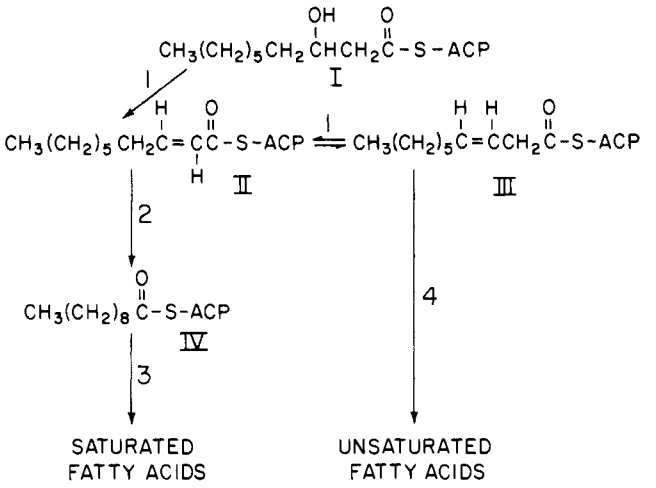

FIGURE 1: Branch point of *E. coli* fatty acid synthesis. The standard pattern of saturated fatty acid synthesis proceeds until the 3-hydroxydecanoyl-ACP (I) is formed. This substrate is then dehydrated to *trans*-2-decenoyl-ACP (II) by β-hydroxydecanoyl thio ester dehydrase (reaction 1). The *trans*-2-decenoyl-ACP can then either be isomerized to *cis*-3-decenoyl-ACP (III) by the dehydrase or reduced to decanoyl-ACP (IV) by enoyl reductase (reaction 2). The *cis*-3-decenoyl-ACP is then converted to palmitoleic acid or *cis*-vaccenic acid by three or four more cycles of fatty acid synthesis whereas (following three cycles of fatty acid synthesis) decanoyl-ACP gives palmitic acid. A second enzyme capable of conversion of I to II has also been described (see text).

unsaturated fatty acids of *Escherichia coli* [for review see Bloch (1971)]. This enzyme converts thio esters of 3-hydroxydecanoic acid to a mixture of *cis*-3-decenoyl and *trans*-2-decenoyl thio esters (Figure 1). The properties of the dehydrase led Bloch and co-workers (Brock et al., 1967) to propose that the ratio of the *cis*-3- to the *trans*-2-decenoates produced by the dehydrase directly determined the ratio of unsaturated to saturated acyl groups synthesized by *E. coli*. Following reduction to the decanoyl thio ester of acyl carrier protein (ACP), the *trans*-2-decenoyl thio ester would be elongated to saturated fatty acids, whereas several cycles of elongation of the *cis*-3-decenoyl-ACP would result in the unsaturated fatty acids of *E. coli*, palmitoleic acid and *cis*-vaccenic acid (Figure 1). The proposal of Brock et al. (1976) was weakened by the isolation of mutants lacking β-hydroxydecanoyl thio ester dehydrase activity (Silbert & Vagelos, 1967). These mutants contain lesions in the structural gene (the *fabA* gene) of the dehydrase (Cronan & Gelmann, 1973) and require only an unsaturated fatty acid for growth; saturated fatty acid synthesis proceeds normally (Silbert & Vagelos, 1967; Cronan & Gelmann, 1973). Similar observations were made with 3-decynoyl-*N*-acetylcysteamine (DNAC), an irreversible and specific inhibitor that is an analogue of the *cis*-3-decenoate substrate (Kass, 1968; Kass & Bloch, 1967). To explain these observations of continued saturated fatty acid synthesis, a second *E. coli* enzyme that dehydrated 3-hydroxydecanoyl-ACP to *trans*-2-decenoyl-ACP was sought and demonstrated (Birge et al., 1967; Birge & Vagelos, 1972). These results, coupled with studies on the mechanism of the dehydrase indicating that *trans*-2-decenoate is an obligate enzyme-bound intermediate in the formation of *cis*-3-decenoate (Rando & Bloch, 1968; Helmkamp & Bloch, 1968; Schwab & Klassen, 1983), suggested that the dehydrase did not produce free *trans*-2-decenoyl-ACP in vivo. β-Hydroxydecanoyl thio ester dehydrase was, therefore, thought not to function in saturated fatty acid biosynthesis.

In this paper, we report the isolation of *E. coli* strains that produce 10–15-fold more dehydrase than normal. Surprisingly, these strains overproduce saturated rather than unsaturated fatty acids, indicating that dehydrase activity is not rate-limiting for unsaturated fatty acid synthesis under normal conditions of growth. We show that the increased saturated fatty acid content is directly due to the overproduction of dehydrase activity by these strains. We also present evidence that the level of β-ketoacyl-ACP synthase I and, to a much lesser extent, the level of dehydrase control the ratio of unsaturated to saturated fatty acids synthesized by *E. coli* in the absence of the thermal regulatory system.

Table I: Bacterial Strains

strain	relevant genotype	source or derivation
K113	*fadR fabA*$^+$	P. Overath
CY52	*pyrD fadE*	*fadE* of CY50 (Cronan, 1974)
CY57	*fabA(Ts)*[a] *recA*	Cronan (1974)
CY59	*fabA*$^+$ *recA*	Cronan (1974)
DC170	*fadR fabAup*	see text
DC192	*fabA*$^+$	P_1 (DC170)[b] × KL185 for *pyr*$^+$
DC193	*fabAup*	P_1 (DC170) × KL185 for *pyr*$^+$
DC308	*fabA(Ts) pyrD zcb*::Tn*10*	see text
DC311	*fabAup recA pyrD*	*c*
DC312	*fabA*$^+$ *recA pyrD*	*c*
DC315	*fabAup fabF1*	P_1 (DC170) × UC1098 for *fab*$^+$
DC317	*fabA*$^+$ *fabF1*	P_1 (DC170) × UC1098 for *fab*$^+$
DC228	*fabA*$^+$ *fadE*	P_1 (DC170) × CY52 for *pyr*$^+$
DC230	*fabAup fadE*	P_1 (DC170) × CY52 for *pyr*$^+$
DC439	*fabA(Ts) fabB5 pyrD zcb*::Tn*10*	see text
DC466	*fabB5 fabAup*	see text
DM105	*fabB*$^+$ *fabA*$^+$	P_1 (K113) × M5 for *fab*$^+$
KL185	*pyrD*	Cronan (1974)
M5	*fabB5*	DeMendoza et al. (1983)
UC1098	*fabA(Ts) fabF1*	Gelmann & Cronan (1972)
plasmids		
F106	F′ carrying *fabA*$^+$ and *pyrD*$^+$	Cronan (1974)
CYF1	F′ carrying *fabA(Ts)* and *pyrD*$^+$	Cronan (1974)
pDM4	derivative of pBR322 carrying *fabB*$^+$ and Amp^R	DeMendoza et al. (1983)
pDM5	derivative of pBR322 carrying *fabA*$^+$ and Amp^R	see text

[a] This allele (*fabA2*) encodes an abnormally temperature-sensitive (Ts) dehydrase (Cronan & Gelmann, 1973). [b] The strain in parentheses is the transduction donor. *fadR* strains are constitutive in β-oxidation whereas *fadE* strains lack β-oxidation activity. [c] Strains DC311 and DC312 were constructed from DC308 by transduction for growth at 42 °C with phage stocks grown on strains DC170 and K113, respectively. Tet^S recombinants carrying the appropriate *fabA* allele were then transduced to *recA* (see Materials and Methods).

Materials and Methods

Bacterial Strains, Media, and Genetic Procedures. The *E. coli* strains (all derivatives of *E. coli* K-12) are given in Table I. In the construction of several strains, an insertion (called *zcb*::Tn*10*) of transposon Tn*10* located close to the *fabA* gene was used. The insertion was isolated by transduction as previously described (Clark & Cronan, 1980) and cotransduced at frequencies of 68 and 87% with the *fabA* and *pyrD* genes, respectively [selection for tetracycline resistance (Tet^R)]. An illustration of the use of this insertion is given by the derivation of strain DC446. Strain DC446 was constructed by transduction of strain DC439 with a P1 phage stock grown on strain DC170. The *pyr*$^+$ recombinants were then screened for tetracycline-sensitive (Tet^S) *fabAup* colonies; strain DC446 is one such colony. Strain DC439 is a *pyrD* Tet^R *fabA*(Ts)

Table II: β-Hydroxydecanoyl Thio Ester Dehydrase Levels and Sensitivity to DNAC

strain	*fabA* characteristic	dehydrase activity (units/mg)	overproduction (x-fold)[a]	MIC to DNAC (μM)[b]
K113	wild type	6.9		5
DC170	*fabAup*	92.1	13	>400
DC192	wild type	1.4		50
DC193	*fabAup*	22.4	16	>400
CY59	wild type	1.6		
pDM5/CY57	*fabA*$^+$ plasmid	17.7	11	

[a] Overproduction relative to the isogenic wild-type strain grown in parallel. The absolute levels of dehydrase varied depending upon the phase partition protocol and the lots of poly(ethylene glycol) used. In each case, the wild-type and dehydrase-overproducing strains were assayed in parallel with the same reagents. [b] Minimal inhibitory concentration needed to halt growth.

made by transduction of strain M5 to Tet^R with a phage stock grown on strain DC308. Strain DC308 was derived from strain KL185 by a series of transductions with *zcb*::Tn*10* to introduce Tet^R and *fabA*(Ts) lesions and retain the *pyrD* marker of the recipient strain. A *recA* mutation was also introduced into various strains with a Tn*10* insertion into the *srl* gene (Clark & Cronan, 1980). The transduction method and the media used were described previously (Clark & Cronan, 1980). Introduction of F′ factors by conjugation has also been described (Cronan, 1974).

The construction of plasmid pDM5 was done by mixing complete restriction nuclease *Bam*HI digests of plasmids pBR322 and pLC29-15 followed by ligation with T4 ligase and transformation of strain CY59 simultaneously to ampicillin resistance and growth at 42 °C (Maniates et al., 1982). All such colonies were sensitive to tetracycline, indicating insertion into the *Bam*HI site of pBR322. Plasmid pDM5 is of these plasmids and is about 14 kilobase pairs in size.

Lipid Analysis. Cells grown to exponential phase in broth or in minimal medium with 0.4% glucose plus appropriate amino acids (50 mg/L) and vitamins (1 mg/L) were used for analysis of fatty acid composition. Analysis was done as described previously (Gelmann & Cronan, 1973; DeMendoza et al., 1982) except that a diethylene glycol succinate column was used for gas chromatography, and the phospholipid fatty acids were converted to the methyl esters by transesterification in the presence of sodium methoxide (Polacco & Cronan, 1977).

Enzyme Assays. β-Hydroxydecanoyl thio ester dehydrase was assayed in cell extracts by the isomerase assay following phase partition to remove nucleic acids as described previously (Cronan & Gelmann, 1973). A unit of dehydrase activity is defined as 1 nmol of *trans*-2-decenoyl-NAC formed per min at 24 °C. The concentration of *cis*-3-decenoyl-NAC in the assay was 0.2 mM.

Assay of Sensitivity to 3-Decynoyl-NAC (DNAC). DNAC resistance was routinely used to score recombinant strains for the *fabAup* mutation. Tubes containing 2 mL of minimal glucose medium and various concentrations of DNAC were inoculated with 0.1 mL of an overnight culture of the test strain. The minimal inhibitory concentration (MIC) was the lowest concentration preventing detectable growth after incubation with shaking for 16 h at 37 °C.

Materials. The syntheses of 3-decynoic and *cis*-3-decenoic acids and their conversion to the NAC esters were done as previously described (Cronan & Gelmann, 1973; Nunn & Cronan, 1974).

Results and Discussion

Isolation of a Chromosomal Mutation Giving Overproduction of β-Hydroxydecanoyl Thio Ester Dehydrase. The acetylenic substrate analogue DNAC is a remarkably potent and specific inhibitor of all β-hydroxydecanoyl thio ester dehydrase catalyzed reactions (Brock et al., 1967; Helmkamp et al., 1968). At concentrations of 1 μM, DNAC completely and irreversibly inhibits both the dehydration and isomerization activities of the purified enzyme. Concentrations of 10–50 μM completely inhibit the growth of wild-type strains of *E. coli* (Kass, 1968), and growth inhibition is specifically relieved by addition of unsaturated fatty acids to the medium. DNAC was the first mechanism-activated "suicide" substrate to be discovered; isomerase activity is required to convert the triple bond to the reactive species, the allene (Endo et al., 1970). Therefore, mutants resistant to DNAC due to a deficiency in inhibitor activation should also lack enzyme activity, and most mutants resistant to inhibitory concentrations of DNAC should be strains possessing increased levels of dehydrase rather than a structurally altered enzyme.

Strain K113 carrying the *fadR* lesion (constitutive for the β-oxidation pathway of fatty acid degradation) was chosen as the parent strain for isolation of resistant mutants because (for unknown reasons) such strains are considerably more sensitive to inhibition by DNAC than isogenic *fadR*$^+$ strains (Nunn et al., 1983). Strain K113 was mutagenized with ethylmethanesulfonate, and mutants resistant to the inhibitory action of DNAC were isolated by overnight growth in glucose minimal medium containing 0.2 mM DNAC. After purification by single-colony isolation, the resistant strains were again screened for resistance to 0.2 mM DNAC, and one, strain DC170, was chosen for further study. Assay of cell-free extracts of strain DC170 and its parent K113 demonstrated that the DNAC-resistant strain DC170 contained over 15-fold more dehydrase activity than strain K113 (Table II). Preincubation of the two extracts with various concentrations of DNAC and subsequent assay of the dehydrase showed that DNAC inhibited the dehydrase activity of the extracts of both strains to the same degree (data not shown). Thus, as expected, the resistant phenotype of strain DC170 was due to overproduction of dehydrase and not to production of a form of the enzyme less sensitive to DNAC inhibition.

These results suggested that the DNAC resistance was due to more efficient transcription and/or translation of the *fabA* gene. Most mutants that overproduce enzymes have alterations of the promoter (RNA polymerase binding site) of the structural gene and thus show close genetic linkage to the structural gene and are cis active (Rosenberg & Court, 1979). The overproduction lesion (referred to as *fabAup*) was very closely linked to the structural gene as shown by cotransduction with phage P1. When phage P1 stocks grown on a strain carrying the *fabAup* lesion and a functional *fabA* gene were used to transduce recipient strains carrying the *fabA2* structural gene lesion to temperature resistance, all 600 recombinants tested were resistant to 0.2 mM DNAC. Moreover, the *fabAup* lesion cotransduced with the *pyrD* locus at the same frequency (70%) as the *fabA2* mutation. Several of these *pyr*$^+$ transductants were assayed for their dehydrase levels. A

Table III: Fatty Acid Compositions of Phospholipids of Strains with Elevated Dehydrase Levels

strain	*fab* character	growth temp (°C)	methyl esters (wt %) C14	C16	C16:1[a]	C18:1	% SFA[b]
DC192	*fab*$^+$	30	5.9	36.7	40.1	17.4	42.6
		37	2.4	44.0	41.1	12.7	46.4
		42	2.3	46.1	39.9	11.7	48.4
DC193	*fabAup*	30	3.3	46.8	37.5	12.5	50.1
		37	5.0	48.8	39.2	7.1	53.8
		42	4.8	52.0	38.5	4.9	56.8
DC317	*fabF1*	30	4.8	52.5	42.6	tr[c]	57.3
		37	3.5	52.6	42.8	tr	56.1
		42	2.8	51.6	45.8	tr	54.4
DC315	*fabAup fabF1*	30	7.9	49.9	42.4	tr	57.8
		37	4.0	57.8	38.1	tr	61.8
		42	3.6	72.5	23.9	tr	75.1
pBR322/CY59	*fab*$^+$[d]	30	6.0	34.7	39.0	20.3	40.7
		42	8.0	48.2	34.3	9.5	56.2
pDM5/CY57	*fabA*$^+$ *plasmid*	30	6.0	37.4	38.8	17.8	43.4
		42	12.8	56.0	28.8	2.4	68.8

[a] This figure includes any C17 cyclopropane acid. [b] C14 plus C16. [c] tr signifies a trace component. [d] A derivative of strain CY57 carrying pBR322 and a functional *fabA* gene on F106 gave essentially identical fatty acid compositions.

DNAC-resistant transductant (DC193) had a level of dehydrase 16-fold greater than that of the transductant (DC192) normally sensitive to the antibacterial compound (Table II). This result indicates that the same genetic lesion causes overproduction of dehydrase and DNAC resistance.

The dehydrase overproduction lesion is cis acting. We constructed an isogenic pair of *pyrD recA* strains (DC311 and DC312, Table I) carrying F′ factors that contained either a normal *fabA*$^+$ gene (F106) or the *fabA2* lesion (CYF1). The dehydrase activity of strains DC311 and DC312 as well as of their F106 and CYF1 derivatives was assayed after growth at 37 °C (the restrictive temperature for the plasmid-coded temperature-sensitive dehydrase). All cultures had similar dehydrase levels (29–43 units/mg of protein), and thus, overproduction of dehydrase due to the presence of the chromosomal mutation was unaffected by the presence of either F′ factor. Moreover, the *fabA* alleles present on the F′ factors did not overproduce dehydrase when present in a *fabAup* host. The overproduction lesion therefore is probably a promoter mutant although it could also be a mutation giving a more efficient ribosome binding site (Gold et al., 1981).

Molecular Cloning of Dehydrase Structural Gene. To achieve overproduction of dehydrase by a second means (for comparison with *fabAup* strains), we utilized a multicopy plasmid carrying the *fabA* gene. We previously reported (Clark & Cronan, 1981) that the recombinant plasmid pLC29-15 of the collection of Clarke & Carbon (1976) complemented the lesion of mutants deficient in the *fabA* gene. Strains carrying this plasmid produced 1.5–5-fold more dehydrase than wild-type strains. However, plasmid pLC29-15 is unstable upon growth at 42 °C. To obtain a more stable plasmid carrying a selectable marker for plasmid maintenance, we subcloned the *fabA* gene from plasmid pLC29-15 into the cloning vector plasmid pBR322 (Materials and Methods). The resulting plasmid, pDM5, gave a 16-fold overproduction of dehydrase (Table II).

Fatty Acid Compositions of Strains Overproducing Dehydrase. The fatty acid compositions of phospholipids from a *fabAup* strain and of an isogenic wild-type strain grown at several temperatures are shown in Table III. Strains carrying the pDM5 plasmid were also examined. To our surprise, the *fabAup* strain DC193 overproduced saturated rather than unsaturated fatty acids when compared to strain DC192, its wild-type *fabA*$^+$ derivative. Strains carrying the pDM5 plasmid also overproduced saturated acids (Table III), indicating a direct correlation between dehydrase overproduction and the increased saturated fatty acid content. The elevated dehydrase level was demonstrated to be the cause of the increased saturated fatty acid content by titration of the intracellular dehydrase levels with DNAC. The wild-type strain DC228 and an isogenic *fabAup* strain DC230 were grown at 42 °C in the presence of varying concentrations of DNAC (Table IV). As expected from the results of Nunn & Cronan (1974), the level of saturated fatty acids in the wild-type strain increased with increased inhibitor concentrations until growth was completely inhibited at 40 μM [due to insufficient unsaturated fatty acid synthesis (Kass, 1968)]. In contrast, addition of 20 μM DNAC to the *fabAup* strain decreased the saturated fatty acid content to give a fatty acid composition virtually identical with that of the untreated wild-type strain (Table IV). Thus, inactivation of a portion of the excess dehydrase restored the fatty acid composition to normal. The unusually high levels of dehydrase present in strains carrying either the *fabAup* mutation or the pDM5 plasmid thus increase the cellular saturated fatty acid content, presumably via increased production of the dehydrase product, *trans*-2-decenoyl-ACP. It should be noted that the increased level of saturated fatty acid in the phospholipids is at the expense of *cis*-vaccenic acid [as expected from the acylation specificity of phospholipid synthesis (Rock & Cronan, 1982)]. The in-

Table IV: Effect of DNAC on Fatty Acid Composition[a]

strain	DNAC (μM)	fatty acid composition (wt %) C14 + C16	C16:1	C18:1	% SFA
DC228 *fab*$^+$	0	54.3	45.7	13.0	54.3
	10	56.7	43.3	7.5	56.7
	20	60.7	39.3	8.7	60.7
	40[b]				
DC230 *fabAup*	0	65.4	34.6	7.5	65.4
	20	54.5	45.5	12.7	54.5
	40	57.5	42.5	10.9	57.5

[a] Cells were grown in minimal glucose medium at 42 °C in the presence of various concentrations of DNAC, and the fatty acid compositions of the phospholipids were determined. [b] No growth.

Table V: Fatty Acid Compositions of Strains Overproducing both Dehydrase and Synthase I

strain	enzyme levels[a]		methyl esters (wt %)[b]				
	dehydrase	synthase I	C14	C16	C16:1	C18:1	% SFA
pBR322/DM105	1	1	2.0	47.8	39.0	11.2	49.8
pBR322/DC446	15	1	7.5	52.5	35.2	4.8	60.0
pDM4/DM105	1	10	1.1	35.0	36.4	27.5	36.1
pDM4/DC446	15	10	5.1	33.7	34.2	27.0	38.8

[a] The enzyme levels are approximations taken from Table II and DeMendoza et al. (1983). [b] The cultures were grown at 42 °C. The C16:1 figure includes any derived C17 cyclopropane acid.

creased saturated content in dehydrase-overproducing strains was consistently observed in cells grown at 37 or 42 °C but was sometimes not obvious in cells grown at lower temperatures. The reason for these results is not known but could be due to differing levels of a fatty acid synthetic enzyme synthesized in cells grown at different temperatures (see below).

The increased saturated fatty acid content is independent of the thermal regulatory mechanism since dehydrase overproduction also gave increased saturated fatty acid contents in strains deficient in the thermal regulation of fatty acid synthesis due to a lack of the *fabF* gene product, β-ketoacyl-ACP synthase II (Table III). We have also found that the presence of increased dehydrase levels results in an increased saturated to unsaturated fatty acid ratio in the free fatty acids produced [in an experiment analogous to that of DeMendoza et al. (1982)] during blockage of phospholipid synthesis, indicating that the effect is also independent of acyl transfer into phospholipid (data not shown).

Effects of Dehydrase and β-Ketoacyl-ACP Synthase I Overproduction on Fatty Acid Composition. Overproduction of dehydrase resulted in increased production of saturated rather than unsaturated fatty acids (Table III). It, therefore, seemed that conversion of *cis*-3-decenoyl-ACP to unsaturated fatty acids was limited by the levels of another fatty acid synthetic enzyme, and hence, the products of the increased dehydrase activity were diverted to saturated fatty acids as *trans*-2-decenoyl-ACP. The fatty acid elongation enzyme, β-ketoacyl-ACP synthase I, catalyzes a rate-limiting step in unsaturated fatty acid synthesis (DeMendoza et al., 1983), and the key step catalyzed by this enzyme (rather than by β-ketoacyl-ACP synthase II) is thought to be the elongation of *cis*-3-decenoyl-ACP to 3-keto-5-decenoyl-ACP (Garwin et al., 1980). It therefore seemed probable that an increased level of synthase I would offset the effect of the increased dehydrase levels. It should be noted that either synthase I or synthase II can catalyze all the condensation reactions required for saturated fatty acids synthesis (Garwin et al., 1980).

We used the pDM4 plasmid, which carries the structural gene (*fabB*) for β-ketoacyl-ACP synthase I (DeMendoza et al., 1983), to increase the levels of this enzyme. Strains harboring this plasmid overproduce synthase I about 10-fold. This overproduction results in increased levels of unsaturated fatty acids due to an increased synthesis of *cis*-vaccenic acid (DeMendoza et al., 1983). We constructed a strain that carried both the pDM4 plasmid and the *fabAup* mutation and thus overproduced both synthase I and dehydrase. Isogenic strains that overproduced dehydrase or synthase I or neither enzyme (a wild-type strain) were also constructed. The four strains were then grown at 42 °C, and their fatty acid compositions were determined (Table V). The strains that overproduced either dehydrase or synthase I gave the results expected (Table III; DeMendoza et al., 1983) whereas the strain that overproduced both dehydrase and synthase I had a saturated fatty acid content essentially identical with that of the synthase I overproducing strain (Table V). Therefore, overproduction of synthase I canceled the effect of dehydrase overproduction.

Our data indicate that in wild-type cells, synthase I is essentially saturated with *cis*-3-decenoyl-ACP. When excess *cis*-3-decenoyl-ACP is produced via overproduction of the dehydrase, it is shunted (as *trans*-2-decenoyl-ACP) to saturated fatty acids.[2] However, if the levels of both synthase I and dehydrase were increased, the overproduction of saturated fatty acids due to dehydrase overproduction was no longer observed. It, therefore, is clear that the dehydrase does not normally catalyze a rate-limiting step in unsaturated fatty acid synthesis. The level of synthase I regulates the saturated to unsaturated ratios (in the absence of thermal regulation) since increasing this activity strikingly affects the ratio (DeMendoza et al., 1983). In contrast, although the dehydrase is required for unsaturated fatty acid synthesis, a 15-fold increase in activity does not give increased unsaturated fatty acid synthesis but only a meager increase in the level of saturated fatty acids.

It should be noted that Cronan (1974) reported that strains carrying two functional copies of the *fabA* gene gave a transient increase (relative to monoploid strains) in the rate of unsaturated fatty acid synthesis upon an abrupt shift of growth from 42 to 15 °C. The effect persisted only briefly (about one generation), and thus dehydrase activity was rate limiting for unsaturated fatty acid synthesis of only a short time after shift. The transient nature of the effect was puzzling. Recent data suggest that the temporary stimulation of unsaturated synthesis was probably due to increased levels of both the dehydrase and synthase I. The increased dehydrase level was due to the increased dosage of the *fabA* gene whereas the level of synthase I was increased (relative to the level found in 15 °C grown cells) by growth at 42 °C. Synthase I has been identified as protein F42.2 of *E. coli* (Garwin et al., 1980), and Herendeen et al. (1979) have reported that the levels of protein F42.2 (synthase I) are 35% greater in 42 °C grown cells than in 15 °C grown cells. Therefore, upon temperature shift from 42 to 15 °C, the elevated synthase level coupled with the elevated dehydrase level could have stimulated unsaturated fatty acid synthesis. Upon growth at 15 °C, however, the synthase I level would decline to the lower level characteristic of 15 °C grown cells, and the effect of dehydrase overproduction would subside. Consistent with this interpretation is the finding that 30 °C grown cells of a strain overproducing both dehydrase and synthase I (pDM5/DC446) had a slightly greater unsaturated content than a strain (pBR322/DC446) that only overpro-

[2] It is difficult to decide if excess *cis*-3-decenoyl-ACP would be produced under conditions of dehydrase overproduction. At equilibrium, the predominant product of the isolated dehydrase reaction with NAC thio ester is the trans-2 isomer (Brock et al., 1967). However, recent evidence indicates that the protein moiety of ACP interacts with the acyl chain of thio ester bound substrates (Cronan, 1982). If the interaction of the cis-3 chain with the protein is stronger than that of the *trans*-2 chain, then the level of *cis*-3-decenoyl-ACP present in vivo may be significantly greater than that suggested by the NAC thio ester data.

duced dehydrase (data not shown).

Conclusions

Although β-hydroxydecanoyl thio ester dehydrase catalyzes the first step in the formation of unsaturated fatty acids in *E. coli*, this enzyme does not catalyze a rate-limiting step in the synthesis of these acids. The step that limits the rate of unsaturated fatty acid synthesis is catalyzed by β-ketoacyl-ACP synthase I and is probably the elongation of *cis*-3-decenoyl-ACP. In the absence of thermal regulation, the ratio of saturated to unsaturated fatty acids in *E. coli*, therefore, is dependent upon the efficiency with which the genes encoding synthase I and β-hydroxydecanoyl thio ester dehydrase are transcribed and translated. The levels of expression of these two genes thus establish a basal ratio of saturated to unsaturated fatty acid synthesis. The thermal regulatory enzyme β-ketoacyl-ACP synthase II modulates the basal ratio by specifically increasing the rate of unsaturated fatty acid synthesis at low temperature.

Registry No. β-Hydroxydecanoyl thio ester dehydrase, 9030-80-2; β-ketoacyl acyl carrier protein synthase, 9077-10-5.

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