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Chemical synthesis of acyl thioesters of acyl carrier protein with native structure

(lipid synthesis/fatty acid synthesis/chemical modification/imidazole catalysis/sulfhydryl groups)

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ABSTRACT **The acyl carrier protein (ACP) of *Escherichia coli* was converted to acyl-ACP by imidazole-catalyzed *S*-acylation with *N*-acylimidazole. The acylation was specific to the sulfhydryl group; no acylation of tyrosine or amino groups of the protein occurred. The acyl-ACP substrates synthesized had a native structure as determined by gel electrophoresis, hydrophobic chromatography, and enzymatic activity. *N*-Acylimidazoles are readily synthesized and permit preparation of those acyl-ACP substrates that cannot be produced enzymatically.**

Acyl carrier protein (ACP) plays an essential role in the synthesis and subsequent metabolism of fatty acids in bacteria and plants (1, 2). To study these reactions in detail, the use of pure native acyl-ACP substrates is of paramount importance. Early attempts to synthesize acyl-ACP substrates used a chemical method that involved acetylation of the amino groups of four lysines and the NH_2-terminal serine of *Escherichia coli* ACP (3). Subsequently, acetylation was shown to result in a drastic loss of secondary structure and alteration of the biological activities of both ACP (4–6) and acyl-ACP (7). These findings coupled with the highly variable substrate activity of the acetylated preparations (8, 9) compromised the interpretation of enzyme studies carried out with these substrates.

The first advance in production of native acyl-ACP substrates was made by Jaworski and Stumpf (9) who synthesized acyl-ACPs by using plant cell extracts and ACP from *E. coli*. However, only long-chain saturated species (palmitoyl-ACP and stearoyl-ACP) could be synthesized. More recently, Ray and Cronan (10) described a novel ligase activity from *E. coli* that synthesized acyl-ACP from free fatty acids and ACP. This enzyme, acyl-ACP synthetase, was subsequently purified (11) and successfully used to produce various native acyl-ACP substrates (12, 13). However, acyl-ACP synthetase is inactive on short-chain saturated ($<C_8$) and unsaturated ($<C_{12}$) fatty acids (10), and thus synthesis of these vital substrates in a native form was not possible.

In this paper we report a chemical procedure to synthesize native acyl-ACP substrates having acyl chain lengths of 2 to 18 carbon atoms.

MATERIALS AND METHODS

Synthesis of *N*-Acylimidazoles. Two synthetic methods (for review, see refs. 14 and 15) were used (Fig. 1). Both methods give an essentially quantitative yield of *N*-acylimidazole (I).

Method A. The acid chloride (II, 20 mmol) was added with stirring to 40 mmol of imidazole (III) partially dissolved in 50–100 ml of dry benzene. This mixture was stirred for 3–6 hr, filtered to remove the insoluble imidazolium chloride, (IV), and flash evaporated. The residue was dissolved in the appropriate solvent and crystallized.

Method B. A solution of 10 mmol of the fatty acid (V) in 10 ml of dry tetrahydrofuran was added to a solution of 10 mmol of *N,N'*-carbonyldiimidazole (VI) in the same solvent. This mixture was refluxed until CO_2 evolution was complete (5–15 min) and then flash evaporated. The residue was dissolved in benzene, filtered to remove the bulk of the imidazole formed, and then crystallized from the appropriate solvent.

The short-chain *N*-acylimidazoles ($<C_8$) were crystallized from benzene or toluene whereas the long-chain compounds were crystallized from ethyl acetate or mixtures of benzene and ethyl acetate. The unsaturated *N*-acylimidazoles were oils at room temperature and hence difficult to crystallize. When prepared by method B, these oils were contaminated with a small amount of imidazole. However, because the acylation of ACP was performed in an imidazole buffer and the *N*-acylimidazole concentrations were determined spectrophotometrically, further purification was not needed. Both the *N*-myristoyl- and *N*-acetylimidazoles had an ε_{245} of 3.1×10^3 in acetonitrile, and this value was used to calculate all concentrations. The saturated *N*-acylimidazoles had melting points either identical to or within 1°C of those in the literature (14, 15). *N*-Myristoylimidazole melted at 73–75°C.

Synthesis of Acyl-ACPs. *E. coli* ACP (2–20 mg) was dissolved in 0.1 M Tris·HCl, pH 9.0/0.1 M hydroxylamine, pH 9/20 mM dithioerythritol. The mixture was incubated at 37°C for 30 min and cooled in ice. Trichloroacetic acid was added to 10% and, after 20–30 min in ice, the ACP precipitate was recovered by centrifugation, and washed twice with 0.2 M citric acid/HCl, pH 4.0, and then twice with water.

For the synthesis of short-chain ($<C_8$) acyl-ACP derivatives, the ACP pellet was resuspended at a final concentration of 2–2.5 mg/ml in 0.5 M imidazole·HCl, pH 6.48–6.51/2 mM Na EDTA containing dithioerythritol (1 mol/mol of ACP). The appropriate volume of 0.5 M *N*-acylimidazole in dry acetonitrile was added to give a final concentration of either 2.5 mM (C_2 or C_4) or 5.0 mM (C_6 or C_8). After 10–15 min at 22°C, trichloroacetic acid was added to 10%, and the acylated protein was recovered as described above and dissolved in 50 mM potassium phosphate buffer (pH 7.0).

The synthesis of long-chain ($<C_{10}$) acyl-ACPs was performed by using a detergent to solubilize the *N*-acylimidazoles (the addition of water-miscible organic solvents slowed the reaction greatly). The detergents (Triton X-100 or Brij 35) were purified to remove endogenous oxidizing agents and acidic components. Solutions of the *N*-acylimidazole and detergent in acetonitrile were added to a few milliliters of water and the acetonitrile was evaporated under nitrogen. To the resulting turbid solution was added an equal volume of 1 M imidazole·HCl, pH 6.34/4 mM

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Abbreviation: ACP, acyl carrier protein.

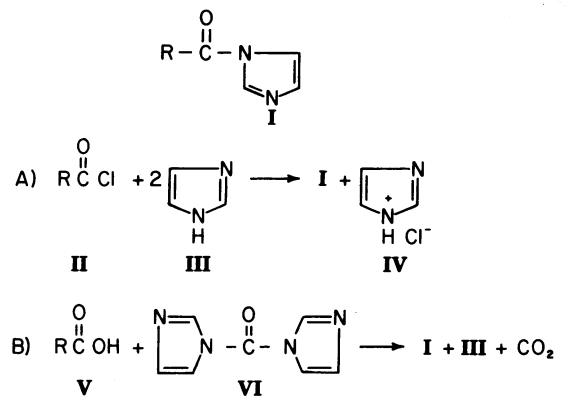

FIG. 1. Structure and synthesis of *N*-acylimidazole. *N*-Acylimidazoles (I) can be readily synthesized by either of two routes starting from the acid chloride (method A) or the free fatty acid (method B).

Na EDTA. ACP and an equimolar amount of dithioerythritol were added and the final concentrations of the components were adjusted to: ACP, 1–2.5 mg/ml; detergent, 10%; and *N*-acylimidazole, 5–20 mM (final pH, 6.50). The final *N*-acylimidazole concentrations used were 5 mM (C_{10} or C_{12}), 10 mM (C_{14}), or 20 mM (C_{16} or C_{18}). After incubation for 4 hr at 22°C (with occasional vigorous mixing), the solutions were applied to DEAE-cellulose and freed of the nonprotein components; the protein was eluted as described by Rock and Garwin (12).

The yields of acyl-ACPs based on ACP were essentially quantitative for chain lengths ≤12. The yields of myristoyl-ACP were about 90%, whereas the yields of the C_{16} and C_{18} acids varied widely (30–90%), probably due to variability in the dispersion of these insoluble compounds. The only unsaturated acyl-ACP synthesized, *cis*-3-decenoyl-ACP, was prepared as described for octanoyl-ACP and gave a similar yield.

Analytical Techniques. Polyacrylamide gel electrophoresis was done as described by Rock *et al.* (6) and Rock and Cronan (16). High-pressure chromatography was performed on a Waters apparatus with a column (50 × 0.46 cm) of Perisorb RP8 (Merck) (C. O. Rock, personal communication). ACP concentrations were determined by absorbance at 280 nm (17), by a microbiuret procedure (8), or by use of ACP radioactively labeled (by biosynthetic incorporation) in the prosthetic group (16).

Materials. ACP was purified from *E. coli* K-12 as described by Rock and Cronan (16, 17). Organic chemicals were from Aldrich. Triton X-100 and Brij 35 (from Sigma) were reduced with $NaBH_4$ and extracted as described by Chang and Bock (18). The chloroform extract was then throughly dried and chromatographed on a column of activated alumina F-1 (Alcoa) to remove possible acidic components. The alumina column was eluted with chloroform, and the purified detergent was stored as described (18). Solvents were dried with molecular sieve 4A.

RESULTS

Rationale of the Method. *N*-Acetylimidazole has been a standard reagent for the modification of protein tyrosine residues without acetylation of protein amino groups (19, 20). However, it has not been generally appreciated that *N*-acetylimidazole is also a powerful reagent for the modification of sulfhydryl groups. Stadtman (21) reported the *S*-acetylation of glutathione by *N*-acylimidazole, and Riordan and coworkers (19, 20) reported the *S*-acetylation of a number of model compounds and proteins. Furthermore, Jencks, and Carriuolo (22) demonstrated that the rate of *S*-acetylation of 2-mercaptoethanol by *N*-acetylimidazole is 6-fold faster than the rate of *O*-acetylation of phenol and is 60- to 80-fold more rapid than the acetylation of the amino groups of glycine and glycylglycine.

Although these data gave some promise of a specific *S*-acylation of ACP by *N*-acylimidazole, the presence of the five amino groups (α terminus and 4ε) and the tyrosine residue (23) meant that either a low conversion of ACP to acyl-ACP or some acylation of residues other than the prosthetic group must be tolerated. The solution to this problem was the observation by Jencks and Carriuolo (22) that the free base of imidazole can specifically increase the rate of *S*-acylation.

Jencks and Carriuolo (22) demonstrated that imidazole buffers greatly increase the rate of *S*-acetylation of 2-mercaptoethanol by *N*-acetylimidazole whereas enhancement of rate of acetylation of phenol is very small. Later results showed no catalysis by imidazole of the acylation of phenol or a number of substituted phenols (24). Jencks and Carriuolo (22) also observed significant imidazole catalysis of the acetylation of amino groups by *N*-acetylimidazole but this catalysis was largely suppressed at pH values below neutrality. The imidazole-catalyzed reaction of *N*-acetylimidazole with sulfhydryl groups is an example of general base catalysis and is thus pH independent, depending only on the concentration of imidazole free base (22). The decreased rate of *S*-acylation by *N*-acylimidazole observed at lower pH therefore can be offset by increasing the imidazole concentration of the buffer. From the data of Jencks and Carriuolo (22) and the assumption that the nucleophilic groups of ACP and those of the model compounds have similar reactivities, the ratio of the reactivity of the ACP sulfhydryl group relative to the sum of the reactivities of the other groups (one phenolic hydroxyl group and five amino groups) could be calculated. The ratio calculated for the acylation of 0.1 mM ACP dissolved in 1 M imidazole at pH 6.5 with 2.5 mM *N*-acetylimidazole was about 50, and thus the specific *S*-acylation of ACP by *N*-acylimidazole seemed possible. Moreover, the actual specificity for *S*-acylation of ACP seemed likely to be greater than that calculated from the model compound data because the amino groups of ACP appear to be involved in salt linkages (4) and the tyrosine residue appears to be in a nonreactive environment (5).

Studies with Model Compounds. *N*-Acylimidazoles are readily synthesized by starting with either the free fatty acid or the acid chloride (14, 15). The yields are essentially quantitative and the concentrations of *N*-acylimidazole can be determined either spectrophotometrically or by conversion to the hydroxamate (14). *N*-Acylimidazoles are readily hydrolyzed at extremes of pH and are unstable even at neutral pH (25). Imidazole free base catalyzes hydrolysis (22, 25). The rates of reaction of *N*-acylimidazoles with nucleophiles had been reported to vary with the acyl moiety (26, 27) but no data on long (>C_4) acyl chains were available. We therefore synthesized a number of *N*-acylimidazoles and compared their reactivities with water and the sulfhydryl of 2-mercaptoethanol in imidazole buffer.

The rates of hydrolysis of the short-chain *N*-acylimidazoles varied with chain length (Table 1). Our value for *N*-acetylimidazole was essentially identical to that previously reported by Jencks and Carriuolo (22) and as expected (26) a similar behavior was observed for *N*-butylimidazole. However, the hydrolysis rate for the C_6 and C_8 *N*-acylimidazoles was more rapid than that of *N*-acetylimidazole, which may reflect the type of micelle these sparingly soluble compounds assume in water. As expected from previous work (28), the addition of a nonionic detergent to disperse the longer-chain *N*-acylimidazoles decreased the rate of hydrolysis. The rates of *S*-acylation by *N*-acylimidazoles decreased with increased chain length and were also slowed by the addition of detergent. Detailed study of the reaction of the *N*-butyl- and *N*-hexanoylimidazoles with sulfhy-

Table 1. Properties of *N*-acylimidazoles in 0.5 M imidazole (pH 6.5)

Chain length	k_1, min^{-1} Water	k_1, min^{-1} Mercaptoethanol (10 mM)	Relative *S*-acylation rate
C_2	0.46	4.1	(100)
C_4	0.46	3.4	84
C_6	0.86	2.9	71
C_8	0.92	3.1	76
Plus detergent:			
C_6	0.53	1.5	37
C_8	0.27	1.2	28
C_{10}	0.14	0.8	20
C_{12}	0.11	0.4	9
C_{14}	0.12	0.25	6
C_{16}	0.07	0.24	6
C_{18}	0.08	0.26	6
$C_{16}\Delta^9$	0.07	0.35	9
$C_{18}\Delta^9$	0.10	0.34	8
$C_{18}\Delta^{11}$	0.09	0.28	7

The k_1 values were calculated from $k_1 = 0.693/t_{1/2}$. The values for the reaction with water or 2-mercaptoethanol were obtained under pseudo-first-order conditions essentially as described by Jencks and Carriuolo (22). The values obtained with various concentrations of 2-mercaptoethanol have been normalized to 10 mM. The detergent used was Brij 35 at a final concentration of 5%.

dryl, amino (both α and ε), and hydroxyl (both phenolic and aliphatic) groups of model compounds and synthetic peptides showed that the *S*-acylation specificity of *N*-acylimidazoles implied by the results of Jencks and Carriuolo (22) on the acetyl derivative held for the longer chain lengths (data not shown). These model compound data were then used as a basis for the preparation of acyl-ACP derivatives.

Acylation of ACP. The acetylation of ACP in 0.5 M imidazole buffer (pH 6.5) with *N*-[*acetyl*-^{3}H]imidazole had a biphasic dependence on the *N*-acetylimidazole concentration (Fig. 2). Increasing concentrations of *N*-acylimidazole give an almost proportional increase in the number of acetyl groups incorporated per molecule of ACP until a ratio of about 0.7–0.8 mol of acetate per mol of ACP was reached. Further increases in the *N*-acetylimidazole concentration gave increased levels of acylation but the concentration dependence was weak. For example, a 4-fold increase in *N*-acetylimidazole concentration (from 5 to 20 mM) gave only a 50% increase in the number of acetyl groups incorporated. A similar behavior was observed with *N*-palmitoylimidazole but much higher concentrations of the *N*-acylimidazole were needed for significant incorporation. The time course of the acylation of ACP depended primarily on the rate of hydrolysis of the *N*-acylimidazole. For example, acylation of ACP with 5 mM *N*-acetylimidazole was complete in 6–8 min (data not shown), at which time the *N*-acetylimidazole concentration had decreased to <0.6 mM due to the hydrolysis catalysed by imidazole (Table 1).

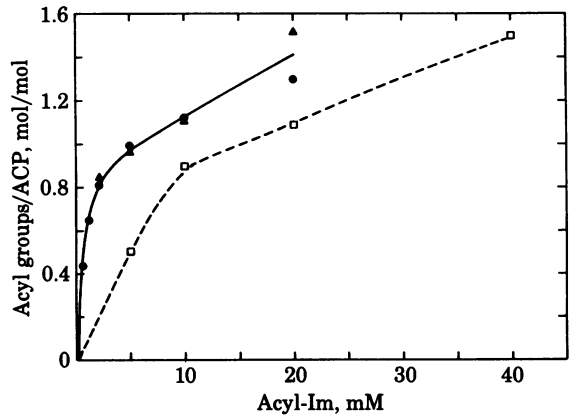

FIG. 2. Effect of *N*-acylimidazole concentration. Acylation of ACP with either *N*-[*acetyl*-^{3}H]imidazole (●, ▲) or *N*-[*palmitoyl*-^{3}H]imidazole (□) was performed as described in *Materials and Methods* except that the *N*-acylimidazole concentrations were varied as shown. The two different symbols for *N*-acetylimidazole denote two different experiments. ACP concentrations were determined by the microbiuret reaction (●, □) or by use of ACP labeled with ^{14}C in the prosthetic group (▲). The abscissa is the molar ratio of acyl groups incorporated to ACP.

Specificity of the Acylation Reaction. The model compound experiments correctly implied that the acylation occurring at high *N*-acylimidazole concentrations was the reaction of phenolic hydroxyl and amino groups after complete *S*-acylation. Greater than 92% of the [^{3}H]acetyl groups incorporated into ACP at concentrations of *N*-[^{3}H]imidazole <5 mM were cleaved from the protein by 1 M hydroxylamine at neutral pH (Table 2). Cleavage by neutral hydroxylamine is consistent with either *S*-acetylation (9, 10) or acetylation of the tyrosine hydroxyl group (19, 20). Tyrosine acylation can be monitored by the resulting decrease to one-sixth in the absorption of tyrosine residues at 275–280 nm (19, 20). ACP lacks tryptophan (1) and thus its absorption at 275–280 nm is almost entirely due to the single tyrosine residue (17). The absorption of the acylated ACP at 278 nm was therefore a sensitive measure of the extent of tyrosine modification. No detectable modification of the tyrosine residue was noted in acyl-ACP preparations having a molar ratio of acyl groups to ACP ≤1.

Amino group modification also did not occur at low concentrations of *N*-acylimidazole as demonstrated by (*i*) the sensitivity of the acyl group cleavage to neutral hydroxylamine, (*ii*) no loss of amino groups as measured by trinitrobenzene sulfonate titration (29), and (*iii*) the insensitivity of the acylated protein to pH induced denaturation (see below). The specificity of *S*-acylation was also demonstrated by (*i*) the finding that, at 10 mM

Table 2. Properties of acyl-ACP derivatives

Acyl group	Preparations	Yield, %	Acyl group/ACP, mol/mol	Tyrosine/ACP, mol/mol	Hydroxylamine cleavage, %
Chemical synthesis:					
C_2	6	95–100	0.85–1.02	0.92–1.2	92–98
C_4	2	95–100	—	0.98–1.06	90
$C_{10}\Delta^3$	2	95	—	0.97	95
C_{14}	3	60–90	—	0.94–1.21	95
C_{16}	2	50–80	0.4–0.6 (0.98)*		90–96
Enzymatic synthesis:					
C_{14}	—	—	1.0	0.96	95
C_{16}	—	—	1.0	—	93

The yields were determined by use of *N*-[*acyl*-^{3}H]imidazoles (C_2 and C_{16}), of ^{14}C- or ^{3}H-labeled (prosthetic group) ACP (followed by hydrophobic chromatography), or by visual inspection of stained polyacrylamide gels. The ratio of acyl group/ACP was determined by use of radioactive *N*-acylimidazoles or by use of enzymatic synthesis (12, 13). The tyrosine/ACP values were obtained by comparing the A_{278} with the protein concentration determined by the microbiuret procedure. The portion of the acyl groups released by neutral hydroxylamine was ascertained as described (10) for radioactive acyl-ACPs (C_2, C_{16}) or by polyacrylamide gel electrophoresis.

* The C_{16} value in parenthesis is the value obtained after removal of ACP by hydrophobic chromatography.

N-[*acetyl*-^{3}H]imidazole, myristoyl-ACP (prepared enzymatically) was labeled to an extent only 15% of that of a parallel sample of ACP and (*ii*) addition of a 10-fold molar excess (over ACP) of the synthetic peptide L-lysyl-L-tyrosyl-L-serine had no effect on the *S*-acetylation of ACP (data not shown).

Physical Properties of the Synthesized Acyl-ACPs. Previous studies from this laboratory demonstrated that acyl-ACPs having an acyl moiety $\leq C_8$ are much more resistant to induced hydrodynamic expansion than is ACP (6, 16, 30). At alkaline pH values the structure of ACP is expanded due to destruction of stabilizing salt linkages by titration of the amino groups of the protein (4–6, 16, 30). This expansion can be assayed at pH 9.4 by gel filtration (30) or, more conveniently, by polyacrylamide gel electrophoresis (6, 16). Acetylation of the amino groups of ACP results in a greatly increased sensitivity to alkaline pH (4–6, 16). This completely acetylated ACP is a random coil at pH 9.4 (4) and hence migrates much more slowly than ACP on gel electrophoresis (6). Mobility on gel electrophoresis is a sensitive measure of the stability of ACP derivatives and is particularly sensitive to amino group acylation (6).

Acyl-ACP molecules synthesized by the *N*-acylimidazole method under conditions giving one acyl group per ACP molecule had electrophoretic mobilities identical to those of enzymatically synthesized acyl-ACP derivatives (Fig. 3). Hydroxylamine treatment of these acyl-ACP preparations gave a quantitative conversion to ACP as assayed by gel electrophoresis.

We also examined the homogeneity of the chemically synthesized acyl-ACP molecules by hydrophobic chromatography. Hydrophobic chromatography of acyl-ACP derivatives on either octyl-Sepharose (12, 13) or by high-pressure chromatography on Perisorb RP-8, another octyl group-substituted chromatographic medium, is sensitive to the chain length of the acyl moiety (12, 13). Overacylation of ACP should result in an anomalously late elution from the hydrophobic matrix. Palmitoyl-ACP synthesized from *N*-palmitoylimidazole had an elution profile identical to that of enzymatically synthesized palmitoyl-ACP on either Perisorb RP-8 (Fig. 4) or octyl-Sepharose (data not given).

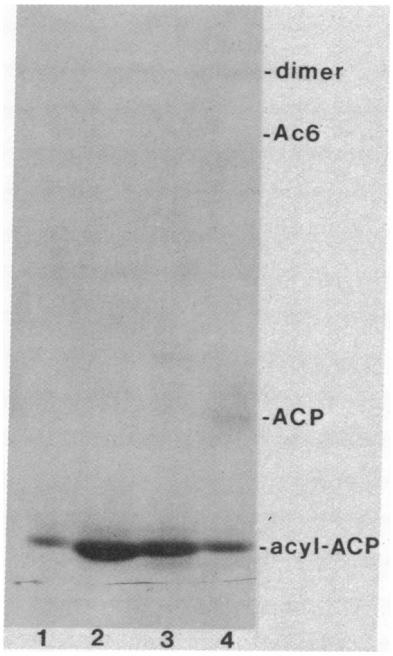

FIG. 3. Polyacrylamide gel electrophoresis of acyl-ACP. Gels (20% polyacrylamide) were run at pH 9.5 (final pH) and stained as described (6, 16). Lanes: 1, myristoyl-ACP enzymatically synthesized (12 μg); 2, decanoyl-ACP (68 μg); 3, lauroyl-ACP (50 μg); and 4, myristoyl-ACP (40 μg). The sample in lane 1 was purified free of ACP by octyl-Sepharose chromatography (12, 13); the samples in lanes 2–4 were synthesized by the imidazole procedure and purified only by DEAE-cellulose chromatography. The faint band at the top of the gel is the disulfide dimer of ACP formed during storage of acyl-ACP (6). The faint bands above and below ACP are probably complexes of ACP and contaminating trace metal ions (6). The migration position of amino-acylated ACP is marked Ac6. All four substrates were active in the β-ketoacyl-ACP synthase reaction.

Enzymatic Properties. Myristoyl-ACP synthesized by the imidazole procedure was fully active in the β-ketoacyl-ACP synthase I reaction. β-Ketoacyl-ACP synthase had identical maximal velocities and Michaelis constants for myristoyl-ACP preparations obtained either enzymatically or via the imidazole procedure (Fig. 5). It should be noted that this enzyme is sensitive to the quality of the acyl-ACP preparations in that the native myristoyl-ACP substrate is at least 10-fold more active than the substrate having acetylated amino groups (31, 32).

Characteristics of the *S*-Acylation Reaction. The experimental conditions for the acylation of ACP with *N*-acylimidazole are of critical importance. The addition of a small amount of reducing agent to the reaction mixture greatly increased the yield of acyl-ACP. The final pH and the concentrations of *N*-acylimidazole and ACP must be carefully checked. The *N*-acylimidazole concentration is particularly critical. Concentrations of *N*-acylimidazole 2- to 3-fold greater than those recommended occasionally gave acylation of tyrosine residues (determined spectrophotometrically) and of amino groups (determined by gel electrophoresis).

The acyl-ACP synthesized must be recovered fairly promptly from the reaction mixture to prevent transfer of the acyl group to imidazole. Stadtman (21) demonstrated that the *S*-acetylation of glutathione by *N*-acetylimidazole was reversible. A similar but much slower transfer of the thioester acyl moiety to imidazole was also observed for acyl-ACPs. The rate of transfer depended on both the imidazole concentration and the acyl chain length. The half-life for acetyl-ACP in 0.5 M imidazole (pH 6.5) was 25 min, whereas the half-life for palmitoyl-ACP (synthesized by either the imidazole or the enzymatic procedure) was

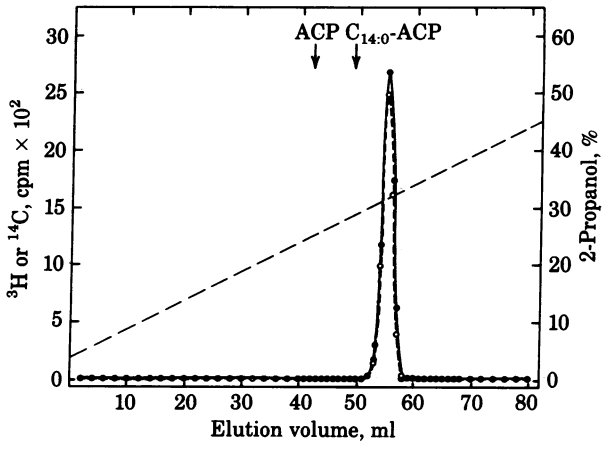

FIG. 4. Hydrophobic chromatography of palmitoyl-ACP. A mixture (≈20 μg, total) of [1-^{14}C]palmitoyl-ACP (●) prepared enzymatically was mixed with [9,10-^{3}H]palmitoyl-ACP (○) prepared by the imidazole method and injected onto a Perisorb RP-8 column equilibrated with 4% 2-propanol in 50 mM potassium phosphate buffer (pH 7.2). A linear gradient (broken line) to 45% 2-propanol in the same buffer was run for 160 min at 0.5 ml/min. Samples (1 ml) were collected and assayed in a scintillation counter set to discriminate between the two isotopes. The recoveries of ^{14}C and ^{3}H were 90% and 87%, respectively. The elution volumes of ACP and myristoyl-ACP (arrows) were established in subsequent runs of the same column.

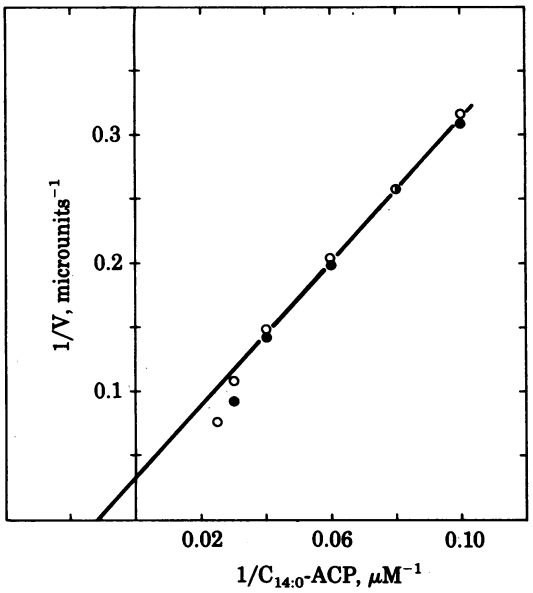

FIG. 5. Enzymatic utilization of myristoyl-ACP. The substrate activities of myristoyl-ACP samples prepared by either the enzymatic (●) or the imidazole (○) procedure were assayed at 37°C with purified β-ketoacyl-ACP synthase I (specific activity, 0.8 unit/mg) as described (31). Both acyl-ACP preparations were purified by Perisorb RP-8 chromatography (Fig. 4) to remove unreacted ACP. The Michaelis constant from the Lineweaver–Burk plot was 82 μM. The reactions with the enzymatic and imidazole synthesized substrates had K_m values of 84 and 78 μM, respectively.

about 4 hr in 2.5 M imidazole (pH 6.5). The reversibility of the acylation reaction precluded utilizing the somewhat greater specificity for *S*-acylation that would theoretically (22) result from increasing the concentration of imidazole buffer.

DISCUSSION

The synthesis of acyl-ACP substrates by the *N*-acylimidazole procedure proceeded smoothly and gave essentially quantitative conversion of ACP to acyl-ACP for short-chain ($\leq C_{12}$) acids. The ACP moiety of these substrates had native structure, and the physical and biological properties of these acyl-ACPs were indistinguishable from those of enzymatically prepared substrates. The *N*-acylimidazole method is complementary to the previous acyl-ACP synthetase method in that short-chain *N*-acylimidazoles are the most efficient acyl donors in the chemical method whereas the enzymatic synthesis functions only with long-chain acids.

The main use of the *N*-acylimidazole method will be in the synthesis of short-chain acyl-ACP substrates and of acyl-ACPs having acyl chains with functional groups. For the long-chain acids, the enzymatic method should remain the method of choice due to its absolute specificity. The synthesis of the short-chain acyl-ACPs will allow more definitive studies of the regulation of fatty acid synthesis and of the influence of the acyl group on the structure of ACP.

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