step fractures into the body of the chopper. Generally such use flakes were formed at the bulbar parts of scars of sharpening flakes, but dulling of the tool was often caused by clogging of its edge with crushed wood fiber.

The weight of the implement had to be preserved, so that when resharpening it we could not remove large flakes; renewal of the edge had to be accomplished by removal of thin flakes which, because of their thinness, could not terminate by feathering but were hinge-fractured instead, so that the edge was thickened at their point of termination. Hitherto scars of this sort have been considered results of use; they are really only products of resharpening. When the working edge of a chopper became too obtuse for further sharpening, the chopper was replaced.

True use-flakes on the ventral side of a stone tool are generally accidental, resulting from either improper use of the implement or striking of the support. These flakes are irregular in size and spacing, have diffused bulbs of force, are rapidly expanding, and terminate in a hinge or step fracture; in no way do they resemble scars left by backing (blunting) of an edge or by intentional retouch. When a scraping or planing tool is unhafted and handheld, only small use-flakes are removed, terminating in step fractures but not in the same way as do those removed individually by either pressure or percussion.

A chopper of Calico Hills chalcedony was used for reducing split oak boards to a rough shape but not for finishing. No functional scars were detected on the flat side of the chopper and along the upper margins of its edges; short flakes may have resulted from slight crushing during resharpening by percussion.

A chopper of Panamint Valley basalt was an even more useful tool than the chalcedony chopper because of the toothy edge and tough qualities of basalt. Used improperly, this chopper struck the support and three use-flakes resulted on the flat surface; they were short and rapidly expanding, and terminated in step fractures.

The working edge of a side scraper of flint (Harrison County, Indiana) was made by simultaneous serration and pressure-retouching of a primary flake; it was used to scrape oak paddles by application of pressure and by drawing of the scraper toward the operator. Use-flakes were removed from the ventral to the dorsal side; they were short, small, and terminated in step fractures. When the tool is held improperly, at an angle less than 90 deg, a complete flake is removed. It was determined that no use-flakes are pressed off when leather or hide is scraped.

A backed obsidian knife is used in whittling, like a pocket knife, but the direction of the cut must be kept in line with a stone knife's edge; if the knife is twisted, short, deep flakes are removed from its edge, almost at right angles. When the knife is repeatedly pulled either toward or away from the user, flakes concave to the edge are snapped off. As each concavity forms, it establishes a new platform; thus subsequent strokes pull off additional flakes, damaging the edge and making it useless.

Drilling was done with a chalcedony point fluted on both faces to facilitate hafting to the shaft of the pump drill. The wood was penetrated by alternate drilling of both sides of the paddle handle. Drilling quickly blunted the drill tip because microflakes detached and embedded themselves in the wood, causing double abrasion. A drill had to be resharpened once for penetration of the handles of two pottery paddles. Use-flakes were diminutive and usually terminated in hinge fractures.

Our tests suggest a number of probabilities in archeology:

1) Working of wood quickly consumes stone tools; for this reason, much roughing-out of wooden gear may have been done by aborigines at quarry workshops, near an abundance of material for primary tools (*4*). Much of the quarry litter that archeologists have dismissed as "quarry blanks and rejects"—or just scrap—may be accumulations of rough-and-ready implements; some of our own best tools would be classed as junk or detritus.

2) Unless the craftsman makes a mistake in direction of cut, or misses the mark and hits another stone, his tools show little or no sign of damage except for a gradual smoothing and rounding of the edges that is best perceived tactually; signs of use should also be visible under a binocular microscope (*5*).

3) There are readily discernible differences between three kinds of deceptively similar flake scars: those produced by intentional pressure retouch, those produced by secondary shatter associated with removal of larger flakes, and nicks caused by misuse of the tool. These very different types of scar have probably been confused by archeologists, with resultant loss of information.

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Acyl Carrier Protein: Effects of Acetylation and Tryptic Hydrolysis on Function in Fatty Acid Synthesis

Abstract. *Acetylation of the four lysine residues and the amino group of the terminal serine residue of* Escherichia coli *acyl carrier protein has no effect on the ability of this protein to function in fatty acid synthesis. Subsequent trypsin hydrolysis resulting in complete inactivation cleaves a single arginyl peptide bond, releasing the amino terminal hexapeptide from the molecule.*

All of the reactions of fatty acid biosynthesis in *Escherichia coli* occur with the substrates bound as thioesters to the acyl carrier protein (ACP) (*1*). This protein functions in fatty acid synthesis in all biological systems tested, including plants, fungi, bacteria, birds, and mammals (*2*). The prosthetic group and substrate binding site of this protein is 4′-phosphopantetheine, which is bound as a phosphodiester to a serine residue of the molecule (*3*). Acyl carrier protein is a heat-stable acid protein which has a molecular weight of

9500 and contains 86 amino acid residues plus the prosthetic group. There are four lysine residues and one arginine residue in ACP, all of which are relatively near the two ends of the molecule. The prosthetic group is bound to a serine residue about 27 amino acids from the amino terminus. Despite the fact that 4′-phosphopantetheine is present in both coenzyme A and ACP, the enzymes of fatty acid biosynthesis show marked specificity for thioesters of ACP. Moreover, previous studies have indicated that a high degree of structural integrity of ACP is required for its function in fatty acid synthesis. The only structural modification which did not severely impair ACP function was the cleavage of the three carboxyl terminal amino acid residues of the molecule with carboxypeptidase A. Trypsin hydrolysis of ACP leaves a large central peptide comprising half of the ACP molecule with an intact prosthetic group. This peptide does not function in fatty acid synthesis, indicating that some site, other than the prosthetic group, essential to ACP function has been lost (*4*). I now report studies which demonstrate that fully acetylated ACP remains active in *E. coli* fatty acid biosynthesis despite a change of 5 charge units. Subsequent tryptic hydrolysis of acetylated ACP results in cleavage of a single arginine bond releasing the amino terminal hexapeptide from the molecule. Cleavage of this bond results in complete inactivation of ACP in fatty acid synthesis, suggesting that some part of the amino terminal hexapeptide is essential to ACP function in fatty acid synthesis.

Carbon-14-ACP [labeled in the β alanine moiety of the prosthetic group (*4*)], was used for acetylation with acetic anhydride. Reaction mixtures contained 1 μmole of ACP and 2 mmole of $KHCO_3$, *p*H 8.0, in 5 ml of water at 0°C. Two hundred micromoles of H^3-acetic anhydride (2.6 μc/mole) were added and nitrogen was bubbled through the mixture for 15 minutes. The acetylated ACP was then precipitated with 2 ml of 10 percent trichloroacetic acid, and after centrifugation the precipitate was washed with 5 percent trichloroacetic acid to remove the excess acetic anhydride. The acetylated ACP was then dissolved in 2 ml of 1*M* neutral hydroxylamine and incubated at 30°C for 30 minutes. This treatment hydrolyzes the acetyl group from the sulfhydryl group of ACP. The acetylated ACP

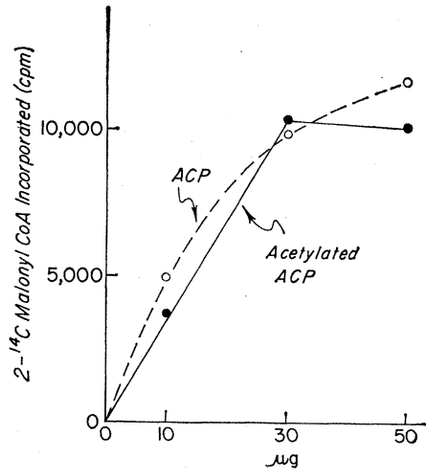

Fig. 1. Fatty acid synthesis using native ACP and acetylated ACP. The 2-C^{14}-malonyl CoA used had an activity of 1.3 μc/μmole.

was again precipitated with trichloroacetic acid and after centrifugation the precipitate was washed free of hydroxylamine with 5 percent trichloroacetic acid and dissolved in 0.1*M* tris buffer *p*H 8.0 containing 0.02*M* 2-mercaptoethanol. This mixture was incubated at 30°C for 15 minutes to insure complete reduction of the sulfhydryl group. Control samples of native ACP were carried through the identical procedure except for the addition of acetic anhydride. Aliquots were then taken for assay by both the malonyl CoA-CO_2 exchange reaction, which depends upon the first three enzymes of the fatty acid synthesis pathway (*5*), and the ACP-dependent incorporation of 2-C^{14}-malonyl CoA into long-chain fatty acids in the presence of the *E. coli* fatty acid synthetase (*4*). Aliquots were also taken for assay by the ninhydrin reaction (*6*) and for determination of C^{14} and H^3 activity in a liquid scintillation counter.

Table 1. Amino acid composition of the two peptides derived from trypsin hydrolysis of acetylated ACP and of a sample of unhydrolyzed acetylated ACP. Amino acid compositions were determined after hydrolysis in constant boiling HCl in sealed evacuated tubes for 22 hours at 105°C.

Amino acid	Amino acid residues per molecule			
	T-1	T-2	Sum, T-1 + T-2	Acetylated ACP control
Lys	3.1		3.1	3.6
His	0.7		0.7	1.1
Arg	0	0.9	.9	0.8
Asp	10.4		10.4	9.9
Thr	5.5	1.0	6.5	6.1
Ser	2.0	1.0	3.0	2.7
Gly	3.4		3.4	4.1
Pro	1.0		1.0	1.0
Glu	17.5	2.0	19.5	19.8
Ala	7.4		7.4	7.5
Val	6.5		6.5	7.0
Meth	1.1		1.1	0.9
Ile	4.5	1.0	5.5	6.7
Leu	5.7		5.7	5.9
Tyr	0.6		0.6	0.9
Phe	2.4		2.4	2.1
β-Ala	1.2		1.2	1.2
H^3 acetate	3.8	1		
C^{14}-ACP	1	0		

The four lysine residues and the free amino group on the amino terminal serine residue can account for 5 moles of H^3 acetate per mole of C^{14}-ACP. In two experiments there were 5.6 and 6.3 moles of H^3 acetate per mole of C^{14}-ACP, suggesting that some other group or groups in the molecule were acetylated to a small extent as well as all of the amino groups. The only other group which is likely to be acetylated at this *p*H is tyrosine. Further evidence for the complete acetylation of amino groups was the lack of reaction of acetylated ACP with ninhydrin. The ninhydrin reaction on control ACP yields 1.7 leucine equivalents per mole, while acetylated ACP has less than 0.05 leucine equivalent per mole (*7*). Despite the apparent complete acetylation of the amino groups of the ACP molecule it remains fully active when assayed by either the malonyl CoA-CO_2 exchange reaction or by the incorporation of 2-C^{14}-malonyl CoA into long-chain fatty acids. Control ACP catalyzed the incorporation of 0.015 μmole of CO_2 into malonyl CoA per minute, per milligram of ACP, compared to values of 0.010 and 0.023 for acetylated ACP. Acetylated ACP and control ACP were found to have parallel activity in long-chain fatty acid synthesis, as shown in Fig. 1. Thus, substrates of acetylated ACP are metabolized by all of the enzymes of fatty acid synthesis, despite the fact that acetylated ACP differs from control ACP by 5 charge units.

Acyl carrier protein contains only one arginine residue, which is the sixth residue from the amino terminus of the molecule (*4*). Thus, trypsin hydrolysis of acetylated ACP should result in cleavage of a single peptide bond releasing a hexapeptide from acetylated ACP.

Acetylated ACP was treated with 2

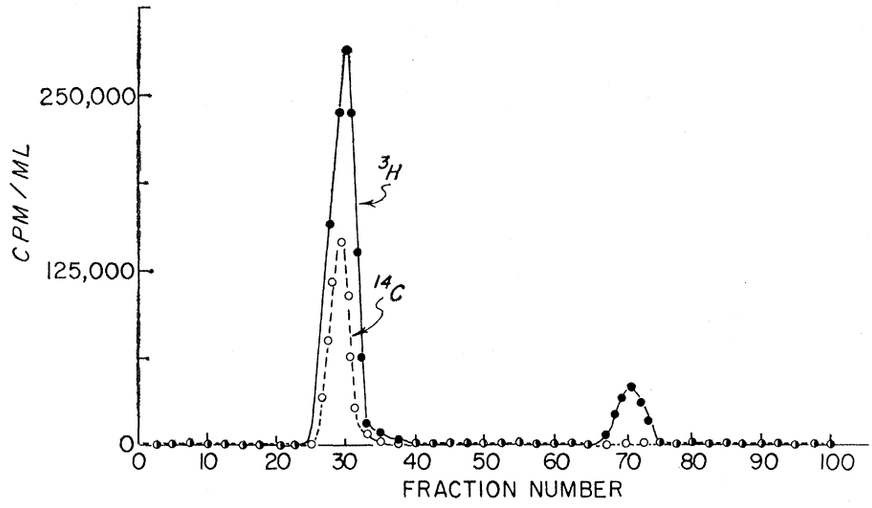

Fig. 2. Elution diagram of Sephadex G-50 column of acetylated ACP after tryptic hydrolysis.

percent trypsin by weight at *p*H 8.0 for 1 hour at room temperature, and the reaction was stopped with soybean trypsin inhibitor. The trypsin used in this experiment was twice crystallized enzyme purchased from Worthington Biochemical Corporation and pretreated with L-(1-tosylamido-2-phenyl) ethyl chloromethyl ketone to remove any chymotrypsin activity (*8*). After trypsin treatment acetylated ACP was completely inactive as assayed both by the malonyl CoA-CO_2 exchange reaction and the fatty acid synthesis assay. The trypsin-hydrolyzed acetylated ACP was next filtered over a 2.5- by 150-cm column of Sephadex G50 fine and eluted with 0.01*M* potassium phosphate, *p*H 7.0, to determine whether a single bond was split during trypsin hydrolysis. As shown in Fig. 2 only two peptides were eluted from this column.

The small tritium-containing peptide (T-2) was identified as the amino terminal hexapeptide of ACP by acid hydrolysis and amino acid analysis (Table 1). The large peptide, containing both C^{14} and H^3, was further purified on a 1- by 15-cm DEAE Sephadex A50 column to separate small amounts of contaminating peptides resulting from the hydroylsis of ACP which was not fully acetylated. This column was eluted with 200 ml of LiCl in a linear gradient from 0.15 to 0.55*M* containing 0.01*M* potassium phosphate, *p*H 7.0. The resulting purified major peptide (T-1) contained 3.8 moles of H^3 acetate per mole of C^{14}-ACP, which agrees well with the predicted value of 4.0 based upon the lysine residues. This peptide was also treated with carboxypeptidase A (*9*) with resulting release of 1 mole of alanine and 0.6 mole of glutamine and histidine. No other amino acids were released, thus indicating that the carboxyl terminal sequence of this peptide corresponds to the carboxyl terminal —His—Gln—Ala sequence of intact ACP and that no other peptides were present which had carboxyl terminal residues susceptible to carboxypeptidase A hydroylsis. The peptide was also subjected to acid hydrolysis, and amino acid analysis was performed. The results of the amino acid analyses of the two peptides derived from trypsin hydrolysis of acetylated ACP as well as the amino acid analysis of unhydrolyzed acetylated ACP are shown in Table 1.

The sum of the amino acid compositions of the two peptides corresponded closely to the amino acid composition of unhydrolyzed acetylated ACP. The only differences between the two are that 0.5 lysine residue, 0.7 glycine residue, and 1.2 isoleucine residues appear to be missing from the sum of the two peptides derived from trypsin hydrolysis. Despite these minor discrepancies, the general correspondence suggests that the cleavage of a single arginyl bond releasing the amino terminal hexapeptide of ACP has resulted in a loss of ACP activity. This loss of activity occurs despite the fact that the serine residue which carries the prosthetic group is more than 20 residues away from this bond. Several attempts to reconstitute ACP activity by mixing peptides T-1 and T-2 under varying conditions were unsuccessful.

These experiments suggest that the amino terminal hexapeptide of ACP is essential to its function. Previous experiments have shown that free ACP competitively inhibits the reduction of acetoacetyl ACP by β-ketoacyl-ACP reductase, while the central tryptic peptide with its intact prosthetic group is not inhibitory. These experiments suggested that ACP interacts with the enzymes of fatty acid synthesis at some site distant from the prosthetic group (*4*). Some part of the amino terminal hexapeptide may represent this site. Since *Clostridium butyricum* and spinach ACP contain no arginine and since these ACP's are at least partially interchangeable with *E. coli* ACP it is unlikely that arginine is the crucial residue (*10*). Sequence analysis of the amino terminal portions of the ACP's from different species may show similarities of primary structure, as is the case in the region of the prosthetic group, thus defining another essential sequence common to ACP's from different species.

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