Plant lipid biosynthesis in 1959 and 1984

P. K. Stumpf

Department of Biochemistry and Biophysics, University of California, Davis, CA 95616

Twenty-five years ago information concerning the synthesis of fatty acids was at a primitive stage. It was known from the work of Wakil, Brady and Gurin that in animal systems the β-oxidation enzymes were not involved, citrate stimulated synthesis remarkably, and CO_2 had a similar effect. The mechanism of these effects was not known (1).

In higher plants, work in 1952 with Newcomb outlined in very general terms the in vivo incorporation of [^{14}C]acetate into fatty acids (2). Barber, in our laboratory, then described a particulate system isolated from avocado mesocarp tissue that readily incorporated [^{14}C]acetate into ^{14}C-labeled palmitate and oleate (3); furthermore, acetone powders of these particulates also incorporated [^{14}C]acetate into C_{16} and C_{18} fatty acids and, whereas CO_2 increased incorporation several fold, citrate had no effect (4). Another very disturbing observation was that, in sharp contrast to the animal data in which [^{14}C]stearate was readily converted to [^{14}C]oleate by tissue slices, in a wide variety of plant systems, stearate was totally inert as a precursor of oleate suggesting indeed that stearate might not even be a precursor of oleate. In contrast, when [^{14}C]acetate was incubated with avocado particles in the presence of oxygen, [^{14}C]oleate was formed, although under anaerobic conditions [^{14}C]stearate accumulated (5). These puzzling observations faced the plant lipid biochemist in 1959.

Rather than recite in chronological order the important events that explained these 1959 observations, it might be simpler to summarize present information on the biosynthesis of fatty acids in 1984.

Needless to say, much progress has been made by a number of laboratories in Canada, Denmark, England, France, and Japan, as well as our group in Davis. Because of lack of space, the many contributions made internationally cannot be cited. The following summary does, however, present the current knowledge concerning fatty acid biosynthesis.

The organic chemistry of all saturated fatty acid synthesis is outlined in reactions 1–6 in **Table 1.** These six reactions are identical in all living cells. However, the molecular structures of the fatty acid synthetases (FAS) differ profoundly depending on the source of the tissue. In eucaryotic yeast cells, the FAS system is a giant complex molecule with a molecular weight of 2.4 $\times 10^6$ and with two dissimilar subunits, α and β, making up the complete FAS molecule as $\alpha_6\beta_6$. In animal tissues, the situation is quite different in that two identical subunits are involved, each with a full complement of the enzymic domains (sites of catalytic activity) required to catalyze the reactions listed in Table 1 and with acyl carrier protein (ACP) being one of the domains in each subunit. The molecular weight is 4.8×10^5. In enteric bacteria, such as *Escherichia coli,* however, all of the enzymic activities are associated not with domains in a polypeptide but with separable proteins that can be purified so that their kinetic properties can be individually studied. This type of system is called a non-associated system.

For a number of years, circumstantial evidence suggested that the plant FAS system was probably quite similar to the procaryotic type. The evidence cited included *a*) the absolute requirement for added ACP in plant systems, as well as in the non-associated *E. coli* system (the yeast and animal systems have ACP incorporated into their peptides) and *b*) exposure of a plant extract to prolonged high gravitational force (100,000 *g* centrifugation for several hours) with no sedimentation of the FAS system under conditions where polymeric eucaryotic systems were sedimented. Within the last 2 years, this problem has been clarified by the almost simultaneous publication of data from a number of laboratories working with diverse plant tissue, i.e., seeds, chloroplasts, and mesocarp tissue. These investigations have come to the same conclusion, namely that the plant FAS system, regardless of its origin in the plant, is a non-associated system responsible for the synthesis of palmitic and stearic acids.

A number of important conclusions have now surfaced as a result of these investigations and they will be listed and discussed briefly.

1) There is good evidence, based on in vivo and in vitro data, that the FAS system (i.e., the system responsible for the synthesis of palmitic (16:0) and stearic

Abbreviations: ACP, acyl carrier protein; FAS, fatty acid synthetase(s).

TABLE 1. Reactions involved in the synthesis of fatty acids in higher plants by the acyl carrier protein-dependent pathway

Ancillary enzymes

A. $CH_3COOH + ATP + CoA \xrightarrow[Mg^{2+}]{\text{acetyl-CoA synthetase}} CH_3COCoA + AMP + PP_i$

B. $CH_3COCoA + ATP + CO_2 \xrightarrow[Mg^{2+}]{\text{acetyl-CoA carboxylase}} COOHCH_2COCoA + ADP + P_i$

C. $\text{Oleoyl-ACP} + H_2O \xrightarrow[\text{oleoyl-ACP hydrolase}]{} \text{oleic acid} + ACP$

FAS enzymes

1. $CH_3COSCoA + ACP \cdot SH \xrightleftharpoons{\text{acetyl transferase}} CH_3CO \cdot S \cdot ACP + CoASH$
2. $HO_2C \cdot CH_2COSCoA + ACP \cdot SH \xrightleftharpoons{\text{malonyl transferase}} HO_2C \cdot CH_2CO \cdot S \cdot ACP + CoASH$
3. $CH_3COS \cdot ACP + HO_2C \cdot CH_2CO \cdot S \cdot ACP \xrightleftharpoons{\beta\text{-ketoacyl-ACP synthetase I}} CH_3COCH_2CO \cdot S \cdot ACP + ACP \cdot SH + CO_2$
4. $CH_3COCH_2CO \cdot S \cdot ACP + NADPH + H^+ \xrightleftharpoons{\beta\text{-ketoacyl-ACP reductase}} \text{D-}CH_3CH(OH)CH_2CO \cdot S \cdot ACP + NADP^+$
5. $CH_3CH(OH) \cdot CH_2CO \cdot S \cdot ACP \xrightleftharpoons{\beta\text{-hydroxyacyl-ACP dehydratase}} CH_3CH \xrightleftharpoons{\text{trans}} CHCO \cdot S \cdot ACP + H_2O$
6. $CH_3CH = CHCO \cdot S \cdot ACP + NADPH + H^+ \xrightleftharpoons{\text{enoyl-ACP reductase}} CH_3CH_2CH_2CO \cdot S \cdot ACP + NADP^+$ butyroyl-ACP
7. Butyroyl·S·ACP now reacts with a second molecule of malonyl·S·ACP and proceeds through reactions 3–6 to form hexanoyl·S·ACP, etc., until palmitoyl·S·ACP is formed.
8. $\underset{\text{palmitoyl-ACP}}{CH_3(CH_2)_{14}CO \cdot S \cdot ACP} + \underset{\text{malonyl-ACP}}{HO_2CCH_2COSACP} \xrightarrow[\beta\text{-ketoacyl-ACP synthetase II}]{\text{enzymes 4, 5, 6}} \underset{\text{stearoyl-ACP}}{C_{17}H_{35}COSACP} + CO_2$

D (in reaction 4), D optical isomer.

(18:0) acids) is solely localized in specific organelles in plant cells. Thus, in spinach leaf tissue, the specific site for de novo synthesis of palmitic (16:0) and oleic (18:1) acids is the chloroplast. (The site of synthesis of linoleic and linolenic acids in the leaf cell is still under intensive investigation.) All the ACP of the spinach leaf cell is localized in this organelle, as are a number of important ancillary enzymes such as acetyl-CoA synthetase and acetyl-CoA carboxylase. Since ACP is thus localized, obviously all ACP-requiring reactions must, by definition, be sited in that same organelle. Thus, acetyl-CoA:ACP transacylase (transferase), malonyl-CoA:ACP transacylase (transferase), β-ketoacyl-ACP synthetase I and II (also called condensing enzymes), β-ketoacyl-ACP reductase, β-hydroxyacyl-ACP dehydrate, and enoyl-ACP reductase, as well as the stearoyl-ACP desaturase and the oleoyl-ACP hydrolase, are associated exclusively with the chloroplast. The chloroplast is an ideal site for fatty acid synthesis since the principal products of photophosphorylation, namely ATP, O_2, and NADPH, are absolute requirements for fatty acid synthesis. Evidence, both direct and indirect, points to the proplastid as the site for the conversion of C_2 to oleic acid in the developing seed. Work with root tissues has not progressed sufficiently to suggest the sites of lipid biosynthesis in root cells.

2) The three enzymes, β-ketoacyl-ACP reductase, β-OH acyl-ACP dehydratase, and enoyl-ACP reductase, have a broad carbon chain specificity in that they participate in the entire range of reactions from the initial C_4 level to the C_{18} level.

3) The two condensing enzymes,β-ketoacyl-ACP synthetase I and β-ketoacyl-ACP synthetase II, are of considerable interest. Synthetase I is very sensitive to the inhibitor cerulenin, whereas synthetase II is relatively insensitive to low concentrations of this antibiotic. Synthetase I has a broad specificity for the condensation of malonyl-ACP to β-ketoacyl-ACP to form eventually palmitoyl-ACP. Palmitoyl-ACP and stearoyl-ACP are completely inactive as acceptor substrates for malonyl-ACP. Synthetase II only functions with myristoyl-ACP (C_{14}) and palmitoyl-ACP as substrates. Stearoyl-ACP is totally inactive. Thus, the levels and activities of these two enzymes play an important regulatory role in determining the formation of palmitic and stearic acids (as ACP derivatives) in all plant systems. That palmitic acid is the principal saturated fatty acid in plants would suggest that there may be a regulatory mechanism that monitors the level of palmitate synthesis by controlling the activities of synthetases I and II. Further support for these functions came from the observation by many that the principal product of fatty acid synthesis in the chloroplast

and the proplastid is free oleic acid. Stearoyl-ACP, the product of the FAS system, becomes the substrate for a very active stearoyl-ACP desaturase which then converts stearoyl-ACP to oleoyl-ACP.

4) The enzyme oleoyl-ACP hydrolase, which in the chloroplast is a stromal enzyme, plays an important role in that it specifically hydrolyzes oleoyl-ACP to free oleic acid and ACP. Thus, ACP is regenerated and recycled into the synthesis of fatty acids, while oleic acid is now readily transported out of the organelle to the cytosol where the enzymes for modifying the acyl component of fatty acids are associated with the endoplasmic reticulum. We refer to the chloroplast or the proplastid as the synthesizing compartment and the endoplasmic reticulum as the modifying compartment.

5) There are several candidates that may be responsible for the regulation of lipid synthesis in the synthesizing organelles. These include the generation of acetate, acetyl-CoA synthetase, acetyl-CoA carboxylase, and the two ACP transacylases. Once the seven (five plus two condensing enzymes) purified enzymes of the FAS system became available, each enzyme was varied with all the others kept constant in a reconstituted system. The results showed quite clearly that varying the concentration of acetyl-CoA:ACP transacetylase had a profound effect on the final composition of newly synthesized fatty acids. With low activities, a normal C_{16}–C_{18} pattern was observed; by increasing the activity, i.e., by increasing tenfold the level of the transacylase with all the other enzymes held constant, a dramatic shift to the formation of shorter chain fatty acids was consistently observed. Since the specific activity of the acetyl-CoA:ACP transacylase is the lowest of all the FAS enzymes examined, a shift of its activity would have a profound effect in the total synthesis of fatty acids, as well as the type of fatty acid formed. The relationship between the activities of the transacylase, synthetases I and II, and hydrolases should be explored more thoroughly in terms of defining the precise mechanism for chain termination in seeds.

6) In addition to specificity of a reaction conferred by an enzyme, there is another type of specificity that relates to compartmentations. Both in vivo and in vitro data now indicate that certain substrates formed in a cell are never allowed to enter another compartment. For example, in the developing jojoba seed, only monoenoic long-chain acids and alcohols (C_{20}–C_{22}) make up the wax esters, and all of these are derived from oleic acid. One compartment, presumably the proplastid, is the site for oleic acid biosynthesis exactly as with the leaf chloroplast; this acid is then transported to the cytosol for elongation by endoplasmic reticular enzymes with malonyl-CoA as the elongation unit. However, if one were to employ stearoyl-ACP as a substrate with the membranous elongation system, rapid formation of saturated C_{20} and C_{22} acids and alcohols surprisingly also occurred. But in vivo, the elongation system would never utilize stearoyl-ACP as substrate since stearoyl-ACP would be confined to the proplastid compartment and only oleic acid would be transferred to the cytosolic compartment. In other words, the jojoba cell establishes a compartmental-imposed specificity in the reaction, i.e., the elongation system sees only oleate, never stearate as the substrate for elongation.

In conclusion, in 1984 we can readily explain all the puzzles observed in 1959. The reason we had particulate preparations, rather than cytosolic systems isolated from liver tissues, was because the compartment containing the fatty acid synthetase in the avocado mesocarp was a proplastid; the lack of citrate activation was because plant acetyl-CoA carboxylase, unlike the animal system, is not activated by citrate; the lack of stearate conversion to oleate in plant system is due to the fact that stearate serves as a substrate for desaturation at physiological levels only as stearoyl-ACP; stearate cannot be converted directly to its activated substrate but can only be generated by the de novo ACP pathway with acetyl-CoA and malonyl-CoA as substrates.

Finally, now that the basic biochemistry of the events describing fatty acid biosynthesis has been resolved, the concepts of molecular biology and regulatory enzyme chemistry will begin to impinge on these problems with the likelihood of revolutionizing changes in the field of lipid biochemistry.

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