Medium-chain fatty acid synthesis

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Most plant and mammalian tissue lipids contain fatty acids with a carbon chain longer than 14 atoms. However, milk lipids from several species like ruminants and rodents (Jenness, 1974), seed lipids from certain plants like coconuts (Child, 1974) and Cuphea (Graham & Kleiman, 1985) and lipids from several insects (Addae-Mensah & Cameron, 1978) and from some bird glands (Downing, 1976; Jacob, 1976) contain fatty acids with from four to 14 carbon atoms. Studies of the mechanisms by which medium- and short-chain fatty acids are synthesized have been primarily performed with enzymes isolated from animal mammary glands, sebaceous glands of water fowl and from aphids.

Rabbit mammary gland fatty acid synthetase produces in vitro predominantly short- (C2 + C3) and long- (C14 - C16) chain fatty acids (Carey & Dils, 1970), whereas in vivo the gland produces mostly medium- (C6 + C7) chain fatty acids (Dils et al., 1977). Long-chain fatty acids synthesized by the rabbit fatty acid synthetase in vitro are released by hydrolytic cleavage of the acyl–enzyme complex by thioesterase I, which is an inherent part of the synthetase complex. This enzyme is, however, only able to cleave long-chain (≥C14) thioester acyl–enzyme complexes (Grunnet & Knudsen, 1978). The short-chain (C2 + C3) product is released as acyl-CoA esters (Hansen & Knudsen, 1980). The transferase involved in the termination of synthesis at short-chain length is most likely the loading transferase, which also is an inherent part of the synthetase complex (Hansen & Knudsen, 1980). The transferase from the rabbit mammary synthetase complex is unable to handle acyl-chains longer than C14 effectively (Knudsen & Grunnet, 1980). The rabbit mammary synthetase is therefore unable to terminate fatty acid synthesis at medium-chain length by either a thioesterase or acyltransferase reaction.

The explanation for rabbit mammary tissue producing mostly medium-chain fatty acid in vivo is that this tissue contains an additional thioesterase (thioesterase II). This enzyme has a molecular weight of 29000, is able to interact with and hydrolyse the fatty acid synthetase acyl–enzyme complex when the chain length has reached 8–10 carbon atoms (Knudsen et al., 1975, 1976).

Similar thioesterases have been found in rat mammary gland (Libertini & Smith, 1978) and mouse mammary gland (Libertini et al., 1980), which also synthesize medium-chain fatty acids. The enzyme has also been found in the sebaceous gland of mallard ducks (Rogers et al., 1982) and in aphids (Ryan et al., 1982).

In contrast, goat mammary gland, which produces short-, medium- and long-chain fatty acids in vivo, does not contain a thioesterase II enzyme (Grunnet & Knudsen, 1979a). Purified fatty acid synthetase from goat mammary gland synthesizes only short- and long-chain fatty acids like the rabbit mammary enzyme (Grunnet & Knudsen, 1979b). However, in the presence of an acyl-CoA-utilizing or -complexing system like the triacylglycerol-synthesizing system from mammary microsomes (Grunnet & Knudsen, 1979b, 1981; Knudsen & Grunnet, 1982) or bovine serum albumin (Knudsen & Grunnet, 1983) and 2,6-di-O-methyl-α-cyclodextrin (K. H. Hansen, unpublished work), 2,6-di-O-methyl-α-cyclodextrin (K. H. Hansen, unpublished work), the purified enzyme synthesizes mainly short- and medium-chain fatty acids and only a few long-chain fatty acids. The products synthesized in the presence of 2,6-di-O-methyl-α-cyclodextrin have been shown to be short- and medium-chain acyl-CoA esters (Knudsen & Grunnet, 1982).

The goat mammary fatty acid synthetase loading transferase can, in contrast to the loading transferase in rabbit and rat mammary synthetase, use both short- and medium-chain acyl-CoAs as substrates (Knudsen & Grunnet, 1980). Amino acid sequence studies of the active site domain of the malonyl-, acetyl- and decanoyltransferases from goat mammary synthetase show that all these activities are catalysed by the same active site (Mikkelsen et al., 1985a, b).

The loading transferase of the goat mammary gland fatty acid synthetase is therefore responsible for loading both acetyl and malonyl moieties on the enzyme and for unloading medium-chain-length acyl moieties back to CoA.

The fact that goat mammary gland fatty acid synthetase requires an acyl-CoA-removing system in order to synthesize medium-chain fatty acid indicates that there must be a close interaction between fatty acid synthetase and triacylglycerol-synthesizing enzymes in vivo. Experiments in vitro also indicate that this is the case. More than 90% of decanoyl-CoA synthesized by goat mammary synthetase in vitro is incorporated into triacylglycerols in the presence of the goat mammary microsomal fraction. In contrast, only 20% of the same amount of decanoyl-CoA added to the microsomal fraction alone under identical conditions is incorporated into triacylglycerols (J. Knudsen & H. G. Hansen, unpublished work). Evidence that this close interaction also occurs in vivo has been obtained from immunohistochemical studies, where it was shown that fatty acid synthetase is localized exclusively on the rough endoplasmic reticulum in dispersed lactating goat mammary cells (J. Knudsen & H. C. Hansen, unpublished work).

Short- and medium-chain fatty acids are found mainly in the sn-2 and sn-3 position in goat milk triacylglycerols. The sn-1 position always contains a long-chain fatty acid (Parodi, 1982). Studies in vitro have shown that the rate of medium- and short-chain fatty acid synthesis de novo is dependent on the rate of activation of exogenously added fatty acids. A sufficient amount of activated exogenous long-chain fatty acid is necessary for the initial acylation of the sn-1 position on the glycerol 3-phosphate backbone. Excess amounts of activated long-chain fatty acids will compete effectively with medium-chain fatty acids for the sn-2 and sn-3 position of triacylglycerols and thereby inhibit the synthesis de novo of these fatty acids (Hansen et al., 1984). The reason for this is that long-chain acyl-CoAs are the preferred substrate for acylation of all three positions on the glycerol phosphate in ruminant mammary gland (Marshal & Knudsen, 1980). Experiments with dispersed goat mammary gland cells confirm the results from the above studies in vitro. Low concentrations of palmitic acid in the incubation medium stimulated fatty acid synthesis de novo but the stimulatory effect was lost when the concentration was increased. In contrast, oleic acid and stearic acid inhibited synthesis de novo at all concentrations tested (J. Knudsen & H. O. Hansen, unpublished work). These results indicate that both the amount and the fatty acid composition of fat supplemented to ruminant feed will influence the synthesis of fatty acid in the mammary gland directly.
Role of cer-cqu in epicuticular wax biosynthesis

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The mixture of rather non-polar, very-long-chain lipids present on the outermost surfaces of plants are called epicuticular waxes. Their carbon chains are believed to be constructed via elongases, enzyme complexes similar to fatty acid synthetase which use fatty acid synthetase intermediates or products as primers. Subsequent action of associated enzyme systems, for example decarboxylative or reductive, gives rise to the different lipid classes. In barley, 1580 eceriferum (cer) mutants localized to 78 genes are known (Søgaard et al., 1984; U. Lundqvist, personal communication) to affect synthesis and/or deactivation of hydroxyl group into the ω-hydroxyl group. In one pair of the three investigated markers, cer-d6, -q5, and -u6, the results led to two important deductions regarding barley wax biosynthesis. The first is that the cer-cqu gene determines a polypeptide with emphasis on three recent results updating previous reviews (von Wettstein-Knowles, 1979, 1982).

Genetic analyses (von Wettstein-Knowles & Søgaard, 1980) of mutations modifying the phenotype of spike epicuticular waxes led to the identification of three complementation (functional) groups, cer-c, -q and -u, located on chromosome 4. Test cross-analyses revealed that any pair of the three investigated markers, cer-c, -q and -u, were within 0.0025 cM of one another, with the best approximation of the distance being 0.0012 cM. Their carbon chains are believed to be constructed via elongases, enzyme complexes similar to fatty acid synthetase which use fatty acid synthetase intermediates or products as primers. Subsequent action of associated enzyme systems, for example decarboxylative or reductive, gives rise to the different lipid classes. In barley, 1580 eceriferum (cer) mutants localized to 78 genes are known (Søgaard et al., 1984; U. Lundqvist, personal communication) to affect synthesis and/or deactivation of hydroxyl group into the ω-hydroxyl group. In one pair of the three investigated markers, cer-d6, -q5, and -u6, the results led to two important deductions regarding barley wax biosynthesis. The first is that the cer-cqu gene determines a polypeptide with emphasis on three recent results updating previous reviews (von Wettstein-Knowles, 1979, 1982).

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