Transacylase-like structure and its role in substrate channeling of 6-hydroxymellein synthase, a multifunctional polyketide biosynthetic enzyme in carrot cell extracts

Fumiya Kurosaki*

Cell Biology Laboratory, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Sugitani, Toyama 930-01, Japan

Abstract 6-Hydroxymellein synthase, a multifunctional poly-ketide biosynthetic enzyme of carrot, lost the binding ability toward its co-substrates, acetyl- and malonyl-CoAs, by the treatment with the blocking reagents for serine-OH. In contrast, the enzyme retained the binding ability even when the two SH groups at the reaction center (cysteine-SH of the condensation enzyme and cysteamine-SH of acyl carrier protein) were blocked, and one substrate bound to the SH-blocked enzyme was readily replaced by the other. It appeared that the cysteine-SH accepted only acetyl moiety while cysteamine-SH was preferentially malonyl-ated in the presence of both of the substrates. These results suggest that transacylase-like domain is involved in the structure of 6-hydroxymellein synthase as a common primary binding site of its co-substrates, and acetyl and malonyl moieties are properly channeled from their CoA esters to cysteine-SH and acyl carrier protein-SH via this domain, respectively.

1. Introduction

Polyketide biosynthetic enzymes (PKSs) have been assumed to share many common properties with fatty acid synthases (FASs) [1–4], and a starter unit, acetyl- or other acyl-CoAs, and several extender units malonyl-CoAs are sequentially introduced into these enzymes. It has been demonstrated that the reaction center of multifunctional FASs of yeast and animal cells is composed of two SH-groups (Cys-SH of the condensation enzyme and pantetheine-SH attached to acyl carrier protein(ACP)). The building units acetyl and malonyl moieties are transferred from the CoA esters to these SH groups prior to the initiation of the condensation reaction, and Ser-OH of transacylase domain is considered to play an important role in the proper entry and channeling of the co-substrates [1,2]. Recent genetic and biochemical approaches have been revealed that ACP- and transacylase-like domains also occur in several polyketide biosynthetic enzymes in fungi and bacteria [3,4]. In sharp contrast to PKSs of microbial origin, it was demonstrated [5,6] that ACP is not involved in the structures of chalcone and stilbene synthases, well characterized PKSs of higher plants, and occurrence of transacylase domain is also not evident. It has been assumed [3,5,6] that the acyl moieties for chain assembly are directly transferred from the CoA esters to the active Cys-SH at the reaction center in these enzymes.

Carrot cells accumulate 6-methoxymellein as the phytoalexin upon the invasion of pathogenic microorganisms [7], and biosynthesis of this compound is catalyzed by two inducible enzymes, 6-hydroxymellein synthase [8,9] and 6-hydroxymellein-O-methyltransferase [10]. We demonstrated [11] that 6-hydroxymellein synthase is a multifunctional PKS, and catalyzes the condensation of 1 acetyl-CoA and 4 malonyl-CoA, and an NADPH-dependent keto reduction takes place at the triketide intermediate stage [8]. Recently, I have reported [12] that 6-hydroxymellein synthase is organized as a homodimer in which two multifuctional subunits (approximately 130 kDa each) involving ACP-like domain [11] are aligned in an antiparallel direction to form the two reaction centers for each enzyme molecule. Therefore, the organization of 6-hydroxymellein synthase resembles type I FAS of animal cells [13], however, unlike in the FAS, two SH groups at the reaction center of this enzyme contribute from the same subunit [12]. In the present study, I attempted to identify the primary binding site(s) of the acyl-CoAs, and to elucidate the channeling pathways of the co-substrates to the two SH groups at the reaction center of 6-hydroxymellein synthase. For this purpose, I prepared several chemically modified enzymes in which the functional groups of amino acid residues were specifically blocked, and determined the binding abilities of these partially masked enzymes toward the acyl moieties.

2. Materials and methods

2.1. Chemicals

6-Hydroxymellein was prepared by demethylating 6-methoxymellein isolated from fungus-infected carrot roots with BBr$_3$ in anhydrous methylene chloride as reported previously [14]. Chloroacetyl-CoA was synthesized according to the method of Kawaguchi et al. [15]. 2-Chloroethylphosphonic acid, acetyl-CoA, malonyl-CoA, NADPH and bovine serum albumin were purchased from Sigma while iodoacetoamide was from Wako. 4-(2-Aminoethyl)benzenesulfonyl fluoride (AEBSF) was obtained from Merck. [2-^{14}C]Acetyl-CoA (spec. act. 2.1. GBq/mmol) and [2-^{14}C]malonyl-CoA (spec. act. 2.2 GBq/mmol) were from New England Nuclear. All other chemicals were reagent grade.

2.2. Induction, purification and assay of 6-hydroxymellein synthase

Carrot roots were purchased from a local market, and induction of 6-hydroxymellein synthase in the root disks was carried out with 2-chloroethylphosphonic acid as the elicitor by the method described previously [9]. Highly purified 6-hydroxymellein synthase was prepared as described previously in detail [9], and the assay of the synthase activity (approximately 1 pkat/assay) was carried out according to the method described previously [8,9]. Protein concentration was determined by the method of Bradford [16]. Purity of the synthase preparation was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with an 8% gel according to the method of Laemmli [17]. A densitometric scan on a dual wavelength chromatoscanner (Shimadzu, CS-910) was performed [9] after the proteins were

*Corresponding author. Fax: (81) (764) 34-4656.

stained with Coomassie Brilliant blue. It was confirmed that the synthase comprised 38–52% of proteins in the enzyme preparation in repeated experiments. The enzyme solution was dialyzed against 20 mM Na-phosphate buffer (pH 7.5) to remove mercaptoethanol, and was appropriately concentrated on an Amicon cell (YM-10 membrane).

2.3. Chemical modification of 6-hydroxymellein synthase

6-Hydroxymellein synthase (approximately 100 pkat/assay) was incubated with various concentrations of iodoacetoamide, chloroacetyl-CoA or AEBSF in 100 μl of 20 mM Na-phosphate buffer (pH 7.5) at 37°C for 15 min. Controls received only the buffer instead of the inhibitors. After the incubation, mercaptoethanol was added to the mixtures at the final concentrations of 0.1% (v/v) to quench the SH inhibitors and reduce the unmodified SH groups, and these modified enzymes were immediately used for the assay of the 6-hydroxymellein synthetic activity or the binding ability toward acyl-CoAs.

2.4. Binding of acetyl- and malonyl-CoAs to 6-hydroxymellein synthase

Binding abilities of the chemically modified 6-hydroxymellein synthase toward its substrates were determined by the incubation with ^{14}C-labeled acyl-CoAs according to the method described previously [12] with several alternations. The assay mixture consisted of 20 mM Na-phosphate (pH 7.5), 10 μM of [^{14}C]acetyl-CoA or [^{14}C]malonyl-CoA (7.4 kBq), 1 mM of NADPH, 5 μg protein of the enzyme preparation (approximately 100 pkat/assay) and 0.1% of mercaptoethanol in a total volume of 100 μl. The reaction was run for 2 min at 37°C, and was terminated by the addition of 500 μl of 2 M trichloroacetic acid. If necessary, [^{14}C]acyl-CoAs were incubated in the presence of 10 μM of unlabeled acyl-CoAs, or the acylated enzymes were post-incubated with 1 mM of unlabeled acyl-CoAs for 30 s prior to the addition of trichloroacetic acid. To the solution was added 100 μg of bovine serum albumin as a carrier, and the proteins were precipitated by centrifugation (11.500 × g, for 2 min). In some experiments, the acylated 6-hydroxymellein synthase was further treated with performic acid [18]. The precipitated proteins were dissolved in 200 μl of formic acid, and 40 μl of 31% hydrogen peroxide was added to the mixture. They were incubated at 37°C for 1 h, and the oxidized proteins were recovered by the addition of (NH₄)₂SO₄ to bring the concentration to 67% saturation. Oxidation of the proteins with performic acid was repeated again in the same way, and the (NH₄)₂SO₄ precipitates were washed with 500 μl of trichloroacetic acid to remove the salt. Resultant pellets were denatured with 5% (v/v) mercaptoethanol and 2% (w/v) SDS, and the samples were subjected to SDS-PAGE (8% gels). It has been shown [12] that the native dimer of 6-hydroxymellein synthase is completely dissociated to the monomeric subunits under these conditions. After electrophoresis, proteins were stained, and the band corresponding to the 6-hydroxymellein synthase subunit (128 kDa) was identified with standard proteins (myosin, 200 kDa; Escherichia coli galactosidase, 116 kDa; rabbit muscle phosphorylase, 97 kDa; bovine serum albumin, 66 kDa; ovalbumin, 43 kDa). Gels slices containing the enzyme protein were excised with a blade, immersed in 2 ml of Protosol (New England Nuclear), and their radioactivities were determined.

3. Results

3.1. Modification of reaction center of 6-hydroxymellein synthase with SH- and OH-blocking reagents

Highly purified 6-hydroxymellein synthase was incubated with various concentrations of the reagents which specifically block Cys-SH, cysteamine-SH of ACP or Ser-OH, and the residual activities of the enzyme were determined in the presence of 0.1% mercaptoethanol (Fig. 1). Iodoacetoamide and chloroacetyl-CoA are known as the specific alkylating reagents for Cys-SH and ACP-SH in FASs, respectively [1,19,20], and these reagents also exhibited their inhibitory activities against 6-hydroxymellein synthase. Activity of the synthase decreased to the levels of approximately 16% with 10 mM iodoacetoamide and 9% with 1 mM of chloroacetyl-CoA. AEBSF, a specific blocking reagent for Ser-OH [21], was also found to appreciably inhibit the synthase activity in a dose-dependent manner, and
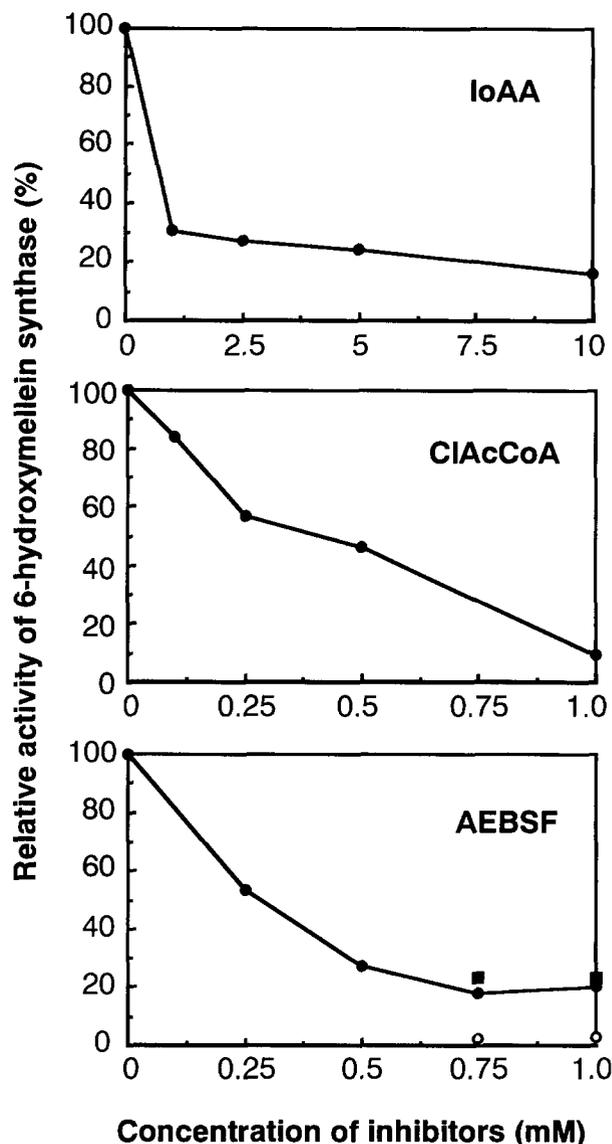
Fig. 1. Inhibition of 6-hydroxymellein synthase activity by the treatment with various blocking reagents. Highly purified 6-hydroxymellein synthase was incubated with various concentrations of iodoacetoamide (IoAA), chloroacetyl-CoA (ClAcCoA) or AEBSF in the absence of mercaptoethanol. After the modification reactions, mercaptoethanol was added at the final concentration of 0.1%, and the residual activities of the synthase were determined (●). In AEBSF treatment, the enzyme activity was also determined in the absence (○) or presence of 1% mercaptoethanol (■). Results were expressed as percentage of the control which received only Na-phosphate buffer instead of the blockers. Means of two independent experiments were presented.

the activity decreased to the level of 18–20% of the control with 0.75–1 mM of the inhibitor. Recently, it has been demonstrated [22] that phenylsulfonyl fluoride derivatives are capable of blocking SH group in some extent as well as Ser-OH group, however, this effect can be readily reversed by SH reagents such as mercaptoethanol and dithiothreitol. As shown in Fig. 1, the activity of 6-hydroxymellein synthase was, apparently, almost completely inhibited by the treatment with AEBSF when the assay was carried out without mercaptoethanol. However, it was restored to the level of about 20% upon the addition of
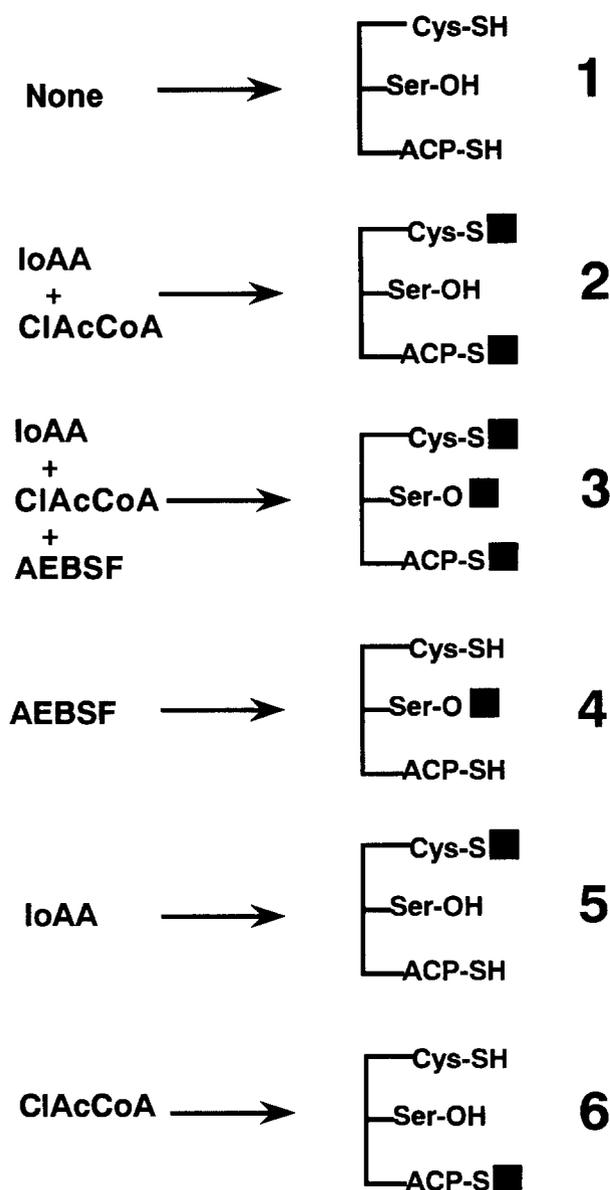
Fig. 2. Expected modification of the reaction center of 6-hydroxy-mellein synthase after the treatment with the blockers. The functional groups at the reaction center of the synthase, Cys-SH, ACP-SH and/or Ser-OH, were appropriately blocked by the combination use of 10 mM of iodoacetoamide (IoAA), 1 mM of chloroacetyl-CoA (ClAcCoA) and/or 1 mM of AEBSF. Expected blocking sites of these reagents were presented as the closed boxes, and the native and the modified enzymes were numbered from 1 to 6.

0.1% of mercaptoethanol, and was essentially not altered when the concentration was elevated to 1%. Therefore, it appears that SH groups of the enzyme possibly blocked by AEBSF are, if any, almost completely rescued upon the addition of 0.1% mer-captoethanol, and the observed inhibition of the enzyme with AEBSF under the present experimental conditions should be responsible for its blocking effect for Ser-OH group. Similar irreversible inhibition was also observed for another Ser-OH blocking reagent, phenylmethylsulfonyl fluoride, however, the inhibitory activity of this reagent was somewhat low as com-pared with that of AEBSF. The synthase activity was decreased

to 32% of the control by the treatment with 1 mM of the inhibitor (data not shown). These observations strongly suggest that Ser-OH, as well as Cys-SH and ACP-SH, plays an impor-tant role in the catalytic reaction of 6-hydroxymellein synthase.

3.2. Characterization of primary binding site of acyl-CoAs in 6-hydroxymellein synthase

In the next experiments, reaction center of 6-hydroxymellein synthase was appropriately modified by the combination use of the blocking reagents, and the binding abilities of these partially masked enzymes toward the acyl-CoAs were deter-mined. Expected modifications of the reaction center of the synthase with these blockers were summarized in Fig. 2, and the native and the modified enzymes were numbered from 1 to 6. 6-Hydroxymellein synthase pre-treated with iodoacetoamide plus chloroacetyl-CoA which was expected to be blocked either Cys-SH or ACP-SH (Fig. 2, 2) was capable of binding apprecia-ble amounts of the acyl-CoAs suggesting that the modified enzyme does not lose the ability of binding the substrates. This observation suggested the possibility that a certain primary binding site(s) other than the two SH groups at the reaction center is involved in the structure of 6-hydroxymellein synthase. As reported previously [12], an attempt to estimate the chemical stoichiometry of the enzyme-bound acyl-CoAs versus 6-hy-droxymellein synthase molecule was unsuccessful because the unstable activity of the synthase was partially lost during the purification processes. Therefore, results obtained in the fol-lowing experiments were expressed as relative values in which amount of acetyl-CoA bound to the modified enzyme 2 was taken as 1.00 in each set of the experiments (Table 1). The binding efficiencies of the modified synthase 2 toward each substrate were comparable even when the enzyme 2 was incu-bated with the mixture of the acyl-CoAs suggesting that the putative primary binding site of 6-hydroxymellein synthase does not recognize these CoA esters. In contrast, the synthase lost the most of the binding activity when the enzyme protein was treated with a mixture of iodoacetoamide, chloroacetyl-CoA and AEBSF (3). The entry of the acyl groups into the synthase was also blocked when only Ser-OH of the enzyme was masked by the treatment with AEBSF (4) suggesting that Ser-OH group(s) in certain structure of the enzyme is a primary binding site of the acyl-CoAs. The Ser-O-bound acyl moieties were readily replaced by the post-treatment with excess amounts of unlabeled acyl-CoAs, and this replacement also observed by the post-incubation with the other CoA ester, respectively. This 'cross-exclusion' of the enzyme-bound acyl moieties by acyl-CoAs implies that the acyl transfer from the CoA esters to the binding site is the reversible reactions, and the two acyl-CoAs share the Ser-OH as a common binding site.

3.3. Channeling of acyl-CoAs to Cys- and ACP-SHs via Ser-OH of 6-hydroxymellein synthase

6-Hydroxymellein synthase was treated with iodoacetoamide or chloroacetyl-CoA to block Cys-SH (Fig. 2, 5) or ACP-SH (6) at the reaction center, respectively. Employing these modi-fied enzymes in which the primary binding site plus one of the two SH groups should function, the substrate channeling path-ways from CoA esters onto Cys-SH and ACP-SH via Ser-OH were examined. As shown in Table 2, approximately twice the number of acetyl moieties were bound to iodoacetoamide-treated synthase (5) as compared with that bound to the mod-
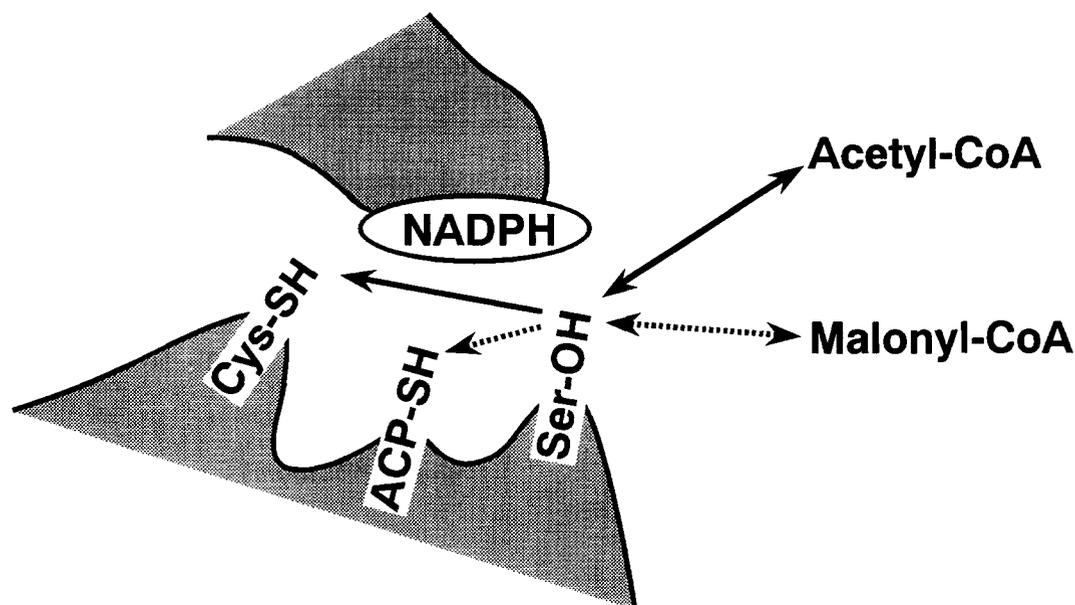
Fig. 3. Schematic presentation of the entry and channeling of acetyl- and malonyl-CoAs at the reaction center of 6-hydroxymellein synthase. Active form of 6-hydroxymellein synthase is organized as a homodimer in which the domains of acyl-CoA condensation and NADPH-dependent keto reduction are associated with each other in functional form, and two SH groups at the reaction center contribute from the same subunit [9,11,12]. The acyl moieties are reversibly transferred from their CoA esters to Ser-OH of the transacylase-like structure as the common primary binding site. The acyl moieties are, then, properly channeled to the two SH groups, acetyl to cysteine-SH of the condensation enzyme and malonyl to cysteamine-SH of ACP, via this domain, respectively.

ified enzyme 2. The amounts of the enzyme-bound acetyl were, however, decreased to the level of approximately 64% upon the post-incubation with performic acid which specifically hydro-lyzes thioesters but not oxyesters [18] suggesting that both Ser-OH and ACP-SH were acetylated in enzyme 5. This modified synthase also accepted twice the number of malonyl moieties, and it decreased to 43% level after the oxidation with performic acid. It appeared, therefore, that ACP-SH is able to accept both acetyl and malonyl moieties from CoA esters via Ser-OH. In constrast, 6-hydroxymellein synthase treated with chloroacetyl-CoA (6) was able to bind about half the number of malonyl groups as compared with that of acetyl. Most part of the en-zyme-bound malonyl were found to be insensitive to the perfor-

mate-treatment suggesting that Ser-OH but not Cys-SH was malonylated. These observations suggested that acetyl moiety is capable of transferring to both Cys-SH and ACP-SH from the primary binding site Ser-OH, while malonyl moiety trans-fers to only ACP-SH. The results also implied that malonyl is channeled from the CoA ester to ACP-SH via Ser-O-ester, however, it remained uncertain whether acetyl is firstly trans-ferred to ACP-SH then channeled to Cys-SH or it is directly channeled to Cys-SH from Ser-O-ester. Therefore, Cys-SH-blocked enzyme (5) was incubated with the mixture of acetyl- and malonyl-CoAs, and the acylated enzyme was post-treated with performic acid. As shown in Table 2, approximately 1.5-fold number of malonyl moieties were associated with this

Table 1
Binding activity of 6-hydroxymellein synthase toward acetyl- and malonyl-CoAs after the treatment with various blocking reagents

Pre-treatment	Substrate	Post-treatment	Enzyme-bound acyl group	
IoAA + ClAcCoA	Acetyl-CoA	–		1.00
IoAA + ClAcCoA	Malonyl-CoA	–		0.78
IoAA + ClAcCoA	Acetyl-CoA + malonyl-CoA	–	Acetyl	0.43
			Malonyl	0.51
IoAA + ClAcCoA + AEBSF	Acetyl-CoA	–		0.17
IoAA + ClAcCoA + AEBSF	Malonyl-CoA	–		0.08
AEBSF	Acetyl-CoA	–		0.09
AEBSF	Malonyl-CoA	–		0.15
IoAA + ClAcCoA	Acetyl-CoA	Acetyl-CoA		0.13
IoAA + ClAcCoA	Acetyl-CoA	Malonyl-CoA		0.06
IoAA + ClAcCoA	Malonyl-CoA	Acetyl-CoA		0.07
IoAA + ClAcCoA	Malonyl-CoA	Malonyl-CoA		0.11

Highly purified 6-hydroxymellein synthase was treated with 10 mM of iodoacetoamide (IoAA), 1 mM of chloroacetyl-CoA (ClAcCoA) and/or 1 mM of AEBSF, and the binding abilities of these modified enzymes toward the substrates were determined by the incubation with ^{14}C-labelled acyl-CoAs. In some experiments, the acylated enzymes were post-treated with excess amount of unlabeled acyl-CoAs. The results were expressed as means of relative values obtained from two independent experiments in which acetyl moiety bound to the IoAA plus ClAcCoA-treated enzyme (modified enzyme 2 in Fig. 2) was taken as 1.00.

Table 2
Binding activity of 6-hydroxymellein synthase toward acetyl- and mlaonyl-CoAs after the blocking of one of the two SH groups at the reaction center

Pre-treatment	Substrate	Post-treatment	Enzyme-bound acyl group	
IoAA	Acetyl-CoA	–		1.76
IoAA	Acetyl-CoA	Performic acid		1.13
IoAA	Malonyl-CoA	–		2.21
IoAA	Malonyl-CoA	Performic acid		0.89
ClAcCoA	Acetyl-CoA	–		1.84
ClAcCoA	Acetyl-CoA	Performic acid		0.86
ClAcCoA	Malonyl-CoA	–		0.76
ClAcCoA	Malonyl-CoA	Performic acid		0.83
IoAA	Acetyl-CoA + malonyl-CoA	–	Acetyl	0.42
			Malonyl	1.61
IoAA	Acetyl-CoA + malonyl-CoA	Performic acid	Acetyl	0.51
			Malonyl	0.62

One of the two SH groups at the reaction center of 6-hydroxymellein synthase was blocked by the treatment with iodoacetoamide (IoAA) or chloroacetyl-CoA (ClAcCoA), and the binding activities of the modified enzymes toward the substrates were determined. In some experiments, the acylated enzymes were oxidized with performic acid to hydrolyze the thioesters. The results were expressed as means of relative values obtained from two independent experiments in which acetyl moiety bound to the IoAA plus ClAcCoA-treated enzyme (modified enzyme 2 in Fig. 2) was taken as 1.00.

modified synthase, and 61% of the malonyl were found to be performate-labile. In contrast, the number of the enzyme-bound acetyl was considerably low, and they were essentially insensitive to the oxidation. These results suggested that malonyl moiety preferentially transfers to ACP domain in the co-presence of the substrates, and acetylation of ACP-SH takes place, if any, in negligible extent.

4. Discussion

In the present experiments, it has been demonstrated that Ser-OH group of a certain structure in 6-hydroxymellein synthase is a common primary binding site of acetyl- and malonyl-CoAs, the co-substrates of the enzyme. It appeared that acetyl and malonyl groups, the building units of ketomethylene chain, are properly channeled from their CoA esters to Cys-SH and ACP-SH via the Ser-containing domain, respectively. These observations strongly suggest that transacylase or the related domain is involved in the structure of 6-hydroxymellein synthase, and, to my knowledge, this is the first report which demonstrates the occurrence of this domain in PKS of higher plants. Hypothetical scheme of the substrate entry and channeling in 6-hydroxymellein synthase is presented in Fig. 3, and one of the most likely explanation for the early events of the synthase catalyzing reactions would be as follows. Acetyl- and malonyl-CoAs firstly acylate Ser-OH of the transacylase-like domain in the enzyme, and the malonyl moiety is channeled from the Ser-O-ester to ACP-SH while acetyl moiety is directly channeled to Cys-SH. When one of the two SH groups is appropriately acylated, the Ser residue of the transacylase-like domain would exchange the acyl groups to the proper ones by rapid replacement. In ACP-malonylated enzyme, acetylated Ser channels it to free Cys-SH while malonylated Ser would exchange the moiety to acetyl. In Cys-acetylated enzyme, Ser-bound malonyl moiety is transferred to ACP-SH while Ser-O-acetyl would exchange the acyl group to malonyl moiety prior to the acyl transfer to ACP-SH. Any way, it seems that combination of the rapid exchange of the acyl moieties at Ser-OH and limited or preferential acylation abilities of Ser-O-acetyl and Ser-O-malonyl toward the two SH groups would be essential for channeling of the acyl groups onto proper thiols.

In FASs, characterization of transacylase domain and chan-

neling pathways of acyl moieties to the SH groups at the reaction center have been investigated in several prokaryotic and eukaryotic cells [1]. It is well known that, in bacterial FAS system composed of separable components, both acetyl- and malonyl-CoAs are transferred to ACP by the action of transacylase, and then, to SH group of the condensation enzyme [1]. Similar channeling pathway has also been reported in multifunctional FAS of animal cells [23]. However, in yeast FAS, it was shown [24] that acetyl moiety is directly channeled to Cys-SH via Ser-OH of transacylase domain without acylating ACP-SH. These findings suggest that even if the general architecture of FASs that catalyzes the entry and channeling of the co-substrates would resemble in a wide variety of organisms, the channeling pathways of the acyl moieties utilizing these domains are varied and depend on the source of FASs. By contrast, in plant PKSs, occurrence of either ACP or transacylase is not evident in chalcone and stilbene synthases, and acyl-CoAs are assumed to transfer directly to Cys-SH of the condensation enzyme [3,5,6]. On the other hand, results obtained in the present study demonstrate that transacylase-like domain is involved in the structure of 6-hydroxymellein synthase, and channeling pathways of acyl groups resembles yeast FAS although location of ACP-like domain in the polypeptide chain of the enzyme is quite different from that in multifunctional FASs [1,12]. Therefore, it is possible that not only the entry and channeling pathways of the substrates but also the functional domains and their arrangement in the protein structure which are essential for catalyzing these processes are diverse in PKSs of higher plants. Further characterization of the partial reactions of 6-hydroxymellein synthase is in progress in my laboratory.

References

[1] Wakil, S.J. (1989) Biochemistry 28, 4523–4530.
[2] Stumpf, P.K. (1984) in: Fatty Acid Metabolism and Its Regulation (Numa, S.Ed.) pp. 155–180, Elsevier Science, Amsterdam.
[3] Hopwood, D.A. and Sherman, D.H. (1990) Annu. Rev. Genet. 24, 37–66.
[4] Katz, L. and Donadio, S. (1993) Annu. Rev. Microbiol. 47, 875–912.
[5] Lanz, T., Tropf, S., Marner, F.J., Schröder, J. and Schröder, G. (1991) J. Biol. Chem. 266, 9971–9976.

[6] Tropf, S., Kächer, B., Schröder, G. and Schröder, J. (1995) J. Biol. Chem. 270, 7922–7928.

[7] Kurosaki, F. and Nishi, A. (1983) Phytochemistry 22, 669–672.

[8] Kurosaki, F., Kizawa, Y. and Nishi, A. (1989) Eur. J. Biochem. 185, 85–89.

[9] Kurosaki, F., Itoh, M., Kizawa, Y. and Nishi, A. (1993) Arch. Biochem. Biophys. 300, 157–163.

[10] Kurosaki, F. and Nishi, A. (1989) FEBS Lett. 227, 183–186.

[11] Kurosaki, F., Itoh, M., Yamada, M. and Nishi, A. (1991) FEBS Lett. 288, 219–221.

[12] Kurosaki, F. (1995) Arch. Biochem. Biophys. 321, 239–244.

[13] Wakil, S.J. and Stoops, J.K. (1983) in: The Enzymes: Structure and Mechanism of Fatty Acid Synthetase (Boyer, P. Ed.), vol. 16, pp. 3–61, Academic Press, New York.

[14] Kurosaki, F., Matsui, K. and Nishi, A. (1984) Physiol. Plant Pathol. 25, 313–322.

[15] Kawaguchi, A., Yoshimura, T. and Okuda, S. (1981) J. Biochem. 89, 337–339.

[16] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.

[17] Laemmli, U.K. (1970) Nature 227, 680–685.

[18] Schuster, H., Rautenstrauss, B., Mittag, M., Stratmann, D. and Schweizer, E. (1995) Eur. J. Biochem. 228, 417–424.

[19] Anderson, G.J. and Kumar, S. (1987) FEBS Lett. 220, 323–326.

[20] McCarthy, A.P., Alastair, A.D., Hardie, D.G., Santikara, S. and Williams, D.H. (1983) FEBS Lett. 160, 296–300.

[21] Markwardt, F., Drawert, J. and Walsmann, P. (1974) Biochem. Pharm. 23, 2247–2256.

[22] Dunn, B.M. (1989) in: Proteolytic Enzymes, a Practical Approach (Beynon, R.J. and Bond, J.S. Eds.), pp. 57–82, IRL Press, Oxford.

[23] Yuan, Z. and Hammes, G.G. (1985) J. Biol. Chem. 260, 13532–13538.

[24] Stoops, J.K., Singh, N. and Wakil, S.J. (1990) J. Biol. Chem. 265, 16971–16977.