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An endogenous proteinacious inhibitor in porcine liver for *S*-adenosyl-L-methionine dependent methylation reactions: identification as oligosaccharide-linked acyl carrier protein

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Abstract

A proteinacious inhibitor of *S*-adenosyl-L-methionine (AdoMet)-dependent transmethylation reactions was purified to homogeneity from porcine liver by size exclusion chromatography and FPLC. The molecular weight of the inhibitor was 12,222 Da. A 7400 Da polypeptide fragment of the purified inhibitor was sequenced by matrix-associated laser desorption ionization; time-of-flight MS, and was found to be identical with the known sequence of spinach acyl carrier protein (ACP). Although the remainder of the molecule was not clearly defined, ^{1}H and H—H correlation of spectroscopy (COSY) NMR analysis revealed the presence of an oligosaccharide with α-glycosidic linkage. The purified oligosaccharide-linked ACP inhibited several AdoMet-dependent transmethylation reactions such as protein methylase I and II, *S*-farnesylcysteine *O*-methyltransferase, DNA methyltransferase and phospholipid methyltransferase. Protein methylase II was inhibited with a K_i value of 2.4×10^{-3} M in a mixed inhibition pattern, whereas a well-known competitive product inhibitor *S*-adenosyl-L-homocysteine (AdoHcy) had K_i value of 6.3×10^{-6} M. Commercially available active ACP fragments (65–74) and ACP from *Escherichia coli* had less inhibitory activity toward *S*-farnesylcysteine *O*-methyltransferase than the purified inhibitor. The biological significance of this oligosaccharide-linked ACP which has two seemingly unrelated functions (inhibitor for transmethylation and fatty acid biosynthesis) remains to be elucidated.

Keywords: Oligosaccharide-linked acyl carrier protein; Endogenous inhibitor; Transmethylation

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1. Introduction

S-Adenosyl-L-methionine (AdoMet) an important biological compound serves as a methyl donor in a wide variety of methylation reactions involving proteins, lipids, nucleic acids, polysaccharides, amino acids and hormones [1–6]. *S*-Adenosyl-L-homocysteine (AdoHcy), the reaction product of AdoMet-dependent transmethylation reactions is considered to be one of the most potent in vivo modulators of transmethylation reactions. The biological importance of AdoMet dependent transmethylation reactions leads to suggestions that the in vivo ratio of AdoMet to AdoHcy plays a crucial role in maintaining the status of biological methylation [7,8].

AdoHcy and several endogenous factors have been found to modulate some transmethylation reactions in vivo [9–12]. Kim and Paik identified an inhibitor for protein methylase II [AdoMet:-protein-carboxyl *O*-methyltransferase; EC 2.1.1.77] in rat liver particulate fractions [9]. The inhibitor was non-dialyzable, thermolabile and partly inactivated by tryptic digestion. Subsequently, Chiva and Mato obtained two proteinacious inhibitors in rat liver cytosol, which inhibited phosphatidylethanolamine methyltransferase activity (EC 2.1.1.17) [12]. These inhibitors with apparent molecular weights of 3200 and 1600 Da were resolved on Bio-Gel P-6 column and found to be resistant to the action of DNase and Rnase but were partly inactivated by subtilisin. In the present study, we identified and purified a proteinacious inhibitor from porcine liver. The molecular weight of the purified inhibitor was 12,222 Da, and the molecule was composed of two components; a polypeptide of 7400 Da and the remainder of oligosaccharide. The amino acid sequence analysis of the protein portion by matrix-associated laser desorption ionization; time-of-flight MS (MALDI-TOF MS) was found to be identical with the known sequence of acyl carrier protein (ACP) and the presence of oligosaccharides in the purified inhibitor was deduced by ^{1}H and H—H correlation of spectroscopy (COSY) NMR analysis.

2. Materials and methods

2.1. Materials

S-Adenosyl-L-[methyl-^{14}C]methionine (specific activity, 54 mCi/mmol in H_2SO_4), [9,10(n)-^{3}H]palmitic acid (specific activity, 50 Ci/mmol), HiPrep Sephacryl S-300 and HiLoad Superdex 75 column were purchased from Amersham Pharmacia Biotech, UK. Histone (type II-AS from calf thymus; a mixture of various histone fractions), *N*-acetyl-*S*-farnesyl-L-cysteine (AFC), ACP from *Escherichia coli*, ACP active fragment (VQAAIDYING) from *E. coli*, *E. coli* acyl-ACP synthetase, bovine serum albumin, Sephadex G-25 (exclusion limit, 5,000 Da), and AdoHcy were obtained from Sigma Chemical Co. The rest of the chemicals were from various commercial sources and of the highest grade available.

2.2. Partial purification of protein methylase II and assay for inhibitor

To test the inhibitory activity of an endogenous inhibitor for AdoMet-dependent transmethylation reactions, protein methylase II [AdoMet: protein-carboxyl *O*-methyltransferase: EC. 2.1.1.77] was chosen. Protein methylase II was partially purified from porcine liver according to the previously described method by Kim [13]. This involved the homogenization of freshly porcine liver in 4 vols of 0.25 M sucrose solution containing 5 mM EDTA, 2.4 mM 2-mercaptoethanol and 1 mM phenylmethylsulfonyl fluoride (PMSF). The whole homogenate was centrifuged at 105,000 *g* for 1 h. The supernatant obtained was then treated with finely powdered ammonium sulfate at 50% saturation. The precipitate obtained at 15,000 *g* for 30 min was dialyzed for 12 h against 20 mM Tris–HCl buffer (pH 8.0) containing 5 mM EDTA and 2.4 mM 2-mercaptoethanol. The enzyme preparation remaining inside the bag was lyophilized and stored at $-70^{\circ}C$ for future use. The preparation had a specific activity of 7 pmol of Ado [methyl-^{14}C]Met transferred/min/mg protein.

The incubation mixture which contained 50 mM citrate–phosphate buffer (pH 6.0), 0.6 mg of histone suspension, protein methylase II and the inhibitor in a total volume of 0.09 ml was preincubated at 37°C for 5 min. This was further incubated for 15 min following the addition of 0.01 ml of 25 μM Ado[methyl-^{14}C]Met (130 dpm/pmol). After incubation, 0.10 ml of 125 mM sodium-borate buffer (pH 11.0) was added to the incubation mixture and the mixture was further incubated for 5 min at 37°C. 1 ml of isoamyl alcohol was added and the mixture vortexed. Following centrifugation 0.70 ml of the supernatant was counted for radioactivity. Protein concentration was determined by the method of Bradford [14], employing bovine serum albumin as a standard.

2.3. Partial purification and assay for S-farnesylcysteine O-methyltransferase

Freshly obtained rat brain was homogenized in 7 vols of 250 mM sucrose solution (pH 7.4) containing 10 mM Tris–HCl and 1 mM EDTA by Bio-homogenizer for 2 min at 1,200 rpm [15]. The whole homogenate was centrifuged at 14,500 *g* for 12 min. The supernatant was further centrifuged at 105,000 *g* for 1 h and the pellet was suspended in the above homogenizing buffer and stored at −70°C until use. The preparation had a specific activity of 4.3 pmol of Ado [methyl-^{14}C]Met transferred/min/mg protein.

An assay for *S*-farnesylcysteine *O*-methyltransferase activity was carried out according to the method of Stephenson and Clake [15] with slight modification. Briefly, the incubation mixture containing 33 mM HEPES (pH 7.6), 33 mM NaCl, 0.1 mM *N*-acetyl-*S*-farnesyl-L-cysteine (AFC), the enzyme preparation and the inhibitor (without the inhibitor in the case of control) in a total volume of 0.09 ml was preincubated at 37°C for 5 min and further incubated for 15 min after the addition of 0.01 ml of 25 μM Ado[methyl-^{14}C]-Met. The reaction was terminated with 0.05 ml of 1.0 N NaOH containing 1% SDS, the mixture was thoroughly vortexed and a 0.12 ml aliquot (total 0.15 ml) was transferred to a pleated filter paper (1.5 × 8 cm, Whatman No.1). The filter paper was placed on the neck of scintillation vial (20 ml-capacity) containing 10 ml of scintillation cocktail solution and left at room temperature for 2 h (base-hydrolysis). After removing the paper, the amount of volatile $^{14}CH_3OH$ formed was measured. The difference in radioactivities between the presence and absence of AFC was taken for enzyme activity.

2.4. Purification of the inhibitor from porcine liver

Forty grams of fresh porcine liver was cut into small pieces and homogenized in 4 vols of 1 mM phosphate buffer (pH 7.4) by Bio-homogenizer for 2 min at 1,200 rpm. The whole homogenate was passed through a double layer of cheesecloth and centrifuged at 12,500 g for 20 min at 4°C (the following procedures were carried out at around 4°C). The supernatant was further centrifuged at 105,000 *g* for 1 h. The supernatant obtained was boiled for 15 min and coagulated protein removed by centrifugation at 15,000 *g* for 20 min. The supernatant was then lyophilized, the lyophilized material was dissolved in 5.0 ml of 1 mM phosphate buffer (pH 7.4) and the suspension loaded onto a Sephadex G-25 column [exclusion limit of 5,000 Da; 1.4 cm (i.d.) × 120 cm] which had been equilibrated with the above phosphate buffer. The column was eluted with the phosphate buffer at a flow rate of 30 ml/h. A 0.5 ml aliquot of each 7.5 ml fraction was concentrated by lyophilization (this was done during the early stage of purification, as the volume of inhibitor was too small to directly examine the activity prior to concentration), and the inhibitory activity on protein methylase II was assayed. Fractions containing the inhibitory activity (peak A sample in Fig. 1) were pooled, lyophilized and dissolved in 1 ml of 1 mM phosphate buffer (pH 7.4). The inhibitor was further purified by rechromatography on Sephadex G-25 column [1.4 cm (i.d.) × 120 cm] under the same condition as in Fig. 1 (Fig. 2a). The fractions containing the inhibitory activity were pooled, lyophilized and reconstituted to 1 ml with the phosphate buffer. The suspension was loaded on a HiPrep Sephacryl S-300 column [1.6 cm (i.d.) × 60 cm] and the column eluted with the above

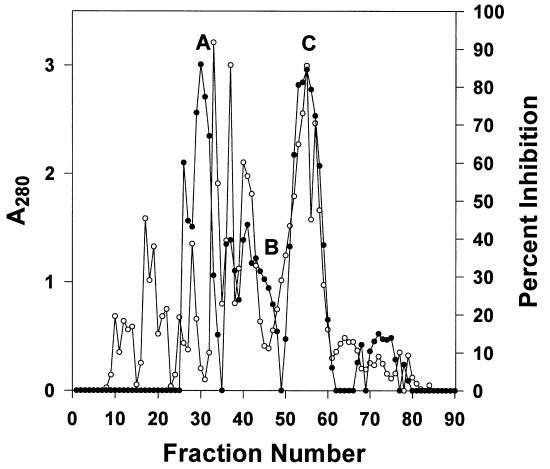

Fig. 1. Chromatogram of porcine liver soluble fraction on Sephadex G-25 column. ○, A_{280}; and ●, inhibitory activity on protein methylase II. Sephadex column [1.4 cm (i.d.) × 120 cm] was used. The experimental procedures are described under "Materials and methods".

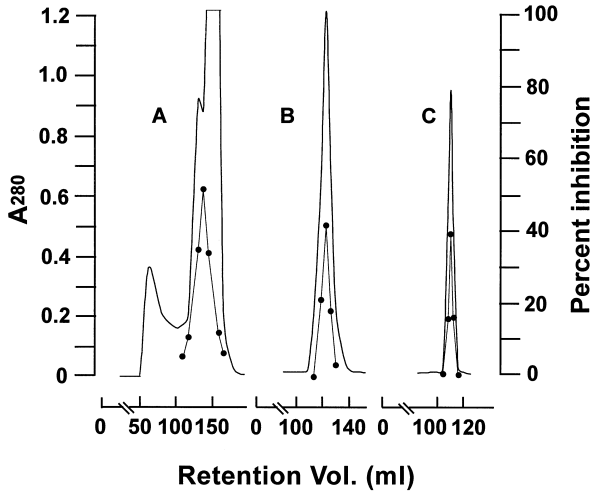

Fig. 2. Chromatogram of the peak A in Fig. 1 on Sephadex G-25 (A), HiPrep Sephacryl S-300 (B) and HiLoad Superdex 75 column (C). (A) Peak A sample in Fig. 1 was lyophilized and suspended in 1 ml of 1 mM phosphate buffer (pH 7.4). The suspension was rechromatographed on Sephadex G-25 column [1.4 cm (i.d.) × 120 cm] under the same condition as in Fig. 1. (B) One ml of pooled suspension containing inhibitory activity was applied onto HiPrep Sephacryl S-300 column [1.6 cm (i.d.) × 60 cm] and the column was eluted with the same buffer. (C) One ml of pooled suspension containing inhibitory activity was applied onto HiLoad Superdex 75 column [1.6 cm (i.d.) × 60 cm] and the column was eluted with the same buffer. The inhibitory fractions were pooled and lyophilized. —, A_{280}; and ●, inhibitory activity.

phosphate buffer. 2.5 ml fractions were collected at a flow rate of 30 ml/h. A 0.5 ml aliquot from each fraction was lyophilized and reconstituted to 0.030 ml for the inhibitory activity. The inhibitory fractions was pooled (Fig. 2b), lyophilized and dissolved in 1 ml of the phosphate buffer. The suspension was applied onto HiLoad Superdex 75 column [1.6 cm (i.d.) × 60 cm] and the column eluted with the same phosphate buffer. 1 ml fractions were collected at a flow rate of 60 ml/h and from each fraction an aliquot of 0.030 ml was assayed for the inhibitor. The inhibitory fractions were pooled (Fig. 2c), lyophilized, and the lyophilized sample was dissolved in 1 ml of water and stored at −70°C.

2.5. Structural identification of the inhibitor by NMR and MALDI-TOF MS

NMR (Bruker AMX-500, Germany), MALDI-TOF MS (Perseptive analytical Elite, USA) and MALDI-TOF MS/MS (Kratos, compact maldi III, UK) were employed for structural assignment of the purified inhibitor.

2.6. Assay for acyl-ACP synthetase (Table 1)

The amino acid sequence of the purified inhibitor was identical to that of ACP. The ACP activity in the inhibitor preparation was therefore measured by determining [^{3}H]palmitic acid incorporation into ACP by *E. coli* acyl-ACP synthetase (EC.6.2.1.20). 380 nM [^{3}H]palmitic acid (50,000 dpm/pmol), 76 mM Tris–HCl (pH 8.0), 3.81 mM ATP, 304 mM LiCl, 7.6 mM $MgCl_2$, 1.5 mM dithiothreitol (DTT), *E. coli* acyl-ACP synthetase and 10 μM of ACP preparation (ACP from *E. coli*, ACP active fragment from *E. coli* or the purified inhibitor) were incubated at 37°C in a 1.5-ml microcentrifuge tube in a final volume of 0.05 ml [16]. After 20 min, the reaction mixture was applied to Whatman 3MM filter paper disks (2 cm^2) which had been dried at room tem-

perature and rinsed three times with a mixture of chloroform:methanol:acetic acid (3:6:1, vol/vol) to remove unbound fatty acid. The filter disk was then transferred to scintillation vials, 5 ml of scintillation cocktail was added, and the amount of [^{3}H]-labelled ACP counted.

3. Results and discussion

3.1. Purification of an endogenous inhibitor

When porcine liver soluble fraction was heated at 100°C for 15 min and the supernatant at 15,000 *g* was applied onto Sephadex G-25 column (exclusion limit: 5,000 Da), numerous A_{280} absorbance peaks were apparent. However, three major peaks for the inhibitory activity of protein methylase II were apparent (Fig. 1). These peaks at fraction numbers 30, 41 and 55 were designated as peak A, B, and C, respectively.[1] The material under the peak A was further purified by procedures involving Sephadex G-25 rechromatography, FPLC HiPrep Sephacryl S-300 and HiLoad Superdex 75 column chromatographies (Fig. 2a–c). Producing about 0.092 mg of highly purified material from 40 g of fresh porcine liver. The material under peak B has not been considered in the present work.

3.2. Chemical characterization of the purified inhibitor by NMR and MALDI-TOF MS

As the first step of characterization the approximate molecular size of the molecule was estimated employing HiLoad Superdex 75 column chromatography and found to be very close to that of cytochrome *c* whose molecular weight was 12,400 Da (data not shown). Subsequently, employing both MALDI-TOF MS and SDS–PAGE, the molecular weight of the inhibitor was accurately determined to be 12,222.4 Da (Fig. 3). In a single-stage TOF-MS, a simple relationship exists between the flight time and fragment-ion mass. Based on such sets of data and the actual flight times of precursor and fragment ions recorded, a computer can calculate the fragment-ion masses to the desired accuracy by iteration [18]. In practice, an algorithm was integrated into our PC-based TOF-MS data acquisition and processing software (Grams/32 Array Basic, Ver 4.0, Galactic industries Corporation, USA). Data acquisition was performed by a transient recorder at 10 ns sampling rate and 10 Hz duty cycle. Fifty-one hundred spectra were summed for each reflector setting [18]. The sequence of 7,400 Da was found to be completely identical to the known sequence of spinach ACP with a single exception, residue-77 (Table 1). It should be mentioned here that MALDI has many advantages over other methods of ion formation; such as high sensitivity and resolution, the extended mass range to over 100,000 Da, and fast analysis [19].

Preliminary analysis of the inhibitor indicated the molecule contained oligosaccharides. The presence and linkage pattern of oligosaccharides to the peptide were deduced by ^{1}H and H—H correlation of spectroscopy (COSY) NMR analysis. Data of ^{1}H-NMR (D_2O) for oligosaccharides analysis were as follows. ^{1}H-NMR (500 MHz, D_2O) δ:5.07 (1H, d, J = 4.0 Hz, glu-1), 3.39 (1H, glu-2), 3.76 (1H, glu-3), 3.68 (1H, glu-4), 3.96 (1H, glu-5), 3.71, 3.72 (1H, glu-6a,b), 4.70 (1H, d, J = 4.0 Hz, gal-1), 3.32 (1H, gal-2), 3.59 (1H, gal-3), 3.94 (1H, gal-4), 3.65 (1H, gal-5), 3.64, 3.74 (1H, gal-6a,b). The coupling of H-1 and H-2 in galactose was determined by 2D H—H COSY NMR analysis. The chemical shift of both H-5 (glu-5 = 3.96; gal-5 = 3.65) and H-6 (glu-6a,b = 3.71, 3.72; gal-6a,b = 3.64, 3.74) and the lack of substitution for H-6a,b in galactose were confirmed by triple quantum-filtered (TQF) NMR analysis. The α-glycosidic linkage pattern of the glucose to galactose was conclusively established by coupling constant (J = 4.0 Hz; if β-glycosidic bond, J = 7.0 Hz). The presence of glucose and galactose was confirmed by TLC analysis of the acid hydrolysate (data not

[1] It should be mentioned here that, in our previous study [17], we purified the material under peak C to homogeneity and found it to be a conjugate of *S*-adenosylhomocycteine with a peptide of molecular mass of 1400 Da.

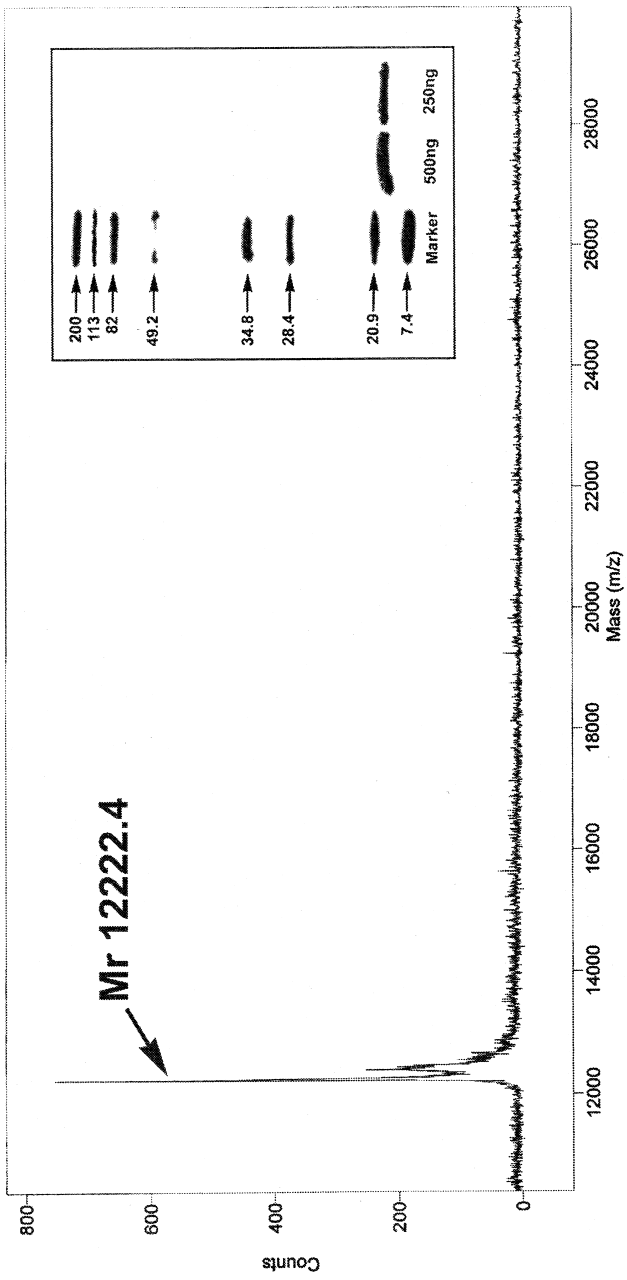

Fig. 3. Analysis of purified inhibitor by MALDI-TOF MS and SDS–PAGE. The molecular weight of the purified oligosaccharide-linked ACP on HiLoad Superdex 75 column chromatography was determined using MALDI-TOF MS (Perseptive analytical Elite, USA) and MALDI-TOF MS/MS (Kratos, compact maldi III, UK). Dihydrobenzoic acid (DHB) was dissolved in a solution mixture of acetone and water (1:1). The purified proteinacious inhibitor to be examined was first dissolved in 0.1% trifluoroacetic acid, and then was added to the matrix solution, so that the final protein concentration of the mixture was less than 0.1 g/L. Typically, 0.5 μl of matrix/protein solution was then applied to a metal probe tip and dried. *Inset:* The molecular weight of the oligosaccharide-linked ACP on SDS–PAGE was estimated to be similar to that on MALDI-TOF MS/MS.

Table 1
Amino acid sequences of ACP and purified inhibitor[a]

I	1	AKKETIDKVSDIVKEKLALGADVVVTADSEFSKLGADSLD	40
A	2	AKKETIDKVSDIVKEKLALGADVVVTADSEFSKLGADSLD	41
I	41	TVEIVMNLEEEFGINVDEDKAQDISTIQQAADVIELLLEKKA	82
A	42	TVEIVMNLEEEFGINVDEDKAQDISTIQQAADVIEGLLEKKA	83

[a] The primary sequences of A (spinach acyl-carrier protein-I, GenBank) and I (the purified inhibitor) show good correspondence to the identities (81/82; 98%) and positives (81/82; 98%) with only one exception of residue 77th.

shown). The coupling of galactose H-1 at 4.50 ppm was assigned and identified in H—H COSY NMR analysis. Therefore, according to a published report [20], it was highly likely that galactose of oligosaccharide portion was linked to serine residue of polypeptide portion in the molecule. However, the full identity of the 12,222 Da molecule and a definitive linkage site of the oligosaccharides to the peptide remains to be found. Our results have therefore established that the purified endogenous inhibitor is a glycoprotein with a molecular weight of 12,222 Da and that the polypeptide segment is identical to ACP.

3.3. Inhibition kinetics

Lineweaver–Burk plot of inhibitory activity of the oligosaccharide-linked ACP toward protein methylase II with AdoMet as a methyl donor is shown in Fig. 4a. It shows a mixed inhibitory pattern with K_i value of 2.4×10^{-3} M. This mixed inhibitory mode describes the overall pattern, but does not indicate the presence of more than one type of inhibitor [21]. For comparison, Fig. 4b shows AdoHcy, a well known potent inhibitor of transmethylation reactions, to be competitive with K_i value of 6.3×10^{-6} M.

3.4. Inhibitory effect of the oligosaccharide-linked ACP on various AdoMet-dependent methyltransferases

The inhibitory effect of the oligosaccharide-linked ACP was the strongest on protein methylase II, followed by *S*-farnesylcysteine *O*-methyltransferase (3rd column of Table 2). The oligosaccharide-linked ACP and AdoHcy additively inhibited all the AdoMet-dependent transmethylation reactions tested (the last column); sums of inhibitory activities by either the oligosaccharide-linked ACP or AdoHcy alone were equal to those observed in combined treatment

Table 2
Inhibitory effects of the oligosaccharide-linked ACP and AdoHcy on various AdoMet-dependent methyltransferases[a]

AdoMet-dependent methyltransferases tested	Inhibition (%)		
Protein methylase II	48.2 ± 3.8	53.1 ± 2.8	93.2 ± 6.4
S-Farnesylcysteine *O*-methyltransferase	39.9 ± 4.5	43.1 ± 6.4	79.0 ± 3.6
DNA methyltransferase	52.7 ± 2.8	31.9 ± 8.3	66.0 ± 4.2
Protein methylase I	44.6 ± 3.2	31.1 ± 6.4	71.3 ± 2.6
Phospholipid methyltransferase[b]	37.5 ± 2.4	29.5 ± 3.7	76.5 ± 8.1
Catechol-*O*-methyltransferase	51.7 ± 9.3	11.3 ± 5.7	75.7 ± 3.3
AdoHcy	+	–	+
Oligosaccharide-conjugated ACP	–	+	+

[a] The final concentrations of AdoHcy and oligosaccharide-conjugated ACP in each incubation mixtures were 10 μM and 2 mM, respectively. Results are expressed as percentage inhibition of the control (without inhibitor) and represent mean ± SE of duplicate determinations from three separate experiments.

[b] Phospholipid methyltransferase was treated with 100 μM AdoHcy.

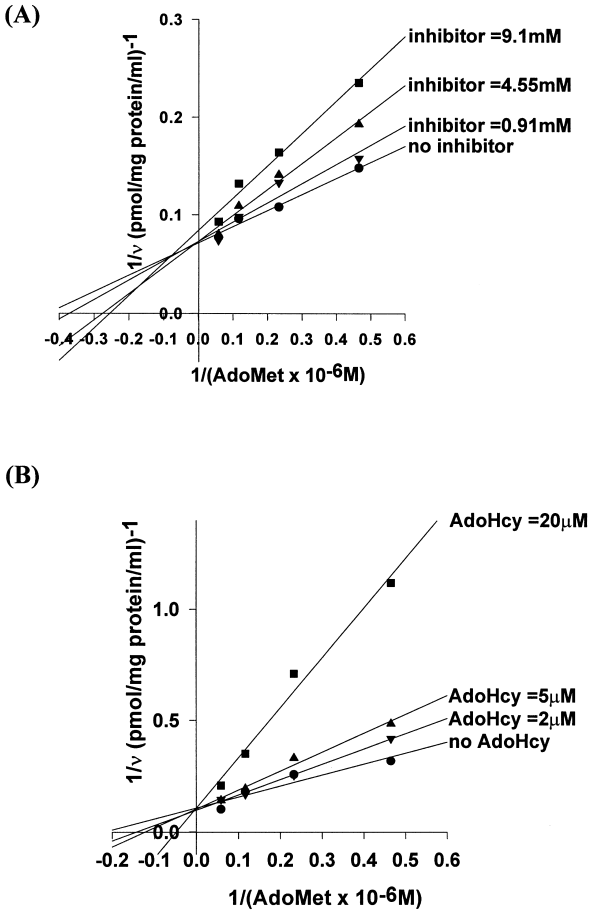

Fig. 4. Lineweaver–Burk plot of the oligosaccharide-linked ACP and AdoHcy on protein methylase II. (A) Assay was performed by varying the concentration of the oligosaccharide-conjugated ACP (0–9.1 × 10^{-3} M) and AdoMet (2.15–17.2 × 10^{-6} M). The K_i value was 2.4 × 10^{-3} M. (B) Assay was performed by varying the concentrations of AdoHcy (0–20 × 10^{-6} M) and AdoMet (2.15–17.2 × 10^{-6} M). The K_i value was 6.3 × 10^{-6} M. Detailed conditions are described under "Materials and methods".

with two agents. The result in Table 2 is compatible with the result in Fig. 4, indicating their modes and sites of inhibition to be quite different.

3.5. Effect of the oligosaccharide-linked ACP, ACP, and an active ACP fragment on rat brain S-farnesylcysteine O-methyltransferase

Employing *S*-farnesylcysteine *O*-methyltransferase, the inhibitory effect of various ACP preparations was examined (Table 3). It was found that both ACP and an active ACP fragment from *E. coli* were far less effective than purified oligosaccharide-linked ACP. This might be due to the difference in amino acid sequences (homology 44%) between the purified ACP and *E. coli* ACP or to the presence of a conjugated carbohydrate segment.

3.6. Various ACP preparations as substrates for acyl-ACP synthetase

Formation of [^{3}H]palmityl ACP catalyzed by

Table 3
Effects of various ACP preparations on *S*-farnesylcysteine *O*-methyltransferase[a]

ACPs tested	Inhibition (%)
Oligosaccharide-conjugated ACP	46.5 ± 6.4
ACP from *E. coli*	0
Active fragment 65–74 of ACP from *E. coli*[b]	18.5 ± 3.8

[a] The final concentration of the preparations in each incubation mixture was 2 mM. Results are expressed as percentage inhibition of the control (without inhibitor; the specific activity of 4.3 pmol of Ado[methyl-^{14}C]Met used/mg protein/min). The values represent mean ± SE of duplicate determinations from three separate experiments.

[b] The amino acid sequence of ACP active fragment 65–74 from *E. coli* is as follows: VQAAIDYING.

E. coli acyl-ACP synthetase is shown in Table 4. The purified oligosaccharide-linked ACP has an inhibitory effect on AdoMet-dependent transmethylation reactions. It also accepts the palmityl group through the action of acyl-ACP synthetase (Table 4). The amount of palmityl ACP produced by acyl-ACP synthetase is very close to that of *E. coli*, whereas that of the ACP active fragment from *E. coli* is quite low. These results demonstrate that the purified oligosaccharide-linked ACP has two functions; typical activity to accept acyl group as well as a regulatory function in transmethylation reactions.

Proteinacious factors which inhibit AdoMet-dependent transmethylation reactions were purified from porcine liver cytosol. The structural analysis of the purified inhibitor with the molecular weight of 12,222 Da was carried out by ^{1}H, H—H COSY NMR and MALDI-TOF MS spectral data. The amino acid sequence of 7,400 Da peptide portion and the presence of α-glycosidic linkage of glucose and galactose were reported. We examined the possibility this inhibitor regulated carboxylmethylation in vitro and found that the inhibitor had a mixed inhibition pattern with AdoMet with K_i value of 2.4×10^{-3} M (Fig. 4). The mode of inhibition of AdoHcy was competitive with AdoMet with K_i value of 6.3×10^{-6} M. To the best of our knowledge, this is the first report on the presence of oligosaccharide-linked ACP in porcine liver and its inhibitory effect on various AdoMet-dependent methyltransferases.

To examine the binding capacity of acyl groups into oligosaccharide-linked ACP, we carried out an acyl-ACP synthetase assay using *E. coli* acyl-ACP synthetase and [^{3}H]palmitic acid. The binding capacity of the oligosaccharide-linked ACP was similar to that of commercially available *E. coli* ACP (Table 4). The result, therefore, indicated dual functionality of purified oligosaccharide-linked ACP, affecting both fatty acid synthesis and transmethylation. However, the exact biological significance of posttranslational modification of ACP by oligosaccharide is unclear at present. Since activation of Ras as a relay between growth factor receptor tyrosine kinases and the serine/threonine kinase network requires carboxymethylation of p21^{ras} protein [22–25], we are at present conducting further study into nature and role of ACP and/or its oligosaccharides-linked form on transmethylation, as an attractive strategy for regulating methylation.

Acknowledgements

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Table 4
Binding of palmityl group to the purified oligosaccharide-linked ACP[a]

ACPs tested	[^{3}H]Palmityl bound to ACP (Unit/ml/min)
Oligosaccharide-conjugated ACP	0.0817
ACP from *E. coli*	0.0762
Active fragment 65–74 of ACP from *E. coli*	0.0027

[a] The final concentration of the ACPs in each incubation mixture was 10 μM. Detailed experimental conditions are described under ''Materials and methods''. The values are averages of two independent determinations.

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