Inhibition of Acyl-CoA : Cholesterol Acyltransferase by Isohalobacillin, a Complex of Novel Cyclic Acylpeptides Produced by *Bacillus* sp. A1238

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A complex of metabolites consisting of two isomeric cyclic acylpeptides was isolated from a culture of *Bacillus* sp. A1238 by successive chromatographies on Amberlite XAD-7, silica gel and silica ODS columns. By a combination of spectroscopic and chemical analyses, the two subcomponents were identified as isomers of halobacillin, and the complex was designated isohalobacillin. Each molecule of isohalobacillin subcomponents contains either a 3-hydroxy-1-oxo-13-methyltetradecyl or a 3-hydroxy-1-oxo-12-methyltetradecyl moiety in place of a 3-hydroxy-1-oxopentadecyl moiety that is found in the halobacillin molecule. In a cell-free assay, isohalobacillin inhibited acyl-CoA : cholesterol acyltransferase by 50% at a concentration of 50 μM. When added to a culture of macrophage J774, the agent inhibited oxidized low density lipoprotein-induced synthesis of cholesteryl ester from [^{14}C]oleate without affecting surface binding, internalization and degradation of the lipoprotein in the cells.

Acyl-CoA : cholesterol acyltransferase (ACAT) (EC 2.3.1.26), a microsomal enzyme that catalyzes the synthesis of cholesteryl esters from acyl-CoA and cholesterol, plays key roles in both intestinal absorption of cholesterol[1] and cholesteryl ester accumulation in macrophages incubated with modified low density lipoprotein (LDL), such as acetylated LDL[2]. In the course of our search for inhibitors of cholesteryl ester formation in macrophages, a complex of novel cyclic acylpeptides, designated isohalobacillin, was isolated from a culture of *Bacillus* sp. A1238. The present communication deals with the isolation, structural characterization and biological activity of isohalobacillin.

Experimental

General Procedures

The UV spectrum was measured in methanol on a model 320 spectrometer (Hitachi, Japan) and the IR spectrum on a IR-810 spectrometer (JASCO, Japan) as a pellet with KBr. Electron impact (EI) and fast atom bombardment (FAB) mass spectra were taken on a SX-102A spectrometer (JEOL, Japan). NMR spectra were measured on a GX-270 spectrometer (JEOL) at 270 MHz (for ^{1}H) and 67.9 MHz (for ^{13}C). The optical rotation was measured on a DIP-360 (JASCO). Elemental analysis was performed on a model MT-3 CHN Corder (Yanagimoto, Japan). Amino acid analysis was carried out on a model 835 analyzer (Hitachi). Gas chromatography-mass spectrometry (GC-MS) was carried out using a capillary column (CP-Sil 88, 0.25 mm × 50 m, GL Sciences, Japan) on a JMS-DX300 spectrometer (JEOL). The column temperature was kept at 50°C for 1 minute after injection of a sample and then increased at a rate of 32°C/minute up to 150°C, subsequently at a rate of 4°C/minute up to 225°C.

Fermentation and Isolation

The producing strain, A1238, was isolated from a soil sample collected in the suburbs of Tokyo. This strain consisted of endospore-forming Gram-positive rods; it was found to belong to the genus *Bacillus* by taxonomic studies based on the BERGEY'S Manual of Systematic Bacteriology[3]. The strain was grown aerobically at 28°C for 4 days in a medium containing 1% glucose, 3% corn starch, 0.5% polypeptone, 1% soy bean meal, 0.5% yeast extract, 0.2% $CaCO_3$ and 0.01% CB442 (antifoam) at pH 7. The culture supernatant (6 liters) was applied to an Amberlite XAD-7 column (60 × 200 mm) after adjusting to pH 8. The column was washed with 3 liters of 10 mM potassium phosphate, pH 8, and active metabolites were eluted with 3 liters of methanol. The methanol eluate was concentrated to dryness and then suspended with 200 ml of water, followed by extracting with butanol (once with 200 ml and twice with 100 ml). The organic extracts were concentrated to dryness, giving 4.76 g of an oily residue, which was then applied to a silica gel column (Wakogel C-200, 30 × 200 mm). The column was successively washed with 1 liter each of dichloromethane, dichloromethane - ethyl acetate (1 : 1) and ethyl acetate. Active compounds were eluted with 1 liter of ethyl acetate - methanol (4 : 1). The active fractions were combined and evaporated to dryness, giving 2.23 g of an oily residue. The residue was dissolved in methanol and applied to a silica ODS column (Inertsil PREP-ODS,

30 × 250 mm, GL Sciences). The column was developed with methanol - 0.1% aqueous phosphoric acid (19 : 1) at a rate of 25 ml/minute. Active fractions were concentrated and extracted with ethyl acetate at pH 3. The organic layer was dried over Na_2SO_4 and concentrated to dryness, giving 178 mg of purified isohalobacillin as a white powder. As will be described in the structure determination, isohalobacillin consisted of an inseparable mixture of two isomers. The composition of the isohalobacillin complex varied somewhat among batches of fermentation: the ratio of the subcomponent containing a 3-hydroxy-1-oxo-13-methyltetradecyl moiety over that having 3-hydroxy-1-oxo-12-methyltetradecyl was in a range of 6 : 4 to 7 : 3, as determined by GC-MS and NMR spectra.

Acid Hydrolysis

Complete acid hydrolysis of isohalobacillin was carried out at 110°C for 20 hours in 6 M HCl in a sealed tube at a concentration of 1 mg/ml.

Amino Acid Oxidase Treatment

In the D-amino acid oxidase treatment, acid hydrolysate of isohalobacillin (90 nmol) was incubated in 1 ml of a mixture containing 50 mM sodium pyrophosphate, pH 8.3, 10 μM FAD, 10 μg/ml hog kidney D-amino acid oxidase[4] (Boehringer Mannheim) and 0.1 μg/ml bovine liver catalase (Sigma) at 37°C for 24 hours. The reaction was terminated by adding 15 μl of 6 M HCl. Under these conditions, over 90% of standard samples of D-Asp, D-Glu, D-Val, D-Ile and D-Leu were oxidized.

L-Amino acid oxidase treatment was carried out as follows. Complete acid hydrolysate of isohalobacillin (53 nmol) was incubated in 1 ml of a mixture containing 0.1 M sodium phosphate, pH 7.5, 10 μg/ml *Crotalus adamanteus* venom L-amino acid oxidase[5] (Sigma) and 0.1 μg/ml catalase at 37°C for 72 hours: both L-amino acid oxidase (10 μg) and bovine liver catalase (0.1 μg) were added after 24- and 48-hour intervals. The reaction was terminated by adding 15 μl of 6 M HCl. Under these conditions, over 90% of standard L-Asp, L-Glu, L-Ile and L-Leu were oxidized, while oxidation of L-Val was ~50%.

Methanolysis of Isohalobacillin and Isolation of the Resulting Fatty Acid Methyl Ester

Isohalobacillin (48 mg) was heated at 90°C for 15 hours in 25 ml of a mixture of 12 M HCl and methanol (3 : 7) in a sealed tube. Subsequently, the mixture was evaporated to remove HCl and then extracted three times with *n*-hexane. The hexane extracts were washed twice with 25 ml of water, dried over anhydrous Na_2SO_4 and then concentrated to dryness, giving 4.9 mg of an oily residue. The residue was dissolved in 100 μl of chloroform and applied to a silica gel column (Wakogel C-200, 0.8 × 5 cm). Fatty acid methyl esters were eluted with 0.8 ml of chloroform.

Preparation of Linear Peptide Derivatives of Isohalobacillin

Finely powdered isohalobacillin (6 mg) was incubated at 25°C for 18 hours with vigorous shaking in 4 ml of a mixture containing 0.5 M NaOH and methanol (1 : 1). Subsequently, the pH of the mixture was adjusted to 3 with HCl, and the mixture was extracted three times with 4 ml of ethyl acetate. After concentration followed by dissolving in methanol, the organic extracts were applied to a silica-ODS column (Inertsil PREP-ODS, 6 × 250 mm, GL Sciences). The column was developed at 40°C with methanol - 0.1% aqueous formic acid (95 : 5) at a rate of 1 ml/minute, and two compounds appearing as peaks at retention times of 8.7 and 9.8 minutes were collected. The former (FAB-MS, *m/z* 1053 $(M+H)^+$) was a linear β-hydroxyacid peptide produced by hydrolysis of the ester bond of isohalobacillin, and the latter was a dehydrated α,β-unsaturated acid peptide derivative of the hydroxyacid peptide (FAB-MS, *m/z* 1035 $(M+H)^+$).

Permethylation

Two mg of the dehydrated α,β-unsaturated acid peptide derivative of linearized isohalobacillin (see above) was dissolved in 0.48 ml of dimethyl sulfoxide (DMSO) and 0.12 ml of DMSO carbanion[6]. The mixture was stirred under nitrogen for 4 hours, and then 64 μl of CH_3I was added to initiate a methylation reaction. The reaction continued for 17 hours at room temperature with stirring. Subsequently, 8 ml of water was added, and the mixture was extracted twice with 8 ml of chloroform. The chloroform extracts were washed twice with water, and the resulting organic layer was concentrated to dryness.

Isolation of a Peptide Fragment of Isohalobacillin

Isohalobacillin (5 mg) was heated at 100°C for 1 hour in 5 ml of a mixture of 6 M HCl and propionic acid (1 : 1) in a sealed tube. After evaporation of the lysate, the resulting residue was washed with water and dissolved in methanol. The sample was applied to HPLC on a silica ODS column (Inertsil PREP-ODS, 6 × 250 mm, GL Sciences). The column was developed at 40°C with acetonitrile - 0.1% aqueous formic acid (19 : 81) at a rate of 1 ml/minute. The peptide fragment appearing as a peak at a retention time of 26 minutes was isolated (FAB-MS, *m/z* 344 $(M+H)^+$; amino acid composition, Leu and Val in a 2 : 1 ratio).

Peptide Synthesis

Linear tripeptides were synthesized according to the Fmoc (9-fluorenylmethoxycarbonyl) chemistry[7] using 2-chloro-tritylchloride-resin (Shimadzu Co., Japan). Briefly, Fmoc-L-valyl-resin was prepared by reacting the resin with Fmoc-L-Val in dimethylformamide - dichloromethane - *N*,*N*-diisopropylethylamine (5 : 89 : 6 by volume) for 20 minutes, followed by adding 0.08 volume of methanol for 10 minutes. To 30 mg of the resin,

Fmoc-amino acids (0.18 mmol each) were consecutively coupled in 0.36 ml of dimethylformamide containing 0.18 mmol *N*-methylmorpholine, 0.18 mmol 1-hydroxybenzotriazole and 0.3 mmol benzotriazol-1-yl-oxy-tris-(pyrrolidino)phosphonium hexafluorophosphate. After removing the amino-terminal Fmoc group, peptides were released from the resin by treating with acetic acid - 2,2,2-trifluoroethanol - dichloromethane (1 : 1 : 8 by volume).

Biochemical Determinations

Cholesteryl ester synthesis from [^{14}C]oleate in macrophage J774 was determined in the presence of oxidized LDL (100 μg protein/ml) as described previously[8,9]. Cell surface binding, intracellular accumulation and degradation of oxidized ^{125}I-LDL in macrophage J774 was determined after incubating the cells with 10 μg protein/ml of oxidized ^{125}I-LDL at 37°C for 3 hours[8,9]. ACAT activity was determined in a cell-free system using rat liver microsomes as described previously[10].

Results and Discussion

Physico-chemical Properties and Structure Elucidation

The physico-chemical properties of isohalobacillin are summarized in Table 1. The molecular formula of the agent was established to be $C_{53}H_{94}N_8O_{12}$ (MW 1,034) from the results of FAB-MS and elemental analyses. In the UV spectrum, isohalobacillin showed no characteristic absorption other than end absorption. The presence of absorption bands at 1650 and 1540 cm^{-1} in the IR spectrum is consistent with the presence of amide carbonyl, and a band at 1730 cm^{-1} ester carbonyl. The existence of a peptide structure was also suggested by ^{1}H NMR spectrum, in which amide proton signals were observed at δ_H 7.3~8.1. The NMR spectrum also showed the presence of several aliphatic methyl and methylene signals at δ_H 0.8~1.0 and ~1.2, respectively. ^{13}C NMR data are consistent with these observations.

Isohalobacillin yielded Asp, Glu, Ile, Val and Leu with an approximate molar ratio of 1 : 1 : 1 : 1 : 3 after complete acid hydrolysis. When the compound was reduced by $LiBH_4$ prior to acid hydrolysis, Ile content was markedly reduced (Table 2), showing that the carboxyl group of Ile is not involved in a peptide bond but in another linkage like an ester bond. In addition, the content of Asp but not of Glu declined markedly if the compound was methylated by diazomethane then reduced by $LiBH_4$, followed by acid hydrolysis, demonstrating the presence of Gln and non-esterified Asp residues in the isohalobacillin molecule. The configuration of constituent amino acids was determined by treating the acid hydrolysate with either D- or L-amino acid oxidase. As shown in Table 3, the amounts of Asp, Glu, Val and Ile were unchanged after D-amino acid oxidase, while the contents of these amino acids other than Val were markedly reduced by the L-amino acid oxidase treatment. Val is known to be resistant to oxidation by L-amino acid oxidase from *C. adamanteus* venom[5], and the content of a standard sample of Val was reduced by ap-

Table 1. The physico-chemical properties of isohalobacillin.

Nature	White powder
MP (°C)	134~140
$[\alpha]_D^{23}$ (MeOH)	−10.3 (*c* 1.0)
FAB-MS (*m/z*)	1,035 $(M+H)^+$
	1,033 $(M-H)^+$
Molecular formula	$C_{53}H_{94}N_8O_{12}$
Elemental analysis (%)	
Found:	C 60.7
	H 8.7
	N 11.1
Calcd	C 60.5
(for $C_{53}H_{94}N_8O_{12}\cdot H_2O$):	H 9.1
	N 10.6
IR ν_{max} (KBr) cm^{-1}	3300, 3060, 2960, 2930, 2870, 1730, 1650, 1540, 1460, 1380, 1290, 1250, 1210, 1140
Amino acid composition	L-Asp (1), L-Gln (1), L-Leu (1), D-Leu (2), L-Ile (1), L-Val (1)

Table 2. Amino acid composition of isohalobacillin before and after treatment with either $LiBH_4$ or diazomethane followed by $LiBH_4$.

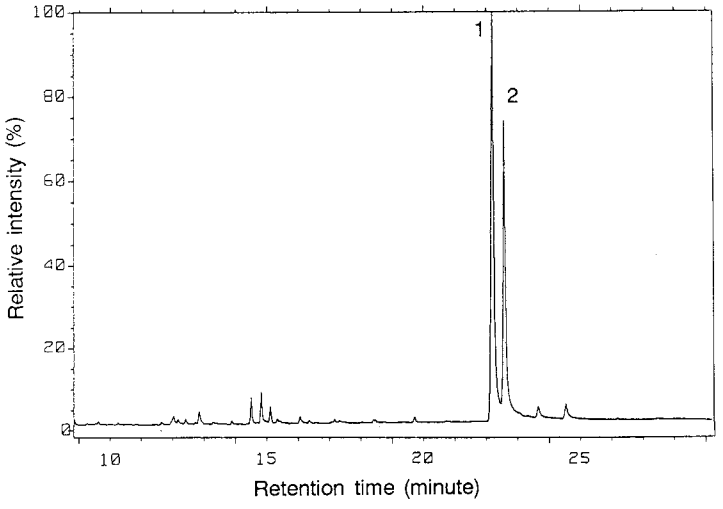
Treatment		Molar ratio				
Diazomethane	$LiBH_4$	Asp	Glu	Val	Ile	Leu
−	−	1.08	1.03	1.10	0.94	3
−	+	0.67	0.67	1.24	0.30	3
+	+	0.10	0.57	1.67	0.28	3

Isohalobacillin (1 mg), either before or after methylation by diazomethane, was treated with $LiBH_4$ (5 mg) in tetrahydrofuran (0.5 ml) under reflux for 6 hours. After addition of 60% aqueous methanol (1 ml), the mixture was concentrated to dryness. The resulting residue was extracted with ethyl acetate at pH 2, and the organic extract was concentrated to dryness. Subsequently, the residues obtained, as well as untreated isohalobacillin, were hydrolyzed prior to amino acid analysis. The molar ratio shown is relative to Leu, which is assigned a value of 3.

Table 3. Amino acid composition of isohalobacillin after treatment with L- and D-amino acid oxidases.

Treatment	Amino acid composition (mol/mol isohalobacillin)				
	Asp	Glu	Val	Ile	Leu
None	1.02	0.99	0.97	0.95	2.94
D-Amino acid oxidase	0.98	0.96	0.97	0.95	0.99
L-Amino acid oxidase	0.13	0.12	0.60	0	1.97

Fig. 1. Gas chromatogram of β-hydroxy fatty acid methyl esters obtained from isohalobacillin by methanolysis.



Peak number	Retention time (minute)	Ion peaks in the MS spectrum (m/z)		
		M^+	Base peak	Diagnostic peak
1	22.2	272	103	254, 241, 199, 74
2	22.5	272	103	254, 241, 199, 74

The molecular and prominent fragment ion peaks observed in the mass spectra of the two components are shown.

Table 4. Assignments of ^{13}C NMR data for β-hydroxy fatty acid methyl esters derived from isohalobacillin by methanolysis.

Chemical shift* (ppm)	Assignment	
	Methyl 3-hydroxy-13-methyltetradecanoate	Methyl 3-hydroxy-12-methyltetradecanoate
173.6	C-1	C-1
68.1	C-3	C-3
51.8	OCH_3	OCH_3
41.1	C-4	C-4
39.1	C-12	—
36.5	C-2	C-2
34.4	—	C-12
29.9	C-11	—
29.5~29.7	C-6, 7, 8, 9, 10	C-6, 7, 8, 9, 10, 11, 13
27.4	C-13	—
25.5	C-5	C-5
22.7	C-14, 13-CH_3	—
19.2	—	12-CH_3
11.0	—	C-14

* The ^{13}C NMR spectrum was measured in $CDCl_3$ with TMS as an internal standard (0 ppm).

proximately 50% in parallel incubations. Thus, it was concluded that the configurations of Asp, Gln, Val and Ile residues of isohalobacillin were in the L form. The content of Leu was decreased to ~1/3 and ~2/3 by the treatments with D- and L-amino acid oxidase, respectively, showing the contents of L-Leu and D-Leu as a 1 : 2 ratio.

By methanolysis, isohalobacillin yielded two lipophilic fragments that could be separated by gas chromatography (Fig. 1). The two fragments gave a molecular ion peak at m/z 272 and a prominent fragment ion peak at m/z 103, a characteristic fragment ion peak of β-hydroxy fatty acid methyl esters. The patterns of mass fragmentation for the two components were very similar and indistinguishable from that for *n*-, *iso*- and *anteiso*-β-hydroxy fatty acid methyl esters. In the ^{13}C NMR spectrum of a mixture of the two β-hydroxy fatty acid methyl ester isomers, three terminal methyl signals were observed in the high field region: signals at δ_C 11.0 and 19.2, characteristics of the terminal methyl groups of *anteiso*-fatty acids, and a signal with a significantly higher intensity at δ_C 22.7, which is found in *iso*-fatty acids (Table 4). Thus, the structures of the two isomers were determined as methyl 3-hydroxy-13-methyltetradecanoate and methyl 3-hydroxy-12-methyltetradecanoate.

The peptide sequence of isohalobacillin was determined by mass spectrometry using linear peptide derivatives (see Experimental). In the FAB-MS spectrum of the dehydrated α,β-unsaturated acid peptide derivative of isohalobacillin, prominent sequence ion peaks corresponding to the carboxy-terminal fragments (Y series[11]) were observed (Fig. 2). From this result, the sequence of the derivative was suggested to be fatty acyl-Gln-Leu-Leu-Val-Asp-Leu-Ile. Similarly, the β-hydroxyacid peptide derivative of isohalobacillin gave the Y series sequence ion peaks at m/z 1052 (31) $[(M+H)^+]$, 813 (8), 685 (38), 572 (21), 459 (31), 360 (34), 245 (100), 132 (55) (relative intensities in parentheses) in the FAB-MS spectrum, confirming the above sequence. In addition, the permethylated dehydrated α,β-unsaturated acid peptide derivative of isohalobacillin gave consistent results in EI-MS (data not shown). To determine the configuration of the tandem leucyl moiety, a peptide fragment containing this moiety,

Fig. 2. FAB-MS spectrum of dehydrated α,β-unsaturated acid peptide derivative of isohalobacillin.

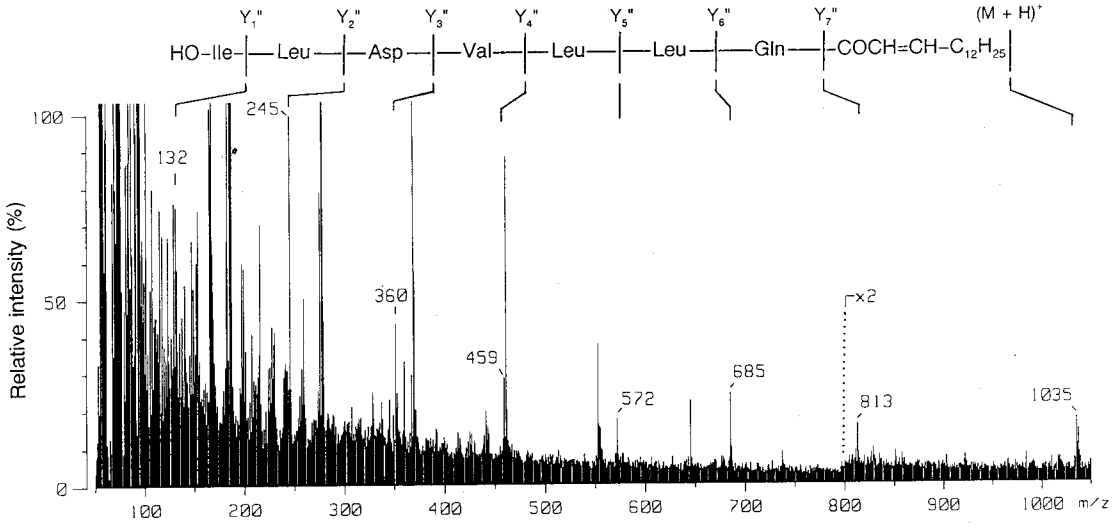


Fig. 3. The proposed structures of the two subcomponents of isohalobacillin.

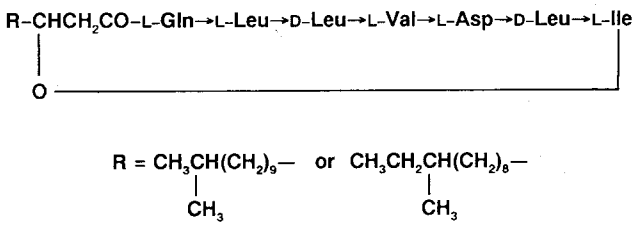
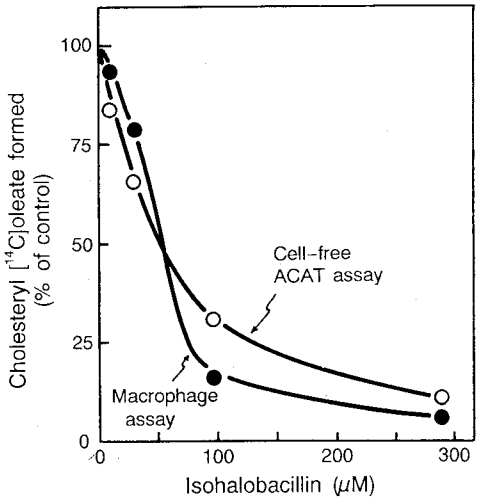


Fig. 4. Inhibition of cholesteryl ester formation by isohalobacillin in both macrophage and cell-free ACAT assays.



Cholesteryl ester formation in macrophages (●) was determined by incubating J774 macrophages with oxidized LDL (100 μg protein/ml) and [^{14}C]oleate (0.1 mM, 10,000 dpm/nmol) at 37°C for 3 hours in the presence of the indicated concentrations of isohalobacillin. In the cell-free assay for ACAT (○), microsomes (16.7 mg/ml) were preincubated at 37°C for 15 minutes in the presence of isohalobacillin at concentrations as indicated. Subsequently, ACAT activity in the mixture was determined. Each value represents the average of duplicate determinations. The mean control values were 75.7 nmol·hour^{-1}·mg cell protein^{-1} for the macrophage assay and 75.6 pmol·minute^{-1}·mg protein^{-1} for the cells-free ACAT assay.

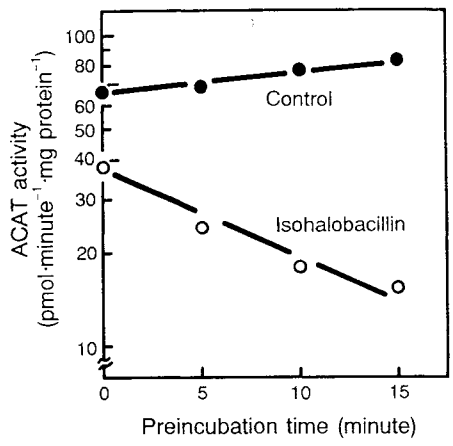
H-Leu-Leu-Val-OH, was isolated after mild acid hydrolysis and compared with synthetic tripeptides, H-L-Leu-D-Leu-L-Val-OH, H-D-Leu-L-Leu-L-Val-OH and H-D-Leu-D-Leu-L-Val-OH, by analytical HPLC under conditions where all the three tripeptides could be separated. The results demonstrated that the isolated peptide fragment was identical to the synthetic H-L-Leu-D-Leu-L-Val-OH.

Taken together, the structures of the two isohalobacillin subcomponents were proposed as shown in Fig. 3. Isohalobacillin belongs to a family of cyclic acylpeptides which include surfactin[12], acylpeptides[13], esperin[14] and pumilacidins[15]. Isohalobacillin has a peptide sequence distinct from the sequences of these agents. TRISCHMAN *et al.*[16] have recently isolated a cyclic acylpeptide, halobacillin, which has the same molecular formula as isohalobacillin has. Except for the configuration of three Leu residues, which have not been determined for halobacillin, its peptide structure is identical to that of isohalobacillin. Each subcomponent of isohalobacillin contains either a 3-hydroxy-1-oxo-13-methyltetradecyl or a 3-hydroxy-1-oxo-12-methyltetradecyl moiety in its molecule, whereas the corresponding group in halobacillin is 3-hydroxy-1-oxopentadecyl.

Biological Activity

When incubated with oxidized LDL, macrophage J774 binds, takes up and degrades the lipoprotein, resulting

Fig. 5. Time-dependent inhibition of ACAT by isohalobacillin.



Microsomes (16.7 mg/ml) were preincubated with 0 (○) or 290 μM (●) isohalobacillin at 37°C. After the intervals indicated, ACAT activity in the mixture was determined. Each value represents the average of duplicate determinations.

in accumulation of cholesteryl esters in the cell. The activity for cholesteryl ester formation was determined by incorporation of [^{14}C]oleate in the cell. Isohalobacillin inhibited this activity 50% at a concentration of 50 μM (Fig. 4). On the other hand, isohalobacillin inhibited neither surface binding, uptake nor degradation of oxidized ^{125}I-LDL (data not shown), suggesting that the agent inhibited the cholesterol esterification reaction, which is mainly catalyzed by ACAT.

ACAT activity was determined in a cell-free system using rat liver microsomes. Isohalobacillin inhibited ACAT activity by 50% at a concentration of 50 μM (Fig. 4). When microsomes were pretreated with isohalobacillin at 37°C prior to determination of ACAT activity, ACAT activity decreased as time of the pretreatment was prolonged, indicating time-dependent inhibition of ACAT by the agent (Fig. 5).

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References

1) STANGE, E. F. & J. M. DIETSCHY: Cholesterol absorption and metabolism by the intestinal epithelium. *In* New Comprehensive Biochemistry. Vol. 12. Sterols and Bile Acids. *Eds.*, H. DANIELSSON & J. SJÖVALL, pp. 121~149, Elsevier Science Publishers, 1985
2) BROWN, M. S. & J. L. GOLDSTEIN: Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. Annu. Rev. Biochem. 52: 223~261, 1983
3) SNEATH, P. H.: Endospore-forming Gram-positive rods and cocci. *In*: BERGERY's Manual of Systematic Bacteriology, Vol. 2, pp. 1104~1139, Williams & Wilkins, Baltimore, 1986
4) YAGI, K.: D-Amino acid oxidase and its complex (hog kidney). Methods Enzymol. 17B: 606~622, 1971
5) MASSEY, V. & B. CURTI: On the reaction mechanism of *Crotalus adamanteus* L-amino acid oxidase. J. Biol. Chem. 242: 1259~1264, 1967
6) HAKOMORI, S.: A rapid permethylation of glycolipid, and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. J. Biochem. 55: 205~208, 1964
7) NOKIHARA, K. & Y. YOKOMIZO: Applications of new linker for Fmoc chemistry. SynProPep Bulletin, No. 10 (Shimadzu Corp.), 1993
8) HASUMI, K.; C. SHINOHARA, S. NAGANUMA & A. ENDO: Inhibition of the uptake of oxidized low density lipoprotein in macrophage J774 by the antibiotic ikarugamycin. Eur. J. Biochem. 205: 841~846, 1992
9) PARK, J. K.; K. HASUMI & A. ENDO: Inhibition of the binding of oxidized low density lipoprotein to the macrophages by iturin C-related compounds. J. Antibiotics 48: 226~232, 1995
10) HASUMI, K.; C. SHINOHARA, T. IWANAGA & A. ENDO: Lateritin, a new inhibitor of acyl-CoA:cholesterol acyltransferase produced by *Gibberella lateritium* IFO 7188. J. Antibiotics 45: 1782~1787, 1993
11) ROEPSTORFF, P. & J. FOHLMAN: Proposal for a common nomenclature for sequence ions in mass spectra of peptides. Biomed. Mass. Spectrom. 11: 601, 1984
12) KAKINUMA, A.; A. OUCHIDA, T. SHIMA, H. SUGINO, M. ISONO, G. TAMURA & K. ARIMA: Confirmation of the structure of surfactin by mass spectrometry. Agric. Biol. Chem. 33: 1669~1671, 1969
13) HOSONO, K. & H. SUZUKI: Acylpeptides, the inhibitors of cyclic adenosine 3',5'-monophosphate phosphodiesterase. II. Amino acid sequence and location of lactone linkage. J. Anibiotics 36: 674~678, 1983
14) THOMAS, D. W. & T. ITO: The revised structure of the peptide antibiotic esperin, established by mass spectrometry. Tetrahedron 25: 1985~1990, 1969
15) NARUSE, N.; O. TENMYO, S. KOBARU, H. KAMEI, T. MIYAKI, M. KONISHI & T. OKI: Pumilacidin, a complex of new antiviral antibiotics. Production, isolation, chemical properties, structure and biological activity. J. Antibiotics 43: 267~280, 1990
16) TRISCHMAN, J. A.; P. R. JENSEN & W. FENICAL: Halobacillin: a cytotoxic cyclic acylpeptide of the iturin class produced by a marine *Bacillus*. Tetrahedron Lett. 35: 5571~5574, 1994