Inhibition of the Binding of Oxidized Low Density Lipoprotein to the Macrophages by Iturin C-related Compounds

JONG KOO PARK, KEIJI HASUMI and AKIRA ENDO

Department of Applied Biological Science, Tokyo Noko University,
Fuchu, Tokyo 183, Japan

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Binding of modified lipoproteins including oxidized low density lipoprotein (oxidized LDL) to cell surface receptors is an initial step of conversion of monocyte-derived macrophages into lipid-laden foam cells, a key cellular component in the early lesions of atherosclerosis. We have searched for microbial metabolites that inhibit oxidized LDL-induced lipid accumulation in macrophages and isolated three compounds from a strain of *Bacillus* sp. as inhibitors of oxidized LDL binding. By chemical and spectroscopic analyses, these metabolites were shown to be related to the cyclic lipopeptide iturin C. Two of these compounds were novel metabolites having long chain β-amino acid moieties of different length. These agents, at concentrations ranging from 5 to 20 μM, inhibited cell surface binding of oxidized ^{125}I-LDL, resulting in reduced intracellular accumulation and degradation of the lipoprotein as well as in reduced cholesteryl ester formation from [^{14}C]oleate in macrophages J774.

One of the early events in atherogenesis is the accumulation of lipid-laden foam cells in the sub-endothelial space[1]. These cells originate mainly from monocyte-derived macrophages[1~3] that have ingested large amounts of plasma lipoproteins, particularly low density lipoprotein (LDL). Native LDL, however, does not cause foam cell transformation, whereas modified LDLs are avidly taken up by macrophages and induce lipid accumulation[4]. There has been growing evidence that physiologically relevant modification of LDL is its oxidation[5]. Specific binding of oxidized LDL to receptor(s) on the surface of macrophages is a prerequisite for subsequent internalization and degradation of oxidized LDL and cholesteryl ester accumulation in the cells[6]. Thus, there is a possibility that inhibitors of oxidized LDL binding to the surface may prevent foam cell transformation of macrophages and development of atherosclerosis.

During the course of our search for microbial metabolites with a potential to inhibit oxidized LDL-induced cholesteryl ester accumulation in macrophages, we have isolated a series of active metabolites from a strain of *Bacillus* sp. One of the metabolites was identified to be iturin C, which is related to a family of cyclic lipopeptides, iturins, produced by *Bacillus subtilis*[7~11]. Iturin A is an antibiotic active against fungi and yeasts[11], while iturin C is an inactive metabolite of iturin family[7,11]. In the present paper, we describe isolation of two novel iturin C-related metabolites and biochemical characterization of these compounds as inhibitors of oxidized LDL binding to macrophages.

Materials and Methods

Materials

The strain, A2822, was isolated from a soil sample collected in Itsukaichi, Tokyo, Japan. This strain was endospore-forming Gram-positive rods and identified to belong to the genus *Bacillus* by taxonomic studies which were based on the Bergey's Manual of Systematic Bacteriology[12]. Carrier-free Na^{125}I was obtained from Amersham. [^{14}C]Oleic acid was purchased from ICN Radiochemicals and was used in complex with albumin as described by GOLDSTEIN *et al.*[13]. Iodogen and polyinosinic acid were obtained from Sigma. Human LDL (1.019~1.063 g/ml) and fetal calf lipoprotein-deficient serum (LPDS) were prepared by ultracentrifugation[13]. LDL was radioiodinated at a specific activity of 100~300 cpm/ng protein by the iodogen method[14]. Both labeled and unlabeled LDL were oxidized by exposure to $CuSO_4$ at 37°C for 48 hours as described previously[15~17].

Isolation of Iturin Cs

Bacillus sp. A2822 was grown aerobically at 28°C for four days in a 500-ml flask containing 100 ml of medium consisting of 1% glucose, 1% soybean meal, 3% corn starch, 0.5% peptone, 0.5% yeast extract and 0.2% $CaCO_3$ (pH 7.0). Cultured broths (51 liters) were centrifuged to separate cells and supernatant. Cell pellets were extracted twice with acetone, and the extract was evaporated to remove acetone. The residue was combined with the culture supernatant, and the mixture was extracted three times with ethyl acetate at pH 3. The

organic extracts were dried over anhydrous sodium sulfate and evaporated to give 46.9 g of an oily residue, which is then applied to silica gel column. After washing with ethyl acetate, the column was developed with a solvent mixture of ethyl acetate - methanol (1 : 1). Active fractions were combined and evaporated. Resulting oily material (2.43 g) was subjected to preparative HPLC on a silica-ODS column (Inertsil PREP-ODS, 30 × 250 mm, GL Sciences, Japan). The column was developed with methanol - aqueous 0.1% phosphoric acid (4 : 1) at 40°C at a rate of 25 ml/minute. Active compounds were eluted in three separate fractions. After evaporation, each fraction was extracted with ethyl acetate, and the organic layer was concentrated to dryness after drying over anhydrous sodium sulfate, giving a purified material.

Methylation and Trifluoroacetylation of β-Amino Acids

Test sample (1 mg) was hydrolyzed in a sealed tube with 6 M HCl at 150°C for 8 hours. After evaporation to dryness, residue was suspended with chloroform. Chloroform extract was washed with water, dried over anhydrous sodium sulfate and treated with diazomethane (1 ml) for 30 minutes. The mixture was evaporated to dryness under a stream of nitrogen and treated with trifluoroacetic acid anhydride for 30 minutes at room temperature to yield *N*-trifluoroacetylated methyl esters of β-amino acids[18].

Spectroscopic Analyses

UV spectrum was measured in methanol on a model 320 spectrometer (Hitachi, Japan). IR spectrum was taken on a IR-810 spectrometer (JASCO, Japan) as a pellet with KBr. Fast atom bombardment mass (FAB-MS) spectrum was measured on a ZAB-HF spectrometer (VG Co., Great Britain) using glycerin as a matrix. NMR spectra were measured in dimethyl sulfoxide-d_6 on a GX-270 spectrometer (JEOL, Japan) at 270 MHz (for ^{1}H) and 67.9 MHz (for ^{13}C).

Other Analyses

Elemental analysis was performed on a model MT-3 CHN Corder (Yanagimoto, Japan). Amino acid composition was determined after acid hydrolysis in a sealed tube (6 M HCl, at 110°C for 20 hours) on a model 835 analyzer (Hitachi). Peptide sequence was determined using a model 477A peptide sequencer (Applied Biosystems, U.S.A.). Gas chromatography-mass spectrometry (GC-MS) was carried out using a capillary column (TC-FFAP, 0.25 mm × 30 m, GL Sciences) on a model SX-102A chromatograph (JEOL). The column temperature was kept at 40°C for 1 minute after injection of a sample and then increased at a rate of 50°C/minute up to 100°C, subsequently at a rate of 10°C/minute up to 230°C.

Cells

Mouse macrophage cell line J774 A.1 was obtained from the Japanese Cancer Resources Bank. Cells were grown at 37°C in monolayer in medium A (DULBECCO's modified EAGLE's medium containing 100 units/ml penicillin and 100 μg/ml streptomycin) supplemented with 10% fetal calf serum. For assays, 1.5×10^5 cells were seeded into 24-well plastic dishes in 0.4 ml of medium A supplemented with 10% fetal calf serum (day 0). On day one, cells were switched to medium B (medium A supplemented with 10% fetal calf LPDS) and used for experiments on day two.

Biochemical Determinations

For all biochemical determinations, test compounds were dissolved in dimethyl sulfoxide and added to medium or buffer at the solvent concentration of less than 1% (v/v). Control incubations contained dimethyl sulfoxide alone at the same concentrations. As described previously, all determinations using macrophage, J774, essentially were carried out[15~17].

Briefly, for determination of cholesteryl ester formation from [^{14}C]oleate, each monolayer of J774 macrophages received 0.3 ml of medium B containing 100 μg protein/ml of oxidized LDL and 0.1 mM [^{14}C]oleate (10,000 dpm/nmol) in complex with albumin. After incubation at 37°C for 3 hours, medium was discarded and cells were washed with buffer A (137 mM NaCl, 2.7 mM KCl, 7.9 mM sodium phosphate and 60 μg/ml of kanamycin, pH 7.4). Cellular lipids were extracted twice with 0.5 ml of *n*-hexane - isopropanol (3 : 2) and subjected to thin layer chromatography to determine [^{14}C]cholesteryl esters[13].

For determination of the amounts of surface-bound, intracellular accumulation and degraded oxidized ^{125}I-LDL, each monolayer of cells received 0.3 ml of medium B containing 10 μg protein/ml of oxidized ^{125}I-LDL. After incubation at 37°C for 3 hours in the absence or presence of 400 μg protein/ml of unlabeled oxidized LDL, medium was removed and assayed for degraded oxidized ^{125}I-LDL. Cells were washed twice at 4°C with buffer A containing 1 mg/ml of albumin, then incubated at 4°C for 10 minutes in 1 ml of the same buffer, following which cells were washed with buffer A and incubated at 4°C for 60 minutes in 0.3 ml of 10 mM Hepes, 50 mM NaCl and 4 mg/ml dextran sulfate (M_r 500,000, Pharmacia). Subsequently, supernatant was removed to determine surface-bound oxidized ^{125}I-LDL. Cells were dissolved in 0.3 ml of 0.2 M NaOH to determine intracellular oxidized ^{125}I-LDL. High affinity (specific) value was calculated by subtracting the value obtained in the presence of excess unlabeled oxidized LDL (nonspecific value) from the value obtained in its absence (total value).

For determination of surface binding of oxidized ^{125}I-LDL at 4°C, each monolayer of cells was chilled on ice for 30 minutes, then received 0.3 ml of a fresh medium (medium B without sodium bicarbonate, supplemented with 20 mM Hepes, pH 7.4) containing 10 μg protein/ml of oxidized ^{125}I-LDL. After incubation at 4°C for 2 hours

in the absence or presence of 400 μg protein/ml of unlabeled oxidized LDL, medium was discarded and cells were washed sequentially as described above. Subsequently, cells were dissolved in 0.3 ml of 0.2 M NaOH to determine the amounts of oxidized ^{125}I-LDL bound.

Acyl-CoA-cholesterol acyltransferase activity was determined using isolated rat liver microsomes as described previously[19]. Protein was determined by the method of LOWRY *et al.*[20].

Results

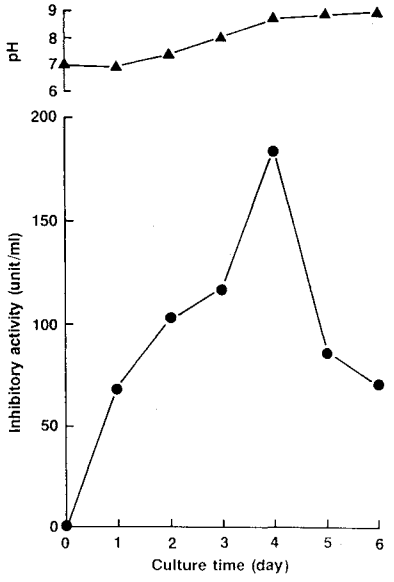
Isolation of iturin Cs

When grown aerobically at 28°C, a culture of *Bacillus* sp. A2822 accumulates metabolites that inhibits cholesteryl ester formation from [^{14}C]oleate in oxidized LDL-treated J774 macrophages. The accumulation of the inhibitory activity increased for up to four days and decreased after five days of culture (Fig. 1). During the cultivation period, pH of the medium gradually increased from 7 to 8.5. From 51 liters of four-day cultures, three active metabolites, which were tentatively designated compounds A2822-a, -b and -c, were isolated by solvent extraction and chromatography as pale yellow powders with yields of 74.0, 86.2 and 62.5 mg, respectively.

Structural Characterization of Iturin Cs

The physico-chemical properties of the three metabolites are summarized in Table 1. Acid hydrolysis of each compound yielded Asp, Glu, Tyr, Ser and Pro with an approximate molar ratio of 3:1:1:1:1. In addition to the α-amino acid components, ^{1}H NMR (overlapping signals at $\sim$1.1$\sim$1.3 ppm) and ^{13}C NMR (multiple signals at $\sim$28$\sim$29 ppm) spectra and IR spectrum (C–H stretching bands at 2920 and 2850 cm^{-1}) suggested the

Fig. 1. Time course for the production of inhibitory activities by *Bacillus* sp. A2822.



Bacillus sp. A2822 was grown aerobically at 28°C as described in *Materials and Methods*. A 1 ml portion of the culture was removed every day and pH (▲) was measured. The culture was then extracted with ethyl acetate at pH 3 and evaporated to dryness, then inhibitory activity (●) was determined after dissolving dried materials in the medium. One unit of inhibitory activity was defined as the volume of culture that was required for 50% inhibition of cholesteryl ester formation from [^{14}C]oleate in macrophages that were incubated with 100 μg protein/ml oxidized LDL for 3 hours at 37°C.

Table 1. Physico-chemical properties of compounds A2822-a (iturin C_2), A2822-b (iturin C_3) and A2822-c (iturin C_4).

	A2822-a (iturin C_2)	A2822-b (iturin C_3)	A2822-c (iturin C_4)
Appearance	Pale yellow powder	←[a]	←
MP (°C)	>350	←	←
FAB-MS (*m/z*)	1,058 $(M+H)^+$, 1,056 $(M-H)^+$	1,072 $(M+H)^+$, 1,070 $(M-H)^+$	1,086 $(M+H)^+$, 1,084 $(M-H)^+$
Molecular formula	$C_{49}H_{75}N_{11}O_{15}$	$C_{50}H_{77}N_{11}O_{15}$	$C_{51}H_{79}N_{11}O_{15}$
Elemental composition			
Found (C:H:N, %)	48.6:6.7:9.8	48.6:6.9:9.8	49.9:6.9:11.3
Calcd. (C:H:N, %)	48.9:7.2:10.6	49.3:7.3:10.5	49.8:7.4:10.3
UV λ_{max}^{MeOH} nm (ε)	225 (23,500), 278 (5,300)	←	←
IR (KBr) cm^{-1}	3300, 2920, 2850, 1660, 1530, 1440, 1240, 990	←	←
Amino acid composition after acid hydrolysis	Asp (3), Glu (1), Tyr (1), Ser (1), Pro (1)	←	←

[a] Arrows denote that the contents of the column are the same as those in the left column.

presence of alkanoyl chain(s) in each molecule. These results suggested that these metabolites belong to a family of iturin complex[11]; the physico-chemical properties of compound A2822-a were consistent with those of one of the iturin C components[7]. That compounds A2822 have a cyclic peptide structure identical to iturin C was demonstrated by chemical and sequence analyses of peptide fragments obtained by mild acid hydrolysis and *N*-bromosuccinimide treatment as described by PEYPOUX *et al.*[21].

That compounds A2822-b and -c have been iturin C-related metabolites with different chain structure of a β-amino acid moiety was revealed by GC-MS analysis of a lipophilic fragment obtained by acid hydrolysis, followed by methyl esterification and trifluoroacetylation. Thus, derivatives of lipophilic fragments from compounds A2822-b and -c as well as A2822-a showed a base ion peak at *m/z* 198, which is characteristic for β-amino acids[18]. These derivatives were discriminated from each other both by retention time in gas chromatography and by mass of molecular and fragment ion peaks in the mass spectra (Table 2). Since mass fragmentation patterns of these derivatives were indistinguishable from that of *n*-, *iso*- and *anteiso*-β-amino acids, the terminal structure of the alkanoyl chain of these β-amino acids was determined by NMR. Two terminal methyl groups in compound A2822-c as well as A2822-a were found at ~11 and ~19 ppm in the ^{13}C NMR spectrum and appeared as an overlapping multiplet at 0.9 ppm in the ^{1}H NMR spectrum (Table 3). In compound A2822-b, both of two terminal methyl carbon signals were at 22.5 ppm, and proton signal at 0.9 ppm as a 6H-doublet (Table 3). These values were consistent with those of *anteiso*- and *iso*-β-amino acid moieties of iturin A[22], respectively. Thus, the long chain β-amino acid moieties of compound A2822-b and -c were identified to be 3-amino-14-methylpentadecanoic acid and 3-amino-14-methylhexadecanoic acid, respectively.

The proposed structures of the current metabolites are shown in Fig. 2. Since iturin C is a mixture of two components[7] and the two new related metabolites were isolated in the present study, for discrimination, we propose to call these compounds iturins C_1, C_2, C_3 and C_4 in the order of carbon chain length of the β-amino acid moiety (Fig. 2).

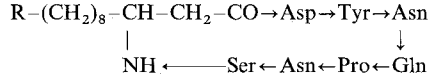
Biochemical Characterization of Iturin Cs as Inhibitors of Oxidized LDL Binding to Macrophages

When macrophage J774 cells were incubated at 37°C for 3 hours in the presence of oxidized LDL (100 μg protein/ml), large amounts of lipids, as visualized by Oil Red O staining after fixation, were seen within the cells. Incubation in the absence of lipoprotein did not result in such lipid accumulation in macrophage J774. Addition

Table 2. GC-MS analysis of *N*-trifluoroacetyl-β-amino acid methyl esters from compounds A2822-a (iturin C_2), A2822-b (iturin C_3) and A2822-c (iturin C_4).

N-Trifluoroacetylamino acid methyl ester from	Retention time (minutes)	Ion peak (*m/z*)		
		M^+	Base peak	Diagnostic peaks
A2822-a (iturin C_2)	22.05	367	198	349, 336, 307, 298, 294, 270
A2822-b (iturin C_3)	22.87	381	198	363, 350, 321, 312, 308, 284
A2822-c (iturin C_4)	25.40	395	198	377, 364, 335, 326, 322, 298

Fig. 2. The structures of iturin Cs.



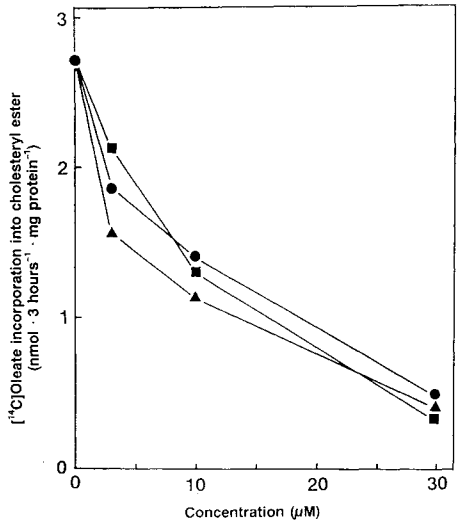
Compound	R
Iturin C (iturin C_1)	$CH_3-\underset{\displaystyle CH_3}{\underset{\vert}{CH}}-$
Iturin C (A2822-a) (iturin C_2)	$CH_3-CH_2-\underset{\displaystyle CH_3}{\underset{\vert}{CH}}-$
A2822-b (iturin C_3)	$CH_3-\underset{\displaystyle CH_3}{\underset{\vert}{CH}}-CH_2-CH_2-$
A2822-c (iturin C_4)	$CH_3-CH_2-\underset{\displaystyle CH_3}{\underset{\vert}{CH}}-CH_2-CH_2-$

Table 3. ^{13}C and ^{1}H NMR data for β-amino acid moiety of compounds A2822-a (iturin C_2), A2822-b (iturin C_3) and A2822-c (iturin C_4).

Compound	^{13}C NMR[a] (δ)						^{1}H NMR[b] (δ)
	C-12	C-13	C-14	C-15	C-16	CH_3[c]	Terminal CH_3
A2822-a (iturin C_2)	35.2	29.4	11.2	—	—	19.1	0.9 (6H, m)
A2822-b (iturin C_3)		34.6	28.6	22.5	—	22.5	0.9 (6H, d)
A2822-c (iturin C_4)			35.2	29.3	11.1	19.0	0.9 (6H, m)

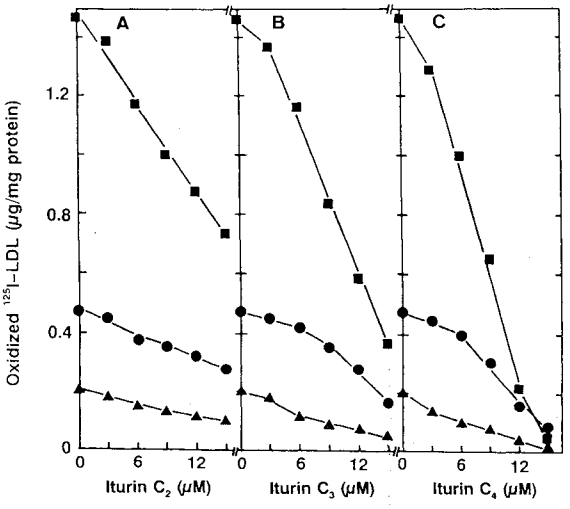
[a] 68 MHz, dimethyl sulfoxide-d_6; [b] 270 MHz, dimethyl sulfoxide-d_6; [c] branch methyl.

Fig. 3. Inhibition of cholesteryl ester formation from [^{14}C]oleate in macrophage J774 by iturins C_2, C_3 and C_4.



Each monolayer of J774 macrophages received 0.1 mM [^{14}C]oleate in complex with albumin and the indicated concentrations of iturin C_2 (●), C_3 (▲) or C_4 (■). After incubation in the presence of 100 μg protein/ml of oxidized LDL at 37°C for 3 hours, [^{14}C]oleate incorporated into cholesteryl ester was determined. Each value represents the average of duplicate determinations.

Fig. 4. Effects of iturins C_2, C_3 and C_4 on the surface binding, intracellular accumulation and degradation of oxidized ^{125}I-LDL in macrophage J774.



Each monolayer of J774 macrophages received 10 μg protein/ml of oxidized ^{125}I-LDL and the indicated concentrations of iturins C_2 (A), C_3 (B) or C_4 (C). After incubation at 37°C for 3 hours in the absence or presence of 40-fold excess of unlabeled oxidized LDL, specific values for the amounts of surface-bound ▲), intracellular (■) and degraded (●) oxidized ^{125}I-LDL were determined. Each value represents the average of duplicate determinations.

of iturin C_4 at a concentration of 5 μM resulted in marked reduction in the oxidized LDL-induced lipid accumulation in macrophages. Under these conditions, viability of cells, as judged from trypan blue exclusion, was not affected significantly. However, at concentrations higher than 30 μM, the agent caused cell detachment. Similar results were obtained with iturins C_2 and C_3 (data not shown).

To explore the mechanism for the inhibition of the lipid accumulation by iturin Cs, their effects on cholesteryl ester synthesis were determined by incubating macrophages with [^{14}C]oleate. In the presence of oxidized LDL, macrophages J774 synthesized 2.71 nmol cholesteryl esters per mg cell protein during a 3-hour incubation period, while the value was 0.69 in the absence of lipoproteins. Iturins C_2, C_3 and C_4 inhibited this oxidized LDL-induced cholesteryl ester synthesis by 50% at concentrations ranging from 5 to 10 μM (Fig. 3). At these concentrations, these agents did not affect acyl-CoA-cholesterol acyltransferase activity in a cell-free assay using isolated rat liver microsomes. These results suggested that iturin Cs inhibit a step(s) of oxidized LDL metabolism leading to lysosomal degradation of the lipoprotein and subsequent release of free cholesterol.

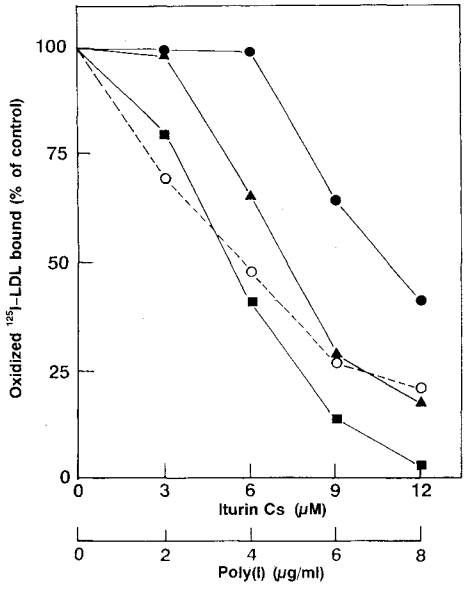
Macrophages J774 were incubated with oxidized ^{125}I-LDL at 37°C for 3 hours in the presence of varying concentrations of iturins C_2, C_3 and C_4, and then the amounts of surface-bound, intracellular and degraded oxidized ^{125}I-LDL were determined. As shown in Fig. 4, all these parameters were reduced in parallel by these compounds. Of the three agents tested, iturin C_4 was most potent and caused 50% reduction at a concentration of ~8 μM. Iturins C_2 and C_3 were slightly less active than iturin C_4, giving 50% inhibition at ~10 and ~15 μM.

To determine the effects of iturin Cs on surface binding of oxidized LDL, macrophages were incubated with oxidized ^{125}I-LDL at 4°C, the conditions where binding, but not subsequent internalization and degradation of the lipoprotein, occurs. As shown in Fig. 5, iturin Cs inhibited this activity at concentrations similar to those required for the inhibition of oxidized LDL metabolism at 37°C. Thus, iturins C_2, C_3 and C_4 caused 50% inhibition at concentrations approximately 11, 7 and 5 μM, respectively. Under the same conditions, polyinosinic acid inhibited oxidized LDL binding by 50% at a concentration of 4 μg/ml (Fig. 5).

Discussion

In the present study, we have isolated three related metabolites from *Bacillus* sp. as inhibitors of oxidized LDL binding to macrophages. These compounds were

Fig. 5. Inhibition of oxidized ^{125}I-LDL binding to macrophage J774 at 4°C by iturins C_2, C_3 and C_4 and polyinosinic acid.



Each monolayer of J774 macrophages received 10 μg protein/ml of oxidized ^{125}I-LDL and the indicated concentrations of either iturins C_2 (●), C_3 (▲) or C_4 (■) or polyinosinic acid (Poly(I), ○). After incubation at 4°C for 2 hours in the absence or presence of 40-fold excess of unlabeled oxidized LDL, the amount of oxidized ^{125}I-LDL specifically bound was determined. Each value represents the average of duplicate determinations. The mean control value was 199 ng/mg cell protein.

cyclic lipopeptides belonging to a family of iturins[11]. This family of compounds, which includes iturins[7,11,23], bacillomycins[21,24] and mycosubtilin[25], are metabolites of *Bacillus subtilis* and were characterized by the presence of a long chain β-amino acid as a constituent amino acid. These metabolites are classified as above according to their α-amino acid compositions. The current compounds had a peptide structure identical to iturin C[7] and contained either of a C_{15}-, C_{16}- or C_{17}-β-amino acid, thus being classified into iturin C. Since two of the current compounds were new metabolites, they have been subdivided into iturins C_3 and C_4 by the order of chain length in the β-amino acid moiety.

We showed that iturin Cs inhibited surface binding of oxidized LDL to macrophages J774. It is likely that this inhibition is attributable to the suppression of lipid accumulation in the macrophages that have been incubated with oxidized LDL, since this lipid accumulation, as well as cholesteryl ester formation and oxidized LDL metabolism, was reduced by similar extent by concentrations of iturin Cs that were required for the inhibition of oxidized LDL binding.

It appeared that the potency of iturin Cs increases as an alkanoyl chain in the β-amino acid moiety extends. Since other peptide structures are the same among these metabolites, this observation suggests that hydrophobicity and/or bulkiness of the compound may affect inhibitory potency. It is interesting if other cyclic lipopeptides with different peptide structures, such as iturin A, bacillomycins and mycosubtilin, affect oxidized LDL binding.

One of the predominant binding sites for oxidized LDL in macrophages is a scavenger receptor[26,27]. It recognizes, in addition to oxidized LDL, a variety of ligands including modified proteins (acetylated LDL and maleylated albumin), certain polyribonucleotides (such as polyinosinic and polyguanylic acids), some polysaccharides, platelet secretory products and bacterial lipopolysaccharide[4,28,29]. Whether or not iturin Cs inhibit binding of these ligands is unknown and further studies will be conducted. On the other hand, native LDL, which is recognized by LDL receptor, is not a ligand for scavenger receptor[4]. In our preliminary experiment, surface binding of ^{125}I-LDL to cultured human skin fibroblasts, which express LDL receptor but not scavenger receptor, was not inhibited by iturin C_4.

The scavenger receptor ligands mentioned above compete with oxidized LDL for the receptor, thus acting as inhibitors of the lipoprotein binding. In our assay, polyinosinic acid was as inhibitory as iturin Cs in oxidized LDL binding to macrophages. However, these substances including polyinosinic acid are of high molecular mass and may be limited in the use, especially in *in vivo* experiments. The relatively low molecular mass compounds like iturin Cs may be of advantage for such purposes and would have a potential to determine if the inhibitors of oxidized LDL binding prevent foam cell formation and development of atherosclerosis.

Acknowledgments

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