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MODE OF ACTION OF THE LANTHIONINE-CONTAINING PEPTIDE ANTIBIOTICS DURAMYCIN, DURAMYCIN B AND C, AND CINNAMYCIN AS INDIRECT INHIBITORS OF PHOSPHOLIPASE A_2

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Abstract—Effects of the lanthionine-containing peptide antibiotics duramycin, duramycin B, duramycin C and cinnamycin on the activity of phospholipase A_2 from six different sources were studied, and their mode of action was investigated. The four antibiotics inhibited potently all tested phospholipases A_2, with IC_{50} values of around 1 μM, using phosphatidylethanolamine or [1-^{14}C]oleate-labelled *Escherichia coli*, whose phospholipids are rich in phosphatidylethanolamine, as substrates. No inhibition was observed when the substrate was phosphatidylcholine. Binding of the antibiotics to the lipid fraction of *E. coli* could be demonstrated by co-sedimentation with whole, but not with lipid-depleted *E. coli*. In addition, preincubation of duramycin B with vesicles of phosphatidylethanolamine, but not those of phosphatidylcholine, prevented the inhibition of phospholipase A_2 activity. The interaction of duramycin B and C, but not that of the biologically inactive compounds actagardine and the duramycin B trisulphoxide, with phosphatidylethanolamine was demonstrated using circular dichroism studies. On the other hand, no interaction of duramycin B with phosphatidylcholine could be demonstrated. A strict correlation between the physico-chemical interaction of the studied lantibiotics, demonstrated by circular dichroism spectroscopy, and their inhibition of phospholipase A_2 was observed. These results suggest that lanthionine-containing peptide antibiotics inhibit phospholipase A_2 indirectly by specifically sequestering the substrate phosphatidylethanolamine. This mode of action is analogous to the one described for the protein lipocortin.

The polycyclic peptides duramycin [1], ancovenin [2] and cinnamycin [3], identical to Ro09-0198 [4], are members of a class of lanthionine-containing antibiotics for which the common name "lantibiotics" has been proposed [5]. Two new members were recently added to this class, duramycin B and duramycin C [6]. Both compounds were detected in the course of a screening program for inhibitors of phospholipase A_2 (PLA_2,† EC 3.1.1.4) among secondary metabolites produced by microorganisms. After isolation of these compounds—guided by following the PLA_2 inhibitory activity—and determination of their chemical structures, the close analogy to duramycin was recognized. Since duramycin and cinnamycin were found to be equipotent with duramycin B and C as inhibitors of PLA_2 [6], we speculate that inhibition of PLA_2 may be a general property of small lantibiotics.

PLA_2 plays a key regulatory role in the arachidonic acid cascade by catalysing the rate-limiting step in a metabolic pathway leading to a variety of potent mediators of inflammation and allergy, i.e. prostaglandins, leukotrienes and hydroxyeicosatetraenoic acids [7–9]. Furthermore, PLA_2 is also essential for the formation of lyso-PAF, the immediate precursor of platelet-activating factor (PAF), another pro-inflammatory mediator [10, 11]. Since inhibition of PLA_2 could serve as a tool to suppress the excessive formation of eicosanoid mediators, e.g. in inflammatory disease, it seemed of interest to investigate further the phospholipase inhibitory activity of lantibiotics by studying their enzyme and substrate specificity and their mode of action. The results of this study are described in the present report.

MATERIALS AND METHODS

Materials. Duramycin B and C, duramycin and cinnamycin were isolated as described previously [6]. A sample of actagardine was generously provided by the Lepetit Research Centre (Gerenzano, Italy). Bio-Sil A (silicic acid for chromatography, 100–200 mesh) was from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Essentially fatty acid-free bovine serum albumin, Tyloxapol, cobra snake venom PLA_2 (*Naja naja naja*, 1300 units/mg) and L-α-phosphatidylethanolamine,dipalmitoyl were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine was synthesized by Dr J. Brokatzky at Ciba-Geigy (Basel, Switzerland). Octyl glucoside (*n*-octyl-β-D-glucopyranoside) was a product of Calbiochem-Behring (La Jolla, CA, U.S.A.). Porcine pancreatic PLA_2 (700 units/mg) was obtained from Boehringer Mannheim (F.R.G.). ^{14}C-Labelled compounds were purchased from Amersham International (Amersham, U.K.).

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† Abbreviations: CD, circular dichroism; HPLC, high performance liquid chromatography; IC_{50}, concentration causing 50% inhibition; PLA_2, phospholipase A_2; PMN, polymorphonuclear leukocytes; SEM, standard error of the mean.

Preparation and assay of phospholipases. PLA_2 was extracted from human polymorphonuclear leukocytes (PMN) by treatment for several hours at 0° with 0.36 N sulfuric acid–2 M NaCl, followed by dialysis of the extract against 10 mM acetate pH 4.5 and removal of precipitated protein by centrifugation [12]. Enzyme activity was assayed by incubation of enzyme with 0.1 M Tris–HCl pH 7.0, 1.0 mM $CaCl_2$ and [1-^{14}C]oleate-labelled autoclaved *Escherichia coli* or sonicated 1-palmitoyl-2-[1-^{14}C]linoleoyl-*sn*-glycero-3-phosphoethanolamine (5–10,000 cpm, phospholipid content 5 nmol) in a volume of 1 mL for 1 hr at 37°. The enzyme concentration was adjusted within the linear range of the assay, i.e. hydrolysis of the substrate of up to 5%. The reaction was stopped by extraction with Dole reagent. Released [1-^{14}C]fatty acid was separated from substrate by passing an aliquot of the upper phase of the Dole extract over a disposable silicic acid (Bio-Sil) column followed by elution with ethyl acetate. Radioactivity of the combined eluates was determined in a Canberra–Packard Liquid Scintillation Counter. IC_{50} values of the test compounds were determined graphically [12].

Human synovial fluid was obtained by biopsy and clarified by centrifugation. PLA_2 was assayed after appropriate dilution of the fluid (approximately 1000-fold) under the conditions described above (substrate *E. coli*).

Human platelet PLA_2 was extracted with octyl glucoside from platelet sonicates according to Kramer *et al.* [13]. The clear supernatant obtained after centrifugation of the extract at 150,000 *g* for 1 hr was stored in small aliquots at −80°. Enzyme activity was assayed with [1-^{14}C]oleate-labelled *E. coli*, as described above, or with [^{14}C]-phosphatidylcholine by a modification of the assay described by Kramer *et al.* [13]. Briefly, the enzyme was incubated for 15 min at 37° in an assay mixture (0.5 mL) containing 0.1 M Tris–HCl pH 9.0, 0.1 mg/mL fatty acid-free bovine serum albumin, 1.0 mM mercaptoethanol, 2.0 mM $CaCl_2$ and freshly prepared substrate consisting of a sonicated mixture of 1-stearoyl-2-[1-^{14}C]arachidonoyl-*sn*-glycero-3-phosphocholine (2 μM, 2.5 nCi) and *sn*-1,2-dioleoylglycerol (0.8 μM). The enzyme concentration was adjusted within the linear range of the assay (up to 30% substrate hydrolysis). The incubation was terminated by Dole extraction and released [^{14}C]-arachidonic acid was measured by radiometry.

PLA_2 from porcine pancreas and cobra venom obtained commercially was assayed with [1-^{14}C]-oleate-labelled *E. coli*, as substrate as described above, with the following modifications: The incubation temperature was 21°, the incubation time 5 min and substrate hydrolysis did not exceed 5%, in order to remain within the linear range of the assays. When 1-stearoyl-2-[1-^{14}C]arachidonoyl-*sn*-glycero-3-phosphocholine (100 μM, 2 nCi) was used as substrate, assay mixtures (1 mL) containing 100 mM Tris–maleate pH 7.5, 1.0 mM $CaCl_2$ and 1.0 mM sodium deoxycholate were incubated for 10 min at 37°.

Rat liver lysosomal PLA_2 was prepared from lysosomes induced in rats with Tyloxapol, according to Franson *et al.* [14]. The lysate obtained by dialysis of the lysosomes against distilled water was assayed with [1-^{14}C]oleate-labelled autoclaved *E. coli* (5 nmol phospholipid) as substrate in 1.0 mL 100 mM sodium acetate pH 4.5 by incubation at 37° for 15 min. The reaction rate was linear when substrate hydrolysis was limited to 5%.

Phospholipase C (EC 3.1.4.10) from human platelets was assayed with the supernatant of a platelet sonicate centrifuged for 1 hr at 115,000 *g* by incubation with 25 mM Tris–maleate pH 6.0, 0.2 mM $CaCl_2$ and 20 μM L-3-phosphatidyl-[U-^{14}C]inositol (2.5 nCi) for 10 min at 37° in a volume of 1.0 mL. The reaction was stopped with 50 μL 0.5 N sulphuric acid and by extraction with 5 mL chloroform/methanol 2:1 (v/v). After centrifugation an aliquot of the upper phase was removed for radiometry.

In all enzyme assays blank values obtained by substituting water or buffer for enzyme were subtracted to correct for non-enzyme-related substrate hydrolysis.

[1-^{14}C]*Oleate-labelled* E. coli. [1-^{14}C]Oleic acid was incorporated into the phospholipids of *E. coli* according to Franson *et al.* [15], as described previously [12]. Incorporation of 40% of the added [1-^{14}C]oleic acid into the *sn*-2 position of cell membrane phospholipids was routinely achieved. The autoclaved *E. coli* is an efficient and convenient substrate for PLA_2 from many sources. It is stable for at least one year when stored at −20°.

Lipids and lipid extraction. Lipids were extracted from non-radioactive autoclaved *E. coli* with chloroform/methanol according to Bligh and Dyer [16], using the modification described by Kates [17]. The extracted cells were washed with methanol and distilled water to remove traces of chloroform and were resuspended with distilled water to the original volume. Vesicles of phospholipids in water were prepared by sonication in a Branson 2200 (bath type) sonicator for 10 min and a Heath Systems W-385 (microtip) sonicator (output 1.5) for 1.5 min.

Binding of lantibiotics to E. coli. To determine a possible binding of lantibiotics to *E. coli*, solutions of the peptides were incubated with buffer, Ca^{2+} and whole or lipid-extracted autoclaved *E. coli* under the conditions described above for the assay of PMN PLA_2, except that the addition of enzyme was omitted. At the end of this preincubation the bacteria were sedimented by centrifugation (5 min at 15,000 *g*) and thus quantitatively removed from the mixture, together with any bound lantibiotic.

The concentration of unbound lantibiotic remaining in solution in the supernatant was estimated in two ways: either enzymatically by determining the inhibition of PMN PLA_2 after supplementation of an aliquot of the supernatant with substrate ([1-^{14}C]-oleate-labelled *E. coli*, to replace the substrate removed by the centrifugation step) and enzyme, or in two experiments by reversed-phase HPLC. Experimental conditions for the latter were as follows [6]: analytical reversed-phase column (Nucleosil 5-C18; 100 A; 4 × 120 mm); solvent A: water–TFA 100:0.1; solvent B: water–acetonitrile–TFA 20:80:0.075; gradient elution from 10% solvent B to 35% in 2 min, then to 50% in 20 min; 1.5 mL/min; monitored at 210 nm; 200 μL injection;

Table 1. Inhibition of phospholipases A_2 and C by lanthionine-containing antibiotics

Enzyme source	Substrate phospholipid	concn (μM)	Inhibition IC_{50} (μM) Duramycin B	Duramycin C	Duramycin	Cinnamycin
Phospholipases A_2						
Human PMN	*E. coli*	5	1.5	0.8	1.1	0.9
	PE*	5	0.7	0.6	1.9	ND†
Human synovial fluid	*E. coli*	5	1.0	1.0	0.6	0.6
Human platelets, OG	*E. coli*	5	1.6	1.3	1.5	1.9
	PC/DG	2	IA (25)‡	IA (23)	ND	IA (62)
Porcine pancreas	*E. coli*	5	1.4	1.3	1.1	1.1
	PC/DOC	100	IA (415)	IA (76)	IA (120)	IA (150)
Rat liver lysosomes	*E. coli*	5	0.7	0.6	0.5	0.5
Cobra venom	*E. coli*	5	1.3	1.2	1.2	1.3
	PC/DOC	100	IA (25)	IA (23)	IA (8.0)	IA (10)
Phospholipase C						
Human platelets	PI	20	IA (415)	IA (38)	IA (40)	IA (31)

Assays were performed as described in Materials and Methods. IC_{50} values are means of at least three independent assays.

* Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; DG, dioleoyl glycerol; DOC, deoxycholate; OG, octyl glucoside extract.

† ND, not determined; ‡ IA, inactive (highest concentration tested).

retention times: duramycin B 10.1 min, duramycin C 7.0 min.

Circular dichroism (CD) studies. CD measurements were made on a JASCO J720 instrument using a circular quartz cell with a path length of 0.1 cm. Each measurement was the average of six repeated scans in steps of 0.1 nm at ambient temperature. Solutions were prepared at least 12 hr before measurements and the lantibiotic concentration was 25 μM. Stock solutions of phospholipids were prepared in ethanol. The solvent was water with 5% EtOH, except as shown in Fig. 3e, when it was water with 6% EtOH. All spectra were background-corrected.

RESULTS

Enzyme and substrate specificity

The inhibition of PLA_2 from human PMN, human synovial fluid, human platelets, porcine pancreas, rat liver lysosomes and cobra venom, and of phospholipase C from human platelets, by the lanthionine-containing antibiotics duramycin B, duramycin C, duramycin and cinnamycin is presented in Table 1. Potent inhibition with IC_{50} values between 0.5 and 1.9 μM was observed with all six PLA_2 studied, when phosphatidylethanolamine or phospholipids of [1-^{14}C]oleate-labelled *E. coli* were used as substrate. By contrast, no inhibition of the same enzymes was detected—even with much higher concentrations of lantibiotic—with the substrate phosphatidylcholine. Inhibition of PLA_2 by lantibiotic was characterized by very steep dose–response curves. A typical example is illustrated in Fig. 1. Phosphatidylinositol-specific phospholipase C from human platelet cytosol was not affected by lantibiotic at concentrations of 31 μM or higher (Table 1).

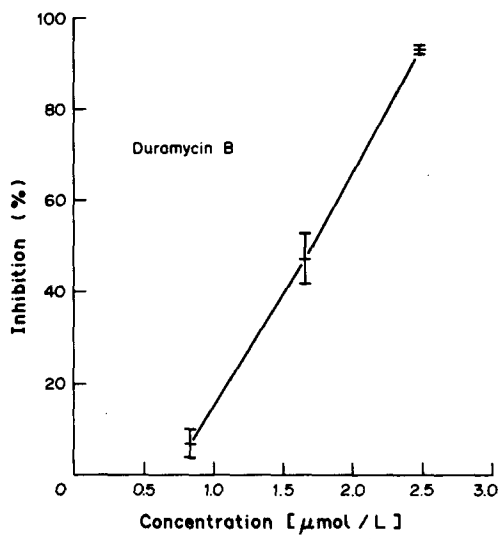

Fig. 1. Inhibition of human PMN phospholipase A_2 by duramycin B. Values given are means ± SEM of four or five separate assays performed as described in Materials and Methods. *E. coli* phospholipids (5 μM) were used as substrate.

Mode of action

The above results indicate that inhibition of PLA_2 by lantibiotic may be linked with a particular kind of substrate (*E. coli* phospholipids, phosphatidylethanolamine), whereas species, tissue or subcellular localization from which the enzymes were derived did not seem to matter. This suggested the possibility of a direct interaction of lantibiotic with certain phospholipids, i.e. with the substrate rather than with the enzyme.

To test this hypothesis, four lantibiotics were preincubated with and without *E. coli* under the conditions used for the assay of human PMN PLA_2—however, in the absence of enzyme. It was observed that a substantial fraction of the added lantibiotic was bound to *E. coli*. This could be demonstrated by measuring a decrease of the concentration of lantibiotic remaining in solution at the end of the preincubation with *E. coli*, after removing the bacteria with bound lantibiotic by centrifugation. When the concentration of unbound lantibiotic in the supernatant was measured enzymatically by determining the inhibition of PMN PLA_2, no significant inhibition was detected in the samples incubated with *E. coli*, whereas inhibition in the range of 65 to 92% was observed when the lantibiotic had been incubated without *E. coli* (Fig. 2a). A different and independent method of measuring the concentration of unbound lantibiotic confirmed these results. Concentrations of free duramycin B and C, determined by HPLC, decreased during incubation with *E. coli* to 35 and 24%, respectively, of the values measured in the samples incubated without *E. coli*. This partial decrease of free duramycins B and C, determined by HPLC, and the virtual total loss of inhibition of PLA_2 measured enzymatically (Fig. 2a) are fully compatible, if the steep dose–response of PLA_2 inhibition by lantibiotic (Fig. 1) is taken into account.

While these data indicate strongly that lantibiotics were able to bind physically to *E. coli* under the conditions used for the assay of PLA_2, they provide no clue as to the component of the *E. coli* cell membrane with which the peptides associated. To clarify this point the above-mentioned incubation procedure was repeated with *E. coli* from which the lipids had been extracted. As shown in Fig. 2b, in clear contrast to the experiment with non-extracted whole *E. coli* (Fig. 2a), the presence of lipid-free bacteria during the incubation with duramycin B or duramycin C did not reduce PLA_2 inhibition in the supernatant after centrifugation of the cells, i.e. no binding of lantibiotic to the lipid-free *E. coli* had occurred.

To investigate the specificity of the association of lantibiotics with lipids, a strongly inhibitory concentration of duramycin B (3.3 μM) was preincubated with sonicated vesicles of phosphatidylethanolamine or phosphatidylcholine (3 μM). While these phospholipids alone had little effect on the activity of PMN PLA_2—determined as usual with [1-^{14}C]oleate-labelled autoclaved *E. coli* (Fig. 2c, left)—phosphatidylethanolamine, in contrast to phosphatidylcholine, largely prevented the inhibition caused by duramycin B (Fig. 2c, right). Thus, the lantibiotic duramycin B seemed to interact specifically with the phospholipid phosphatidylethanolamine.

These data suggest that inhibition of PLA_2 by lantibiotics may result from masking and depletion of the substrate by association of the peptides with certain phospholipids. If correct, increasing the substrate concentration should reduce or abolish the inhibition. The results of Table 2a indeed show that a marked inhibition (85–100%) of human PMN PLA_2 by low micromolar concentrations of lantibiotic was reduced to insignificant values when the concentration of *E. coli* was increased 10-fold, i.e. from a phospholipid concentration of 5 to 50 μM. Likewise, inhibition of the enzyme by duramycins B and C was also abolished with vesicular phosphatidylethanolamine as substrate when its concentration was increased from 5 to 50 μM (Table 2b).

Additional evidence of a specific physico-chemical interaction of lantibiotics with phospholipids was obtained from circular dichroism (CD) studies. As shown in Fig. 3a, b and e, CD spectra of duramycin B, duramycin C and actagardine [18] display a negative maximum of around 204 nm. While the molar ellipticity of duramycin C is almost twice that of duramycin B, actagardine shows a much weaker signal. In the spectrum of the trisulphoxide the maximum is shifted to 200 nm and the molar ellipticity increased markedly. Only duramycin B gives rise to a pronounced second maximum at 190 nm. Addition of phosphatidylethanolamine to duramycin B and C, in contrast to the trisulphoxide of duramycin B or to actagardine, dose-dependently affected the CD signal (Fig. 3a, b, d and e, respectively), whereas addition of phosphatidylcholine to duramycin B did not have any effect (Fig. 3c).

To determine whether duramycin B acted as a competitive inhibitor of PLA_2, an inhibition experiment was performed where the substrate (*E. coli* phospholipid) concentration was varied within the range of 2.5 to 15 μM. PLA_2 inhibition by 1.2 μM duramycin B decreased from virtually complete inhibition at 3.3 μM phospholipid to about 20% at 7.5 μM (Fig. 4a). The abrupt change in inhibition within this narrow range of substrate concentration is borne out even more clearly by a double-reciprocal plot of the data ($1/V$ vs $1/S$; Fig. 4b). These results are not compatible with a competitive type inhibition but support the concept that binding to certain phospholipids (phosphatidylethanolamine), leading to substrate depletion, is the likely mode of action of the PLA_2-inhibitory activity of lantibiotics.

DISCUSSION

This study extends our earlier observation, that the newly discovered polycyclic peptide antibiotics duramycin B and C and their previously known analogs duramycin and cinnamycin are potent inhibitors of PLA_2 from human PMN and synovial fluid [6], to include inhibition of additional phospholipases A_2 from human platelets, porcine pancreas, rat liver lysosomes and cobra venom. While all six phospholipases A_2 studied were inhibited with similar potency when the substrate used for the assays was either phosphatidylethanolamine or endogenous phospholipids in the cell membrane of *E. coli*, no inhibition was observed when three of these enzymes were assayed with phosphatidylcholine as substrate (Table 1). It was surprising to find that inhibition of PLA_2 was virtually independent of the species, tissue or subcellular localization from which the enzymes were derived, and seemed to depend only on the type of substrate used in the assay. This suggested strongly

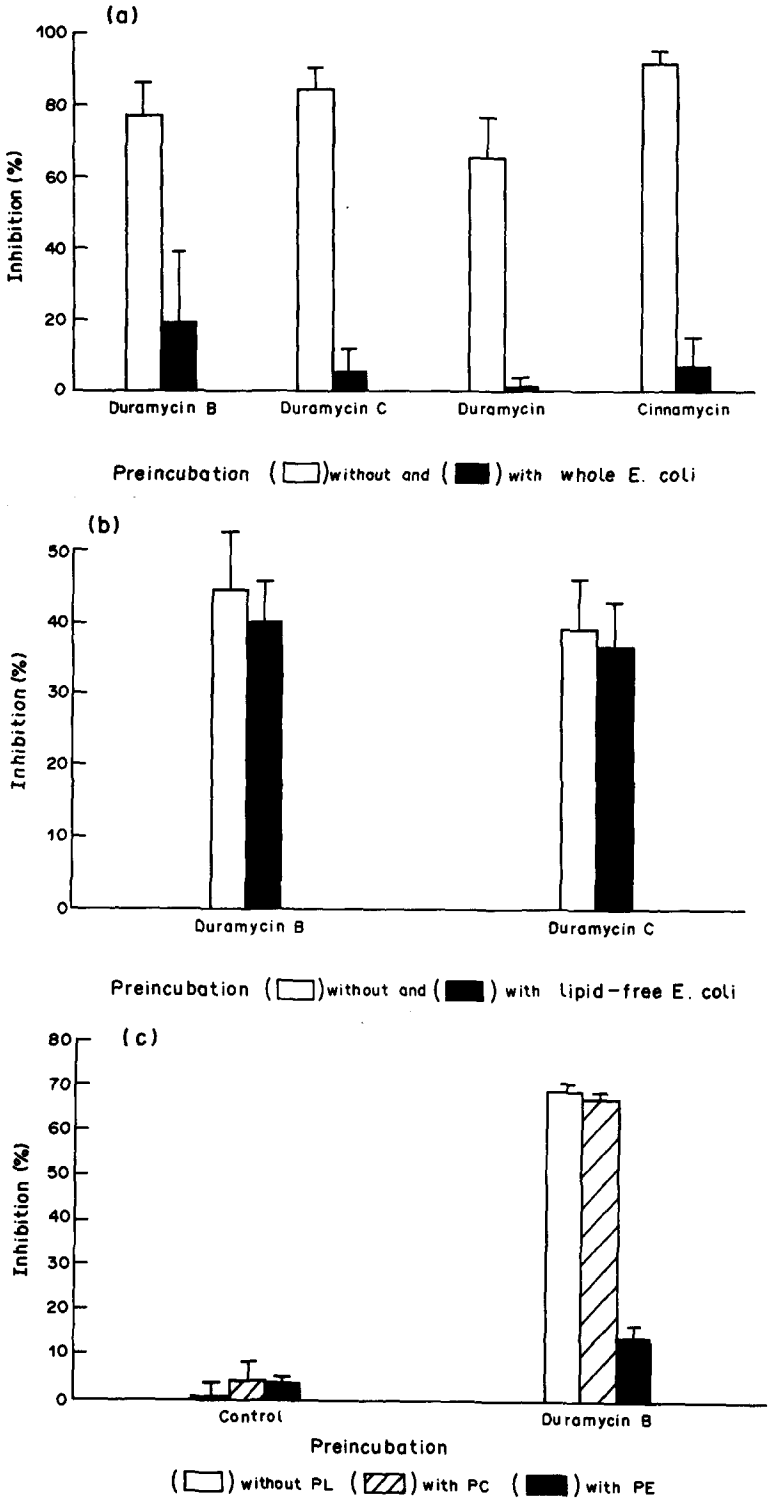

Fig. 2. Binding of lanthionine-containing antibiotics to membrane phospholipids of *E. coli* and to vesicles of phosphatidylethanolamine or phosphatidylcholine. (a, b) The peptides (0.9–1.4 μM) were incubated with or without whole, non-extracted (a) or lipid-extracted (b) autoclaved *E. coli* as described in Materials and Methods. After sedimentation of lantibiotic bound to *E. coli*, the concentration of unbound lantibiotic in the supernatant was estimated by determining the inhibition of PMN PLA_2 after supplementation with substrate and enzyme. (c) Inhibition of PMN PLA_2 was determined after preincubation (15 min at 37°) of duramycin B (3.3 μM) or a solvent control without phospholipid (PL), or with vesicles of phosphatidylcholine (PC, 3 μM) or phosphatidylethanolamine (PE, 3 μM). Values given are means (+SEM) of three (a) or four (b) independent experiments, or one representative experiment with four replicate determinations each (c).

Table 2. Reversal of the inhibition of PLA_2 by lanthionine-containing antibiotics upon increasing the substrate concentration

Compound	Concn (μM)	% Inhibition with substrate concn 5 μM	50 μM	N
(a) Substrate: *E. coli* phospholipids				
Duramycin B	1.4	85.3 ± 7.4	−6.0 ± 6.0	3
Duramycin C	2.3	92.3 ± 1.3	3.0 ± 1.5	3
Duramycin	2.4	100	−1.0 ± 4.5	3
Cinnamycin	1.0	87.3 ± 3.7	0.7 ± 1.7	3
(b) Substrate: phosphatidylethanolamine vesicles				
Duramycin B	1.5	31.1 ± 7.2	−2.0 ± 4.5	6
	2.5	92.7 ± 4.1	7.3 ± 6.1	3
Duramycin C	1.0	43.0 ± 4.9	9.2 ± 4.7	4
	1.9	72.2 ± 3.2	13.2 ± 9.9	4

Inhibition of PLA_2 from human PMN by the test compounds was determined at two concentrations of *E. coli* membrane phospholipids (a) or phosphatidylethanolamine vesicles (b). Other conditions were as described in Materials and Methods.

Values given are means ± SEM of N independent experiments.

that lantibiotics may cause inhibition of PLA_2 by interaction with certain substrates rather than with the enzyme.

We were able to show in three different ways that lantibiotics may indeed associate specifically with certain phospholipids. First, we demonstrated that duramycin B, duramycin C, duramycin and cinnamycin attach to a lipid component in the cell membrane of *E. coli* to the extent that the bound fraction of the peptides can be physically separated from the unbound, soluble fraction by co-sedimentation with the *E. coli* particles (Fig. 2a). The essential role of the lipid component in the binding of lantibiotics was proven in the experiment with lipid-extracted *E. coli* (Fig. 2b).

Second, inhibition of PLA_2 by duramycin B could be prevented by preincubation with an approximately equimolar concentration of pure phosphatidylethanolamine in the form of vesicles (Fig. 2c). This effect is remarkably specific, since it did not occur with phosphatidylcholine.

Third, the CD studies (Fig. 3) clearly demonstrated an interaction between phosphatidylethanolamine (but not phosphatidylcholine) and some, but not all of the lantibiotics investigated. It is important to note that those lantibiotics whose CD spectra were affected by the addition of phosphatidylethanolamine (i.e. duramycin B and C) potently inhibited PLA_2, whereas those whose CD spectra were not changed in the presence of phosphatidyethanolamine (i.e. duramycin B trisulphoxide and actagardine) did not inhibit PLA_2 at all (Table 1; data for actagardine not shown). Thus, inhibition of PMN PLA_2 by lantibiotic and physico-chemical interaction with phosphatidylethanolamine are strictly correlated.

The fact that a lipid component extracted from *E. coli* and pure phosphatidylethanolamine share the ability to bind to lantibiotic is not coincidental. Analysis of the lipid fraction of *E. coli* cell membranes revealed phosphatidylethanolamine as being the main component, contributing as much as 60–95%, depending on the type of membrane and the strain of *E. coli* [19, 20]. It is, therefore, likely that the interactions of *E. coli* with lantibiotics, as described above, were in fact due to the content of phosphatidylethanolamine.

The remarkable specificity of the interaction between phospholipids and lantibiotics is in line with data from the literature. Navarro *et al.* [21] have reported that duramycin induced aggregation of lipid vesicles containing unsaturated phosphatidylethanolamine and monogalactosyl diglyceride. The effect was rather specific, since no interaction was seen with dioleoylphosphatidylcholine, phosphatidylserine, phosphatidic acid or cardiolipin. Selective interaction of Ro09-0198, a lantibiotic recently shown to be identical to cinnamycin [6], with phosphatidylethanolamine in liposome membranes and in dimethyl sulphoxide solution was described by Choung *et al.* [22] and Wakamatsu *et al.* [23], respectively. While this peptide aggregated liposomes containing phosphatidylethanolamine, dialkylphosphatidylethanolamine and lysophosphatidylethanolamine, it had no effect on liposomes of alkylphosphoethanolamine and phosphatidyl-*N*-monomethylethanolamine. Thus, lantibiotics are able to bind to phosphatidylethanolamine but not to phosphatidylcholine, with remarkable selectivity, reminiscent of that usually achieved only by much larger and more complex molecules such as enzymes or antibodies.

Finally, a striking analogy regarding inhibition of PLA_2 seems to exist between lantibiotics and lipocortins. Lipocortin I and II are members of a family of structurally related proteins with molecular mass of 36 kDa, whose synthesis may be induced by glucocorticosteroids. They are reported to inhibit pancreatic PLA_2 and have been claimed to exhibit anti-inflammatory activity in the carrageenin-induced rat paw oedema model (for review see Ref. 24). Recent studies by several groups [25–29] showed clearly that PLA_2 inhibition by lipocortin is the result of binding of lipocortin to the phospholipid substrate, leading to substrate depletion. Despite

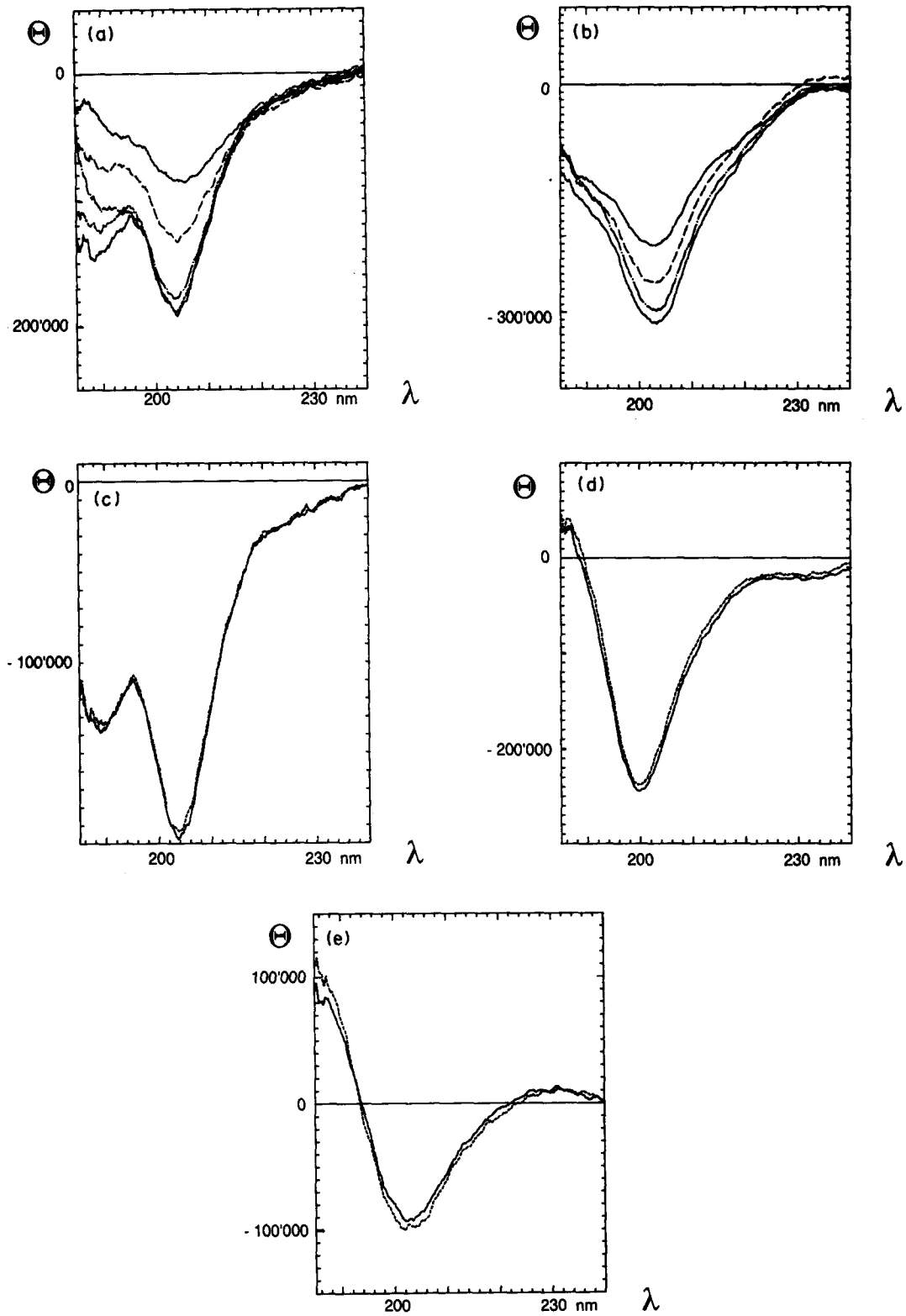

Fig. 3. CD spectra of the lantibiotics duramycin B, duramycin C, duramycin B trisulphoxide and actagardine with or without phosphatidylethanolamine (PE) or phosphatidylcholine (PC). Conditions are described in Materials and Methods. (a) From top: (——) Duramycin B + PE, ratio 1:2; (— —) ratio 1:1; (— · — ·) ratio 2:1; (- - - -) ratio 10:1; (——) duramycin B. (b) From top: (——) Duramycin C + PE, ratio 1:2; (— —) ratio 1:1; (— · — ·) ratio 2:1; (——) duramycin C. (c) (——) Duramycin B; (- - - -) duramycin B + PC, ratio 1:2. (d) (——) Trisulphoxide of duramycin B; (- - - -) trisulphoxide + PE, ratio 1:2. (e) (——) Actagardine; (- - - -) actagardine + PE, ratio 1:2.

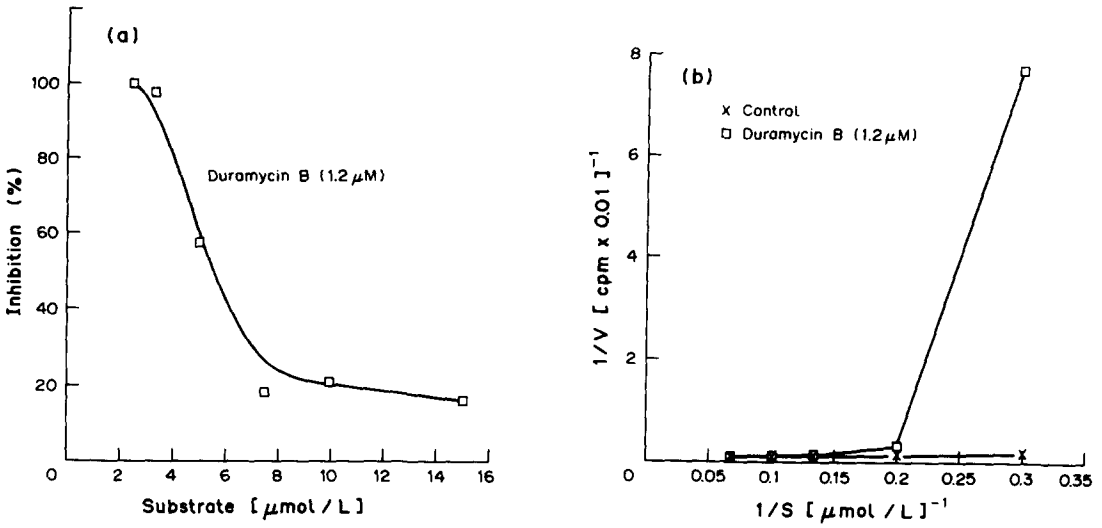

Fig. 4. Inhibition of human PMN phospholipase A_2 by duramycin B at different substrate concentrations. The concentration of the substrate (*E. coli* phospholipid) was varied between 2.5 and 15 μm/L. Values given are means of duplicates. (a) % inhibition vs substrate concentration; (b) double-reciprocal plot, 1/V vs 1/S. The reaction velocity, V, is expressed as [^{14}C]oleate (cpm × 0.01) released from [^{14}C]-oleate-labelled *E. coli*. Conditions are described in Materials and Methods.

such a general analogy between lantibiotics and lipocortins concerning the mechanism of action on PLA_2, differences exist with respect to details. Phospholipid binding of lipocortin is strictly Ca^{2+}-dependent [24, 27–29]. This does not seem to be the case for lantibiotics; we found that binding of duramycin B and C to *E. coli*, determined as illustrated in Fig. 2a, was the same in the presence and absence of added calcium (data not shown). Furthermore, lipocortins seem to bind phospholipids with somewhat lower selectivity, although this point has not been studied in detail [28, 29].

In view of the basically similar mode of action of lantibiotics and lipocortins as indirect inhibitors of PLA_2, the reservations concerning a potential role of lipocortins as anti-inflammatory agents [30] may be equally valid for lantibiotics. Yet, preliminary experiments in our laboratories with mouse peritoneal macrophages *in vitro* demonstrated clearly a dose-dependent inhibition of zymosan-stimulated arachidonic acid release by duramycin B (IC_{50} 10 μM; J. M. Pfeilschifter, personal communication). This result may be interpreted as evidence for an inhibitory effect of duramycin B on the arachidonic acid cascade in an intact cell model. However, it still remains to be proven that the effect is totally or predominantly due to inhibition of PLA_2.

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