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Demonstration of a 12-lipoxygenase activity in bovine polymorphonuclear leukocytes

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In this study we present evidence for the existence of an intrinsic 12-lipoxygenase in the bovine polymorphonuclear leukocyte which differs from the well-known platelet 12-lipoxygenase. Intact bovine polymorphonuclear leukocytes synthesize predominantly 5-lipoxygenase products. However, this 5-lipoxygenase activity disappears completely upon sonication of the cells, whereas a 12-lipoxygenase activity then becomes apparent. This 12-lipoxygenase resembles the platelet 12-lipoxygenase in metabolizing arachidonic acid into 12(*S*)-hydroxyeicosatetraenoic acid and in being independent of Ca^{2+} as well as of ATP. The most striking difference between the two 12-lipoxygenases is their behaviour towards linoleic acid. While the platelet 12-lipoxygenase does not convert linoleic acid, the 12-lipoxygenase from bovine polymorphonuclear leukocytes, apparent only in the cell-free system, converts linoleic acid into 13-hydroxyoctadecadienoic acid as efficiently as it converts arachidonic acid into 12-hydroxyeicosatetraenoic acid. This provides a convenient method to distinguish both 12-lipoxygenase activities. The fact that this new 12-lipoxygenase is able to metabolize linoleic acid into 13-hydroxyoctadecadienoic acid suggests that this enzyme, in contrast to platelet 12-lipoxygenase, resembles 5-lipoxygenases in showing a preference for hydrogen abstraction at a position which is determined by the distance to the carboxylic end of the fatty acid.

Introduction

Lipoxygenases (EC 1.13.11.12) of various origin can convert arachidonic acid into 5-, 12- or 15-hydroperoxyeicosatetraenoic acid (HPETE). Most research in animal systems has been focused on the formation of 5-HPETE, because this compound is the precursor of leukotrienes, metabolites with very pronounced biological activities. There is much evidence that leukotrienes play important roles in, for example, inflammatory processes and asthma (for a review see Refs. 1 and 2). For this reason, the leukotriene formation of many cell types has been studied. Recently, we showed that bovine polymorphonuclear leukocytes are capable of synthesizing 5-series leukotrienes [3]. Together with human eosinophils [4] and human mast cells [5] they belong to the most potent producers of the spasmogenic leukotriene C_4 known so far.

Abbreviations: Leukotriene B_4, 5(*S*),12(*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid; leukotriene C_4, 5(*S*)-hydroxy-6(*R*)-*S*-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid; HETE; monohydroxyeicosatetraenoic acid; HOD, hydroxyoctadecadienoic acid.

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Although 5-series leukotrienes are the predominant lipoxygenase products formed by intact bovine polymorphonuclear leukocytes, variable amounts of 12-HETE could also be detected. This was attributed to a platelet contamination, which was difficult to avoid. Although platelets were thought to be the only 12-lipoxygenase-containing blood cells [6,7], recently reports have appeared of the occurrence of 12-lipoxygenases in other cell types [8–11]. This finding, in combination with the fact that we observed no good correlation between the platelet contamination present and the amounts of 12-HETE formed, prompted us to investigate the possible presence of an intrinsic 12-lipoxygenase in the bovine polymorphonuclear leukocyte.

Materials and Methods

Materials. Arachidonic acid (> 99%) and linoleic acid (99%) were purchased from Fluka AG (Buchs, Switzerland). Calcium ionophore A23187, glutathione, prostaglandin B_2, ATP and the radical scavenger 4-hydroxy-2,2,6,6-tetramethylpiperidinooxy free radical were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.). Ficoll-Paque was from Pharmacia Fine Chemicals AB (Uppsala, Sweden). Sample filters (pore size 0.45 μm) were from Nihon Millipore Kogyo K.K. (Yonezawa, Japan). Methanol, water (Merck, Darmstadt, F.R.G.), hexane (Janssen, Beerse, Belgium) and tetrahydrofuran (Baker, Deventer, The Netherlands) were of HPLC quality. Octadecyl solid-phase extraction columns (6 ml) were obtained from Baker. Synthetic leukotriene B_4 and leukotriene C_4 were a kind gift of Dr. J. Rokach (Merck-Frosst Laboratories, Pointe Claire, Dorval, Quebec, Canada). Racemic 5-HETE was generously provided by Mr. G.A.A. Kivits (Unilever Research Laboratory, Vlaardingen, The Netherlands). 12-HETE was prepared from arachidonic acid by incubation with bovine platelets. 12-*epi*,6-6-*trans*,8-*cis*-leukotriene B_4, often referred to as 5(*S*),12(*S*)-dihydroxyeicosatetraenoic acid was biosynthesized from 12-HETE (Verhagen et al., unpublished data). 13-HOD and 9-HOD were prepared by reduction of the hydroperoxides which had been biosynthesized from linoleic acid with soybean and corn germ lipoxygenase, respectively, according to Ref. 12. All other reagents were of pro analyse quality.

Purification of polymorphonuclear leukocytes and platelets. Polymorphonuclear leukocytes and platelets were isolated from bovine blood, obtained from a local slaughterhouse. Polymorphonuclear leukocytes were purified as described previously [2]. The yield was $(20 \pm 2) \cdot 10^8$ cells from 0.7 litre bovine blood (mean value ± S.E., $n = 19$).

For isolation of platelets, the anticoagulated blood (containing 0.1 litre of a 0.15 M trisodium citrate solution per litre blood) was centrifuged at $485 \times g$ for 20 min, after which the platelet-rich plasma was carefully removed by aspiration. The platelet-rich plasma was again centrifuged for 10 min at $485 \times g$ to remove contaminating red and white blood cells. The supernatant was then centrifuged at $2450 \times g$ for 20 min. The pellet was resuspended in approx. 50 ml of isotonic Tris buffer (140 mM NaCl/10 mM Tris/1 mM EDTA, adjusted to pH 7.35 with HCl) and centrifuged again at $2450 \times g$ for 10 min. The platelet pellet was finally resuspended in 5 ml of isotonic Tris buffer. The yield was $(20 \pm 3) \cdot 10^9$ cells from 0.7 litre bovine blood (mean value ± S.E., $n = 19$).

Preparation of the cell-free systems. Purified polymorphonuclear leukocytes or platelets were suspended in phosphate-buffered saline (0.9% (w/v) NaCl in 8.6 mM phosphate buffer (pH 7.4)) containing 0.01% (w/v) sodium deoxycholate. The cells were sonicated twice for 10 s at 60 W with a Branson Sonifier B-12 at 0 °C. The cell-free system was obtained by centrifuging this suspension for 20 min at $12000 \times g$ at 4 °C.

Incubation procedure and sample preparation. Polymorphonuclear leukocytes were suspended in phosphate-buffered saline at a final concentration of $4.0 \cdot 10^7$ cells/ml. To estimate the contribution of contaminating platelets to the 12-HETE formation, purified platelets were suspended in phosphate-buffered saline at the concentration at which they were present as a contamination in the corresponding polymorphonuclear leukocyte preparations. Cell-free systems were used, prepared from the same number of polymorphonuclear leukocytes or platelets as were present in 4 ml of the corresponding intact-cell suspensions, and brought to a final incubation volume of 4 ml.

The suspensions and supernatants were prein-

cubated at 37 °C for approx. 10 min. Incubation was for 10 min at 37 °C and pH 7.4 in the presence of 80 μM arachidonic acid or linoleic acid, 20 μM calcium ionophore A23187, 5 mM glutathione and 2 mM $CaCl_2$ unless stated otherwise. Reactions were stopped by the addition of ice and the incubation mixture was centrifuged at 12000 × *g* for 20 min at 4 °C. The supernatants were applied to octadecyl solid-phase extraction columns which had previously been washed with 10 ml of methanol, 5 ml of water (both HPLC quality) and 5 ml of 0.5% (w/v) EDTA (pH 5.5). The latter washing was carried out to improve the recovery of the sulphidopeptide leukotrienes [13,14]. After applying the supernatant to the extraction column and washing with 10 ml of water, the adsorbed leukotrienes and monohydroxy acids were eluted with 3 ml of methanol. Recovery of leukotrienes and 5-HETE was better than 94% [13]. After addition of 2 μg of the radical scavenger 4-hydroxy-2,2,6,6-tetramethylpiperidinooxy free-radical, the eluates were stored under nitrogen at −20 °C. Before HPLC-analysis, the eluates were concentrated and filtered through sample filters with a pore size of 0.45 μm.

HPLC analysis. Reversed-phase HPLC was carried out using a CP™ Spher 8-C18 column (250 × 4.6 mm, Chrompack, Middelburg, The Netherlands) attached either to a Kratos 783 detector combined with a Kratos 400 pump or attached to a HP-1040A diode-array detector combined with a HP-1090 solvent delivery system. Isocratic elution was carried out with the solvent system tetrahydrofuran/methanol/water/acetic acid (25 : 30 : 45 : 0.1, v/v) which had been brought to pH 5.5 with ammonia [15]. The water phase contained 0.1% EDTA. A flow-rate of 0.9 ml/min was used and the detection was at 280 nm or at 237 nm (Kratos 783) or from 220 to 350 nm (HP-1040A). Data were processed by either a Shimadzu C-R3A integrator or a HP-310 SPU workstation. Leukotrienes were quantified by relating peak areas to that of the internal standard prostaglandin B_2. Molar absorption coefficients are: 28650 $M^{-1} \cdot cm^{-1}$ at 280 nm for prostaglandin B_2, 40000 $M^{-1} \cdot cm^{-1}$ at 280 nm for the leukotrienes, 29500 $M^{-1} \cdot cm^{-1}$ at 237 nm for 12-HETE and 25000 $M^{-1} \cdot cm^{-1}$ for 13-HOD.

Straight-phase HPLC analysis was carried out using a Paritsil-5 column (Chrompack, Middelburg, The Netherlands) of 250 × 4.6 mm. The solvent system was hexane/2-propanol/acetic acid (93 : 7 : 0.05, v/v).

Purification and derivatization of 12-HETE for the determination of the absolute configuration. Polymorphonuclear leukocyte- or platelet-derived 12-HETE was purified by reversed-phase HPLC using methanol/water/acetic acid (70 : 30 : 0.1, v/v) as a solvent system. Fractions containing 12-HETE were collected, the methanol was removed by evaporation and 12-HETE was extracted from the remaining water phase with octadecyl solid-phase extraction columns.

For the determination of the absolute configuration of 12-HETE, the 5(*S*)-hydroxy derivatives of the purified 12-HETE samples were biosynthesized according to Verhagen et al. (unpublished results). The reference compounds 5(*S*),12(*S*)-dihydroxy-6-*trans*,8-*cis*,10-*trans*,14-*cis*-eicosatetraenoic acid and 5(*R*),12(*S*)-dihydroxy-6-*trans*,8-*cis*,10-*trans*,14-*cis*-eicosatetraenoic acid were biosynthesized from racemic 5-HETE by incubation with bovine platelets according to Marcus et al. [16]. The above-mentioned dihydroxy acids all had the characteristic leukotriene ultraviolet spectrum.

GC-MS analysis. For GC-MS analysis, two 20 ml incubations were performed with the supernatant of sonicated polymorphonuclear leukocytes, one with arachidonic acid as a substrate and one with linoleic acid as a substrate. After C18 solid-phase extraction and concentration, the samples were esterified with diazomethane. The major compound with the ultraviolet spectrum of a conjugated diene, was purified on reversed-phase HPLC. The solvent system was methanol/water/acetic acid (80 : 20 : 0.1, v/v) with a flow-rate of 0.9 ml/min. From the collected fractions, methanol was removed by evaporation and the fatty acid metabolite was extracted with diethyl ether. Further purification was performed using a Partisil-5 column (250 × 9mm). The solvent system was hexane/2-propanol/acetic acid (99.5 : 0.5 : 0.05, v/v) with a flow-rate of 3.0 ml/min. After removal of the solvent from the pooled fractions, the compound was dissolved in methanol (approx. 2 ml) and hydrogenated by treatment with H_2 and PtO_2 as a catalyst. After removal of the catalyst and evaporation of methanol, the compound was

trimethylsilylated by dissolving it in 0.1 ml pyridine to which 0.05 ml trimethylchlorosilane was added. After 2 h at room temperature, the derivatized product was extracted with dry hexane. GC-MS spectra were recorded on a Kratos MS 80 system using a BP-1 capillary column (Techmation, Utrecht, The Netherlands) with a temperature gradient of 2 K/min from 180 °C to 260 °C.

Results

Preparations of bovine polymorphonuclear leukocytes are capable of producing 5-series leukotrienes [3] in conjunction with varying amounts of 12-HETE (Fig. 1A). We initially assumed that the 12-HETE was derived from contaminating platelets. The platelet contamination of our polymorphonuclear leukocyte preparations varied between 1 and 12 platelets per polymorphonuclear leukocyte with an average of 4.6 ± 0.8 (S.E., $n = 19$). Incubation of purified bovine platelets showed that the 12-HETE formed by the intact polymorphonuclear leukocyte preparation can indeed be attributed to the platelet contamination (Table I). When we sonicated polymorphonuclear leukoctyes and looked for arachidonic acid metabolites in the cell-free system after stimulation, we could not detect any 5-lipoxygenase products, whereas large amounts of 12-HETE were present (Fig. 1B). In contrast, the 12-lipoxygenase activity from platelets was found to be reduced 9-fold after sonication (Table I). This indicates the presence of an intrinsic 12-lipoxygenase activity in the bovine polymorphonuclear leukocyte, which is distinct from that in bovine platelets.

The 12-HETE formed by the supernatant of sonicated polymorphonuclear leukocytes was characterized by its mass spectrum, by its ultraviolet spectrum and by coelution in two different HPLC systems with 12-HETE originating from bovine platelets. The mass spectrum of the silylated derivative of the hydrogenated methyl ester showed two abundant ions at *m/z* 301 and at *m/z* 215, due to the fragmentation next to the trimethylsilyl ether group at the C12 position, and ions of lower abundance at *m/z* 367, *m/z* 272 and *m/z* 73, in accordance with literature data [17].

TABLE I

THE FORMATION OF 12-HETE AND 13-HOD BY INTACT AND SONICATED BOVINE POLYMORPHONUCLEAR LEUKOCYTES AND PLATELETS

Cells of cell-free systems were incubated in the presence of 80 μM arachidonic acid (A) or linoleic acid (B), 20 μM calcium ionophore A23187, 5 mM glutathione and 2 mM Ca^{2+} in phosphate-buffered saline (pH 7.4) for 10 min. Polymorphonuclear leukocyte concentration was $4.0 \cdot 10^7$ cells/ml with an average of 4.6 ± 0.8 (S.E., $n = 14$) platelets per polymorphonuclear leukocyte. The platelet concentration in the incubations of purified platelets was the same as it was in the corresponding polymorphonuclear leukocyte preparations. The starting concentration of the cells in the cell-free system was the same as in the intact cell incubations. Results are given as nmoles hydroxy acid formed in a 4 ml incubation $\pm$S.E., $n = 14$. I-PMN, intact polymorphonuclear leukocytes; S-PMN, supernatant of sonicated polymorphonuclear leukocytes; I-PLT, intact platelets; S-PLT, supernatant of sonicated platelets.

	I-PMN	S-PMN	I-PLT	S-PLT
12-HETE	7 ±1	28±3	21 ±4	2.3±0.7
13-HOD	3.1±0.7	32±3	2.6±0.4	1.3±0.2

We determined the absolute configuration of the hydroxyl group at C12 by conversion of 12-HETE into the 5(*S*)-hydroxy-derivative and comparing the HPLC behaviour with that of reference compounds. In Fig. 2A an HPLC chromatogram of the reference compounds 5(*S*),12(*S*)-dihydroxy-6-*trans*,8-*cis*,10-*trans*,14-*cis*-eicosatetraenoic acid and 5(*R*),12(*S*)-dihydroxy-6-*trans*,8-*cis*,10-*trans*,14-*cis*-eicosatetraenoic acid is shown. The 5(*S*)-hydroxy derivative of platelet-derived 12(*S*)-HETE coeluted with peak I (Fig. 2B), showing that this peak has to be attributed to 5(*S*),12(*S*)-dihydroxy-6-*trans*,-8-*cis*,10-*trans*,14-*cis*-eicoatetraenoic acid. The 5(*S*)-hydroxy derivative of polymorphonuclear leukocyte-derived 12-HETE (Fig. 2C) also coeluted with peak I (Fig. 2D). This proves the *S*-configuration of the hydroxyl group at C12 of bovine polymorphonuclear leukocyte-derived 12-HETE. It should be noted that the 5(*S*)-hydroxy derivative of 12(*R*)-HETE coelutes with its enantiomer, 5(*R*),12(*S*)-dihydroxy-6-*trans*,8-*cis*,10-*trans*,14-*cis*-eicosatetraenoic acid (peak II).

Since both platelet and polymorphonuclear leukocyte 12-lipoxygenase produce 12(*S*)-HETE, we tried to distinguish both enzymes on their

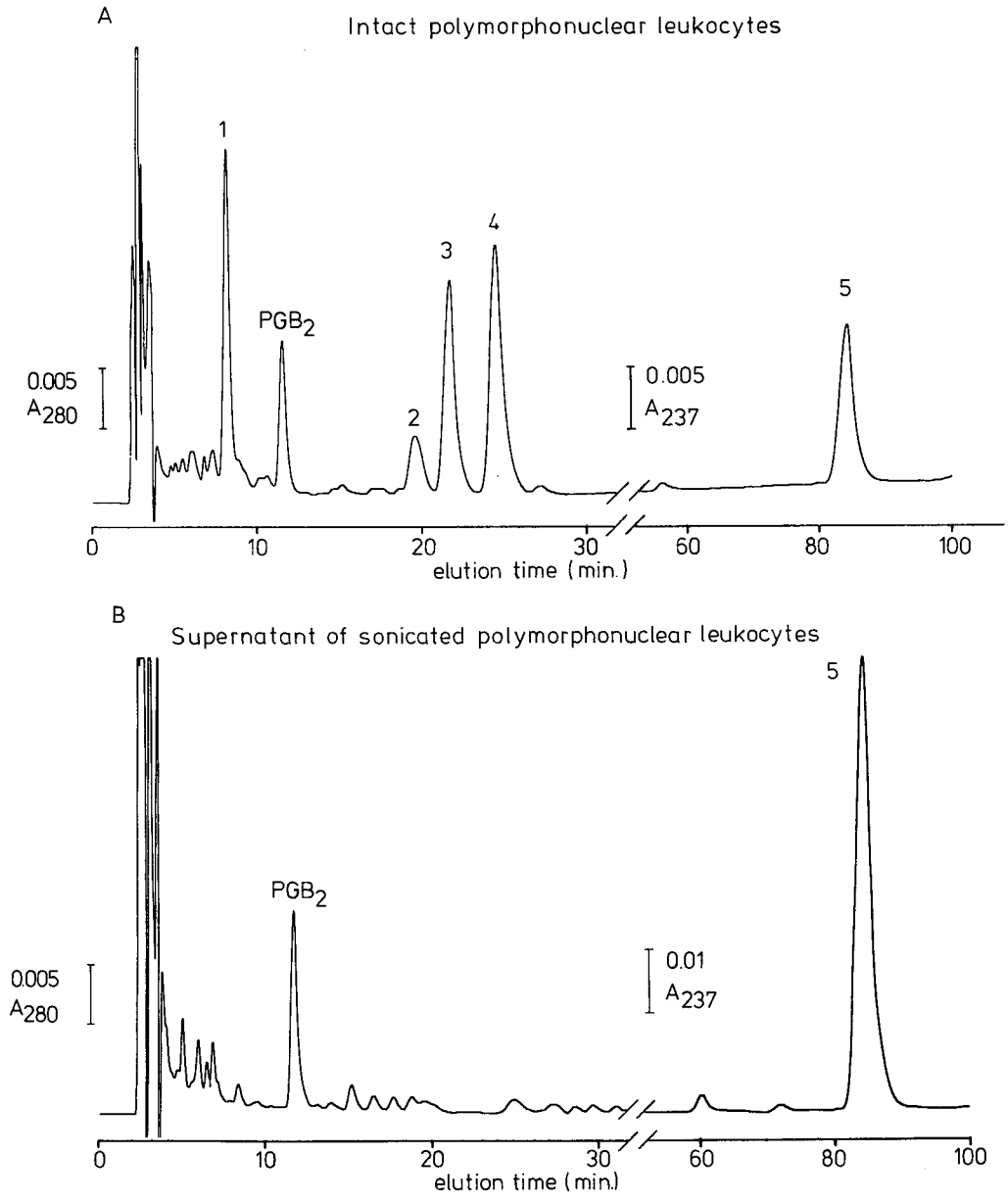

Fig. 1. Reversed-phase HPLC profiles of the leukotrienes and monohydroxy acids formed by intact bovine polymorphonuclear leukocytes (A) and sonicated bovine polymorphonuclear leukocytes (B). $16 \cdot 10^7$ polymorphonuclear leukocytes or the supernatant of $16 \cdot 10^7$ sonicated polymorphonuclear leukocytes were brought to 4 ml. Incubation was in the presence of 80 μM arachidonic acid, 20 μM calcium ionophore A23187, 5 mM glutathione and 2 mM Ca^{2+} in phosphate-buffered saline (pH 7.4) for 10 min. Solvent system was tetrahydrofuran/methanol/water/acetic acid (25 : 30 : 45 : 0.1, v/v) (pH 5.5) with a flow-rate of 0.9 ml/min. Detection was at 280 nm or 237 nm as indicated, using prostaglandin B_2 as an internal standard. Peaks were identified by HPLC behaviour, coelution with synthetic standards and ultraviolet spectra. Peak 1: leukotriene C_4; peak 2: 12-*epi*,6-*trans*-leukotriene B_4 and 6-*trans*-leukotriene B_4; peak 3: leukotriene B_4; peak 4: 12-*epi*,6-*trans*,8-*cis*-leukotriene B_4; peak 5: 12-HETE.

substrate specificity by using linoleic acid instead of arachidonic acid as a substrate [10]. As shown in Table I, linoleic acid is not converted by the platelet 12-lipoxygenase, which is in accordance with Ref. 7. However, for the 12-lipoxygenase from sonicated bovine polymorphonuclear leuko-

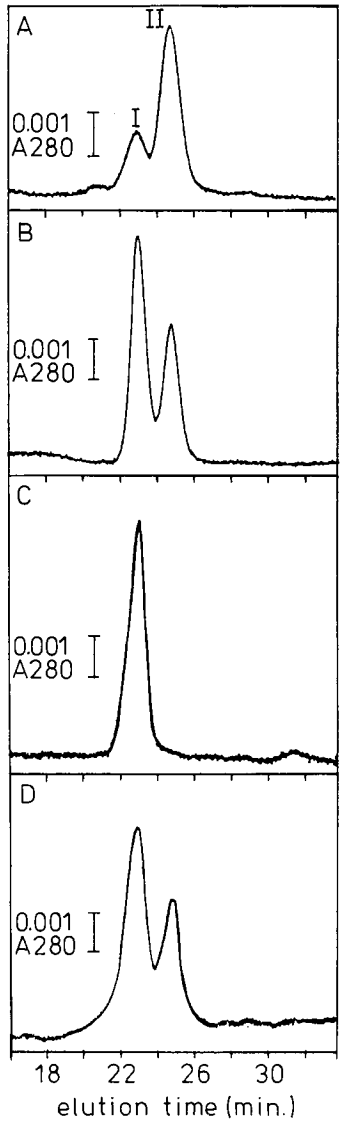

Fig. 2. Straight-phase HPLC profiles of: (A) the reference compounds 5(*S*),12(*S*)-dihydroxy-6-*trans*,8-*cis*,10-*trans*,14-*cis*-eicosatetraenoic acid and 5(*R*),12(*S*)-dihydroxy-6-*trans*,8-*cis*,10-*trans*,14-*cis*-eicosatetraenoic acid synthesized as described in Materials and Methods. (B) The 5(*S*)-hydroxy derivative of bovine platelet derived 12(*S*)-HETE together with the reference compounds. (C) The 5(*S*)-hydroxy derivative of bovine polymorphonuclear leukocyte derived 12-HETE. (D) The 5(*S*)-hydroxy derivative of bovine polymorphonuclear leukocyte derived 12-HETE together with the reference compounds. Solvent system, hexane/2-propanol/acetic acid (93:7:0.05, v/v); flow-rate, 1.0 ml/min. Detection was at 270 nm.

cytes, linoleic acid was found to be as good a substrate as arachidonic acid. Linoleic acid was converted into a compound with an absorption maximum at 235 nm, characteristic of a conjugated diene system. The compound coeluted with 13-HOD in two different HPLC systems, in which 13-HOD and 9-HOD could be well separated. Finally, the mass spectrum of the trimethylsilylated and hydrogenated methyl ester showed two abundant ions at *m/z* 173 and *m/z* 315 and ions of lower abundance at *m/z* 339, *m/z* 286 and *m/z* 73, in accordance with literature data for 13-HOD [17]. This proves that linoleic acid is converted into 13-HOD by the 12-lipoxygenase from bovine polymorphonuclear leukocytes. This finding clearly demonstrates that the 12-lipoxygenase from the bovine polymorphonuclear leukocytes is distinct from the platelet 12-lipoxygenase.

In addition we studied the ATP and Ca^{2+} dependency of both 12-lipoxygenases. It is known that 5-lipoxygenases are stimulated by Ca^{2+} [19] and ATP [20,21]. We found that the 12-lip-

TABLE II

THE INFLUENCE OF ATP ON 12-HETE AND 13-HOD PRODUCTION BY INTACT AND SONICATED BOVINE POLYMORPHONUCLEAR LEUKOCYTES AND PLATELETS

Cells and supernatants were incubated according to the conditions described in the legend to Fig. 2 except for the Ca^{2+} concentration, which was 4 mM, and the ATP cocentration, which was 2 mM. Results are given as nmol hydroxy acid formed in a 4 ml incubation ± S.E., $n = 3$. The ratio of platelets to polymorphonuclear leukocytes was 3 ± 1 (mean ± S.E., $n = 3$). I-PMN, intact polymorphonuclear leukocytes; S-PMN, supernatant of sonicated polymorphonuclear leukocytes; I-PLT, intact platelets; S-PLT, supernatant of sonicated platelets.

	I-PMN		S-PMN		I-PLT		S-PLT	
	12-HETE	13-HOD	12-HETE	13-HOD	12-HETE	13-HOD	12-HETE	13-HOD
−ATP	6.5 ± 0.9	3.1 ± 0.3	26 ± 4	32 ± 8	16 ± 11	2 ± 2	2 ± 1	0.6 ± 0.2
+ATP	3.0 ± 0.5	1.6 ± 0.9	33 ± 7	36 ± 2	15 ± 8	2 ± 2	1 ± 1	0.5 ± 0.1

oxygenases from both bovine polymorphonuclear leukocytes and from bovine platelets are not stimulated significantly by 2 mM ATP (Table II). It has been reported that human platelet 12-lipoxygenase is not dependent on Ca^{2+} [6,22]. We observed that bovine platelet 12-lipoxygenase (results not shown), and also the 12-lipoxygenase from sonicated bovine polymorphonuclear leukocytes is independent of Ca^{2+}. The production of 12-HETE when no Ca^{2+} was added was 11 ± 2 nmol formed in a 4 ml incubation, compared to 10 ± 1 nmol when 2 mM Ca^{2+} was added and 11 ± 2 nmol when 10 mM EDTA was added. When linoleic acid was used as a substrate for this enzyme, 25 ± 8 nmol 13-HOD was formed in the absence of Ca^{2+}, 22 ± 4 nmol in the presence of 2 mM Ca^{2+} and 28 ± 8 nmol when 10 mM EDTA was added (mean value $\pm$ S.E., $n = 4$).

Discussion

In this paper we have shown that the bovine polymorphonuclear leukocyte possesses a 12-lipoxygenase which becomes apparent only after sonication of the cells and is distinct from the platelet 12-lipoxygenase. Although this 12-lipoxygenase resembles the platelet enzyme, since it also forms 12(*S*)-HETE from arachidonic acid and is also independent of ATP and Ca^{2+}, both 12-lipoxygenases also show evident differences. Under our conditions, sonication of the cells results in an increase of the polymorphonuclear leukocyte 12-lipoxygenase-activity and in a decrease of the platelet 12-lipoxygenase-activity. The most striking difference is their behaviour towards linoleic acid. While this fatty acid is not metabolized by 12-lipoxygenase from platelets (cf. Ref. 7), it appeared to be an excellent substrate for the 12-lipoxygenase from polymorphonuclear leukocytes, resulting in the formation of 13-HOD. Platelet 12-lipoxygenase belongs, together with, for example, soybean lipoxygenase 1 [23,24] and reticulocyte lipoxygenase [25], to the group of lipoxygenases, which show a preference for hydrogen abstraction at a position which is determined by the distance to the methyl end of the fatty acid molecule. The 12-lipoxygenase from platelets abstracts hydrogen from arachidonic acid at $n - 11$, as can be seen in Fig. 3. As linoleic acid does not

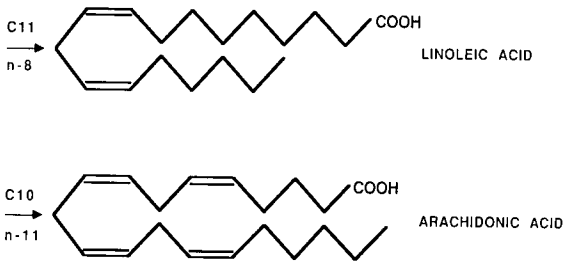

Fig. 3. Comparison of linoleic acid and arachidonic acid with respect to the site of hydrogen abstraction.

possess a methylene group with adjacent *cis* double bounds in this position, it cannot be metabolized by platelet 12-lipoxygenase. 5-Lipoxygenases, for example, from RBL-1 cells [26] and wheat lipoxygenase [27], seem to recognize the site of hydrogen abstraction by its distance to the carboxylic end of the molecule. Assuming that the 12-lipoxygenase from bovine polymorphonuclear leukocytes belongs to this latter group, the conversion of linoleic acid can be explained satisfactorily. Counting from the carboxylic end of the fatty acid, hydrogen abstraction occurs at C10 in arachidonic acid, and at C11 in linoleic acid (Fig. 3), forming 12-HETE and 13-HOD, respectively.

To date, it is not clear why this new 12-lipoxygenase activity becomes apparent only upon sonication fo the cells. An explanation could be that the substrate is not able to reach the enzyme in the intact cell, similar to the suggestion of McGuire et al. [28] for the 15-lipoxygenase in human polymorphonuclear leukocytes. However, concomitantly with the appearance of the 12-lipoxygenase activity, a complete disappearance of the 5-lipoxygenase activity is observed. These findings might indicate that both the 5- and the 12-lipoxygenase activity are associated with the same protein.

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References

1 Hammarström, S. (1984) Biochem. Actions Hormones 11, 1–20
2 Stjernschantz, J. (1984) Med. Biol. 62, 215–230
3 Walstra, P., Verhagen, J., Veldink, G.A. and Vliegenthart, J.F.G. (1984) Biochim. Biophys. Acta 795, 499–503
4 Verhagen, J., Bruynzeel, P.L.B., Koedam, J.A., Wassink, G.A., De Boer, M., Terpstra, G.K., Kreukniet, J., Veldink, G.A. and Vliegenthart, J.F.G. (1984) FEBS Lett. 168, 23–28
5 Peters, S.P., MacGlashan, D.W., Schulman, E.S., Schleimer, R.P., Hayes, E.C., Rokach, J., Adkinson, N.F. Jr. and Lichtenstein, L.M. (1984) J. Immunol. 132, 1972–1979
6 Hamberg, M. and Samuelsson, B. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 3400–3404
7 Nugteren, D.H. (1975) Biochim. Biophys. Acta 380, 299–307
8 Shinjo, F., Yoshimoto, T., Yokoyama, C., Yamamoto, S., Izumi, S., Komatsu, N. and Watanabe, K. (1986) J. Biol. Chem. 261, 3377–3381
9 Hamasaki, Y. and Tai, H.-H. (1984) Biochim. Biophys. Acta 793, 393–398
10 Claeys, M., Kivits, G.A.A., Christ-Hazelhof, E. and Nugteren, D.H. (1985) Biochim. Biophys. Acta 837, 35–51
11 Yoshimoto, T., Miyamoto, Y., Ochi, K. and Yamamoto, S. (1982) Biochim. Biophys. Acta 713, 638–646
12 Verhagen, J., Bouman, A.A., Vliegenthart, J.F.G. and Boldingh, J. (1977) Biochim. Biophys. Acta 486, 114–120
13 Metz, S.A., Hall, M.E., Harper, T.W. and Murphy, R.C. (1982) J. Chromatogr. 233, 193–201
14 Verhagen, J., Wassink, G.A., Kijne, G.M., Viëtor, R.J. and Bruynzeel, P.L.B. (1986) J. Chromatogr. 378, 208–214
15 Verhagen, J., Walstra, P., Veldink, G.A., Vliegenthart, J.F.G. and Bruynzeel, P.L.B. (1984) Prostaglandin Leukotriene Med. 13, 15–21
16 Marcus, A.J., Broekman, M.J., Safier, L.B., Ullman, H.L., Serhan, C.N., Rutherford, L.E., Korchak, H.M. and Weissmann, G. (1982) Biochem. Biophys. Res. Commun. 109, 130–137
17 Woolard, P.M. and Mallet, A.I. (1984) J. Chromatogr. 306, 1–21
18 Claeys, M., Coene, M.-C., Herman, A.G., Jouvenaz, G.H. and Nugteren, D.H. (1982) Biochim. Biophys. Acta 713, 160–169
19 Jakschik, B.A., Sun, F.F., Lee, L. and Steinhoff, M. (1980) Biochem. Biophys. Res. Commun. 95, 103–110
20 Ochi, K., Yoshimoto, T., Yamomoto, S., Taniguchi, K. and Miyamoto, T. (1983) J. Biol. Chem. 258, 5754–5758
21 Rouzer, C.A. and Samuelsson, B. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 6040–6044
22 Lagarde, M., Croset, M., Authi, K. and Crawford, N.C. (1984) Biochem. J. 222, 495–500
23 Hamberg, H. and Samuelsson, B. (1967) J. Biol. Chem. 242, 5329–5335
24 Holman, R.T., Egwim, P.O. and Christie, W.W. (1969) J. Biol. Chem. 244, 1149–1151
25 Bryant, R.W., Bailey, J.M., Schewe, T. and Rapoport, S.M. (1982) J. Biol. Chem. 257, 6050–6055
26 Jakschik, B.A., Sams, R., Sprecher, H. and Needleman, P. (1980) Prostaglandins 20, 401–410
27 Kühn, H., Heydeck, D., Wiesner, R. and Schewe, T. (1985) Biochim. Biophys. Acta 830, 25–29
28 McGuire, J., McGee, J., Crittenden, N. and Fitzpatrick, F. (1985) J. Biol. Chem. 260, 8316–8319