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Stereochemistry of Leukotriene B_4 Metabolites Formed by the Reductase Pathway in Porcine Polymorphonuclear Leukocytes: Inversion of Stereochemistry of the 12-Hydroxyl Group†

Sandra Wainwright,‡ J. R. Falck,§ Pendri Yadagiri,§ and William S. Powell*,‡

Endocrine Laboratory, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec, Canada H3A 1A1, Department of Medicine, McGill University, Montreal, Quebec, Canada H3A 1A1, and Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, Texas 75235

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ABSTRACT: Leukotriene B_4 (LTB_4), a potent proinflammatory agent, is a major metabolite of arachidonic acid in polymorphonuclear leukocytes (PMNL). When porcine PMNL were incubated with LTB_4 and the products purified by reversed-phase high-pressure liquid chromatography (HPLC), we previously identified two metabolites: 10,11-dihydro-LTB_4 and 10,11-dihydro-12-oxo-LTB_4 [Powell, W. S., & Gravelle, F. (1989) *J. Biol. Chem. 264*, 5364-5369]. Further analysis of the reaction products by normal-phase HPLC has now revealed the presence of a third major metabolite of LTB_4. This product is not formed in detectable amounts in the first 5 min of the reaction but accounts for about 20-30% of the reaction products after 60 min, when LTB_4 has been completely metabolized. The mass spectrum and gas chromatographic properties of the new metabolite are identical with those of 10,11-dihydro-LTB_4, suggesting that it is a stereoisomer of this compound. This product was identified as 10,11-dihydro-12-epi-LTB_4 [i.e., 5(*S*),12(*R*)-dihydroxy-6,8,14-eicosatrienoic acid] by comparison of its chromatographic properties with those of the authentic chemically synthesized compound. Both 10,11-dihydro-LTB_4 and 10,11-dihydro-12-oxo-LTB_4 were enzymatically converted to 10,11-dihydro-12-epi-LTB_4 by porcine PMNL, the former compound being the better substrate. The reaction was reversible, since both 10,11-dihydro-12-epi-LTB_4 and 10,11-dihydro-12-oxo-LTB_4 could be converted to 10,11-dihydro-LTB_4. When dihydro metabolites of LTB_4 labeled with tritium in the 12-position were incubated with porcine PMNL, only about 15% of the tritium was retained in the 12-position of the product, suggesting that epimerization due to replacement of the 12-hydroxyl group itself was unlikely. Our results would be consistent with an epimerase-catalyzed reacton in which 10,11-dihydro-12-oxo-LTB_4 is an intermediate. This would explain the lack of stereospecificity in the reduction of 10,11-dihydro-12-oxo-LTB_4 as well as the partial retention of tritium in the 12-position after epimerization. Alternatively, it is possible that the reactions could be catalyzed by a combination of stereospecific 12-hydroxy dehydrogenases and 12-keto reductases. These results indicate that the stereochemistry of 12-hydroxy eicosanoids can be reversed by PMNL, which could have important implications for their biological activities.

Leukotriene B_4 (LTB_4) is a potent mediator of inflammation which is synthesized primarily by polymorphonuclear leukocytes (PMNL)[1] (Borgeat & Samuelsson, 1979). It promotes the adhesion of leukocytes to the vascular endothelium, followed by diapedesis (Dahlén et al., 1981), and plays an important role in attracting PMNL to inflammatory sites (Higgs et al., 1981). LTB_4 is metabolized by three pathways initiated by 20-hydroxylation (Lindgren et al., 1981; Powell, 1984), 19-hydroxylation (Maas et al., 1982; Powell, 1987), and reduction to dihydro metabolites (Powell, 1987). The major route of metabolism of LTB_4 in human PMNL is the LTB_4 20-hydroxylase pathway (Lindgren et al., 1981; Powell, 1984), resulting in the formation of 20-hydroxy-LTB_4 and subsequently 20-oxo-LTB_4 and ω-carboxy-LTB_4 (Soberman et al.,

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* Correspondence should be addressed to this author at the Royal Victoria Hospital.
‡ Royal Victoria Hospital and McGill University.
§ University of Texas Southwestern Medical Center.

[1] Abbreviations: LT, leukotriene, 12-HETE, 12-hydroxy-5,8,10,14-eicosatetraenoic acid; RP-HPLC, reversed-phase high-pressure liquid chromatography; NP-HPLC, normal-phase high-pressure liquid chromatography; ODS, octadecylsilyl; GC-MS, gas chromatography-mass spectrometry; PMNL, polymorphonuclear leukocytes.

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1988). Rat PMNL, on the other hand, possess relatively little 20-hydroxylase activity but metabolize LTB_4 principally by the reductase and 19-hydroxylase pathways (Powell, 1987). The reductase pathway has also been demonstrated as the major route for the metabolism of LTB_4 in other types of cells in the rat (Kaever et al., 1987) as well as in human macrophages (Schönfeld et al., 1988).

ω-Oxidation of LTB_4 by either 19- or 20-hydroxylase does not occur to a significant extent in porcine PMNL, which metabolize this substance almost exclusively to dihydro products. We have recently identified the major metabolites of LTB_4 by these cells as 10,11-dihydro-LTB_4 and 10,11-dihydro-12-oxo-LTB_4 (Powell & Gravelle, 1989). Although 10,11-dihydro-LTB_4 could be formed directly from LTB_4, without the requirement for a 12-oxo intermediate, the dihydro and dihydrooxo metabolites were interconverted by PMNL. In the present study, we have identified a third dihydro metabolite of LTB_4 by comparison of its chromatographic and mass spectral properties with those of authentic chemically synthesized standards.

Experimental Procedures

Materials. Unlabeled LTB_4 was prepared by incubation of arachidonic acid and A23187 (10 μM) with porcine PMNL in the presence of 5,8,11,14-eicosatetraynoic acid as previously described (Powell, 1983; Borgeat et al., 1981). 10,11-Dihydro-12-oxo-LTB_4 was synthesized by incubation of LTB_4 with a preparation of mixed porcine leukocytes for 60 min and separation of the products by reversed-phase (RP) high-pressure liquid chromatography (HPLC) (Powell & Gravelle, 1989). 10,11-Dihydro-LTB_4 [5(*S*),12(*S*)-dihydroxy-6(*Z*),8(*E*),14(*Z*)-eicosatrienoic acid] and 10,11-dihydro-12-epi-LTB_4 [5(*S*),12(*R*)-dihydroxy-6(*Z*),8(*E*),14(*Z*)-eicosatrienoic acid] were chemically synthesized as previously described (Yadagiri et al., 1989). It should be noted that due to the priority rules in assigning *R* and *S* configurations, LTB_4 has the 5*S*,12*R* configuration, whereas 10,11-dihydro-LTB_4 has the 5*S*,12*S* configuration, even though the stereochemistry of the 12-hydroxyl group has not been inverted.

10,11-Dihydro[12-^{3}H]LTB_4 and 10,11-dihydro-12-epi[12-^{3}H]LTB_4 were prepared by reduction of 10,11-dihydro-12-oxo-LTB_4 (25 μg) with sodium [^{3}H]borohydride (30 mCi; 15 Ci/mmol) in methanol (0.15 mL). The mixture was kept at 0 °C for 15 min and at room temperature for a further 45 min prior to extraction on a cartridge (Waters C_{18} Sep-Pak) of octadecylsilyl (ODS) silica (Powell, 1980). After the sample had been loaded onto the cartridge, it was washed with 15% ethanol in water (20 mL), water (20 mL), and petroleum ether (10 mL). Reaction products were eluted with redistilled methyl formate (Aldrich) (10 mL) and further purified by normal-phase (NP) HPLC as described below.

Preparation of Porcine PMNL. Porcine PMNL were prepared as previously described (Powell, 1984). Porcine blood was treated with Dextran T-500 (Pharmacia Fine Chemicals) to remove most of the red blood cells. Any remaining red blood cells in the leukocyte fraction were lysed by treatment with NH_4Cl. PMNL were obtained after centrifugation over Ficoll-Paque (Pharmacia Fine Chemicals) and were resuspended in Dulbecco's phosphate-buffered saline, containing 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 0.5 mM $MgCl_2$, and 0.9 mM $CaCl_2$.

Incubation Conditions. Porcine PMNL (10 mL; 5×10^7 cells/mL) were incubated with LTB_4 (2 μM), 10,11-dihydro-LTB_4 (2 μM), 10,11-dihydro-12-epi-LTB_4 (2 μM), or 10,11-dihydro-12-oxo-LTB_4 (2 μM) at 37 °C for various times. The incubations were terminated by addition of methanol (0.4 volume) followed by cooling to −20 °C.

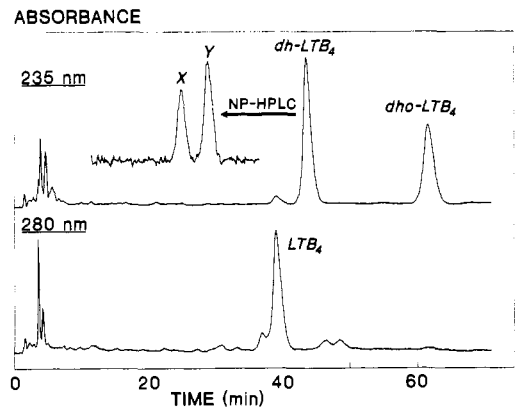

FIGURE 1: Reversed-phase HPLC of LTB_4 metabolites. LTB_4 (2 μM) was incubated with porcine PMNL (50×10^6 cells/mL) for 60 min at 37 °C. The products were extracted on a cartridge of octadecylsilyl silica and analyzed by RP-HPLC on a Waters-Millipore Novapak C_{18} column with water/acetonitrile/acetic acid (63:37:0.02) as the mobile phase. The flow rate was 2 mL/min. The product absorbing at 235 nm with a retention time of 43 min was rechromatographed by NP-HPLC (inset) on a silicic acid column (RoSil; Alltech Associates) with hexane/2-propanol/acetic acid (95:5:0.1) at a flow rate of 2 mL/min as the mobile phase. Abbreviations: dh-LTB_4, 10,11-dihydro-LTB_4; dho-LTB_4, 10,11-dihydro-12-oxo-LTB_4.

Extraction and Analysis of Products by NP-HPLC. PGB_2 (430 pmol) was added to each of the incubation mixtures as an internal standard. The incubation medium was then extracted with ODS silica as described above, and the products in the methyl formate fraction were quantitated by NP-HPLC using a Waters solvent delivery system and a Waters Model 490 UV detector coupled to a Raytest Ramona data system. The stationary phase was a column (300 × 4.6 mm) of silicic acid (5-μm RoSil, Alltech Associates, Inc., Deerfield, IL). It was eluted isocratically with hexane/2-propanol/acetic acid (95:5:0.1) at a flow rate of 2 mL/min.

The amounts of 10,11-dihydro-LTB_4, 10,11-dihydro-12-epi-LTB_4, and 10,11-dihydro-12-oxo-LTB_4 were calculated from the ratios of their peak areas at 235 nm to that of PGB_2, the internal standard, at 280 nm. The extinction coefficients used for the dihydro compounds and PGB_2 were 22 500 and 28 680, respectively.

Gas Chromatography–Mass Spectrometry (GC–MS). Electron impact GC–MS was performed on a VG ZAB instrument located in the Biomedical Mass Spectrometry Unit of McGill University. Gas chromatography was performed on a capillary column (20 m × 0.32 mm) coated with DB-1 (J and W Scientific, Inc.). Prior to analysis, compounds were converted to their methyl ester, trimethylsilyl ether derivatives by treatment with diazomethane and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide, respectively.

Results

When LTB_4 was incubated with porcine PMNL for 60 min at 37 °C and the products analyzed by RP-HPLC, two major metabolites, which absorbed at 235 nm but not at 280 nm, were observed (Figure 1). The product with the retention time (t_R) of 43 min was identified as 10,11-dihydro-LTB_4, whereas that with a t_R of 61 min was identified as 10,11-dihydro-12-oxo-LTB_4 (Powell & Gravelle, 1989). Rechromatography of the 10,11-dihydro-LTB_4 fraction by NP-HPLC revealed the presence of two components (compounds X and Y) in a ratio of about 1:1.4 (Figure 1, inset). Analysis of the methyl ester, trimethylsilyl ether derivatives of these products by GC–MS revealed that they had identical *C* values (23.4). The mass spectra of products X and Y were also identical with

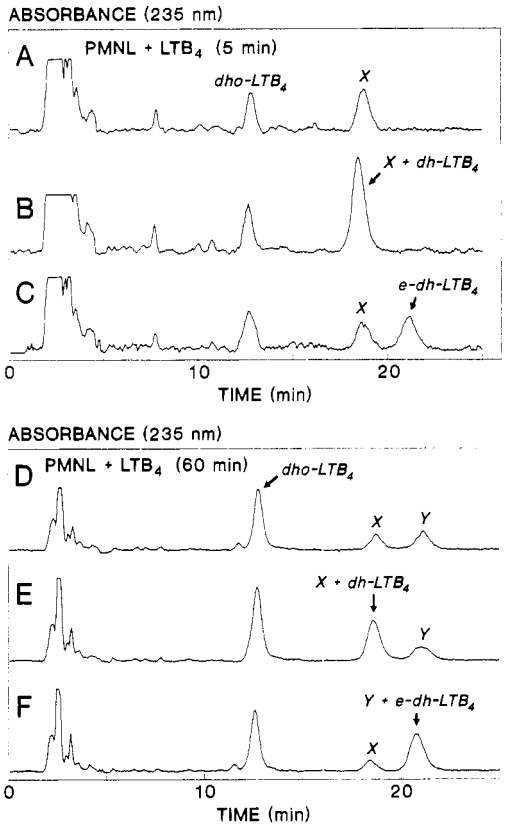

FIGURE 2: Normal-phase HPLC of metabolites of LTB_4 formed after 5 min (A–C) and 60 min (D–F). LTB_4 (2 μM) was incubated with porcine PMNL (50×10^6 cells/mL) for either 5 or 60 min. The products were extracted on a cartridge containing octadecylsilyl silica and aliquots were analyzed by NP-HPLC on a silicic acid column with hexane/2-propanol/acetic acid (95:5:0.1) at a flow rate of 2 mL/min as the mobile phase. Only products absorbing at 235 nm are shown. (A) and (D) show chromatograms obtained from aliquots of the extract without any additions. The other chromatograms are of aliquots of the extract to which has been added 400 ng of either 10,11-dihydro-LTB_4 (B and E) or 10,11-dihydro-12-epi-LTB_4 (C and F). Abbreviations: dh-LTB_4, 10,11-dihydro-LTB_4; e-dh-LTB_4, 10,11-dihydro-12-epi-LTB_4; dho-LTB_4, 10,11-dihydro-12-oxo-LTB_4.

one another, as well as with that which we previously reported (Powell & Gravelle, 1989) for 10,11-dihydro-LTB_4, purified by RP-HPLC alone.

Identification of Products X and Y. The HPLC and GC–MS results suggested that compounds X and Y are stereoisomers of 10,11-dihydro-LTB_4. We previously showed that LTB_4 could be directly reduced to the latter compound, in which case the stereochemistry of the 12-hydroxyl group of the substrate should be preserved. If this is the case, we would expect only one of the two stereoisomers to be formed in the early stages of the reaction, whereas the second one would be formed more slowly. To test this hypothesis, LTB_4 was incubated for different times with porcine PMNL, and the products were analyzed by NP-HPLC. After incubation of LTB_4 with PMNL for 5 min, only one dihydro product (product X) was formed (Figure 2A) whereas incubation for 60 min led to the formation of both products X and Y (Figure 2D). Cochromatography of these samples with chemically synthesized standards indicated that product X cochromatographed with authentic 10,11-dihydro-LTB_4 (Figure 2B,E), whereas product Y cochromatographed with authentic 10,11-dihydro-12-epi-LTB_4 (Figure 2C,F). Authentic 10,11-dihydro-LTB_4 and 10,11-dihydro-12-epi-LTB_4 also cochromatographed with products X and Y upon RP-HPLC, but the two stereoisomers were not separated from one another with either methanol/water/acetic acid (58:42:0.02; t_R, 25 min) or acetonitrile/water/acetic acid (38:62:0.02; t_R, 36 min) as the mobile phase.

Time Courses for the Formation of Metabolites from LTB_4. The time courses for the formation of 10,11-dihydro-LTB_4, 10,11-dihydro-12-epi-LTB_4, and 10,11-dihydro-12-oxo-LTB_4 from LTB_4 are shown in Figure 3A. 10,11-Dihydro-LTB_4 and 10,11-dihydro-12-oxo-LTB_4 are both formed very rapidly and can be detected after incubation for 2 min. There is clearly a delay in the formation of 10,11-dihydro-12-epi-LTB_4, however, and this product could not be detected until 12 min. Its concentration in the incubation mixture gradually increased up to 60 min, at the expense of 10,11-dihydro-LTB_4 and 10,11-dihydro-12-oxo-LTB_4.

Mechanism for the Formation of 10,11-Dihydro-12-epi-LTB_4. The lag phase in the formation of 10,11-dihydro-12-epi-LTB_4 from LTB_4 suggests that it is formed from either 10,11-dihydro-LTB_4 or 10,11-dihydro-12-oxo-LTB_4. Incubation of 10,11-dihydro-LTB_4 with porcine PMNL and analysis of the reaction products by NP-HPLC indicated that this substrate was converted to both 10,11-dihydro-12-epi-LTB_4 and 10,11-dihydro-12-oxo-LTB_4, with the latter product predominating (Figure 3B). Similar results were obtained when 10,11-dihydro-12-epi-LTB_4 was incubated with porcine PMNL (Figure 3C). As with 10,11-dihydro-LTB_4, the major metabolite after 60 min was 10,11-dihydro-12-oxo-LTB_4. Substantial amounts of 10,11-dihydro-LTB_4 were also formed, indicating that the two dihydro stereoisomers are interconvertible.

The most obvious mechanism for the formation of 10,11-dihydro-12-epi-LTB_4 from LTB_4 would be via reduction of 10,11-dihydro-12-oxo-LTB_4. To determine whether this occurred, the latter product was incubated with porcine PMNL for different times, and the products were analyzed by NP-HPLC (Figure 3D). This resulted in the formation of both 10,11-dihydro-LTB_4 and the corresponding 12-epi stereoisomer in comparable amounts. However, on the basis of these kinetic experiments (Figure 3B,D), 10,11-dihydro-LTB_4 appeared to be a considerably better substrate than 10,11-dihydro-12-oxo-LTB_4 for the formation of 10,11-dihydro-12-epi-LTB_4, raising the possibility that the two dihydro-LTB_4 stereoisomers could be directly interconverted.

To determine whether the hydrogen atom in the 12-position of 10,11-dihydro-LTB_4 was retained after it had been converted to 10,11-dihydro-12-epi-LTB_4, we prepared 10,11-dihydro-LTB_4 labeled with tritium specifically in the 12-position and incubated this product with porcine PMNL. This tritium atom would be lost if 10,11-dihydro-12-oxo-LTB_4 was required as an intermediate in the epimerization reaction. After incubation for various times, the reaction products were analyzed by NP-HPLC. After 60 min, the substrate (t_R, 21 min) was still highly labeled with tritium, whereas the product, 10,11-dihydro-12-epi-LTB_4 (t_R, 23 min), was only slightly labeled (Figure 4A). The second product, 10,11-dihydro-12-oxo-LTB_4 (t_R, 14 min), did not possess any detectable radioactivity, confirming the presence of tritium almost exclusively at the 12-position of the substrate. Similar results were obtained when 10,11-dihydro-12-epi[12-^{3}H]LTB_4 was incubated with porcine PMNL for 60 min at 37 °C (Figure 4B).

The specific activities of the products formed after incubation for different times are shown in Table I. Insufficient amounts of products were formed after 10 min to determine their specific activities accurately. However, after 20 min, the specific activity of 10,11-dihydro-12-epi-LTB_4, formed from 10,11-dihydro-LTB_4, was only 17% that of the initial substrate. Similar results were obtained when 10,11-dihydro-12-epi-LTB_4

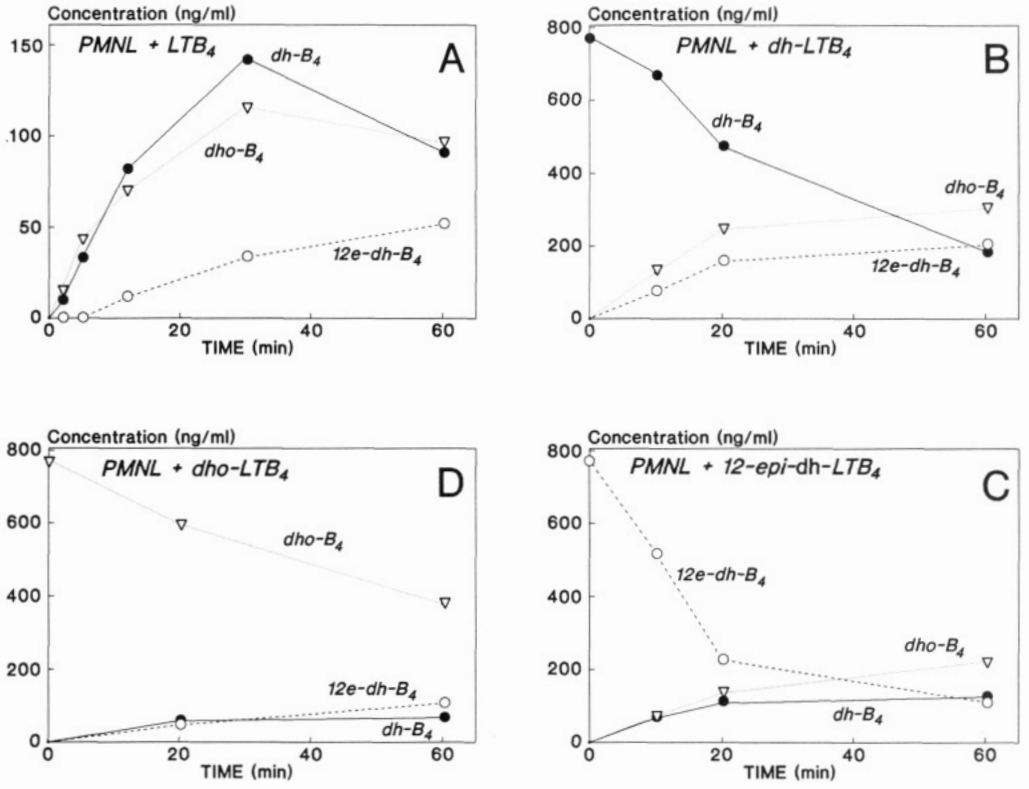

FIGURE 3: Time courses for the formation of metabolites from LTB_4 (A), 10,11-dihydro-LTB_4 (B), 10,11-dihydro-12-epi-LTB_4 (C), and 10,11-dihydro-12-oxo-LTB_4 (D) by porcine PMNL. Porcine PMNL (50×10^6 cells/mL) were incubated with the above substrates (2 μM) for various times at 37 °C. The incubations were terminated by the addition of methanol, and PGB_2 (430 pmol) was added as an internal standard. After solid-phase extraction, the amounts of 10,11-dihydro-LTB_4 (●), 10,11-dihydro-12-epi-LTB_4 (○), and 10,11-dihydro-12-oxo-LTB_4 (▽) were determined by NP-HPLC, using PGB_2 as an internal standard, as described in the legend to Figure 2.

Table I: Specific Activities of 10,11-Dihydro-LTB_4 (dh-LTB_4) and 10,11-Dihydro-12-epi-LTB_4 (12e-dh-LTB_4) after Incubation of either 10,11-Dihydro[12-^{3}H]LTB_4 or 10,11-Dihydro-12-epi[12-^{3}H]LTB_4 with Porcine PMNL for Various Times As Described in the Legend to Figure 4[a]

time (min)	sp act. (dpm/pmol) dh-LTB_4	12e-dh-LTB_4
	dh-LTB_4 → 12e-dh-LTB_4	
0	6.94	
10	6.75	*b*
20	6.69	1.16
60	7.82	1.23
	12e-dh-LTB_4 → dh-LTB_4	
0		5.90
10	*b*	6.57
20	0.80	7.01
60	1.36	9.40

[a] The products were extracted and analyzed by NP-HPLC as described under Experimental Procedures. [b] Only small amounts of products were formed after 10 min, and it was not possible to determine their specific activities accurately under the conditions employed.

was the substrate. The specific activities of both substrates increased with time and were highest after 60 min, consistent with the expected isotope effect for loss of the hydrogen atom in the 12-position.

Discussion

Porcine PMNL convert LTB_4 into two 5,12-dihydroxy compounds in which the 10,11-double bond has been reduced. The stereochemistry of the two chiral centers of LTB_4 at carbons 5 and 12 is unaltered in one of these products, which we have referred to as 10,11-dihydro-LTB_4. The 12-hydroxyl group of the second product has the opposite configuration to that of the corresponding hydroxyl group of LTB_4, and this product has been referred to as 10,11-dihydro-12-epi-LTB_4. These two products have identical gas chromatographic properties and mass spectra. They cannot be separated by RP-HPLC with the conditions we used but are readily separated by NP-HPLC.

Previous experiments showing that deuterium-labeled LTB_4 can be convertd directly to 10,11-dihydro-LTB_4 (Powell & Gravelle, 1989), along with the data in Figure 3B, indicate that 10,11-dihydro-12-oxo-LTB_4 can be formed by a combination of reactions 1 and 2 (Figure 5). However, the time courses for the formation of 10,11-dihydro-LTB_4 and 10,11-dihydro-12-oxo-LTB_4 from LTB_4 are quite similar to one another, and there does not appear to be a significant lag phase in either case (Figure 3A). This suggests that 10,11-dihydro-12-oxo-LTB_4 could also be formed by another mechanism (i.e., reactions 4 and 5; Figure 5), independently of 10,11-dihydro-LTB_4, although we do not as yet have any direct evidence for the formation of the required intermediate, 12-oxo-LTB_4.

From the time courses shown in Figure 3A, it is apparent that 10,11-dihydro-12-epi-LTB_4, unlike 10,11-dihydro-LTB_4, is not an initial reaction product. As shown in Figure 3B,D, the 12-epi isomer can be formed directly or indirectly from either 10,11-dihydro-LTB_4 or 10,11-dihydro-12-oxo-LTB_4. There are several possible mechanisms that could explain the inversion of stereochemistry at C_{12} of 10,11-dihydro-LTB_4. One of the most obvious might be the initial oxidation of the latter substance to 10,11-dihydro-12-oxo-LTB_4 by a dehydrogenase, followed by reduction to 10,11-dihydro-12-epi-LTB_4 by a keto reductase (reactions 2 and 3; Figure 5). Alternatively, it is possible that the 12-hydroxy dehydrogenase could catalyze the reversible reaction in both directions. However, a number of observations raise some questions about this mechanism. First 10,11-dihydro-LTB_4 is converted more rapidly than 10,11-dihydro-12-oxo-LTB_4 to 10,11-dihydro-

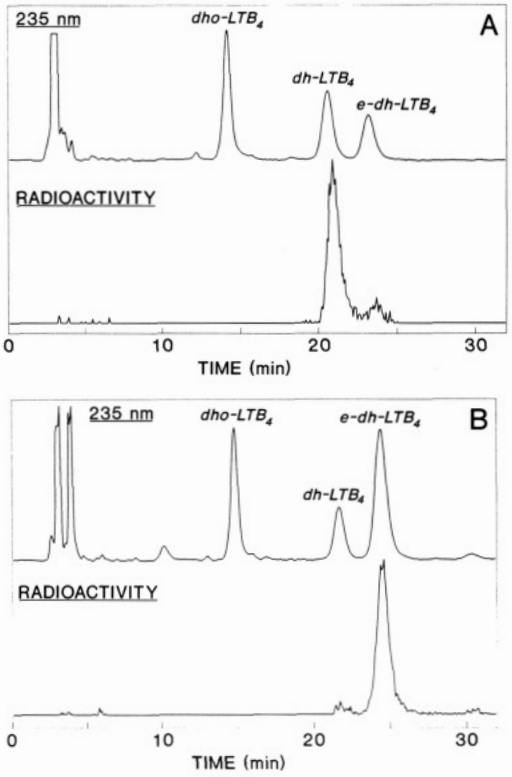

FIGURE 4: Normal-phase HPLC of metabolites of 10,11-dihydro-LTB_4 (A) and 10,11-dihydro-12-epi-LTB_4 (B) labeled with tritium in the 12-position. Porcine PMNL (5×10^8 cells in 10 mL) were incubated with 10,11-dihydro[12-^{3}H]LTB_4 (2 μM; 138 000 dpm/incubation) or 10,11-dihydro-12-epi[12-^{3}H]LTB_4 (2 μM; 118 000 dpm/incubation) for 60 min at 37 °C. The products were extracted and purified by NP-HPLC as described under Experimental Procedures.

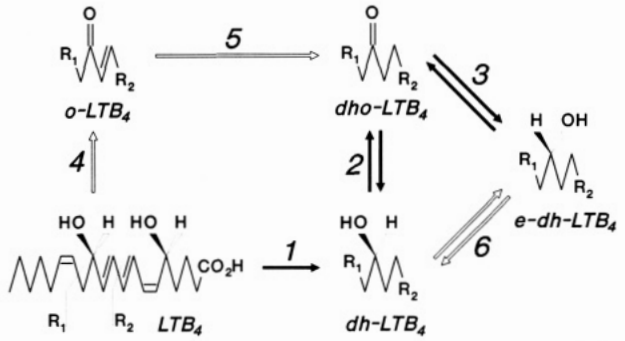

FIGURE 5: Scheme for the formation of dihydro metabolites of LTB_4. The solid arrows indicate the pathways that are most consistent with the observations described here, whereas the open arrows indicate alternative pathways that may also exist. Reactions 1 and 5 would be catalyzed by a 10,11-reductase. An epimerase could catalyze either reaction 6 or reactions 2 and 3 in both directions. Alternatively, reactions 2 and 3, resulting in the formation of 10,11-dihydro-12-oxo-LTB_4, could be catalyzed by a 12-hydroxy dehydrogenase. It is possible that the same enzyme could also catalyze the reverse reactions or that this could be accomplished by one (nonstereospecific) or two (stereospecific) ketoreductases. Reactions 4 and 5 represent an alternative pathway for the synthesis of 10,11-dihydro-12-oxo-LTB_4, but we have not as yet obtained any evidence for the existence of the required intermediate, 12-oxo-LTB_4 (o-LTB_4).

12-epi-LTB_4, raising the possibility that the latter compound could be formed from 10,11-dihydro-12-oxo-LTB_4 via reactions 2 and 6 (Figure 5) as follows:

10,11-dihydro-12-oxo-LTB_4 → 10,11-dihydro-LTB_4 → 10,11-dihydro-12-epi-LTB_4

On the other hand, the fact that 10,11-dihydro-12-oxo-LTB_4 is a poorer substrate could also be explained if it is not transported into the cell as well as 10,11-dihydro-LTB_4.

Another concern about the involvement of a keto reductase is the observation that 10,11-dihydro-12-oxo-LTB_4 is reduced to 12*R* and 12*S* isomers in approximately equal amounts (Figure 3D). With the exception of racemases and epimerases, enzymatic reactions are generally stereospecific. One might therefore expect a keto reductase to stereospecifically reduce the 12-oxo group of the substrate to either a 12(*R*)- or a 12(*S*)-hydroxyl group. For example, prostaglandin D_2 is reduced stereospecifically to 9α,11β-PGF_2 by a keto reductase in the lung (Seibert et al., 1987). However, it has recently been shown that 12-oxo-5,8,10,14-eicosatetraenoic acid (i.e., the 12-oxo derivative of 12-HETE) can be reduced by rat liver microsomes in the presence of NADH or NADPH to a mixture of 12(*S*)-HETE and 12(*R*)-HETE in a ratio of about 2:1 (Falgueyret et al., 1988). Although it is not clear whether a single keto reductase is responsible for the formation of both stereoisomers, these results raise the possibility that oxo eicosanoids may not always be reduced stereospecifically. It is also conceivable that two distinct ketoreductases exist both in liver and in PMNL, one of which catalyzes the formation of a 12(*S*)-hydroxy product whereas the other catalyzes the formation of the 12(*R*)-hydroxy isomer.

Finally, the experiment with 12-^{3}H-labeled substrates indicated that about 15% of the tritium was retained after inversion of the stereochemistry of the 12-hydroxyl group (Table I). This suggests either that 10,11-dihydro-12-oxo-LTB_4 is not an obligatory intermediate or that a cofactor or enzyme is labeled during the oxidation of the 12-hydroxyl group of the substrate and that this tritium is then partially transferred back during the reduction of the 12-oxo intermediate. Thus it is possible that NAD^+ or $NADP^+$ could have been converted to tritiated NADH or NADPH and that this tritium could have then been incorporated into 12-oxo-10,11-dihydro-LTB_4 by a ketoreductase.

Another explanation for the inversion of the stereochemistry at C_{12} that must be considered is the action of an epimerase-like enzyme. Eicosanoids can undergo chemical epimerization due to the loss of a hydroxyl group and the formation of an intermediate carbonium ion (Merritt et al., 1980). However, the reduction of 10,11-dihydro-12-oxo-LTB_4 to 10,11-dihydro-LTB_4 and 10,11-dihydro-12-epi-LTB_4 and the interconversion of the two latter products all appear to be enzymatically catalyzed, since none of these reactions occur if the cell suspensions have previously been placed in a boiling water bath. Moreover, the formation of a carbonium ion due to the loss of a hydroxyl group is unlikely, since the experiment with tritium-labeled substrates showed that it is the hydrogen atom that is lost from the 12-position.

Racemases and epimerases generally act by removing a hydrogen atom from the chiral center of the substrate, in the form of either H^+ or H^- (Adams, 1976). Removal and addition of a proton can be accomplished due to the presence of two basic groups at the active site of the enzyme, as in the case of proline racemase (Cardinale & Abeles, 1968; Albery & Knowles, 1986), or by a single basic residue as illustrated by mandelate racemase (Whitman et al., 1985). When the chiral center possesses a hydroxyl group, as in nucleotide sugars, a hydride anion may be removed by a tightly bound cofactor (Adams, 1976). For example, NAD^+ is tightly bound at the active site of UDP-D-glucose 4′-epimerase (Langer & Glaser, 1974), resulting in abstraction of a hydride anion from the substrate and the formation of an oxo intermediate (Wee & Frey, 1973). This hydride anion is not exchanged with the medium but is added back to the oxo intermedite to give a product with the opposite stereochemistry at the 4′-carbon

(Glaser & Ward, 1970). However, in some cases, as with D-ribulose-5-phosphate 3-epimerase, the abstracted hydride anion is exchanged with the medium rather than transferred back to the substrate (McDonough & Wood, 1961).

Since oxo compounds can be formed as intermediates in epimerase-catalyzed reactions, it is conceivable that a single enzyme could catalyze both reactions 2 and 3 (Figure 5). This could explain (i) the preference for a 12-hydroxy over a 12-oxo substrate (if 10,11-dihydro-LTB_4 and 10,11-dihydro-12-epi-LTB_4 are bound more tightly by the enzyme than 10,11-dihydro-12-oxo-LTB_4), (ii) the lack of stereospecificity in the reduction of 10,11-dihydro-12-oxo-LTB_4, and (iii) the partial retention of tritium in the product after epimerization of either 10,11-dihydro-LTB_4 or 10,11-dihydro-12-epi-LTB_4. It should be noted, however, that the above hypothetical mechanism would differ somewhat from those of other enzymes catalyzing the epimerization of chiral hydroxyl groups, which bind the intermediate oxo compounds quite tightly (Adams, 1976).

From the present results it is not possible to conclude which of the above two mechanisms is responsible for the epimerization of 10,11-dihydro-LTB_4 and the formation of its 12-oxo metabolite. Futher clarification of the mechanism of these reactions awaits purification of the relevant enzymes, which will be the object of future investigations in our laboratory.

To our knowledge, this is the first demonstration of the enzymatic racemization of the 12-hydroxyl group of 12-hydroxy eicosanoids. Inversion of the stereochemistry at C_{12} of these compounds could be an important mechanism for regulating their biological activities. The biological response to LTB_4, for example, is quite specific for the 12*R* configuration (Lewis et al., 1981), and conversion to the 12*S* configuration would result in considerable loss of activity. Similarly, the lipoxygenase product 12(*S*)-hydroxy-5,8,10,14-eicosatetraenoic acid [12(*S*)-HETE] possesses considerably less biological activity than 12(*R*)-HETE (Cunningham & Woollard, 1986). Finally, 12(*R*)-hydroxy-5,8,14-eicosatrienoic acid [i.e., the 10,11-dihydro derivative of 12(*S*)-HETE, which was formed by a different mechanism] has very potent proinflammatory effects not shared by the corresponding 12*S* isomer (Murphy et al., 1988). Interestingly, we recently found that porcine PMNL convert 12(*S*)-HETE to a dihydro metabolite (Wainwright & Powell, 1989). The product was identified as a mixture of the 12*R* and 12*S* isomers of 10,11-dihydro-12-HETE, with the 12*R* isomer predominating (Wainwright et al., unpublished results).

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Registry No. 10,11-Dihydro-LTB_4, 120904-46-3; 10,11-dihydro-12-oxo-LTB_4, 121244-14-2; 10,11-dihydro-12-epi-LTB_4, 122221-76-5.

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