Studies on the substrate specificity and inhibition of prostaglandin biosynthesis with methyl branched isomers of eicosa-8,11,14-trienoic acid

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Abstract Seven radioactive methyl branched isomers of eicosa-8,11,14-trienoic acid, in which a methyl branch was located at carbon 2, 5, 10, 13, 17, 18, or 19, were used as substrates to study their enzymatic conversion into homologues of prostaglandin E_1 by microsomes from bovine vesicular glands. The products of this reaction were partially characterized. When the methyl branch was moved towards position 13, the site of stereospecific hydrogen removal by prostaglandin synthetase, the rate of prostaglandin formation declined rapidly. When a methyl branch was at positions 10, 13, 17, 18, or 19, the rates of prostaglandin formation were less than 25% of that from eicosa-8,11,14-trienoic acid. However, when a methyl branch was at positions 2 or 5, these isomers were converted to prostaglandins at least 50% as rapidly as eicosa-8,11,14-trienoic was converted to prostaglandins. The apparent K_m and V_{max} values for eicosa-8,11,14-trienoic acid were respectively 224 μM and 4.62 nmoles/min/mg protein. Kinetic data suggest that the enzyme converted eicosa-8,11,14-trienoic acid to prostaglandins 3.6 times faster than the 5-methyl branched isomer, although the enzyme bound the branched isomer twice as firmly as eicosa-8,11,14-trienoic acid. The concentration-dependent inhibition of prostaglandin formation from eicosa-8,11,14-trienoic acid by the 13-methyl branched isomer was also demonstrated.

Supplementary key words radiochemical assay · methyl branched isomers of PGE_1 · thin-layer chromatography · bovine prostaglandin synthetase · initial velocity · I_{50}

The substrate specificity of prostaglandin synthetase has been extensively studied at the Unilever Research Laboratories in the Netherlands (1–3). These studies have largely been concerned with determining how variations in the position of double bonds and alterations in the chain length of the substrate influence the rate of PG formation. The rate of PG synthesis was also related to how well these various fatty acids function as essential fatty acids. Recently this same research group prepared a variety of substituted derivatives of eicosa-8,11,14-trienoic acid (4, 5) and then used these acids to determine how substrate modification influences the rate of PG biosynthesis (6).

Recently we reported a comparative study in which we determined the rates of desaturation of seven isomeric methyl branched isomers of eicosa-8,11,14-trienoic acid and then compared those rates of desaturation with that found for the conversion of eicosa-8,11,14-trienoic acid to arachidonic acid (7). Eicosa-8,11,14-trienoic acid and arachidonic acid serve both as essential fatty acids and as precursors for PG. It is thus important to correlate how structural modification of eicosa-8,11,14-trienoic acid influences both desaturation at the 5-position and PG biosynthesis. In the study reported here we incubated these seven radioactive methyl branched isomers of eicosa-8,11,14-trienoic acid with microsomes from bovine vesicular glands to determine whether methyl branching influences the rate of PG biosynthesis in the same way as desaturation at the 5-position was influenced. Since some naturally occurring unsaturated fatty acids (8) and their

Abbreviations: 8,11,14–20:3, eicosa-8,11,14-trienoic acid; PGE_1, 11,15-dihydroxy-9-oxo-13-*trans*-prostenoic acid; PGE_2, 11,15-dihydroxy-9-oxo-5-*cis*-13-*trans*-prostadienoic acid; $PGF_{2\alpha}$, 9α,11α,15α-trihydroxy-5-*cis*-13-*trans*-prostadienoic acid; $PGF_{2\beta}$, 9β,11α,15α-trihydroxy-5-*cis*-13-*trans*-prostadienoic acid; PGB_2, 15α-hydroxy-9-oxo-5-*cis*-8(12),13-*trans*-prostatrienoic acid; 2-Me 20:3, 2-methyl eicosa-8,11,14-trienoic acid; 5-Me 20:3, 5-methyl eicosa-8,11,14-trienoic acid; 10-Me 20:3, 10-methyl eicosa-8,11,14-trienoic acid; 13-Me 20:3, 13-methyl eicosa-8,11,14-trienoic acid; 17-Me 20:3, 17-methyl eicosa-8,11,14-trienoic acid; 18-Me 20:3, 18-methyl eicosa-8,11,14-trienoic acid; 19-Me 20:3, 19-methyl eicosa-8,11,14-trienoic acid; GSH, reduced glutathione; TYA, eicosa-5,8,11,14-tetraynoic acid; K_m, the Michaelis constant; V_{max}, maximum velocity; PG, prostaglandin(s).

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structural analogs (9, 10) inhibit PG biosynthesis, we also used some of the methyl branched isomers to determine whether they would inhibit PG biosynthesis.

MATERIALS AND METHODS

Materials

[1-^{14}C]8,11,14-20:3 (0.56 μCi/μmole, 99% radiochemical purity) was prepared as previously described (11). [1-^{14}C]5-Me 20:3 (0.62 μCi/μmole, 98% radiochemical purity), [1-^{14}C]10-Me 20:3 (0.60 μCi/μmole, 97% radiochemical purity), [1-^{14}C]13-Me 20:3 (0.50 μCi/μmole, 97% radiochemical purity), [1-^{14}C]17-Me 20:3 (0.58 μCi/μmole, 94% radiochemical purity), [1-^{14}C]18-Me 20:3 (0.56 μCi/μmole, 98% radiochemical purity), [1-^{14}C]19-Me 20:3 (0.59 μCi/μmole, 98% radiochemical purity), and the identical nonlabeled fatty acids were prepared by total organic synthesis (12). The nonlabeled and the 1-^{14}C-labeled 2-Me 20:3 (0.81 μCi/μmole, 98% radiochemical purity) were synthesized by using the Favorsky rearrangement from unlabeled and 1-^{14}C-labeled 8,11,14–20:3 (13), respectively. PGE_2 and $PGF_{2\alpha}$ were gifts from Dr. John E. Pike of the Upjohn Co., Kalamazoo, Mich. PGB_2 was prepared by treating PGE_2 with 1 N KOH in methanol according to Zusman (14). $PGF_{2\beta}$ was prepared by treating PGE_2 with sodium borohydride (15). L-Epinephrine, reduced glutathione, and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, Mo. TYA was a gift from Hoffmann-La Roche Inc., Nutley, N.J.

Preparation of substrate and inhibitor solutions

All fatty acids were converted to their potassium salts by adding two equivalents of 0.1 N KOH and diluted to a final concentration of 5 mM. TYA was dissolved in ethanol and adjusted to a final concentration of 2 mg/ml in ethanol. The stock solution was kept at −20°C and further diluted just before use.

Preparation of microsomes

Microsomes from frozen bovine vesicular glands were prepared by the procedure of Takeguchi, Kohno, and Sih (16). The microsomes were lyophilized and stored at −20°C until used. The yield of lyophilized microsomes, based on the wet weight of the vesicular glands was 1.3%. The lyophilized preparation contained about 55% protein as measured by the method of Lowry et al. (17).

Incubation and assay conditions

The incubation conditions of Takeguchi, Kohno, and Sih (16) and the assay methods of Panganamala et al. (18) were modified as follows. Incubations were carried out in 25-ml vials in a metabolic shaker at 37°C in a total volume of 1.5 ml. All incubations contained 1 mM L-epinephrine, 1 mM GSH, and 0.1 M potassium phosphate buffer, pH 8.0. The concentration of protein and substrate varied with the type of incubation and will subsequently be outlined for individual experiments. Incubations were routinely run by adding all cofactors and microsomes to the vials at 4°C. The vials were then preincubated at 37°C for 1 min and the reactions were initiated by addition of substrate. When inhibition studies were carried out, the inhibitors were added along with the cofactors. Duplicate enzyme reactions and zero time controls were always run. The zero time controls were terminated prior to substrate addition and were then allowed to incubate for the same time period as the enzyme reactions. All reactions were stopped by addition of 10 ml of ethyl acetate plus 0.2 ml of 1 M citric acid. Each reaction mixture was extracted three times with 10 ml of ethyl acetate. The ethyl acetate extracts were pooled, washed three times with 2 ml of H_2O, dried over anhydrous Na_2SO_4, and the ethyl acetate was removed on a rotary evaporator at 30°C. The recovery of added radioactive PGE_2 was 97% when the reaction mixture was extracted in the above manner.

The residue was dissolved in chloroform–methanol 2:1 (v/v) and aliquots were streaked on activated silica gel G thin-layer plates and developed in the solvent system chloroform–methanol–acetic acid 180:12:10 (v/v). Authentic PGB_2, PGE_2, $PGF_{2\alpha}$, linolenic acid, and β-hydroxy stearic acid were spotted in a separate single lane on the plate. With this solvent system we found that PGB_2 and β-hydroxystearic acid have virtually the same R_f. After development, the plates were exposed to iodine vapor, the various zones corresponding to standards were marked, the iodine vapor was allowed to evaporate in the hood, and the various zones were scraped into scintillation vials containing 15 ml of Scintisol (Isolabs, Akron, Ohio). Radioactivity was measured in a Packard liquid scintillation spectrometer, Model 3380. Efficiency of counting was determined from a quench curve.

Data from kinetic experiments were first plotted as reciprocal velocities versus reciprocal substrate concentrations and then fitted by computer for the reciprocal form of the Michaelis-Menton equation by the least squares method assuming equal variance for the velocities when these plots were linear.

TABLE 1. Thin-layer chromatographic separation of radioactive products from an incubation mixture

Zone Designation	Percent of ^{14}C-Label
Solvent front	0.8
Free fatty acid	52.7
Hydroxy fatty acid + PGB_2	2.4
Unknown	1.4
PGE_2	41.0
PGF_2	0.9
Origin	0.7

The incubation contained 0.1 mM [1-^{14}C]8,11,14–20:3, 1 mM L-epinephrine, 1 mM GSH, and 4 mg of microsomal protein in 1.5 ml of 0.1 M phosphate buffer (pH 8.0). After incubation at 37°C for 10 min., the products were assayed as described in Methods.

Partial characterization of PG products formed after incubations with methyl branched isomers of 8,11,14–20:3

The products formed during the incubations of some of the [1-^{14}C]methyl branched fatty acids were extracted with ethyl acetate and streaked on the activated plates with authentic standards as described above. The plates were developed with chloroform–methanol–acetic acid 180:12:10 (v/v) and the lane containing standards was visualized by spraying the plate with a 0.2% solution of 2′,7′-dichlorofluorescein in ethanol. The area containing radioactivity that corresponded to the reference PGE_2 was scraped into a sintered glass funnel and then the ^{14}C-labeled material was eluted with ethyl ether. The eluate was evaporated to dryness under a stream of nitrogen. The recovered ^{14}C-labeled material was redissolved in chloroform–methanol 2:1 (v/v) and further purified by thin-layer chromatography using the solvent system, benzene–dioxane–acetic acid 20:20:1 (v/v) (19). The area corresponding to authentic PGE_2 was then extracted with ether.

One-half of this purified ^{14}C-labeled material and the standard PGE_2 were treated with 2 ml of 1 N KOH in methanol and the reaction mixture was incubated at 37°C for 30 min to convert the PGE to PGB (14, 20). The reaction mixture was acidified to pH 5 and extracted with 10 ml of ethyl ether three times. The combined extracts were evaporated to dryness under a stream of nitrogen. The residue was dissolved in chloroform–methanol 2:1 (v/v) and streaked on a silica gel G plate with authentic PGB_2, PGE_2, $PGF_{2\alpha}$, and linolenic acid. The plate was developed in benzene–dioxane–acetic acid 20:20:1 (v/v). After visualizing the components by exposing the plate to iodine vapor the zones corresponding to standards were scraped into counting vials and the radioactivity was measured.

The other half of the purified PGE_1-like ^{14}C-labeled material and the standard PGE_2 were reduced by addition of 2 mg of sodium borohydride in 2 ml of 96% ethanol to convert PGE to PGF (15). After 30 min at room temperature, the reaction mixture was diluted with water, acidified to pH 3, extracted with ethyl ether, and the solvent was removed on a rotary evaporator. The residue was dissolved in chloroform–methanol 2:1 (v/v) and applied to a silica gel plate with standards. The plate was then developed in the solvent system, benzene–dioxane–acetic acid 20:20:1 (v/v) and the areas corresponding to standards were scraped into counting vials that were counted after the addition of 15 ml of Scintisol.

RESULTS

Table 1 depicts the radioactive distribution pattern obtained when the products of an incubation were fractionated by thin-layer chromatography using chloroform–methanol–acetic acid 180:12:10 (v/v) as the developing solvent. When incubations contained 4 mg of microsomal protein and the concentration of [1-^{14}C]8,11,14–20:3 was 0.1 mM, the only major radioactive product had an identical R_f with PGE_2. In other experiments, which are not shown here, we found that when higher concentrations of substrate were used a considerable amount of radioactivity was found in the $PGF_{2\alpha}$ zone. In all later experiments the radioactivities found in both the PGE_2 and $PGF_{2\alpha}$ zones were summed and represent the total amount of prostaglandins formed.

When incubations contained 3 mg of microsomal protein and 1 mM [1-^{14}C]8,11,14–20:3 the rate of PG synthesis was linear with time for at least 4 min. After a 15 min incubation period PG synthesis stopped. Further experiments were carried out to

TABLE 2. Effect of preincubation periods on the rate of prostaglandin synthesis

Preincubation Periods	Cofactors Added After Preincubation	PG Formed
Min.		*nmoles*
0	none	12.0
20	1 mM L-epinephrine	5.0
20	1 mM GSH	5.7
20	1 mM L-epinephrine + 1 mM GSH	5.2
30	1 mM L-epinephrine	3.4
30	1 mM GSH	2.9
30	1 mM L-epinephrine + 1 mM GSH	3.2

The incubation contained 1 mM epinephrine, 1 mM GSH and 3 mg microsomal protein in 1.5 ml of 0.1 M phosphate buffer (pH 8.0). After indicated periods of preincubation, the mixture was incubated at 37°C for 3 min immediately after the addition of the desired cofactor(s) and 0.67 mM [1-^{14}C]8,11,14–20:3.

determine whether this was due to cofactor depletion or destruction or, alternatively, to enzyme inactivation. Incubations with all cofactors, but without substrate, were preincubated at 37°C for either 20 or 30 min. After these preincubation periods additional amounts of cofactors were added. As shown in **Table 2** further addition of L-epinephrine and GSH did not restore enzyme activity. These findings suggest that the enzyme is partially inactivated after preincubation periods of 20 and 30 min. Since all preincubations were carried out in the presence of both L-epinephrine and GSH, these data do not eliminate the possibility that the inactivation was due to some type of cofactor–enzyme interaction. This experiment does emphasize that when enzyme and cofactors are added it is essential to carry out the incubation within a short period of time. Other experiments established that there is a linear relationship between PG biosynthesis and protein up to 5 mg of protein per incubation when incubations were carried out for 3 min with 1 mM [1-^{14}C]8,11,14–20:3.

On the basis of these preliminary studies all kinetic and inhibitor experiments were carried out with 3 mg of protein and an incubation time of 3 min. The dependence of substrate concentration on PG synthesis is shown in **Fig. 1**. From the Lineweaver-Burk plot the apparent K_m for 8,11,14–20:3 and 5-Me 20:3 were 224 μM and 109 μM, respectively. The apparent V_{max} for 8,11,14–20:3 was 4.6 nmoles/min/mg protein and the V_{max} for 5-Me 20:3 was 1.3 nmoles/min/mg protein.

These two kinetic plots were measured under identical conditions with a single concentration of

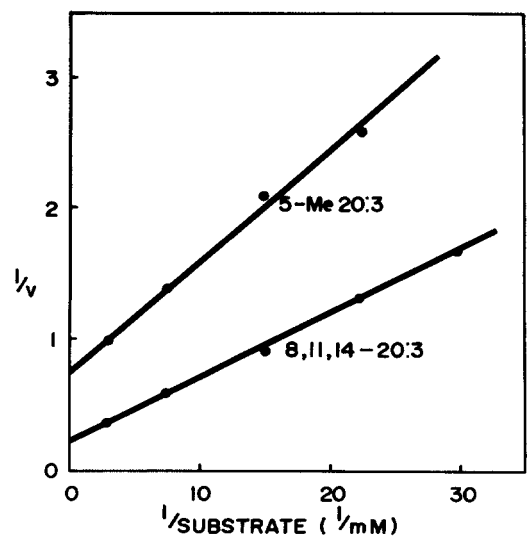

Fig. 1. The effect of substrate concentrations on the rate of prostaglandin synthesis. Conditions of incubation and assay are described in Methods. The velocity, v, is expressed as nmoles of prostaglandin formed per minute per mg of microsomal protein.

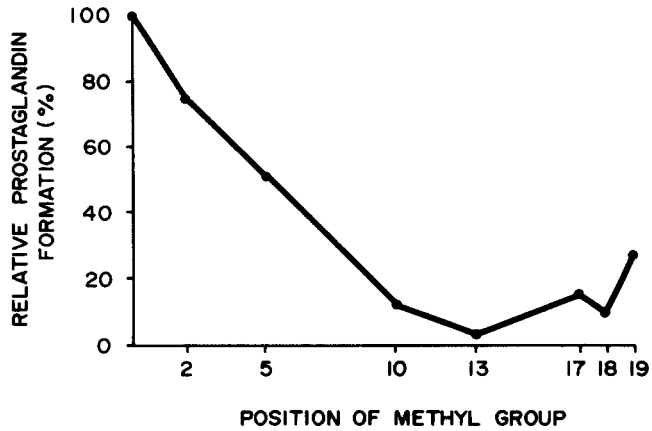

Fig. 2. The effect of a methyl branch on prostaglandin synthesis. Conditions of incubation and assay are described in Methods. The observed rate of PG synthesis from 8,11,14–20:3 under these conditions was 66.2 nmoles per 4 mg of microsomal protein during the 10 min incubation.

protein. The values so obtained may be influenced by the micellar state of the substrate, the binding of substrate to proteins in the incubation, the purity of the microsomal preparation, and by side reactions that would deplete the amount of substrate available for PG biosynthesis. the kinetic parameters obtained are considered as comparative values that may be valid only for this preparation using the assay techniques employed in this study.

Effect of a methyl branch on PG synthesis

Preliminary experiments established that the rate of PG synthesis from some of the methyl branched isomers was very slow. These incubations were thus all carried out under noninitial velocity conditions. All incubations contained 4 mg of microsomal protein, the substrate concentration was 0.1 mM and the incubations were run for 10 min. Under these conditions 66.2 nmoles of PG were produced from [1-^{14}C]8,11,14–20:3.

The rates of PG biosynthesis from the branched isomers relative to PG production from [1-^{14}C]8,11,-14–20:3 are shown in **Fig. 2**. When the methyl branch was located on carbon 13, which is the site of stereospecific hydrogen removal (21), only negligible amounts of PG were formed. When the methyl branch was at position 2 or 5, these isomers yielded PG at 75% and 51% of the rate of PG synthesis from [1-^{14}C]8,11,14–20:3, respectively. When the methyl branch was at positions 10, 17 or 18 the relative rates of PG synthesis ranged 10–15% of that found for [1-^{14}C]8,11,14–20:3. When the methyl branch was at position 19 the rate of PG synthesis was 25% of that found for [1-^{14}C]8,11,14–20:3. The results indicate that methyl branching from

TABLE 3. Radioactive distribution among products after treatment of the PGE_1-like incubation product from [1-^{14}C]19-Me 20:3 with KOH and $NaBH_4$

Zone Designation	% ^{14}C in Products After KOH Treatment	% ^{14}C in Products After $NaBH_4$ Treatment
Free fatty acid	10.8	3.0
PGB_2	83.4	7.6
PGE_2	3.7	5.1
$PGF_{2\alpha}$	0.9	50.1
$PGF_{2\beta}$		33.6
Unknown	0.5	
Origin	0.8	0.5

Conditions of incubation and assay are described in Methods.

positions 2 through 19 decreased the rate of PG synthesis and that the rate of PG biosynthesis was reduced more dramatically when the methyl branch was located near the terminal methyl group of the hydrocarbon chain rather than towards the carboxyl group.

Partial characterization of methyl branched isomers of PGE_1

When the radioactive product (with an R_f equal to that for PGE_2) from the incubation with [1-^{14}C]19-Me 20:3 was treated with KOH and $NaBH_4$ and fractionated by thin-layer chromatography, the radioactive distribution pattern shown in **Table 3** was obtained. These results strongly suggest that 19-Me 20:3 was converted to 19-Methyl PGE_1. After treatment with KOH 83% of the radioactivity was in a component that had the same R_f as authentic PGB_2. In a similar way, after treatment with $NaBH_4$, 50% and 34% of the radioactivity was found in compounds that had R_f values identical with authentic $PGF_{2\alpha}$ and $PGF_{2\beta}$, respectively.

After isolation and purification the radioactive PGE_1-like products, formed from [1-^{14}C]2-Me 20:3 and [1-^{14}C]5-Me 20:3, were also treated with KOH and $NaBH_4$. When the purified ^{14}C-labeled product from [1-^{14}C]2-Me 20:3 was treated with KOH, 91% of the total radioactivity migrated with authentic PGB_2. When the same product was reduced with $NaBH_4$, 48% and 36% of the radioactivity was found in components that migrated with authentic $PGF_{2\alpha}$ and $PGF_{2\beta}$, respectively. In a similar way when the purified incubation product of [1-^{14}C]5-Me 20:3 was treated with KOH, 95% of the radioactivity was in the PGB_2 zone. When the same product was treated with $NaBH_4$, 50% and 21% of the total radioactivity was found in the $PGF_{2\alpha}$ and $PGF_{2\beta}$ zones. Although the PGE_1-like material from the incubations containing [1-^{14}C]10-Me 20:3, [1-^{14}C]17-Me 20:3, and [1-^{14}C]18-Me 20:3 were not characterized due to the low rate of PG biosynthesis, it is reasonable to assume, on the basis of the above results, that the major products were methyl branched isomers of PGE_1.

The inhibitory effect of 13-Me 20:3 on PG synthesis

Since only negligible amounts of PG were formed from the incubation of [1-^{14}C]13-Me 20:3, its possible inhibitory effect on PG synthesis was compared with that of linolenic acid and TYA. As shown in **Table 4**, all three fatty acids showed concentration-dependent inhibition of PG synthetase activity. TYA was a very potent inhibitor with $I_{50} = 6.6$ μM (the inhibitor concentration that gives 50% enzymatic activity), confirming the results of Ahern and Downing (10) and Downing, Ahern, and Bachta (22). Linolenic acid also inhibited PG synthetase with $I_{50} = 0.32$ mM, which is in agreement with the report by Pace-Asciak and Wolfe (8). 13-Me 20:3, whose I_{50} was equal to 1.88 mM, was about six times less potent as an inhibitor than was linolenic acid. Therefore, the concentration of 13-Me 20:3 required for the inhibition of PG synthesis was similar to those values reported for naturally occurring fatty acids such as oleic, linoleic and linolenic acids (8).

DISCUSSION

The radiochemical assay method described here was simple and applicable to measuring the rate of PG synthesis under initial velocity conditions. It is well established that when PG synthesis is measured in the presence of GSH, these conditions favor the

TABLE 4. Inhibitory effect of fatty acids on prostaglandin synthesis

Fatty Acid	Concentration	Rate of PG Synthesis
	μM	*nmoles/min/mg protein*
Linolenic acid	0.0	2.5
	250.0	1.4
	500.0	0.8
	1,000.0	0.4
	1,500.0	0.2
13-Me 20:3	0.0	2.5
	500.0	2.2
	1,000.0	2.0
	1,500.0	1.3
	2,000.0	1.2
Eicosa-5,8,11,14-tetraynoic acid (TYA)	0.0	2.8
	4.5	1.7
	9.0	1.1
	13.5	0.8
	22.5	0.5

Microsomal protein (3 mg) was added to the reaction mixture containing 1 mM epinephrine, 1 mM GSH, and indicated concentrations of inhibitors at 37°C. After a 1 min preincubation period, 0.15 μmoles of [1-^{14}C]8,11,14–20:3 was added and the incubations were run for 3 min. Conditions of assay are described in Methods.

production of PG of the E series (16, 23, 24). The ratio of PGE to PGF produced is, however, dependent on substrate concentration. Flower, Cheung, and Cushman (24) have shown that at constant GSH concentration, the ratio of $PGF_{2\alpha}$ to PGE_2 production increased as the concentration of arachidonic acid increased. These same investigators showed that the ratio of $PGF_{2\alpha}$ to PGE_2 production is relatively independent of GSH concentration providing the concentration of GSH is greater than about 0.05 mM. Since a variety of different substrate concentrations were used in this study all our results were expressed as the total amount of PG formed. The time-dependent loss of enzyme activity observed when cofactors and enzyme were preincubated together at 37°C suggests that preincubation periods should be carried out only long enough to reach temperature equilibrium. For this reason all of our preincubations were for 1 min.

When 5-Me 20:3 was used as a substrate for PG biosynthesis, we found that the apparent K_m and V_{max} were both lower than those calculated for 8,11,14–20:3. The lower K_m for 5-Me 20:3 suggests that PG synthetase may bind 5-Me 20:3 more tightly than 8,11,14–20:3. The higher apparent V_{max} found for 8,11,14–20:3 versus that calculated for 5-Me 20:3 may be due to a more rapid dissociation of the products from the enzyme–product complex since the other substrate, molecular oxygen, was held at constant concentration throughout the incubation period.

These studies clearly show that the rate of PG biosynthesis declined as the methyl branch was moved towards the 13-position, which is the site of stereospecific hydrogen abstraction in PG biosynthesis (21). PG synthetase apparently requires the intact terminal end of the substrate in order to observe a reasonable rate of PG biosynthesis. When the methyl branch was on carbons 10, 13, 17, 18, or 19 these isomers were converted to PG at a rate less than 25% of that found for 8,11,14–20:3. Conversely when the methyl branch was on carbons 2 or 5 these isomers were converted to PG at least 50% as rapidly as was 8,11,14–20:3. Struijk et al. (1) and Beerthuis et al. (2, 3) have demonstrated that the introduction of a double bond at either position 17 or 18 of the substrate results in a decreased rate of PG synthesis, while the insertion of a double bond at positions 2, 3, or 4 in 8,11,14–20:3 did not affect the rate of PG biosynthesis. The recent studies by van Dorp and Christ (6) also show that structural modification of the terminal part of 8,11,14–20:3 is more effective in depressing the rate of PG biosynthesis than is modification of the carboxyl end of 8,11,14–20:3. This series of studies and our studies both show that the rate of PG biosynthesis is depressed by substrate modification and that this effect is most pronounced when the terminal part of the substrate is modified.

It is now established that tissues that carry out PG biosynthesis also produce hydroperoxy fatty acids via a lipoxygenase-type reaction. Although 2-Me 20:3 is a relatively good substrate for PG synthesis Holman, Egwin and Christie (25) have shown that soybean lipoxygenase does not act on 2-methyl arachidonic acid. These findings suggest that 2-Me 20:3 might be a unique substrate in that it can be converted into PG but may not serve as a substrate for lipoxygenase. Further studies will be necessary to evaluate this hypothesis since it has been shown that the substrate specificity of the lipoxygenase of sheep vesicular glands (26) is different from that in platelets (27, 28) and that both of these lipoxygenases have a different substrate specificity than that found for soybean lipoxygenase (25, 26, 29).

Recently, we reported how these various methyl branched isomers were desaturated at the 5-position by rat liver microsomes (7). When the methyl branch was at positions 2, 5, or 10 these isomers were desaturated at the 5-position at a rate less than 20% of that found for the conversion of 8,11,14–20:3 to arachidonate. When a methyl branch was at position 17, 18, or 19 the rate of desaturation was 60–100% of that found for desaturation of 8,11,14–20:3. These results demonstrated that methyl branching of the substrate towards the carboxyl end of the molecule retarded the rate of desaturation, while methyl branching on the terminal part of the molecule did not markedly influence the rate of desaturation. Therefore, two microsomal enzyme systems that use the same acids as substrate have completely opposite substrate specificities.

Since 13-methyl 20:3 was not a substrate for PG synthesis, we thought it might be a potent inhibitor for PG biosynthesis. Although 13-Me 20:3 did inhibit PG synthetase in a concentration-dependent manner, the I_{50} value was about the same as those found for common unsaturated fatty acids (8).

Recent studies have shown that methyl branched PG are physiologically active (30, 31). The present study shows that methyl branched PG can be produced from the respective methyl branched acids but, in all cases, the rate of PG biosynthesis is lower than that found for 8,11,14–20:3.

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