Tetrahedron Letters,Vol.26,No.33,pp 3939-3942,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain 

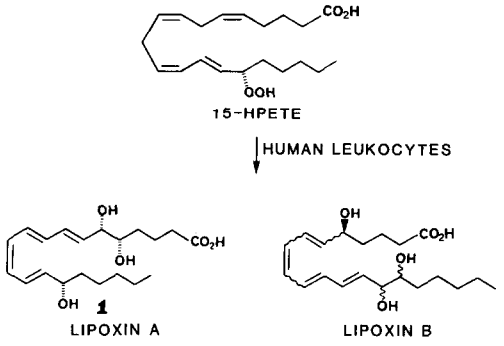
# ON THE BIOSYNTHESIS AND THE STRUCTURE OF LIPOXIN B

Brian J. Fitzsimmons* and Joshua Rokach
Merck Frosst Canada Inc. P.O. Box 1005, Pointe Claire-Dorval, Quebec, H9R 4P8

Data are presented which shows that the lipoxins are formed via a tetraene epoxide, supporting the assignment of the all trans tetraene geometry to lipoxin B.

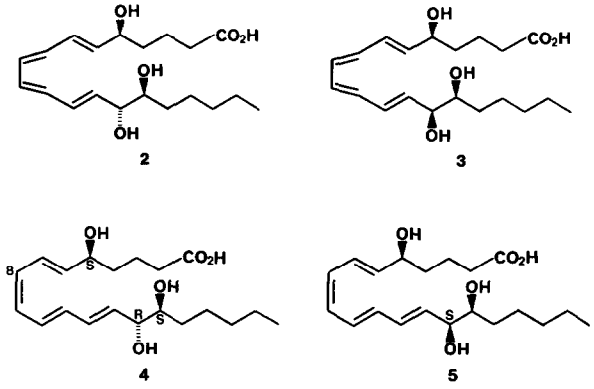
The lipoxins are novel metabolites of arachidonic acid first reported by Serhan, Hamberg and Samuelsson in the Spring of 1984.[1] Two types of lipoxins were identified at that time: lipoxin A and lipoxin B (Fig. 1). Recently the structure of lipoxin A was determined to be the 5S, 6S, 15S, 11-cis isomer 1[2,3] by comparison to synthetic standards.

Figure 1



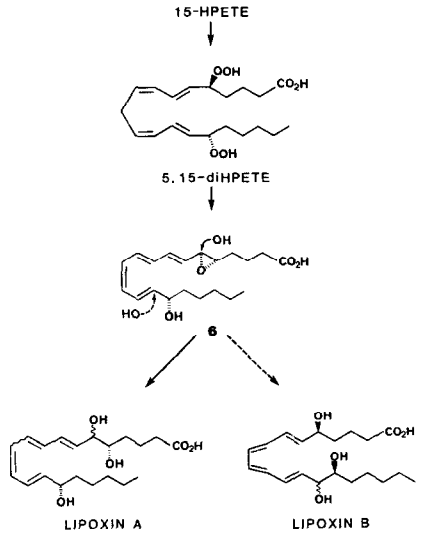

Lipoxin B has also been identified by the same protocol[4,5], however, in this case there is a discrepancy between the structures assigned to this interesting metabolite by two different groups (Fig. 2). The Merck Frosst group assigned the two all trans structures 2 and 3 to naturally-derived lipoxin with the 14R isomer 2 being the major component[4]. While the Harvard group has assigned the two 8-cis structures 4 and 5 to the natural material with the 14S isomer 5 being the major component[5]. In this paper we present further data that support our assignment of the all trans structures 2 and 3 to lipoxin B and elucidates the biosynthesis of the lipoxins.

Figure 2: LIPOXIN B ISOMERS




The lipoxins could be generated by one of two possible biosynthetic pathways described in detail in our assignment of the structure of lipoxin A[2]. The first pathway would involve the generation of the tetraene epoxide 6 from 15-HPETE, as shown in Scheme I, analogous to the formation of $LTA_4$ from arachidonic acid, and its hydrolysis to give the lipoxins. The second pathway would also pass via a 5,15 dioxygenated arachidonic acid derivative. Generation of a carbon centered radical at C-10 of 5,15 DiHPETE (or DiHETE) and trapping of molecular oxygen at C-6 would yield lipoxin A, while trapping at C-14 would yield lipoxin B. The intermediacy of a 5,15 dioxygenated arachidonic acid metabolite is implicated by the detection of substantial amounts of 5,15 diHETE in the original isolation of the lipoxins.

Scheme I

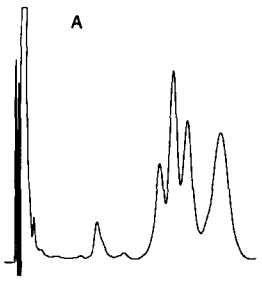



To test these possible biosynthetic routes, two experiments were performed. In the first experiment the tetraene epoxide 6[6] was incubated with human leukocytes under the conditions originally used to generate the lipoxins from 15-HPETE[1], to determine if the same products resulted. The second experiment consisted of attempting three successive lipoxygenations of

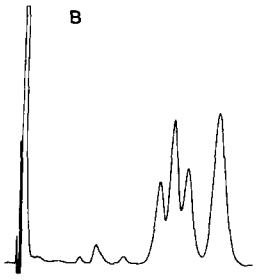
arachidonic acid using a purified lipoxygenase enzyme, which is known to perform the first two oxygenations analogously to the human enzyme.[9] The purpose of the latter experiment was to determine what the products of triple lipoxygenation of arachidonic acid would be.

Incubation of the tetraene epoxide 6 with human leukocytes provided the identical lipoxins in similar relative proportions to those obtained from the incubation of 15-HPETE under the same conditions. This is strong evidence for the intermediacy of this epoxide in the biosynthetic scheme, however, we wished further confirmation. Since the incubations are quenched with methanol and acidified, the less polar fraction of both incubations were examined in an attempt to identify methanol opening products of the intermediate tetraene epoxide (methoxy-lipoxins). Indeed these products were detected in both the incubation of 15-HPETE and of the tetraene epoxide 6 with human leukocytes in similar relative proportions (Fig. 3). Furthermore, treatment of the tetraene epoxide 6 with acidic methanol yielded a virtually identical chromatogram to traces A and B (Fig. 3).[7] These experiments provide direct and conclusive evidence for the intermediacy of the tetraene epoxide 6 in the biotransformation of 15-HPETE to the lipoxins. This biosynthetic pathway via the tetraene epoxide 6, should lead to 11-cis lipoxin A's by vicinal hydrolysis of the epoxide. It should also lead to the all trans lipoxin B's by non-enzymatic hydrolysis of the epoxide, by analogy to non-enzymatic hydrolysis of $LTA_4$.

Figure 3: RP-HPLC of the methoxy-lipoxins. Waters C-18 μBondpak (7.8 mm x 30 cm), 65:35:0.05 methanol/water/acetic acid, flow = 1.5 mL/min.




Methoxy-lipoxins from the incubation of 15-HPETE with human leukocytes.




Methoxy-lipoxins from the incubation of the tetraene epoxide 6 with human leukocytes.

The incubation of arachidonic acid with soybean lipoxygenase in pH9 borate buffer yielded two lipoxins. These were the 5S, 6R, 15S 11-cis lipoxin A and the 5S, 14R, 15S 8-cis lipoxin B 4 . Therefore the third oxygen is introduced in such a manner to create a R stereocenter. This is consistent with the lipoxygenase enzyme's action on other substrates and an analogous reaction in the literature using human enzyme.[8] These data indicate that the 5S, 14R, 15S 8-cis and not

the 5S, 14S, 15S 8-cis lipoxin B isomer is likely to be formed by the triple lipoxygenation pathway.

In addition to these experiments the synthetic and natural lipoxin B isomers were re-examined by RP-HPLC using the column and conditions described by the Harvard group. The retention times obtained for these isomers are shown in Table 1. The identification of leukocyte-derived lipoxin B using these conditions was the same as previously reported by us. The UV spectra of natural lipoxin B was indistinguishable from that of our synthetic all-trans lipoxin B isomers. In addition, in our hands no identifiable amounts of either the 8-cis lipoxin B isomers 4 or 5 were found.

TABLE 1

| Lipoxin B Isomer Methyl Ester | Retention Time (Waters 15 cm C18-Novapak 60:40 Methanol/Water, 2 mL/min) |
|---|---|
| 6 S, 14 S, 15 S all trans 3 | 8.5 min |
| 5 S, 14 R, 15 S all trans 2 | 9.6 min* |
| 5 S, 14 R, 15 S 8-cis 4 | 9.6 min* |
| 5 S, 14 S, 15 S 8-cis 5 | 17.2 min |

*These isomers are easily separated using 40:60:0.05 acetonitrile/water/acetic acid.

In conclusion, it has been shown that the lipoxins are formed via the tetraene epoxide 6 and that the lipoxin B produced by triple lipoxygenation would be the 5S, 14R, 15S 8-cis isomer 4 . These data support the assignment of the all trans structures 2 and 3 to lipoxin B and counter indicates the assignment of the 5S, 14S, 15S 8-cis isomer 5 as the major lipoxin B produced naturally. Also re-examination of the leukocyte-derived lipoxins yielded no identifiable amounts of the 8-cis lipoxin B's. The details of the biosynthetic experiments will be reported in a full paper elsewhere.

Acknowledgement

B. Fitzsimmons thanks N.S.E.R.C.(Canada) for a post-doctoral fellowship..

References

1. Serhan, C.N., Hamberg, M. and Samuelsson, B. Biochem. Biophys. Res. Commun. 1984 118, 943-949.
2. Adams, J., Fitzsimmons, B.J., Girard, Y., Leblanc, Y., Evans, J.F. and Rokach, J. J. Am. Chem. Soc. 1985 107, 464-469.
3. Corey, E.J. and Wei-guo, S. Tetrahedron Lett. 1985 26, 281-284.
4. Leblanc, Y., Fitzsimmons, B.J., Adams, J. and Rokach, J. Tetrahedron Lett. 1985 26, 1399-1402.
5. Corey, E.J., Mehrotra, M.M. and Su, W. Tetrahedron Lett. 1985 26. 1919-1925.
6. Adams, J., Fitzsimmons, B.J. and Rokach, J. Tetrahedron Lett. 1984 25, 4713-4716.
7. The products of acidic methanolysis of the tetraene epoxide 6 were characterized by $^{1}$H-NMR (250 MHz), U.V. spectroscopy ($\lambda_{max}$302 nm) and G.C.-M.S.
8. Maas, R.L., Brash, A.R. Proc. Natl. Acad. Sci. U.S.A. 1984 80, 2864-2868.
9. Van Os, C.P.A., Rijke-Schilder, G.P.M., Van Halbeek, H., Verhagen, J. and Vliegenthart. Biochim. Biophys. Acta 1981 663, 177-193.

(Received in USA 24 May 1985)