

**ARACHIDONATE 15-LIPOXYGENASE FROM HUMAN LEUKOCYTES:
Purification and structural homology to other
mammalian lipoxygenases**

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The enzyme 15-lipoxygenase catalyzes the hydroperoxidation of arachidonic acid to form 15-hydroperoxyeicosatetraenoic acid. This is the first step in the formation of the mono-hydroxy acid 15-hydroxyeicosatetraenoic acid (15-HETE), the dihydroxy acids 8,15 diHETE and 14,15 diHETE and the trihydroxy acids, lipoxin A and B(1). The biological functions of these 15-lipoxygenase metabolites may include the stimulation of mucus release from cultured human airway(2), chemotaxis of human neutrophils(3), inhibition of natural killer cell activity(1) and contraction of guinea pig lung strips(1). The 15-lipoxygenase pathway is the predominant pathway for arachidonic acid metabolism in human lung homogenates(4), isolated human airway epithelial cells(5), human eosinophils(6), and human keratinocytes(7). We have recently described a method for purifying human 15-lipoxygenase to homogeneity from eosinophil-enriched leukocytes (8). In this paper, we summarize the results of the purification procedure and report the N-terminal amino acid sequence of the human 15-lipoxygenase. Sequence similarity with other mammalian lipoxygenases suggests that the enzymes of the lipoxygenase family are related structurally.

Cells were obtained from the peripheral blood of subjects with hypereosinophilia. Arachidonate 15-lipoxygenase was purified from the 100,000 x g supernatant of these leukocytes using ammonium sulfate precipitation, hydrophobic-interaction chromatography, and high pressure liquid chromatography on hydroxyapatite and cation exchange columns. The details of these procedures and the enzyme assays are described elsewhere(10). One unit of enzyme activity is defined as the amount of enzyme required to generate one nmol of 13-OH linoleic acid under standard assay conditions.

Enzyme activity was localized to the 100,000 x g supernatant, suggesting a cytosolic origin. The 15-lipoxygenase in the 30-60% ammonium sulfate fraction was very hydrophobic as indicated by its elution from phenyl-Sepharose at approximately 0% ammonium sulfate. The

protein's elution from the Mono S column at pH 7 suggests that the enzyme is cationic at neutral pH. The results of a purification experiment combining differential centrifugation, ammonium sulfate precipitation, hydrophobic-interaction, hydroxyapatite and cation-exchange chromatography are shown in Table 1. A single protein peak, co-eluting precisely with a peak of lipoxxygenase activity, was obtained in the final chromatographic step. This purification began with 3×10^9 leukocytes (25% eosinophils and 75% neutrophils) and approximately 1800-fold purification of 15-lipoxxygenase was achieved.

TABLE 1. Purification of 15-lipoxxygenase from human leukocytes

Fraction	Vol (ml)	Total protein (mg)	Total activity (units)	Specific Activity (units/mg)	Yield (%)	Purification (fold)
Supernatant (100,000 x g)	60	152	16,419	108	100	--
Precipitate (30-60%)	15	52.5	10,946	208	67	1.9
Phenyl-Sepharose	10	0.891	6,055	6,796	37	62.9
Hydroxyapatite	9	0.066	1,187	17,985	7.2	160
Mono S	2	0.001	198	198,000	1.2	1800

The activity of the purified enzyme was unchanged in the presence or absence of calcium. In contrast, calcium enhanced the activity of the crude enzyme(9), suggesting that either calcium stabilizes the crude enzyme, or the purification procedure removes a calcium dependent stimulatory factor.

The major products produced from linoleic acid and arachidonic acid by the action of the purified lipoxxygenase were identical to that of synthetic 13-OH linoleic acid and 15-HETE, respectively, as determined by ultraviolet spectroscopy, normal phase- and reverse phase-HPLC, and mass spectrometry.

The results from SDS/polyacrylamide gel electrophoresis of samples obtained from each purification step are shown in Fig 1. In the purified preparation, one major protein band (apparent M_r , 70,000) was observed (lane 4). In addition, one major amino acid sequence was obtained from the purified protein. Comparison of the amino-terminal sequence of the human 15-lipoxxygenase with known sequences of other lipoxxygenases (Fig 2) reveals 71% sequence identity with the rabbit reticulocyte lipoxxygenase(10) and 36% sequence identity with the human 5-lipoxxygenase(11,12). Furthermore, when one considers the sequence similarity at positions 5,8,11,13 and 14, there is 60% sequence similarity among all three mammalian lipoxxygenases. In contrast, a search of the entire sequence of the soybean lipoxxygenase isoenzyme 1(13) failed to locate any sequence identity to

the N-terminal sequence of the human 15-lipoxygenase. These results suggest that the mammalian lipoxygenases are members of an homologous family of proteins.

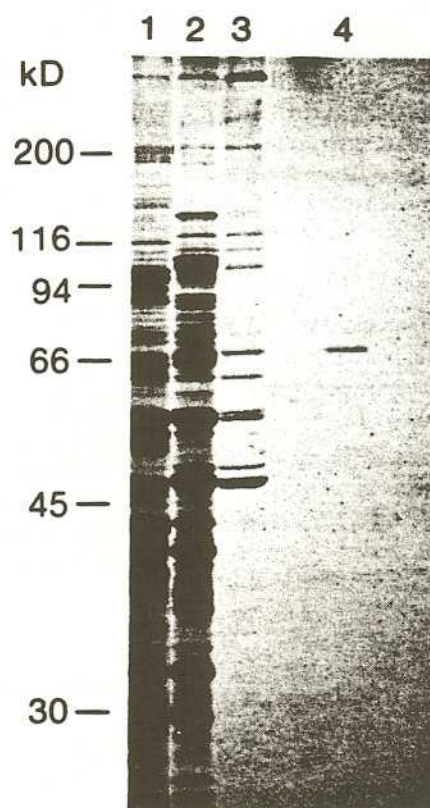


Fig. 1. Polyacrylamide gel electrophoresis of fractions obtained during the purification of human 15-lipoxygenase. The samples are 30-60% precipitate (lane 1), phenyl-Sepharose (lane 2), hydroxyapatite (lane 3), and Mono S cation exchange (lane 4). (Reproduced with permission from [8])

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Human 15-lipoxygenase	—	Leu	Tyr	Arg	Ile	Arg	Val	Ser	Thr	Gly	Ala	Ser	Leu	Tyr	Ala
Rabbit reticulocyte lipoxygenase	Gly	Val	Tyr	Arg	Val	Cys	Val	Ser	Thr	Gly	Ala	Ser	Ile	Tyr	Ala
Human 5-lipoxygenase	Pro	Ser	Tyr	Thr	Val	Thr	Val	Ala	Thr	Gly	Ser	Glu	Trp	Phe	Ala
Soybean lipoxygenase	Met	Phe	Ser	Ala	Gly	His	Lys	Ile	Lys	Gly	Thr	Val	Val	Leu	Met

Fig. 2. N-terminal amino acid sequences for human leukocyte 15-lipoxygenase, rabbit reticulocyte lipoxygenase, human 5-lipoxygenase and soybean 15-lipoxygenase. Sequence identity is indicated by the bold lines. (Reproduced with permission from [8])

The complete structural relationship between the rabbit reticulocyte lipoxygenase and the human 15-lipoxygenase

remains unknown. Despite the fact that others have presented functional and immunological data(14) suggesting that the reticulocyte lipoxygenase is unique to reticulocytes, we speculate, based on the strong sequence identity shown here, that these two lipoxygenases, from different species and different tissues, are closely related in their structure. In view of this, the human 15-lipoxygenase of eosinophils, airway epithelial cells and keratinocytes should be evaluated for the degradative functions of the reticulocyte lipoxygenase such as the ability to degrade mitochondrial membranes selectively and to inhibit cellular respiration by decreasing the synthesis of ATP. Such degradative properties may be important in the pathophysiology of inflammatory and allergic responses.

In summary, we have purified the human 15-lipoxygenase to homogeneity and obtained N-terminal amino acid sequence which suggests that the mammalian lipoxygenases are structurally related. The availability of homogeneous 15-lipoxygenase is expected to play a key role in elucidating other relationships among the various lipoxygenases as well as permitting the study of 15-lipoxygenation of arachidonic acid at the molecular level.

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