IDENTIFICATION AND CHARACTERIZATION OF AN ENHANCER SEQUENCE IN
THE PROMOTER REGION OF HUMAN 15-LIPOXYGENASE (15-LO) GENE

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INTRODUCTION:

Lipoxygenases are lipid-peroxidating enzymes that are implicated in the pathogenesis of a
variety of inflammatory disorders [1-3], membrane remodeling [4-6] and atheroma formation [7-9].
Formation of 15-(S)-hydroxyeicosatetraenoic acid [15-(S)-HETE] and lipoxin (LX) A4 in human
leukocytes, mediated by 15-LO dependent catalysis of arachidonic acid, likely represents a
component of endogenous pro- and anti-inflammatory influences that ultimately regulate the extent
and severity of inflammatory reactions [1-4,10]. 15-(S)-HETE and LXA4 have been proposed as endogenous anti-inflammatory molecules
that suppress white cell chemotaxis, adherence, and activation and to specifically antagonize the
functional responses of proinflammatory 5-LO derivatives, the leukotrienes [1-4,10]. The role and
functions of leukotriene C4 (LTC4), leukotriene D4 (LTD4) and leukotriene B4 (LTB4) have
been described earlier [1-3]. 15-(S)-HETE decreases LTB4 generation in leukocytes [11], antagonizes
LXA4 exerts similar effects as it inhibits natural killer cytotoxicity, attenuates LTB4-induced
chemotaxis, and decreases leukocyte-endothelial cell adhesion [1,10]. The effects of 15-(S)-HETE
on cellular activation and of LXA4 to trigger selective responses have been discussed [11,13,14].

Lymphokines secreted by helper T-lymphocytes (TH) also modulate the inflammatory
response through their actions on monocytes. Evidences in the recent past have established that
lymphokines derived from TH-lymphocyte populations differentially regulate lipoxygenase
enzymatic pathways [15-21]. IL-4 was the only cytokine known to induce 15-LO in human
monocytes/macrophages [22,23] until recently. Nassar et al., [24] have demonstrated that IL-13, but not
IL-10, induces 15-LO mRNA and protein synthesis in human blood monocytes leading to
enhanced production of 15-HETE. This was inhibited by IFN-γ, as described in IL-4 induced
monocytes [22]. Though 12- and 15-LO from human and 15-LO from rabbit have been cloned and
sequenced \(^{25-27}\) and a human \(5-LO\) promoter \(^{28}\) characterized, little is known about the 5' promoter region of human 15-LO.

The regulation of genes encoding for the enzymes involved in arachidonate oxygenation pathways is a recently recognized aspect of cytokine biology. It is particularly intriguing in view of the apparent "logic" in the pattern of specificity between cytokines such as IL-13 which exert anti-inflammatory actions in macrophages and their respective target enzymes in the lipoxygenase pathway, namely 15-LO. It is possible that this aspect of cytokine biology involves mechanisms for transcriptional regulations which are distinct from those of Stat proteins, \(^{29-32}\) which have been implicated in cytokine modulation of phenomena such as cell surface receptor expression and immunoglobulin class switching during allergic-type reactions \(^{32}\).

To identify the cis-acting element that is responsible for the cell-specific expression of the 15-lipoxygenase gene, we analyzed the promoter activity of its 5'-flanking region by transient expression assays. The fusion genes were constructed by inserting the 5'-flanking region of the 15-LO gene upstream from the firefly luciferase gene and were introduced into HeLa cells. We thus found an enhancer element, located between -2.0 and -0.8 kilobase pairs (kb) upstream from the transcription initiation site, that enhances the transient expression of the luciferase reporter gene in HeLa cells. Using the fusion genes containing putative enhancer elements under the control of the heterologous simian virus 40 promoter, we identified the IL-13 induced cell-specific (in monocytes) enhancer of approximately 500 base pairs (bp) between -1.2 Kb and -790 bp and further localized the core sequence to a 41 bp region. This element was then confirmed to direct the HeLa cell-specific expression of the reporter gene under the 15-LO gene promoter. We thus propose that this core element is responsible for the IL-13 induced cell-specific expression of the human 15-LO gene. In this paper, we describe the characterization of the 5' flanking enhancer region of human 15-LO for the first time.

MATERIALS AND METHODS:

5' Rapid Amplification of cDNA Ends (5' RACE): 5' RACE was carried out using the Gibco-BRL 5' RACE System Kit (Cat.No 18374-025). The gene-specific antisense primer was 5'-GCCATATTCAGAATTAACCCGT-3' (436 to 458 bp of 15-LO cDNA)(GSP1), and the nested gene-specific primer was 5'-GGTAGTTCCACCTTGAGTTCTGTCT-3' (136 to 158 bp of 15-LO cDNA) (GSP2). Poly(A)\(^+\) RNA prepared using RNAzol method \(^{24}\), from the human peripheral monocytes induced by IL-13, was reverse transcribed using the primer GSP1. The anchor-ligated cDNA was then PCR amplified using the anchor primer and an internal primer, GSP2. PCR amplified, products were purified from the agarose gel and subcloned into a plasmid vector pCRTMII using the TA Cloning Kit (Invitrogen) and sequenced by dideoxynucleotide chain-termination method as described by Sambrook et al. \(^{33}\).

Isolation of Genomic Clones: A 185 bp (5' RACE product) insert was excised by EcoRI from the plasmid as the probe for screening a human genomic library constructed in Lambda FIX II vector. Dr. Cam Patterson, Harvard School of Public Health, Massachusetts, USA kindly provided this library. The probe was labeled with [\(\alpha^{32}\)P]dCTP with Oligolabeling Kit (Pharmacia Biotech). Plaques were transferred onto nylon immobilization membranes (Schleicher and Schuell) and hybridization was carried out at 42°C for 16-20 h in standard hybridization solution. Final Washing of the membranes was carried out in 2X SSC containing 0.2% SDS at 65°C for 30 min. The membranes were then exposed to X-ray film. Secondary screening was performed to identify and confirm positive plaques. The phage DNA was extracted by the method described by Sambrook et al. \(^{33}\).