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Role of Phospholipid Hydroperoxide Glutathione Peroxidase in Hepoxilin A_3 Biosynthesis in Human Platelets and Biological Actions of Hepoxilin A_3 on Human Neutrophils

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SUMMARY

The biosynthesis of hepoxilins in human platelets, the epidermoid carcinoma cell line A431 and the megakaryoblast cell line UT-7 was investigated. To determine which enzymes play a role in regulating the 12-lipoxygenase pathway, and thus hepoxilin synthesis, the roles of the selenoenzymes cytosolic glutathione peroxidase (GPx-1) and phospholipid hydroperoxide glutathione peroxidase (PHGPx) were examined. This study presents for the first time the presence of PHGPx protein, a membrane bound protein, and its activity in human platelets. The presence of PHGPx protein was revealed using two different antibodies that exhibited a positive reaction corresponding to the expected molecular weight of PHGPx in both human platelets and A431 cells. The activity of PHGPx was determined using the PHGPx-specific substrate 1-palmitoyl-2-[(15S)-hydroperoxy-(5*Z*,8*Z*,11*Z*,13*E*)-eicosatetraenoyl]-phosphatidyl-choline. RT-PCR failed to detect PHGPx mRNA in human platelets which was, however, detected in megakaryocytes. Analysis of mRNA beakdown rates in differentiated UT-7 cells revealed a short half-life for PHGPx mRNA as compared to 12-LOX mRNA. Both GPx-1 and PHGPx, seperated on a Sephadex G-100 (SF) column, exhibited 12-HpETE reductase activity. The ratio of GPx-1:PHGPx activity using 12-HpETE as substrate was found to be approximately 60:1 in human platelets, in megakaryoblasts UT-7 and in epidermoid cell line A431. Hepoxilin formation was not observed in the three cell types when arachidonic acid (100 μM) was used as substrate. In the presence of the selenoenzyme inhibitor iodoacetate, which inhibits both PHGPx and GPx-1, an ~80% inhibition of 12-HETE formation together with a concomitant increase of 12-HpETE by two orders of magnitude was observed. This was accompanied by the formation of hepoxilin A_3 and B_3. Both HXA_3 and HXB_3 were detected, in the absence of iodoacetate, when 12-HpETE was used as substrate. Selenium deficient UT-7 cells, which exhibited PHGPx activity but no measurable GPx-1 activity, reduced 12-HpETE, albeit at a slower rate as compared to wild-type cells. A 10-fold increase in HXA_3 formation was observed in UT-7 cells in the presence of iodoacetate. It is therefore proposed that both GPx-1 and PHGPx are involved in regulating the 12-LOX pathway in platelets and other cells by reducing the hydroperoxide tone and diverting the isomerisation route to the reduction route, thus controlling hepoxilin biosynthesis. Moreover, hepoxilin A_3 was found to up-regulate the expression of PHGPx mRNA in both A431 and HeLa cells, suggesting that HXA_3 may function as a stress-induced protective eicosanoid.

In earlier reports and reviews, it was reported that the free acid form of hepoxilin A_3, unlike its methyl ester, does not enter neutrophils and other cells. We report in the present study that the free acid of HXA_3 is capable of stimulating intact cells. Various experiments were performed to determine its biological activity. Thus, hepoxilin A_3 was found to induce chemotaxis at concentrations as low as 30-40 nM. At concentrations around 1 μM, HXA_3 gave rise to an instantaneous release of intracellular calcium that caused a slight liberation of arachidonic acid. Pretreatment of neutrophils with submicromolar concentrations of HXA_3 significantly blunted fMLP-induced arachidonic acid release and resulted in a 2-3 fold increase in fMLP-induced cAMP release. However, HXA_3 did not induce respiratory burst, oxygen uptake or aggregation. The free acid was found to induce A431 and HeLa cell proliferation as determined by thymidine incorporation assays. The present study thus provides ample evidence that HXA_3 is biologically active towards cells in its unesterified form.

INDEX

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

12-HETE	- 12*S*-**H**ydroxy-5*Z*,8*Z*,10*E*,14*Z*-**e**icosa**t**tetra**e**noic acid
12-HHTrE	- 12*S*-**H**ydroxy-5*Z*,8*E*,10*E*-**h**eptadeca**tri**enoic acid
12-HpETE	- 12*S*-**H**ydro**p**eroxy-5*Z*,8*Z*,10*E*,14*Z*-**e**icosa**t**tetra**e**noic acid
13-HODE	- 13*S*-**H**ydroxy -9*Z*,11*E*-**o**cta**d**ecadi**e**noic acid
5-HpETE	- 5*S*-**H**ydro**p**eroxy-6*E*,8*Z*,11*Z*,14*Z*-**e**icosa**t**tetra**e**noic acid
8,9,12-TriHETrE	- 8,9,12-**Trih**ydroxy-5*Z*,10*E*,14*Z*-**e**icosa**tri**enoic acid
8,11,12-TriHETrE	- 8,11,12-**Trih**ydroxy-5*Z*,10*E*,14*Z*-**e**icosa**tri**enoic acid
AA	- Arachidonic acid (5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid)
BSA	- Bovine serum albumin
cAMP	- Cyclic-adenosine monophosphate
DMEM	- Dulbeco`s Modified Eagles Medium
EDTA	- Ethylenediaminetetraacetic acid
ELISA	- Enzyme linked immuno-sorbent assay
ETYA	- 5,8,11,14-Eicosatetraynoic acid
FCS	- Fetal calf serum
fMLP	- *N*-Formyl-Methionine-Leucine-Phenylalanine
GC-MS	- Gas chromatography – Mass spectrometry
Glc	- Glucose
GM-CSF	- Granulocyte Macrophage Colony-Stimulating Factor
GPx-1	- Cytosolic glutathione peroxidase
HPLC	- High pressure liquid chromatography
HXA_3	- Hepoxilin A_3 8(*S*,*R*)-hydroxy-11,12-epoxyeicosa-5*Z*,9*E*,14*Z*-trienoic acid
HXB_3	- Hepoxilin B_3 10(*S*,*R*)-hydroxy-11,12-epoxyeicosa-5*Z*,8*Z*,14*Z*-trienoic acid
IMEM	- Iscove's Modified Eagles Medium
LOX	- Lipoxygenase
LTB_4	- Leukotriene B_4
PAPC	- 1-palmitoyl-2-arachidonoyl phosphatidylcholine
PBS	- Phosphate-buffered saline (phosphate buffer)
PCR	- Polymerase chain reaction
PHGPx	- Phospholipid hydroperoxide glutathione peroxidase

PMA	- Phorbol 12-Myristate 13-Acetate
PMN	- Polymorphonuclear leukocytes
RT-PCR	- Reverse transcription – polymerase chain reaction
TLC	- Thin-layer chromatography
Tris	- Tris(hydroxymethyl)aminomethane
UV	- Ultraviolet