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Existence of "25 kDa Thiol Peroxidase" in Retina: Evidence for An Antioxidative Role

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We isolated and sequenced a human retina cDNA fragment that encodes 25 kDa thiol peroxidase. A search of a databank showed that the 25 kDa thiol peroxidase from retina is the same type of thiol peroxidase which exists in human brain and red blood cells. This type of thiol peroxidase was distributed in all of the tested tissues including retina. This result suggests a physiological role for the 25 kDa thiol peroxidase as an important antioxidant.

Keywords: Antioxidant, Eye, Retina, Thiol peroxidase, Tissue distribution.

Introduction

In aerobic environment, reactive oxygen species (O_2^-, H_2O_2, ROOH, and HO·) are generated by many physiological processes such as incomplete reduction of molecular oxygen during respiration, NADPH oxidation linked to respiratory burst during phagocytosis, and redox cycling of xenobiotics (Halliwell and Gutterridge, 1989). To prevent the deterious effect of oxygen species, cells have equipped with a number of antioxidant enzymes including catalases, peroxidases, and superoxide dismutases (SOD).

Recently, a 25 kDa antioxidant enzyme was purified from various eukaryotes including yeast (Kim *et al.*, 1988; 1989; Chae *et al.*, 1993), human erythrocyte (Lim *et al.*, 1994b), brain (Lim *et al.*, 1994a), and liver (Cha and Kim, 1996). These enzymes prevent the oxidative damage induced by an oxidation system capable of generating reactive oxygen species in the presence of a thiol reducing equivalent such as DTT (Kim *et al.*, 1988; 1989; Chae *et al.*, 1993). Previously, we have reported that the antioxidant enzyme has a capability to destroy H_2O_2 in the presence of DTT (Lim *et al.*, 1993), and such a peroxidase activity was greatly enhanced by the *in vivo* thiol-regenerating system (thioredoxin-thioredoxin reductase-NADPH) (Chae *et al.*, 1994; Kwon *et al.*, 1994; Cha *et al.*, 1995). This peroxidase has a cysteine residue as a functional group instead of functional selenocysteine residue in selenium-dependent peroxidase such as well-known glutathione peroxidase. This peroxidase is thus named "thiol peroxidase", which could act as an antioxidant enzyme removing peroxides. However, its physiological significance is still debatable because of the existence of catalases and peroxidases in eukaryotic cytoplasm.

In this paper, we first report the existence of 25 kDa thiol peroxidase in the eye tissue including retina, and then discuss its physiological function.

Materials and Methods

Cloning and sequencing A human retina cDNA library in λgt11 (Clontech Lab., Inc., Palo Alto, USA) was screened with rabbit polyclonal antibodies prepared against purified 25 kDa thiol peroxidase from human red blood cell. The sequence determination was done by the dideoxy nucleotide chain-termination method (Sanger *et al.*, 1977).

Other methods Immunoblot analysis of thiol peroxidase in eye tissue was performed by using rabbit polyclonal antibodies against human thiol peroxidase. Procedures for transfer of proteins from 12% SDS-polyacylamide gels to nitrocellulose and for the processing of nitrocellulose blots have been previously described (Kim *et al.*, 1989). Monospecific antibodies for thiol peroxidase were prepared from the γ-globulin fraction using thiol peroxidase from human red blood cell immobilized on nitocellulose strips as described previously (Kim *et al.*, 1989). SDS-PAGE was performed by the method of Laemmli. Southern blot analysis was performed with brain cDNA fragment encoding thiol peroxidase. DNA was digested with restriction enzyme, and separated on a 0.8% agarose gel. DNA on agarose gel was

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transferred to nylon paper, and hybridized with digitogenin-labeled DNA fragment as a probe.

Results

Amino acids sequence of human retina thiol peroxidase A human retina cDNA library was screened with rabbit antibodies to thiol peroxidase from human red blood cell. A positive clone with a 0.7 kb insert was isolated. Southern blot analysis of the 0.7 kb DNA fragment with DNA fragment encoding brain thiol peroxidase revealed the same restriction enzyme-digested patterns for two restriction enzyme digests as those of human brain thiol peroxidase, which indicates that the cloned 0.7 kb DNA fragment is a gene for thiol peroxidase (Fig. 1). Its nucleotide sequence was determined. Figure 2 shows its nucleotide and deduced amino acids sequences. The amino acid alignment of the human thiol peroxidase family shown on Fig. 3 indicates that the open reading frame was identified as a partial gene for 25-kDa thiol peroxidase and found to encode a polypeptide of 151 amino acids. The amino acids sequence of the thiol peroxidase contains highly conserved two cysteine residues. The nucleotide and amino acids sequences are the same as that of 25 kDa from human brain and erythrocyte thiol peroxidases previously reported (Lim *et al.*, 1994b). This result confirms the existence of the same form of thiol peroxidase as brain and erythrocyte forms in human retina cell.

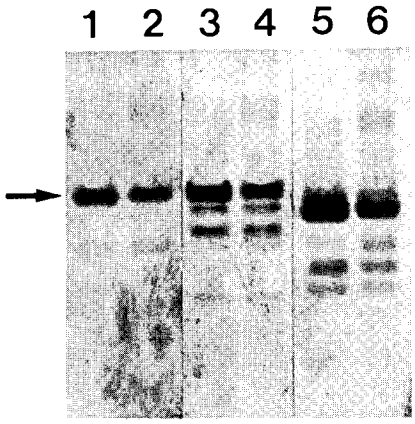

Fig. 1. Southern blot analysis of the gene fragment encoding human retina thiol peroxidase. A 0.7 kb DNA insert was digested with each *Sty*I and *Stu*I, electrophoresed on 0.8% agarose gel, and then transferred to a nylon paper. The paper was hybridized with digitogenin-labeled human brain cDNA fragment encoding thiol peroxidase. Lane 1: 0.7 kb DNA insert encoding human brain thiol peroxidase. Lane 2: 0.7 kb DNA insert encoding human retina thiol peroxidase. Lane 3: the *Sty*I-digested DNA from human brain. Lane 5: the *Stu*I-digested DNA from human brain. Lane 4: the *Sty*I-digested DNA from human retina. Lane 6: the *Stu*I-digested DNA from human retina. Arrow indicates a 0.7 Kb DNA fragment.

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ATG GCC TCC GGT AAC GCG CGC ATC GGA AAG CCA GCC CCT GAC TTC 45
Met Ala Ser Gly Asn Ala Arg Ile Gly Lys Pro Ala Pro Asp Phe 15

AAG GCC ACA GCG GTG GTT GAT GGC GCC TTC AAA GAG GTG AAG CTG 90
Lys Ala Thr Ala Val Val Asp Gly Ala Phe Lys Glu Val Lys Leu 30

TCG GAC TAC AAA GGG AAG TAC GTG GTC CTC TTT TTC TAC CCT CTG 135
Ser Asp Tyr Lys Gly Lys Tyr Val Val Leu Phe Phe Tyr Pro Leu 45

GAC TTC ACT TTT GTG TGC CCC ACC GAG ATC ATC GCG TTC ACA ACC 180
Asp Phe Thr Phe Val Cys Pro Thr Glu Ile Ile Ala Phe Thr Thr 60

GTG AAG AGG ACT TCC GCA AAG CTG GGC TGT GAA GTG CTG GGC GTC 225
Val Lys Arg Thr Ser Ala Lys Leu Gly Cys Glu Val Leu Gly Val 75

TCG GTG GAC TCT CAG TTC ACC CAC CTG GCT TGG ATC AAC ACC CCC 270
Ser Val Asp Ser Gln Phe Thr His Leu Ala Trp Ile Asn Thr Pro 90

CGG AAA GAG GGA GGC TTG GGC CCC TTG AAC ATC CCC CTG CTT GCT 315
Arg Lys Glu Gly Gly Leu Gly Pro Leu Asn Ile Pro Leu Leu Ala 105

GAC GTG ACC AGA CGC TTG TCT GAG GAT TAC GGC GTG CTG AAA AAC 360
Asp Val Thr Arg Arg Leu Ser Glu Asp Tyr Gly Val Leu Lys Asn 120

GAT GAG GGC ATT GCT TAC AGG GGC CTC TTT ATC ATC GAT GGC AAG 405
Asp Glu Gly Ile Ala Tyr Arg Gly Leu Phe Ile Ile Asp Gly Lys 135

GGT GTC CTT CGC CAG ATC ACT GTT AAT GAT TTG CCT GTG GGA CGC 450
Gly Val Leu Arg Gln Ile Thr Val Asn Asp Leu Pro Val Gly Arg 150

TCC GTG GAT GAG GCT CTG CGG CTG GTC CAG GCC TTC CAG TAC ACA 495
Ser Val Asp Glu Ala Leu Arg Leu Val Gln Ala Phe Gln Tyr Thr 165

GAC GAG CAT GGG GAA GTT TGT CCG GCT GCT TGG AAG CCT GGA CGT 540
Asp Glu His Gly Glu Val Cys Pro Ala Ala Trp Lys Pro Gly Arg 180

GAC ACG ATT AAG CCG AAC GTG GAT GAC AGC AAG GAA TAT TTC TCC 595
Asp Thr Ile Lys Pro Asn Val Asp Asp Ser Lys Glu Tyr Phe Ser 195

AAA CAC AAT TAG GCTGGCTAACGGATAGTGAGGTGAGCTTGTGCCCCTGCCTAGG 650
Lys His Asn ***                                             198
TGCCTGTGCTGGGTGTCCACCTGTGCCCCCACCTGGGTGCCCTATGCTGACCCAGGAAA 709
GGGCAGACCTGCCCCTCCAAACTCCACAAGTATGGGACCCTGGAGGGGTAGGGCAAGGG 768
CCTTCTCAATGCCTGCACCTAGAAGTTGAATTGTGAGGCCCTCCCCCAAGCCCAACCCA 827
GGCGGACAAAAGGCCTAGAGGTAACCAATAAAGTATTAGGGAAAGGAAAAAAAAAAAAA 886
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Fig. 2. Nucleotide sequences and deduced amino acids sequence of the 25 kDa thiol peroxidase gene from human retina cDNA. Nucleotides are numbered (right margin) beginning with the first base of the ATG initiator codon. The two VCP regions containing highly-conserved cysteines are shaded. The nucleotide sequence of human retina cDNA fragment encoding thiol peroxidase is underlined.

Tissue distribution of 25 kDa thiol peroxidase Distribution of 25 kDa thiol peroxidase in bovine tissues and eye was analyzed by Western blotting with antibodies against 25 kDa thiol peroxidase from human red blood cell. All of the immunoblot bands showed an apparent molecular mass of 25 kDa (Fig. 4A). The 25 kDa thiol peroxidase exists ubiquitously in all tested bovine tissues and cells including red blood cell, brain, heart, kidney, liver, lung, skeletal muscle, pancreas, retina, and spleen. This result showed that this form of thiol peroxidase is not a tissue specific form, but rather a ubiquitous form. The protein levels vary significantly between different tissues, with red blood cell > brain ≅ retina ≅ lung > pancreas ≅ heart ≅ kidney ≅ skeletal muscle ≅ spleen > liver. The protein levels in the cells such as red blood cells, brain, retina, and lung cell are relatively higher than those in other cells. These cells, except liver cell, have a potential

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HUMBTPX   MAS----------------------------------------------------  3
HUM372    MEAL--PLLAATTPDHGRHRRLLLLPLLLFLLPAGAVQGWETEERPRTRE  48
HUMMER5   MAAAVGRLLRASVA------RHVSAIP-------------WGISATAALRP  32
HUMPAG    MSS-------------------------------------------------  3
HUMORF06  MPGG----------------------LLL---------------------  7
          *

HUMHBPRP  ---------------------------GNARIGKPAPDFKATAVV-DGA  24
HUM372    EECHFYAGGQVYPGEASRVSVADHSLHLSKAKISKPAPYWEGTAVI-DGE  97
HUMMER5   AACGRTSLTNLLCSGSSQAKLFSTSSSCHAPAVTQHAPYFKGTAVV-NGE  81
HUMPAG    ---------------------------GNAKIGHPAPNFKATAVMPDGQ  25
HUMORF06  ---------------------------------GDVAPNFEANTTVGRIR  24
                                          **

HUMBTPX   FKEVKLSD-YKGKYVVLFFYPLDFTFVCP-TEIIAFSNRAEDFRKLGCEV  72
HUMRTPX                                 FVCP-TEIIAFSNRAEDFRKLGCEV  24
HUM372    FKELKLTD-YRGKYLVFFFYPLDFTFVCP-TEIIAFGDRLEEFRSINTEV  145
HUMMER5   FKDLSLDD-FKGKYLVLFFYPLDFTFVCP-TEIVAFSDKANEFHDVNCEV  129
HUMPAG    FKDISLSD-YKGKYVVFFFYPLDFTFVCP-TEIIAFSDRAEEFKKLNCQV  73
HUMORF06  FHDF-LGD-SWG---ILFSHPRDFTPVCT-TELGRAAKLAPEFAKRNVKL  68
          *  * *  *    *  * *** **  **

HUMBTPX   LG-VSVDSQFNHLAW---INTPRKEGGLGPLNIPLLGDVTRRLSEDYGVL  118
HUMRTPX   LG-VSVDSQFNHLAW---INTPRKEGGLGPLNIPLLGDVTRRLSEDYGVL  70
HUM372    VA-CSVDSQFTHLAW---INTPRRQGGLGPIRIPLLSDLTHQISKDYGVY  191
HUMMER5   VA-VSVDSHFSHLAW---INTPRKNGGLGHMNIALLSDLTKQISRDYGVL  175
HUMPAG    IG-ASVDSHFCHLAW---VNTPKKQGGLGPMNIPLVSDPKRTIAQDYGVL  119
HUMORF06  IA-LSIDSVEDHLAWSKDINAYNCEEPTEKLPFPIIDDRNRELAILLGML  117
              * **   ****    *                 *

HUMBTPX   ----KTDEGIAY--RGLFIIDGKGVLRQITVNDLPVGRSVDEALRLVQAF  162
HUMRTPX   ----KTDEGIAY--RGLFIIDGKGVLRQITVNDLPVGRSVDEALRLVQAF  114
HUM372    ----LEDSGHTL--RGLFIIDDKGILRQITLNDLPVGRSVDETLRLVQAF  235
HUMMER5   ----LEGSGLAL--RGLFIIDPNGVIKHLSVNDLPVGRSVEETLRLVKAF  219
HUMPAG    ----KADEGISF--RGLFIIDDKGILRQITVNDLPVGRSVDETLRLVQAF  163
HUMORF06  DPAEKDEKGMPVTARVVFVFGPDKKLKLSILYPATTGRNFDEILRVVISL  167
                  * *                 **   * ** **

HUMBTPX   QYTDEHGEVCPAGWKPGSDTIK----PNVDDSKEY----FSK--------  196
HUMRTPX   QYTDEHGEVCPAGWKPGSDTIK----PNVDDSKEY----FSK--------  148
HUM372    QYTDKHGEVCPAGWKPGSETII----PDPAGKLKY----FDKL-------  270
HUMMER5   QYVETHGEVCPANWTPDSPTIK----PSPAASKEY----FQKV-------  254
HUMPAG    QFTDKHGEVCPAGWKPGSDTIK----PDVQKSKEY----FSK--------  197
HUMORF06  QLTAEKRVATPVDWKDGDSVMVLPTIPEEEAKKLFPKGVFTKELPSGKKY  217
          *         * *           *           *

HUMBTPX   -----HN  198
HUMRTPX   -----HN  150
HUM372    -----N-  271
HUMMER5   -----NQ  256
HUMPAG    -----QK  199
HUMORF06  LRYTPQP  224
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Fig. 3. Amino acids alignment of human thiol peroxidase family. The partial amino acids sequences of human thiol peroxidases (HUMBTPX and HUMRTPX) which are perfectly conserved are shown in shaded character. Symbol * indicates perfect conserved amino acids. Abbreviations: HUMBTPX (accession number L14286), human brain thiol peroxidase; HUMRTPX, human retina thiol peroxidase. HUM372 (U25182), HUMMER5 (D49396), HUMPAG (X67951), and HUMORF06 (D14662) are of the human thiol peroxidase family.

problem resulting from high consumption of oxygen or exposure to oxygen, which requires a corresponding need for protection against oxidative stress. This result suggests that thiol peroxidase is a housekeeping type of antioxidant enzyme. To clarify the antioxidative role of thiol peroxidase in eye, the level of the thiol peroxidase in eye tissue including lens, cornea, aqueous humor, vitreous humor, choroid, and retina was determined by Western blotting. As shown in Fig. 4B, significant amount of thiol peroxidase was detected in cornea, vitreous humor, choroid, and retina cell. Taken together, these data shown in Fig. 4 indicate the antioxidative role of this type of peroxidase in eye tissue.

Discussion

Recently, studies of a family of thiol-specific antioxidant (TSA) proteins, more recently referred to as "thiol peroxidase", have been rapidly growing (Lim *et al.*, 1994b). The similarity among these proteins extend over the entire sequence, especially in the domains (VCP1 and VCP2 domains) which contain highly conserved cysteine(s) (Fig. 3) (Lim *et al.*, 1994b). Therefore, thiol peroxidase has been suggested to be a new type of peroxidase which may be an important antioxidant enzyme. We have identified five types of thiol peroxidases in human cDNA that exhibit homology to thiol peroxidase from human brain and red blood cell (Fig. 3). There are few reports concerning the tissue distributions of thiol peroxidase isoenzymes, especially in eye. In this paper, we focused on the presence and the tissue distribution of one form of the mammalian isoenzymes, the 25 kDa thiol peroxidase, for the purpose of understanding a physiological function of ubiquitous 25 kDa thiol peroxidase.

We isolated and identified a human gene encoding thiol peroxidase. From the amino acids alignment among the human thiol peroxidase family, including human retina thiol peroxidase, it appears that the gene for retina thiol peroxidase is the same type as that of human brain and red blood cells (Fig. 3).

The anterior of the lens is covered by metabolically highly active epithelial cells which are sensitive to damage by reactive oxygen species (Halliwell and Gutterridge, 1989). Reactive oxygen species can damage and cross-link

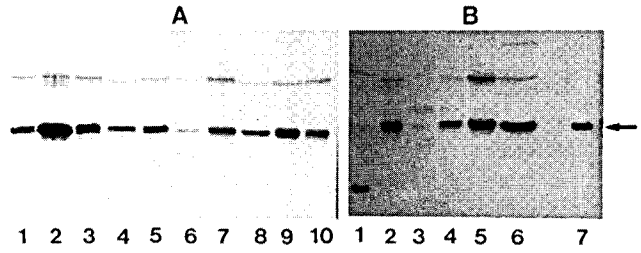

Fig. 4. Western blot analysis for tissue distribution of 25 kDa thiol peroxidase. Each 100 μg of crude proteins from tissue was electrophoresed in 12% SDS-PAGE gel, transferred to nitrocellulose paper, and then immunoblot analysis was performed with the polyclonal antibodies against 25 kDa thiol peroxidase from human red blood cells. From lanes A1 to A10: red blood cells, brain, heart, kidney, liver, lung, skeletal muscle, pancreas, retina, and spleen, respectively. From lanes B1 to B7: lens, cornea, aqueous humor, vitreous humor, choroid, retina, and 50 ng of 25 kDa thiol peroxidase from human red blood cell as a standard, respectively. Arrow indicates a molecular mass of 25 kDa.

lens protein, which causes cataract. The vitreous humor contains hyaluronic acid, which is attacked by reactive oxygen species, which causes severe visual impairment. The lipids present in the membrane of retina cells contain a high percentage of polyunsaturated fatty acids, and are thus susceptible to lipid peroxidation. The retina pigment, rhodopsin, can sensitize the formation of singlet oxygen. The eye has the problem caused by light. Therefore, the eye has a lot of potential oxidative stress-related problems, and one would expect a corresponding degree of protection. Indeed, the concentration of antioxidant in the eye is high. Therefore, the presence and wide distribution of 25 kDa thiol peroxidase in the eye suggests the physiological role for the thiol peroxidase in protecting the oxidative damage caused by reactive oxygen species.

In conclusion, a new type of 25 kDa thiol peroxidase antioxidant was found to be widely distributed in all the tested tissues including eye, which revealed an antioxidative role of the 25 kDa thiol peroxidase. A lot of investigations on the other four types of mammalian thiol peroxidase isoenzymes remain to be done in order to understand the physiological role for each "thiol peroxidase" isoenzyme.

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