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FOR THE RECORD

Common structural features of *MAPEG*—A widespread superfamily of membrane associated proteins with highly divergent functions in eicosanoid and glutathione metabolism

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Abstract: A novel superfamily designated MAPEG (Membrane Associated Proteins in Eicosanoid and Glutathione metabolism), including members of widespread origin with diversified biological functions is defined according to enzymatic activities, sequence motifs, and structural properties. Two of the members are crucial for leukotriene biosynthesis, and three are cytoprotective exhibiting glutathione S-transferase and peroxidase activities. Expression of the most recently recognized member is strongly induced by p53, and may therefore play a role in apoptosis or cancer development. In spite of the different biological functions, all six proteins demonstrate common structural characteristics typical of membrane proteins. In addition, homologues are identified in plants, fungi, and bacteria, demonstrating this superfamily to be generally occurring.

Keywords: 5-lipoxygenase activating protein; LTC_4 synthase; MAPEG; MGST1; MGST1-L1; MGST2; MGST3; microsomal glutathione-S-transferase

The MAPEG family consists of six human proteins, summarized in Table 1. The microsomal glutathione S-transferase 1 (MGST1) is involved in the cellular defense against harmful xenobiotics as well as metabolites produced as a consequence of oxidative stress (Morgenstern et al., 1982; Dejong et al., 1988; Mosialou et al., 1995). Substrates for the GST activity of MGST1 are lipophilic and electrophilic compounds with potential carcinogenic properties. MGST1 also possesses glutathione-dependent peroxidase activity toward various lipid hydroperoxides. Electron crystallography, at high resolution (3 Å), recently established the quaternary structure of the enzyme (trimer) and was consistent with multiple transmembrane regions (Hebert et al., 1997).

Leukotrienes are important mediators of inflammation (Samuelsson, 1983). 5-Lipoxygenase catalyzes the formation of the epoxide intermediate leukotriene (LT) A_4 from arachidonic acid. In the cell, this reaction requires the presence of FLAP (5-lipoxygenase activating protein), which functions as substrate provider for 5-lipoxygenase (Dixon et al., 1990; Miller et al., 1990; Mancini et al., 1993). Leukotriene A_4 can be further metabolized by leukotriene C_4 synthase, which catalyzes the specific conjugation of LTA_4 with glutathione leading to the formation of LTC_4 (Samuelsson, 1983). Subsequent removal of one amino acid residue from the glutathione moiety of LTC_4 leads to the formation of LTD_4 and LTE_4, respectively. Together, these compounds are potent mediators of inflammation and play an important role in the pathophysiology of bronchial asthma. FLAP and LTC_4 synthase were found to be homologous (Lam et al., 1994; Welsch et al., 1994). Based on this homology, other related proteins, i.e., microsomal glutathione S-transferases 2 and 3 (MGST2 and MGST3), were identified and characterized (Jakobsson et al., 1996, 1997). MGST2 and MGST3 were both found to catalyze the conjugation of LTA_4 with glutathione. MGST2 could also conjugate the xenobiotic substrate 1-chloro-2,4-dinitrobenzene with glutathione. In addition, both enzymes were able to catalyze the glutathione dependent reduction of 5-hydroperoxy-eicosatetraenoic acid to 5-hydroxy-eicosatetraenoic acid. Therefore, MGST1, MGST2 and MGST3 are all glutathione S-transferases as well as glutathione dependent peroxidases. Using Western blot and activity assays, MGST2, but not LTC_4 synthase, was demonstrated to be the principal source of LTC_4 synthase activity in liver and endothelial cell microsomes, whereas the opposite was observed for lung and platelet microsomes (Scoggan

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Table 1. *Human members of the MAPEG family*

Gene symbol	Name	Properties	Acc. no.
FLAP	5-Lipoxygenase activating protein	Activation of cellular leukotriene biosynthesis	M60470
LTC_4S	Leukotriene C_4 synthase	Glutathione S-transferase specific for LTA_4	U09353, U11552
MGST1	Microsomal glutathione S-transferase 1	Glutathione S-transferase Glutathione peroxidase	J03746
MGST1-L1	Microsomal glutathione S-transferase 1–like 1	Induced by p53	AF010316, AF027740
MGST2	Microsomal glutathione S-transferase 2	Glutathione S-transferase Glutathione peroxidase	U77604
MGST3	Microsomal glutathione S-transferase 3	Glutathione S-transferase Glutathione peroxidase	AF026977

et al., 1997). Although extensively investigated, no enzymatic activity has been found for FLAP underscoring the functional diversity of this superfamily.

Recently, the gene expression of a novel homologue to MGST1 as well as other enzymes involved in redox regulation was reported to be under the control of p53 (Polyak et al., 1997). This novel MGST1 homologue will be referred to as MGST1-L1 (microsomal glutathione-S-transferase 1–like 1). This protein was independently identified as an expressed sequence tag (EST) and the full length sequence deposited to Genbank database (P.J. Jakobsson, J.A. Mancini, A.W. Ford-Hutchinson, unpubl. results). The finding that MGST1-L1 was upregulated after p53 expression in a colorectal cancer cell line (Polyak et al., 1997) suggests an important biological function possibly associated with cancer or apoptosis. In addition, nonvertebrate members, including those from bacteria, have been identified and are included in this study allowing for comparative analysis.

Results and discussion: The members of the MAPEG superfamily were aligned and found to be distantly homologous, with the relationships supported by the hydropathy plots (Fig. 1). All proteins clearly have similar hydropathy profiles, especially for the region between alignment positions 80 and 120. Previous interpretation of hydropathy plots indicated three membrane spanning segments in MGST1, FLAP, and LTC_4 synthase. However, the pattern in Figure 1B actually indicates the possibility of four membrane spanning segments. Furthermore, four membrane spanning segments are predicted with two different algorithms (Persson & Argos, 1994; Cserzo et al., 1997), one of which was designed specially for prokaryotic membrane proteins (Cserzo et al., 1997). This alternative will need experimental verification and forms an attractive hypothesis at this stage. The resulting topology would be consistent with information on amino acids that are important for activity in LTC4 synthase (Lam et al., 1997) and chemical modification studies on MGST1 (Andersson & Morgenstern, 1990). Structural determination of MGST1 using electron crystallography has yielded a projection structure at high resolution (Hebert et al., 1997). This structure is also consistent with a polytopic membrane configuration. An important issue that needs to be addressed is the quaternary structure of the different members of the superfamily. Not only are the functional characteristics very distinctive, but it appears that one member forms a trimer (Weinander et al., 1996; Hebert et al., 1997) and another forms a dimer (Nicholson et al., 1993; Lam et al., 1997).

The alignment in Figure 1A demonstrates only two strictly conserved residues, Asn78 and Arg115. A catalytic role for Asn78 (Asn55 in LTC_4 synthase) was ruled out since an alanine exchange mutant of the LTC_4 synthase is fully active (Lam et al., 1997). Therefore, if there would be a common reaction mechanism of all these enzymes, and one common catalytic residue, only Arg115 remains as an active site candidate.

The alignment also gives information regarding residues strictly conserved within each of the discernible subfamilies (marked in Fig. 1A with grey background). These sequence patterns reflect residues of structural and functional importance of each subfamily, and since they constitute sequence fingerprints, they can also be used in future database searches for further family members.

For the MGST1 subfamily (top 2 sequences in Fig. 1A), the motifs "FANPED" at positions 44–49 and "VERxxRAH" at 69–76 constitute two such patterns. The MGST2/FLAP/LTC_4S subfamily (sequence rows 3–6 in Fig. 1A) is characterized by the "FERV" pattern at position 46–49. Adjacent to this motif, Arg74 (corresponding to position 51 in LTC_4 synthase) has recently been suggested to function as a proton donor in LTC_4 synthase for the opening of the LTA_4 epoxide (Lam et al., 1997). This arginine is found in all but the FLAP sequences in accordance with the observation that FLAP has no known enzyme activity. In this region, the MGST3 subfamily instead has the pattern "$FNCx_1QRx_2H$" where x_1 is a hydrophobic residue and x_2 is a small residue.

The tyrosine residue at position 122 (corresponding to Tyr97 in LTC_4 synthase) is conserved in all subfamilies but that of the *Escherichia coli* and *Vibrio cholerae* forms. If all enzymes of the MAPEG superfamily have an enzymatic mechanism in common, it is likely that this residue also plays a role. Tyrosine is a residue type frequently found at active sites, as for instance in the large superfamily of short-chain dehydrogenases/reductases (SDR) with highly divergent members having typically 15–30% pairwise residue identity but which all have a catalytic Tyr in common (Jörnvall et al., 1995).

Since most cytosolic GSTs have a catalytic Tyr, such a role could be possible for the conserved Tyr122. However, this residue has been exchanged for Phe in MGST1 without any effect on the catalytic activity (Weinander et al., 1997).

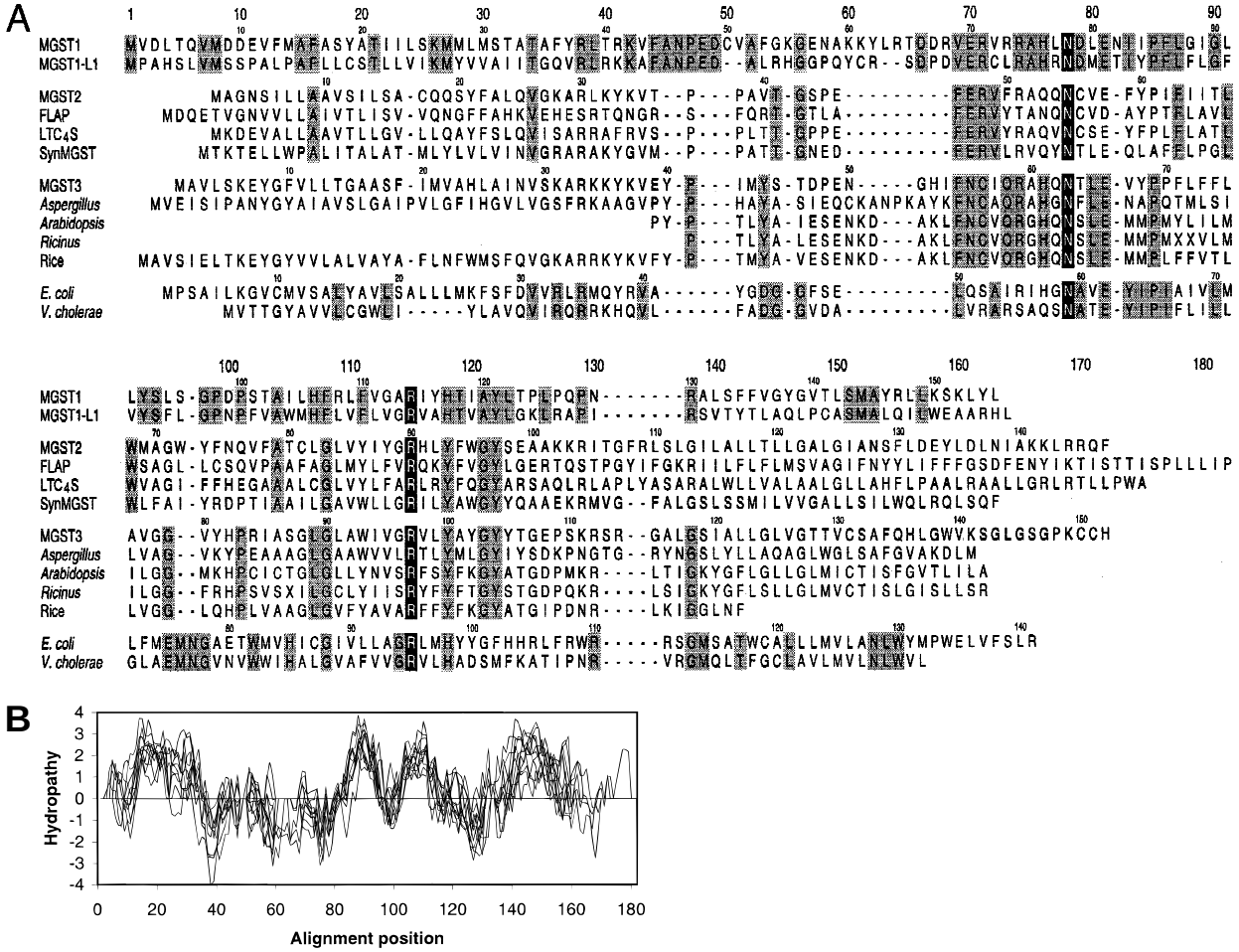

Fig. 1. Hydropathy curves and multiple sequence alignment of 13 members of MAPEG. **A:** Multiple sequence alignment. Strictly conserved residues are marked against a black background. Residues conserved in one of the four subfamilies are marked against a grey background. Big-size numbers represent alignment positions. Small-size numbers represent positions of the top sequence in each subfamily. The alignment was obtained using the ClustalW software (Thompson et al., 1994). Abbreviations as in Table 1. **B:** Hydropathy curves corresponding to the alignment in **A** were calculated according to the Kyte and Doolittle procedure (Kyte & Doolittle, 1982), using a six-residue sliding window.

There is also another tyrosine four residues N-terminally (at alignment position 118), which is strictly conserved among all MGST2/FLAP/LTC_4S/MGST3 forms. Tyr118 was suggested to function as a base for the formation of the thiolate anion of glutathione (Lam et al., 1997). In the MGST1/*E. coli + V. cholerae* subfamilies, this tyrosine is replaced by a histidine residue.

Near the Tyr118, the strictly conserved Arg115 is found. This situation bears similarities with the SDR enzymes having a Lys four residues downchain the active-site Tyr. Since these residues are in an α-helix, their side chains will appear close in the three-dimensional structure of the enzymes. By analogies, if this region in the MGST2/FLAP/LTC_4S/MGST3 proteins forms an α-helix, the Tyr and Arg would be adjacent in space and could constitute a catalytic diad.

Clearly, the similarities are centered around the middle of the protein chains, partly coinciding with a suggested arachidonic acid binding site in FLAP (amino acids 48–61) (Vickers et al., 1992; Mancini et al., 1993, 1994). The N- and C-terminal regions are less strictly conserved, in agreement with two observations on MGST1 and LTC_4 synthase: (1) amino acids 1–41 can be removed from MGST1 by proteolysis without loss of function (Andersson et al., 1994) and (2) C-terminal segments can be exchanged between LTC_4 synthase and FLAP without alteration of protein function (Lam et al., 1997).

All proteins have similar lengths (137–161 amino acid residues) and all except FLAP have a deduced pI between 10 and 11. The pairwise residue identity ranged from 9 to 73%. Excluding species variants, the closest pairs are LTC_4 synthase and MGST2 with 44% residue identity followed by MGST1 and MGST1-L1 with 38% residue identity. FLAP, MGST3, and SynMGST are linked to LTC_4 synthase and MGST2 with 29–35% residue identity. It is evident that the MAPEG superfamily can be divided into four different subgroups, bearing clearly detectable sequence similarities with typically 20–30% residue identity in pairwise comparisons. The MGST1/MGST1-L1 subfamily reveals distantly but clear sequence and structural similarities to the subfamily of LTC_4 synthase, MGST2 and SynMGST. Thus, since MGST1 and MGST2 have similar enzymatic properties (Jakobsson et al., 1996), the family relationships are now corroborated.

In summary, the MAPEG superfamily is supported by sequence alignments and hydropathy plots suggesting similar three-dimensional and membrane-spanning properties. MAPEG thus links proteins important in the endogenous metabolism of physiologically important reactive lipophilic intermediates (leukotrienes) with proteins in the detoxification of highly reactive lipophilic compounds of exogenous and endogenous origin.

Materials and methods: The amino acid sequences were aligned using ClustalW (Thompson et al., 1994). Hydrophilicity profiles were calculated according to Kyte and Doolittle (Kyte & Doolittle, 1982) with six-residue spans. The consensus sequence patterns derived from the alignment were used for database screenings.

TBLASTN searches revealed several overlapping expressed sequence tag (EST) clones from *Aspergillus nidulans* (10 clones: AA786119/AA788211/AA785264/AA788210/AA783834/AA785263/AA783835/AA788217/AA788216/AA786116), *Oryza sativa* (rice) (six clones: C29080/C74120/C73687/D49218/D49242/C29006), *Arabidopsis thaliana* (two clones: W43223/AA712909), and one from *Ricinus communis* (T24301). Sequences were aligned for each species, and a consensus sequence was deduced. The *R. communis* sequence included nondetermined bases where X is given in the amino acid translation. Open reading frames (obtained from genome sequencing projects) similar to MAPEG queries were found in *Synechocystis Sp.* (D90909, PID:g1652931), *E. coli* (AE00280, PID: g1788176, b1869), and *V. cholerae* (GVCLA50TH).

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