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Human Myeloperoxidase and Thyroid Peroxidase, Two Enzymes With Separate and Distinct Physiological Functions, Are Evolutionarily Related Members of the Same Gene Family

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ABSTRACT **Human myeloperoxidase and human thyroid peroxidase nucleotide and amino acid sequences were compared. The global similarities of the nucleotide and amino acid sequences are 46% and 44%, respectively. These similarities are most evident within the coding sequence, especially that encoding the myeloperoxidase functional subunits. These results clearly indicate that myeloperoxidase and thyroid peroxidase are members of the same gene family and diverged from a common ancestral gene. The residues at 416 in myeloperoxidase and 407 in thyroid peroxidase were estimated as possible candidates for the proximal histidine residues that link to the iron centers of the enzymes. The primary structures around these histidine residues were compared with those of other known peroxidases. The similarity in this region between the two animal peroxidases (amino acid 396–418 in thyroid peroxidase and 405–427 in myeloperoxidase) is 74%; however, those between the animal peroxidases and other yeast and plant peroxidases are not significantly high, although several conserved features have been observed. The possible location of the distal histidine residues in myeloperoxidase and thyroid peroxidase amino acid sequences are also discussed.**

Key words: evolution, proximal histidine, distal histidine, heme enzyme

INTRODUCTION

Peroxidases are widely distributed throughout plants, animals, and microorganisms[1] and carry out a variety of biosynthetic and degradative functions related to the consumption of hydrogen peroxide. The structure and reaction mechanism of the plant and yeast enzymes have been well studied.[2–5] The ferric enzyme first reacts with a hydroperoxide to form compound I, which has two oxidizing equivalents more than the ferric enzyme. In most cases, this compound I undergoes a one-electron reduction by a hydrogen donor (AH) to compound II, followed by a further one-electron reduction, to return to the native ferric enzyme. The prosthetic group of the plant and yeast peroxidases is a protoporphyrin IX, and the fifth coordination position of the heme iron is occupied by an imidazole group of the proximal His.[6,7] A geometrically well-situated distal His residue serves as an acid-base catalyst during the reaction. The existence of a nearby Arg residue, which stabilizes the transition state of the peroxidase during compound I formation, has been indicated as an important feature of peroxidase, distinguishing it from an oxygen-carrying globin.[8]

Contrary to the plant and yeast peroxidases, little is known about the structure of animal peroxidases. Among them, myeloperoxidase (MPO) and thyroid peroxidase (TPO) have been extensively studied, especially in terms of their well-established physiological functions. MPO is found in granulocytes and monocytes and plays a major role in the H_2O_2-dependent microbicidal system of neutrophiles.[9,10] In the presence of H_2O_2 and halide, chiefly Cl^- in vivo, MPO catalyzes the two-electron oxidation of Cl^-, leading to the formation of HOCl, which is ultimately toxic to the ingested organisms.[9] Human MPO is primarily synthesized as a glycosylated precursor protein of 89–91 kilodaltons (kDa),[11–13] which is subsequently cleaved into the heavy and light chains of 55–60 kDa and 10–15 kDa, respectively.[14] The mature protein is composed of two of each subunit in a tetrameric structure of 120–160 kDa and contains two identical prosthetic groups per tetramer.[15] The enzyme exhibits a characteristic absorption spectrum with a relatively intense peak at 700 nm and a red-shifted Soret band, which is attributed to the unique iron-chlorin (dihydroporphyrin) prosthetic group.[16–19] Evidence for the coordination of His to the iron center of MPO has been provided.[17,20] The presence of the distal His residue has also been proposed.[21–23]

On the other hand, TPO plays a central role in the biosynthesis of thyroid hormones: the iodination of tyrosine residues on thyroglobulin and the intramolecular coupling reaction of iodinated tyrosines, leading to the formation of T3 (3-3'-5-triiodothyronine) and T4 (thyroxine).[24,25] The iodination reaction occurs via a two-electron oxidation of I^-[26]. The human enzyme is a monomeric protein although it has been

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demonstrated to have two polypeptides of around 100 kDa on NaDodSO$_4$-polyacrylamide gels; the relationship of these two immunochemically related proteins has not been clarified.[27,28] The prosthetic group is likely to be protoporphyrin IX,[24,28,29] which is reflected in the dissimilar absorption spectrum between TPO and MPO. While the physicochemical and physiological properties of these two animal peroxidases are quite different, their halogenation reactions share a unique feature in that compound I undergoes a two-electron reduction to the native ferric state, without passing through compound II.

Recently the nucleotide and the deduced amino acid sequences of human MPO[30,31] and TPO[32] have been established. Unexpectedly we have found significant similarities in their nucleotide and amino acid sequences despite the fact that the halogenation reaction mechanism is the only apparent common feature. We report herein the first evidence tht MPO and TPO diverged from a common ancestral gene and make up a gene family. We further discuss features of the primary structure around the proximal His residue that may be unique to all peroxidases.

MATERIALS AND METHODS

The sequence data used in this paper are the following: human MPO,[30,31] human TPO,[32] cytochrome C peroxidase (CCP),[33] horseradish peroxidase C (HRP C),[34] turnip peroxidase 7 (TP 7),[35] ligninase,[36] and chloroperoxidase (CPO).[37]

Nucleotide and protein sequences were compared by computer analysis using the programs developed by Wilbur and Lipman[38] and Lipman and Pearson,[39] respectively. Secondary structure was analyzed by using the algorithm of Garnier et al.[40] Hydropathy indices were determined by the method of Kyte and Doolittle[41] using a program written by Stephens.[42]

RESULTS AND DISCUSSION

Nucleotide Sequence Comparison Between MPO and TPO

The human MPO nucleotide sequence[30,31] is 3,213 nucleotides in length and contains two different polyadenylation signals in the 815-nucleotide-long 3′ noncoding region, producing two mRNAs of different sizes. The primary translation product has 745 amino acids, which consists of a prosequence (166 amino acids) and a small (112 amino acids) and a large (467 amino acids) subunit of the MPO functional protein. On the other hand, the human TPO nucleotide sequence is 3,048 nucleotides in length, and its 3′ noncoding region is only 177 nucleotides long.[32] Alternate splicing occurs in the middle of the coding sequence, producing a 171-nucleotide shorter second mRNA. The open reading frame of the longer TPO sequence encodes 933 amino acids. Since two different sized nucleotide sequences are available in both peroxidases, we chose only longer sequences for the present comparative studies.

When human MPO and TPO nucleotide sequences were compared for the best alignment (Fig. 1), a counterpart to the entire TPO sequence was found in the MPO sequence, leaving 114 base pairs of the 5′ and 47 base pairs of the 3′ end of the MPO sequence unmatched. Of interest is that the 171-nucleotide stretch, which is absent in the shorter second TPO sequence,[32] also significantly aligned with a part of the MPO sequence coding for the heavy subunit. Within the 3,048 base pairs of sequence aligned between MPO and TPO, the average similarity is 46%. This similarity becomes 58% within the sequence encoding the functional MPO protein (MPO nucleotide 662–2,398).

Amino Acid Sequence Comparison Between MPO and TPO

A comparison of the deduced amino acid sequences (Fig. 2) revealed 44% similarity between MPO and TPO. The similarity is again more evident in the sequence encoding the MPO functional domain (MPO amino acid 167–745, 47% similarity) compared to that in the prosequence (MPO amino acid 27–166, 29% similarity). This global amino acid similarity of 44% clearly indicates that MPO and TPO are members of the same gene family and diverged from a common ancestral gene. This is rather surprising since both enzymes are quite different in terms of the number of polypeptides composing the mature protein and therefore molecular weight, prosthetic group, and physiological function in their specific target tissues.

A computer program that predicts secondary structure[40] suggests that four regions have long continuous α-helical stretches, which are common in both MPO and TPO sequences. These regions are located at TPO amino acid residue 1–23, 38–61, 85–105, and 403–445, and MPO amino acid residue 24–37, 51–85, 107–130, 410–430, and 442–452. The latter two α-helical stretches in MPO overlap the last α-helical region of TPO. If the similarity is calculated within the first through last base of the α-helical match-up in each domain, the second and fourth α-helical regions have higher values of 44% and 74%, respectively, than those of the surrounding residues (29% and 47%, respectively; see above). It is, therefore, conceivable that these regions may have a common function in both enzymes.

The hydropathy indices of both enzymes in the region corresponding to the carboxyl half of the MPO

Fig. 1. Nucleotide sequence comparison of human TPO and MPO. The complete TPO sequence is presented and aligned with the MPO sequence.[38] Mismatches are presented, and homologous nucleotides are absent from the alignment. Gaps (——) are introduced to maximize the similarity between the two sequences. The numbers at the top and bottom of each line refer to TPO and MPO, respectively. The initiation and termination codons are boxed, and polyadenylation signals are underlined. The beginning of the small and large subunits of MPO is indicated by horizontal arrows. The 171-nucleotide sequence, which is not present in the second TPO mRNA, is denoted with brackets.

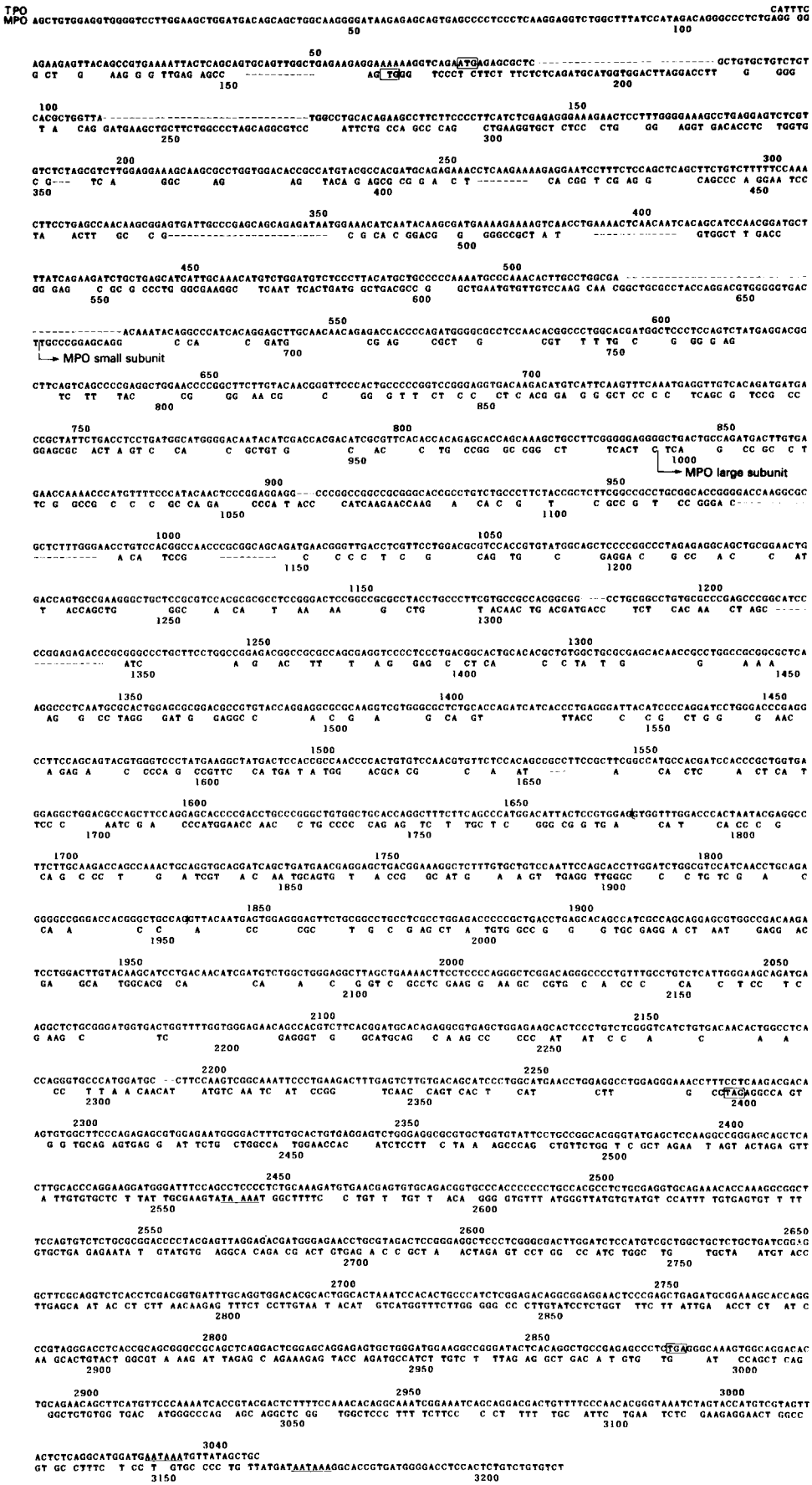

Figure 1

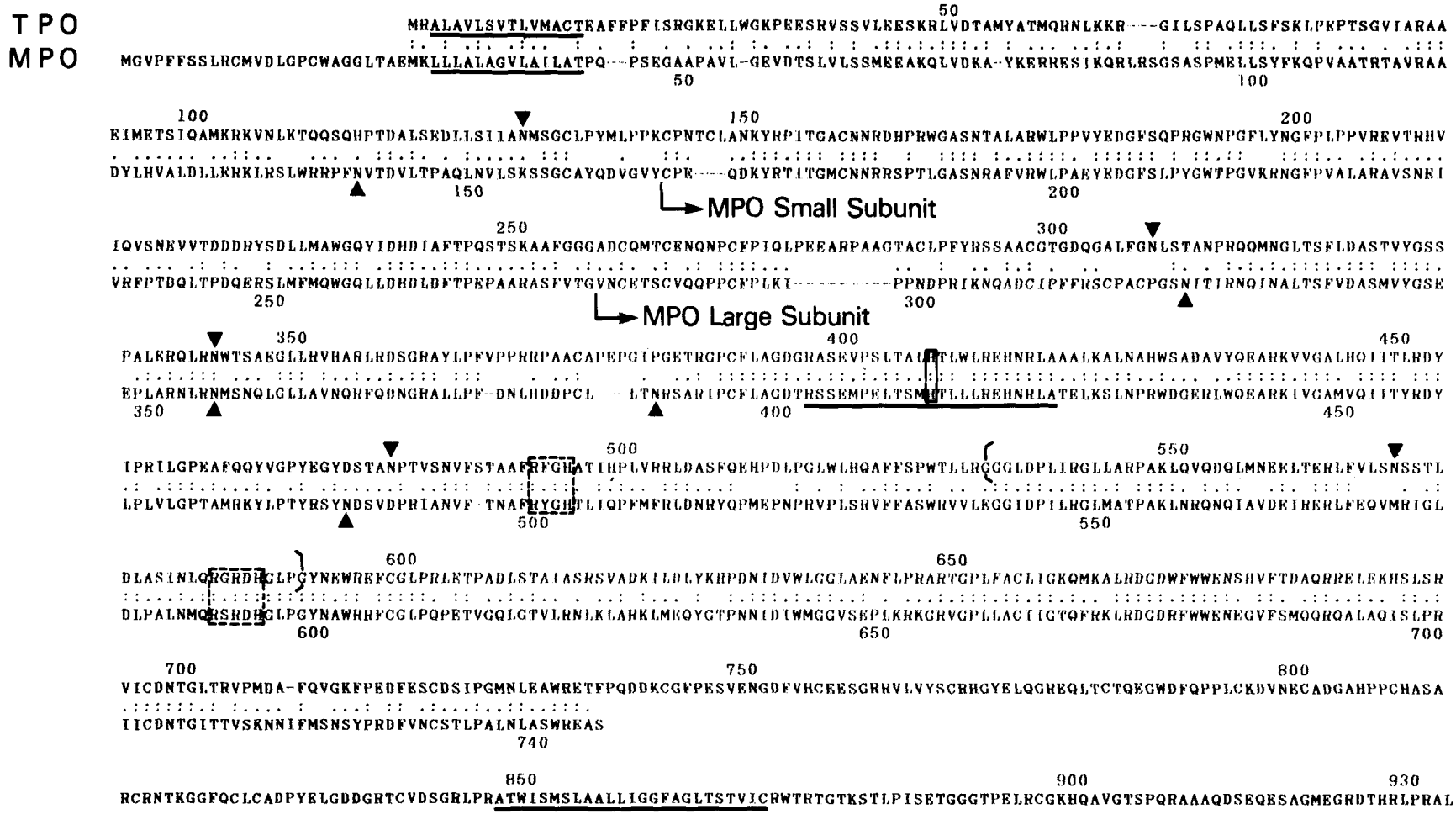

Fig. 2. Protein sequence comparison of human TPO and MPO. The complete TPO and MPO sequences are presented, and the optimized alignment between two sequences is denoted by a colon for an identity and a dot for a conservative replacement (for details, see ref. 39). Gaps (----) are introduced to maximize the similarity between the two sequences. The numbers at the top and bottom of each line refer to TPO and MPO, respectively. The putative His residue, which binds to the iron center, and the possible distal His-containing regions are boxed with solid and broken lines, respectively. The beginning of the small and large subunit of MPO is indicated by horizontal arrows. The 57-amino acid sequence encoded by the 171-nucleotide sequence, which is absent in the second TPO mRNA, is denoted with brackets. Putative asparagine-linked glycosylation sites are marked with solid triangles. The amino acid sequence surrounding the possible proximal His residue, which is used in Figure 4 for the comparison with other sequences, and the N-terminal and the C-terminal hydrophobic segments are underlined.

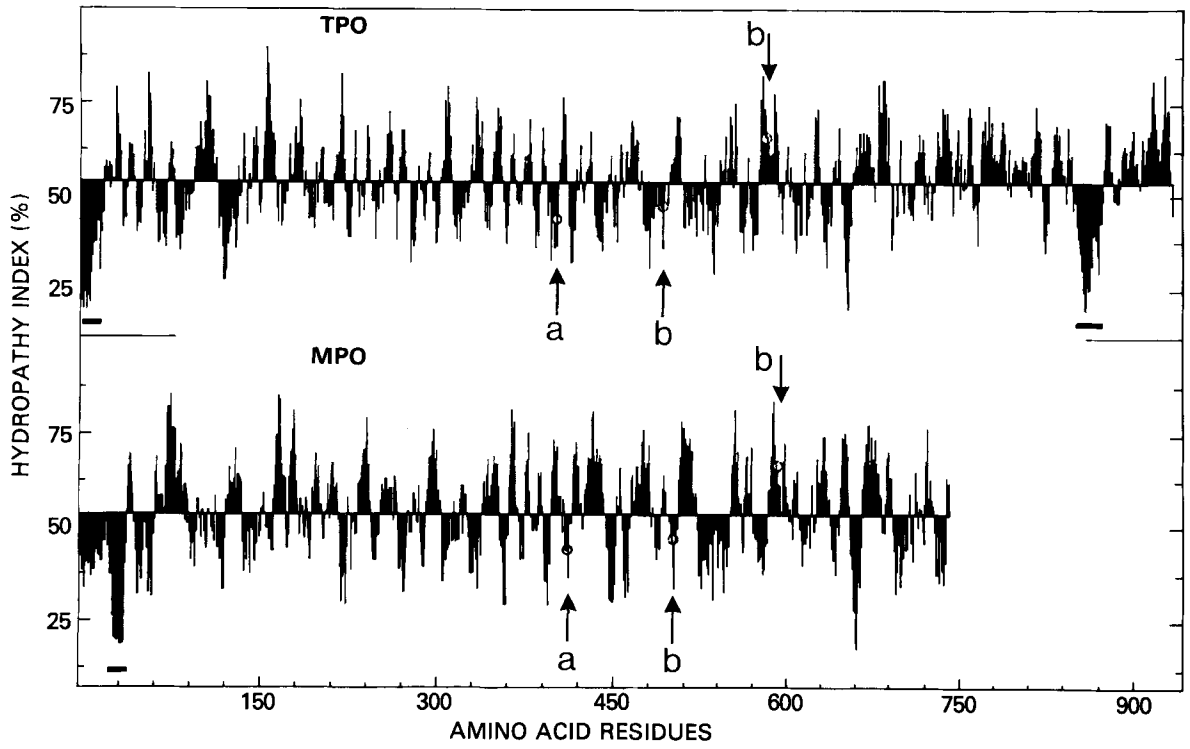

Fig. 3. Hydropathy profiles of human TPO and MPO protein sequences. The areas below and above the center line represents hydrophobic and hydrophilic areas, respectively.[41,42] The putative proximal His residue and the two possible distal His residues are circled, and the positions are indicated with arrows a and b, respectively. The N-terminal and the C-terminal hydrophobic transmembrane regions are indicated with solid bars.

sequence are similar (Fig. 3). In the profiles, a very hydrophobic region that is similar in structure to a signal sequence is seen at the N-termini of both enzymes.[43,44] This region, which is in the first common α-helical domain, is proceeded by a positively charged amino acid (Arg-2 in TPO and Lys-28 in MPO) and terminates with either a negatively charged residue (Glu-17 in TPO) or a helix-breaking residue (Pro-43 in MPO).[45] No direct evidence for the signal peptide cleavage has been available in both enzymes, although in the study of MPO biosynthesis in HL-60 cells, this has been suggested by the demonstration that the precursor protein is found in the extracellular medium.[11–13]

A most interesting question is how one sequence codes for a single polypeptide protein (TPO) and the other similar sequence encodes subunits (MPO). MPO is in this regard very similar to many other lysosomal enzymes, which exist intracellularly as larger precursor polypeptides that appear to be converted slowly by proteolytic cleavage to the mature enzymes.[46,47] The importance of phosphorylated mannose residues in lysosomal enzymes as the recognition marker required for incorporation of the enzymes into lysosomes has been suggested.[47,48] MPO[12,13] and TPO[49,50] are glycoproteins that both have five potential asparagine-linked glycosylation sites, one of which is located at the same position in each sequence. One main difference between the TPO and MPO amino acid sequences is that TPO has a 200-amino acid longer C-terminus (Figs. 2, 3). Interestingly, in this 200-amino acid region, close to the carboxyl end, a broad hydrophobic region is observed (Fig. 3), which is immediately followed by highly charged amino acid residues (Fig. 2). This structure is very similar to a halt transfer signal that prevents passage of the C-terminal portion of the polypeptide through the membrane[43,44] and therefore allows the polypeptide to remain attached to the membrane. This structure cannot be found in the MPO sequence. The presence of the halt transfer signal in TPO is in very good agreement with the reported result that TPO is an integral membrane protein that faces the luminal side of the microsomal vesicle.[50] The halt transfer signal might play an important role in determining the fate of the protein.

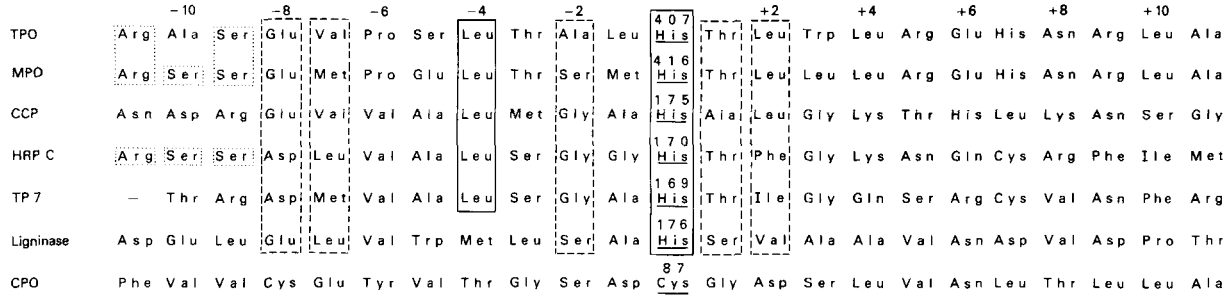

Fig. 4. The primary structure comparison of the proximal His-containing region. The sequence data used in the figure are the following: human MPO,[30,31] human TPO,[32] cytochrome C peroxidase (CCP),[33] horseradish peroxidase C (HRP C),[34] turnip peroxidase 7 (TP 7),[35] ligninase,[36] and chloroperoxidase (CPO).[37] All sequences are aligned and numbered in reference to the proximal His (Cys in the case of CPO). The number above the proximal His (Cys) is the amino acid residue in the original sequence. The amino acid residues that are conserved or that have similar properties among all the peroxidases are boxed with either solid or broken lines, respectively. The amino acids that are conserved among MPO, TPO, and HRP C are boxed with dotted lines.

Primary Structure of the Peroxidase Active Site

The forth common α-helical domain may contain the peroxidase active site, especially the proximal His residue[17,20] for the following three reasons: 1) Seventy-four percent amino acid similarity in this region (TPO amino acid 403–421, MPO amino acid 412–430) is exceptionally high compared with the global similarity (44%). This was also seen in another well-studied hemeprotein, cytochrome P-450, in which the sequence surrounding the heme binding Cys residue is very well conserved for all known P-450s including bacterial P-450.[51] 2) In this α-helical region, there are two His residues exactly aligned in both MPO and TPO; and 3) this α-helical domain is located in the MPO large subunit, which is known to contain the prosthetic group.[9,15] Further support for the location of the proximal His and the surrounding region may be provided by the results reported by Schultz et al.[52] on canine MPO. They isolated the iron chlorin-binding peptide fragment after tryptic and chymotryptic digestion and found that the fragment had the following amino acid composition; 2 Ser, 2 Glx, Leu, Pro, and Ala, with the Leu being the C-terminal residue of the fragment. They proposed that the two hydroxyls of the Ser residues form ester bonds with the propionyl side chains of the iron-chlorin prosthetic group. These data can be accounted for by a short stretch of sequence (MPO amino acid residue 406–412), which is located close to the forth α-helical region, except that Met replaces Ala. This neutral amino acid difference could reflect a species difference. We, therefore, propose that one of the His residues either at 416 in MPO (407 in TPO) or at 423 in MPO (414 in TPO) can be the proximal ligand, binding to the iron centers. Although the amino acid sequences surrounding the His residue at 423 in MPO (414 in TPO) match better between the two enzymes, we feel that the His residue at 416 in MPO (407 in TPO) is more likely to be the proximal His based on the comparison with other known peroxidase proximal His-containing sequences (Fig. 4). The amino acid sequence around the proximal His has been conserved in plant (HRP C, TP 7), yeast (CCP), and fungi (ligninase) peroxidases.[36] If the His residue at 416 in MPO and that at 407 in TPO is aligned to the known proximal His of these nonmammalian peroxidases, some common features are observed for all proximal His-containing sequences although the amino acid similarity in this region as well as the total sequence of animal and other peroxidases is very low. Their shared characteristic features are the following: 1) Leu is found at the −4 position (relative to His) in all the peroxidases except for ligninase, which has a Met residue instead of Leu[36]; 2) an acidic amino acid occupies position −8; 3) a hydrophobic amino acid is located at +2 and −7; and 4) a small amino acid such as Gly, Ala, Ser, Thr is found at positions −2 and +1. In addition, common amino acid residues are seen among MPO, TPO, and HRP C. These results could suggest the existence of a peroxidase supergene family. Chloroperoxidase (CPO) whose proximal axial ligand is not His, shares only a small amino acid characteristics at positions −2 and +1. CPO may belong to a different supergene family.

Another important feature for peroxidase has been suggested by Poulos and Kraut[8] that His and Arg residues should be located at the distal site of the heme iron. They found a consensus sequence (Arg-X-Y-Z-His) for this distal His-containing region in the yeast and plant peroxidases. Assuming this is true of animal peroxidases, this exact consensus sequence and a second similar sequence (Arg-X-Y-His) can be found in TPO sequence bounded by residues 582–586 and 491–494, respectively, and their counterparts in MPO. This exact consensus sequence, however, is absent in the protein encoded by the shorter TPO

mRNA, which is produced as a result of alternate splicing.[32] In spite of the high sequence similarity between MPO and TPO in this region, therefore, the distal His is more likely to be found in the less conserved region. This is also favored by the hydropathy analysis; only His in the less conserved sequence shows the hydropathy indices similar to those in yeast and plant peroxidases (data not presented). Experiments are currently underway by using cDNA expression to identify which distal His-containing sequence is actually important for peroxidase activity.

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