Molecular Cloning and Characterization of cDNA for Human Myeloperoxidase*

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Partial amino acid sequence of human myeloperoxidase was determined, and a 41-base oligonucleotide containing deoxyinosines at four positions was chemically synthesized. By using the oligonucleotide as a probe, cDNA clones for human myeloperoxidase were isolated from a cDNA library constructed with mRNA from human promyelocytic leukemia HL-60 cells. One of the clones containing a 2.6-kilobase insert was subjected to nucleotide sequence analysis. The sequence was found to contain an open reading frame of 467 amino acids, which was located on the COOH terminus half of the protein. The RNA specified by the cDNA was prepared using SP6 RNA polymerase and translated in rabbit reticulocyte lysates, and the product was identified as human myeloperoxidase by immunoprecipitation with rabbit anti-human myeloperoxidase antibody. By Northern hybridization analysis of RNA from leukemic cells, it was shown that myeloperoxidase mRNA is abundantly expressed in human promyelocytic HL-60 and mouse myeloid leukemia NFS-60 cells. Furthermore, the results of Southern hybridization analysis of human genomic DNA suggest that there are one or two genes for myeloperoxidase in the human haploid genome.

Myeloperoxidase (donorhydrogen peroxide, oxidoreductase, EC 1.11.1.7) is a heme-containing glycoprotein present in azurophilic (primary) granules of neutrophilic granulocytes (1) and plays a major role in the oxygen-dependent microbicidal system of granulocytes. The deficiency of this enzyme activity results in a marked microbicidal defect (2) and, in some cases, the deficiency is transmitted inheritably (3).

Myeloperoxidase has been purified from various sources including human granulocytes (4-9) and human leukemic cells (8, 10, 11). The enzyme from human mature granulocytes has a molecular mass of 120,000-160,000 daltons, composed of two heavy and two light chains (5, 8-12). On the other hand, myeloperoxidase from human leukemic cells contains a smaller form with $M_0$ of 72,000-79,000 consisting of one heavy and one light chain in addition to the larger form (8, 10). The sizes of subunits were estimated to be 55,000-60,000 daltons for the heavy chain and 10,000-15,000 daltons for the light chain (4-12) in both the larger and smaller forms.

An electron microscopic analysis of human bone marrow cells has shown that synthesis of myeloperoxidase occurs at the early stage of the myeloid cell differentiation, specifically in promyelocytes, and decreases during further differentiation (13). Human promyelocytic leukemia HL-60 cells express myeloperoxidase constitutively (14), and the relation of the enzyme synthesis with the myeloid cell differentiation was observed in vitro with HL-60 cells (14-16). Furthermore, analysis of myeloperoxidase synthesis in HL-60 cells has shown that human myeloperoxidase is primarily synthesized as a precursor protein with $M_0$ of 89,000-91,000, and the precursor protein is subsequently cleaved into the heavy chain and the light chain to yield the mature protein (16-20).

To study the expression of myeloperoxidase during differentiation of myeloid cells and also to understand the maturation process of this enzyme, we have undertaken the structural analysis of the protein. In this report, the partial amino acid sequence of human myeloperoxidase was determined and, using an oligonucleotide as a probe, cDNAs for human myeloperoxidase were isolated from a recombinant λ phage library prepared from human HL-60 cells. Nucleotide sequence analysis of the cDNA has revealed the structure of the enzyme precursor protein. The RNA specified by the myeloperoxidase cDNA could direct the synthesis of a polypeptide which was immunoprecipitated with anti-human myeloperoxidase antibody.

EXPERIMENTAL PROCEDURES

Materials—Avian myeloblastosis virus reverse transcriptase was obtained from Seikagaku Kogyo Co. Echerichia coli DNA polymerase I and EcoRI methylase were from New England Biolabs. T4 polynucleotide kinase, E. coli RNase H, E. coli and T4 DNA ligases, RNase inhibitor from human placenta (RNasin), Klenow fragment of E. coli DNA polymerase I, and EcoRI linker were products from Takara Shuzo Co. Restriction endonucleases used were purchased from Takara Shuzo Co., Toyobo Co., and New England Biolabs. SP6 RNA polymerase was obtained from Boehringer Mannheim. TPCK-treated trypsin and RNase-free DNAse were products of Worthington. Oligo(dT)$_{12-18}$ and oligo(dT)-cellulose (type VII) were purchased from Collaborative Research Co. and Pharmacia, P-L Biochemicals, respectively. [$\alpha$-32P]dATP (specific activity 3000 Ci/mmol) and [35S]methionine (specific activity 800 Ci/mmol) were from Amersham Corp., and [γ-32P]ATP was prepared as described (21) from carrier-free [32P]H$^3$PO$_4$ (New England Nuclear).

Amino Acid Sequence Determination of Myeloperoxidase—Human myeloperoxidase was purified from human leukemic cells as described previously (8). 300 μg of the purified protein was treated with 0.1% sodium dodecyl sulfate (SDS) and 10 mM dithiothreitol for 30 min at

1 The abbreviations used are: TPCK-1,1-tosylamido-2-phenylethyl chloromethyl ketone; SDS, sodium dodecyl sulfate; PTH, phenylthiohydantoin; HPLC, high pressure liquid chromatography; G-CSF, granulocyte-colony stimulating factor.
room temperature and chromatographed on a TSK G-3000 SW (Toyo Soda Co.) column (0.75 × 60 cm) which was equilibrated with phosphate-buffered saline (PBS) containing 0.1% SDS. Two peaks containing either the heavy chain or the light chain were obtained and concentrated by Centricon 10 (Amicon). On the other hand, 150 pg eluted with a linear gradient of 5-50% of acetonitrile.

37°C for 48 h. The digests were then applied on a C18 reverse-phase high pressure liquid chromatography (HPLC) column (PepRPC HR5/5, Pharmacia P-L Biochemicals). The adsorbed materials were eluted with a linear gradient of 5-50% of acetonitrile.

3.7

HR5/5, Pharmacia P-L Biochemicals). The adsorbed materials were subjected to Edman degradation using a gas-phase protein sequenator (Applied Biosystems, Inc., Model 470A), and the resulting phenylthiohydantoin (PTH)-derivatives were identified by reverse-phase HPLC.

Cell Lines and Preparation of mRNA—Human promyelocytic HL-60 cells (14), human monocytic leukemia U-937 cells (22), and murine myeloid leukemia M-1 cells (23) were maintained in RPMI 1640 medium (Nissui Seiyaku, Tokyo) supplemented with 10% fetal calf serum (GIBCO). Murine myeloid leukemia NFS-60 cells (24) were grown in RPMI 1640 medium containing 10% fetal calf serum and 10% of the medium conditioned by murine WEHI-3 cells.

Total cellular RNA was extracted from cells by the guanidine thiocyanate/cesium chloride method (25) and poly(A) RNA was selected by oligo(dT)-cellulose column chromatography (26).

Construction of cDNA Library—The cDNA library was constructed essentially as described (27). In brief, the double-stranded DNA complementary to HL-60 mRNA was synthesized using avian myeloblastosis virus reverse transcriptase for the first strand, and E. coli DNA polymerase I, RNase H, and DNA ligase for the second strand. Flushing ends were generated using Klenow fragment of DNA polymerase I. The double-stranded DNA was treated with EcoRI methylase and ligated with EcoRI linker. The double-stranded DNA was then digested with EcoRI, passed through Ultrogel AcA34 (LKB) to remove the excess linker, and ligated with EcoRI-digested Xgt10 vector DNA. Recombinant DNA was packaged in vitro (28), and the phage was grown on E. coli C600 Hfl (27).

Screening of the cDNA Library and DNA Sequence Analysis—Two sets of the 41-base oligonucleotides (Fig. 1b) were synthesized by the phosphoramidite method using a DNA synthesizer (Applied Biosystems Inc., Model 380A) and labeled at the 5' end with T4 polynucleotide kinase and [Y-~32P]ATP. About 1.0 × 106 recombinant phages were screened by plaque hybridization (29) using the 32P-labeled oligonucleotide as a probe (specific activity 3-6 × 106 cpm/pmol).

Hybridization was carried out according to Wahl et al. (30) except that the hybridization temperature was lowered to 28°C, and the filters were washed at 42°C with 1 × SSC (1 × SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.0). For rescreening the longer myeloperoxidase cDNA clone, about 3.5 × 1010 recombinant phages were plaque-hybridized with the 12-kb EcoRI fragment of AGM7 (see "Results") which was labeled with 32P by nick-translation. Hybridization was done as described (30) and filters were washed with 0.1 × SSC.

Nucleotide sequences were determined by the dyeoxynucleotide chain termination method after subcloning into M13 mp8 or mp9 (31).

In Vitro Transcription and Translation—The 2.6-kb EcoRI fragment of AGM706 containing the myeloperoxidase cDNA (see "Results") was subcloned at the EcoRI site of pSP65 (32). The resultant plasmid DNA (pSP706) was digested with BamHI and transcribed by SP6 RNA polymerase essentially as described (33). The reaction mixture contained 40 mM Tris-HCl (pH 7.5), 6 mM MgCl2, 2 mM spermidine, 10 mM dithiothreitol, 10 units of RNasin, 100 µg/ml bovine serum albumin, 50 µM GTP, 200 µM each of UTP, CTP, and ATP, 180 µM m7G(5')ppp(5')Gp, 0.5 µg of BamHI-digested pSP706 DNA, and 0.5 units of SP6 RNA polymerase. After incubation at 40°C for 60 min, RNasin and RNase-free DNase were added to give 500 units/ml and 50 µg/ml, respectively, and further incubated at 37°C for 10 min to remove the template DNA.

RNA synthesized by transcription with SP6 RNA polymerase was translated in the reticulocyte lysate system (34) and immunoprecipitated by using anti-human myeloperoxidase serum and protein A adsorbent as described previously (35, 36). Anti-human myeloperoxidase serum was prepared by immunizing rabbits with the homogeneous human myeloperoxidase purified from leukemic cells (8).

Northern and Southern Hybridization—RNA from various cell lines was denatured by heating at 60°C for 5 min in 2.2 M formaldehyde, 50% deionized formamide and electrophoresed through 1% agarose gel containing 2.2 M formaldehyde (28). The fractionated RNA was transferred to a nitrocellulosefilter (Schleicher & Schuell) and hybridized with the nick-translated EcoRI fragment of AGM706 (see "Results") as described (30).

Total cellular DNA was prepared from normal human peripheral leukocyte and HL-60 cells as described (28). DNA was completely digested with several restriction enzymes and subjected to electrophoresis on 0.7% agarose gel. DNA fragments were then transferred to a nitrocellulose filter as described (28) and hybridized as above using the 32P-labeled EcoRI fragment of AGM706.

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**Fig. 1. Partial amino acid sequences and synthetic oligonucleotide probe.** a, the NH2-terminal amino acid sequence of the heavy chain of human myeloperoxidase. X represents unidentified residues, and at five positions there is a possibility of either Ile or Lys since the PTH-derivatives of these amino acids could not be separated. b, the NH2-terminal amino acid sequence of the tryptic peptide and the oligonucleotide used to screen myeloperoxidase cDNA. On the oligonucleotide sequence, deoxyinosine was inserted at four positions and underlined. Asterisks indicate amino acids and nucleotides which did not match the sequence of human myeloperoxidase.
RESULTS

Amino Acid Sequence and Probe Design—Previously, we purified human myeloperoxidase to homogeneity from normal human granulocytes and human leukemic cells (8). When this protein was analyzed by polyacrylamide gel electrophoresis in the presence of SDS, it showed two bands of the heavy chain (M, 55,000) and the light chain (M, 12,000) (8). To determine the NH$_2$-terminal amino acid sequences of both subunits, the subunits were dissociated by treatment with SDS and dithiothreitol and purified on a gel filtration column. As shown in Fig. 1a, the amino acid sequence analysis of the heavy chain revealed 32 residues of the NH$_2$-terminal amino acid sequence. In this sequence, 5 amino acids are shown as isoleucine/lysine because PTH-derivatives of these amino acids could not be separated by the reverse-phase HPLC. Moreover, 5 other amino acid residues could not be assigned, probably due to poor recovery of the PTH-derivatives. The NH$_2$-terminal amino acid sequence of the light chain was not detected because PTH-derivatives of these amino acids could not be separated by the reverse-phase HPLC. Moreover, 5 other amino acid residues could not be assigned, probably due to poor recovery of the PTH-derivatives. The NH$_2$-terminal amino acid sequence of the light chain was not detected despite repeated attempts, which may suggest that the NH$_2$-terminal amino acid of the light chain is blocked.

Additional information on the primary sequence of human myeloperoxidase was obtained by isolating and microsequencing several tryptic peptides of the enzyme. One of the peptides, eluted at 27% acetonitrile (Fig. 2) from the C18 reverse-phase HPLC column, contained a single amino acid sequence of 14 residues as shown in Fig. 1b.

Based on the amino acid sequence of the tryptic peptide, two sets of 41-base oligonucleotides were chemically synthesized (Fig. 1b). The DNA sequence was designed partly on the basis of codon usage frequencies in human proteins (37), and four deoxyinosines were inserted where four different nucleotides can be assigned due to codon degeneracy (38, 39).

Myeloperoxidase cDNA Clones—Oligo(dT)-primed cDNA libraries were prepared from mRNA derived of human HL-60 cells using the λgt10 vector system. Initial screening of the cDNA library (1.0 × 10$^6$ clones), using the $^{32}$P-labeled 41-base oligonucleotides as probes, yielded one positive clone which was designated as λGM7. The clone λGM7 contained an insert of about 1200 base pairs (Fig. 3). The nucleotide sequence of the insert confirmed the presence of a region homologous to the oligonucleotide probes, and the amino acid sequence deduced from the nucleotide sequence of the cDNA of λGM7 was identical, except for 2 residues, to those determined from the purified human myeloperoxidase protein (Figs. 1 and 4).

Since the length of the cDNA of λGM7 was about half of the expected length of the complete myeloperoxidase cDNA, the cDNA library (3.5 × 10$^6$ clones) was rescreened using the 1.2-kilobase (kb) EcoRI fragment of λGM7 as a probe. Thirteen clones were positive and five of them were plaque-purified. The cDNAs of these clones were 1.0–2.6-kb-long, as judged by EcoRI restriction enzyme digestion. The longest cDNA (~2.6 kb) of the clone λGM706 (Fig. 3) was further characterized by restriction enzyme analysis and sequence determination. The cDNA of λGM706 appeared to be of full length, as Northern hybridization analysis of HL-60 mRNA with the cDNA of λGM706 revealed a band of ~2800 nucleotides (Fig. 6a).

Nucleotide Sequence and Predicted Amino Acid Sequence—Fig. 3 shows the physical restriction map and the strategy for the DNA sequence analysis. The nucleotide sequence was determined for both strands, and each restriction site which served for cloning into M13 vector was sequenced using the fragment spanning it. The nucleotide sequence (2568 bp excluding the poly(A) tract) thus obtained is shown in Fig. 4 together with the predicted amino acid sequence.

There is a long reading frame starting from the initiation codon ATG at the nucleotide position 148–150 and ending at
FIG. 4. Nucleotide sequence and deduced amino acid sequence of human myeloperoxidase cDNA. Numbers above each line refer to nucleotide position, and numbers below each line refer to amino acid position. The solid lines under the amino acid sequences show the sequences determined from purified human myeloperoxidase protein. The starting point for the heavy chain is indicated by a box, and the putative polyadenylylation signal near the poly(A) tail is indicated by a dotted line.
the termination codon TAG of the nucleotide number 2,384–2,386. Two other ATG codons can be found in the frame at the nucleotide positions 182–184 and 227–229. Because the first ATG codon is usually the initiation codon of eukaryotic mRNAs (40), the first ATG (nucleotide position 148–150) was tentatively assigned as the initiation codon for the human myeloperoxidase precursor protein. The open reading frame in uitro translation of human HL-60 of 83,868. The calculated molecular weight reported for the heavy chain (55,000–60,000 daltons including the sugar moiety). On the other hand, the light chain is probably on the NH2-terminal half of the precursor protein, but the exact location cannot be assigned since no sequence information is available for the light chain (see “Discussion”). In accordance with the fact that human myeloperoxidase is a glycoprotein (12), six potential N-glycosylation sites (Asn-X-Ser/Thr) can be found at the amino acid residues 139, 323, 355, 391, 483, and 729 of which five are on the heavy chain (Fig. 4). The amino acid composition of the myeloperoxidase precursor protein and the heavy chain are summarized in Table I. Human myeloperoxidase is abundant in proline (7.7% in the heavy chain), which is characteristic for many peroxidases (41). The total amount of the basic amino acids (histidine, lysine, and arginine) is 13.2% in the heavy chain while that of the acidic amino acids (Glu and Asp) is 9.5%. The calculated pl value of the heavy chain is 10.8, which agrees with the value obtained with the purified myeloperoxidase (pl > 9.5) (6).

As shown in Fig. 4, the cDNA of λGM706 contains the poly(A) tract at the 3’ end, and there is a sequence TATAAA at the nucleotide position of 2546–2551 which resembles the consensus sequence ATATAAA for polyadenylation (42). Therefore, the TATAAA sequence is probably used as a polyadenylation signal for the human myeloperoxidase gene. It was reported that the hepatitis B surface antigen gene (43) and the hamster scrapie PrP gene (44) also use TATAAA as the signal for polyadenylation.

Expression of Myeloperoxidase cDNA in Vitro—To prove that the cloned cDNA of λGM706 actually encodes human myeloperoxidase, the cDNA was placed under the promoter of SP6 RNA polymerase on pSP65 plasmid DNA (32). The resultant plasmid DNA (pSP706) was truncated at the BamHI site, and RNA specified by the cDNA was synthesized in uitro using SP6 RNA polymerase. When the RNA was translated in the rabbit reticulocyte cell-free system in the presence of [35S]methionine, a protein of M, 82,000 was observed by polyacrylamide gel electrophoresis in the presence of SDS. As shown in Fig. 5, the protein of M, 82,000 could be immunoprecipitated by rabbit anti-human myeloperoxidase serum but not by rabbit normal serum. The M, of the protein agrees with that calculated from the amino acid sequence of the myeloperoxidase precursor protein. Furthermore, when the

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<td><strong>Amino acid composition of human myeloperoxidase</strong></td>
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<td>Total residues</td>
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*From Fig. 4.

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K. Morishita and S. Nagata, unpublished results.
cDNA of XGM706 was expressed in monkey COS cells under
the control of the simian virus 40 early promoter, a protein
of M, 82,000 could be observed by Western blotting analysis
using rabbit anti-myceloperoxidase serum (data not shown).
From these results, it was concluded that the cloned cDNA
in XGM706 is the cDNA for human myeloperoxidase.

**Myeloperoxidase mRNA in Myeloid Leukemia Cells**—Mye-
loperoxidase protein is synthesized in cells which are at
the early stage of the myeloid cell differentiation and can be
detected in some myeloid leukemia cells (8, 10, 11, 14). In
order to study the expression of the myeloperoxidase gene
in myeloid cells, RNA was prepared from human HL-60 cells,
human U937 cells, murine NFS-60 cells, and murine M-1
cells. Fig. 6a shows the Northern hybridization analysis
of RNAs using human myeloperoxidase cDNA as probe. The
mRNA of about 2.8 kb hybridized very strongly with RNA
from HL-60 cells while no signal was detected with U937
cells. On the other hand, mRNA of about 2.3 kb could be
detected in RNA from murine NFS-60 cells but not M-1 cells.
This result indicates that the synthesis of myeloperoxidase
protein in HL-60 cells is regulated at the transcriptional level.
Furthermore, the strong hybridization of myeloperoxidase
mRNA in murine NFS-60 cells suggests that murine myelo-
peroxidase mRNA is highly homologous to the human mye-
loperoxidase mRNA.

**Southern Hybridization Analysis of Chromosomal DNA**—
Purified DNA from human leukocytes was cleaved with
BamHI, EcoRI, or HindIII, electrophoresed through an agar-
rose gel, and transferred to a nitrocellulose filter. As shown
in Fig. 6b, hybridization with the nick-translated myeloper-
oxidase cDNA revealed six distinct bands in BamHI-, two
bands in EcoRI- and three bands in HindIII-cleaved DNA.
This result suggests that there may exist one or two genes for
myeloperoxidase in the haploid human genome. Similar anal-
ysis with DNA from HL-60 cells showed the bands identical
to those observed in normal leukocytes DNA (data not
shown), which indicates that the constitutive expression of
myeloperoxidase in HL-60 cells is not due to amplification or
gross rearrangement of the myeloperoxidase chromosomal
gene.

**DISCUSSION**

In order to isolate the human myeloperoxidase cDNA clone,
we have used the 41-base oligonucleotide containing deoxy-
inosine at four positions (Fig. 1). This technique was developed
by Ohtsuka et al. (38) and was successfully used to identify
the human cholecystokinin gene (39) and the cDNA for
human granulocyte-colony stimulating factor (G-CSF) cDNA
(27, 45). At the following points, this method seems to be
superior to the more commonly used techniques which involve
screening with pools of short oligonucleotides or a long oli-
gonucleotide probe designed on codon usage frequencies.
Preparation of oligonucleotide containing deoxyinosine is easi-
er than preparation of many mixed probes. The dissociation
temperature of the oligonucleotide can be predicted by assum-
ing deoxyinosine to be an “inert” base (38), and rather strin-
gent conditions can be used for hybridization. In fact, screen-
ing of the cDNA library at 28 °C in 6 x SSC containing 50%
formamide gave a clearly positive clone. Comparison of the
41-base probe to the corresponding nucleotide sequence of
myeloperoxidase cDNA has revealed a 31-nucleotide stretch
having a perfect homology (Fig. 1b).

Sequence determination of the cloned cDNA for the mye-
loperoxidase precursor protein has shown that it codes for a
polypeptide of 745 amino acids. The heavy chain of mye-
loperoxidase consisting of 467 amino acids was located at the

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**FIG. 6. Characterization of the mRNA and genomic gene
coding for human myeloperoxidase.** a, Northern hybridization
analysis of myeloperoxidase mRNA. 15 μg of total RNA from the
following cells was electrophoresed on 1% agarose gel and analyzed
by Northern hybridization as described under “Experimental Proce-
dures.” Lane 1, human HL-60 cells; lane 2, human U937 cells; lane 3,
mouse M-1 cells; lane 4, NFS-60 cells. As size markers, 32P-labeled
DNA fragments and rabbit ribosomal RNA were run in parallel and
sizes of DNA fragments are shown in kilobases. b, Southern hybridi-
ization analysis of myeloperoxidase chromosomal gene. 10 μg of high
molecular weight DNA from normal leukocytes were digested either
with BamHI (lane 1), EcoRI (lane 2), or HindIII (lane 3) and analyzed
by Southern hybridization by using myeloperoxidase cDNA as probe.
HindIII-digested λDNA was used as a size marker and the sizes are
shown in kilobases. Ori indicates the origin of the electrophoresis.
COOH-terminal half of the protein (Fig. 4). Since the heavy and light chains of myeloperoxidase are generated from a single polypeptide (17, 18), the light chain should be on the NH2-terminal half of the precursor protein. Recently, Nauseef (19) and Akin and Kinkade (20) have reported by the pulse-chase experiments using HL-60 cells that a primary translation product of M, 80,000 is modified cotranslationally by cleavage of the signal peptide and glycosylation to produce a protein of M, 91,000–89,000. Subsequently, the precursor is processed to mature heavy and light chains of myeloperoxidase through an intermediate of M, 74,000 (20). From these results and the structure of myeloperoxidase precursor protein revealed from the cDNA (Fig. 4), the maturation process of human myeloperoxidase is diagramed as shown in Fig. 7. This maturation process is essentially identical to that used in maturation of other lysosomal enzymes such as cathepsin D (46) and predicts the existence of the signal peptide and consequence at the NH2-terminal of the myeloperoxidase precursor protein. In this regard, it is noteworthy that analysis of the hydrophatic character of the myeloperoxidase precursor protein shows a stretch of hydrophobic amino acids near the NH2-terminal (Fig. 4). At this location, a property that is typical for signal sequences (47) can be recognized, and the signal sequence for human myeloperoxidase protein probably consists of about 40 amino acids. In order to prove the maturation process proposed for human myeloperoxidase, it will be necessary to determine the NH2-terminal amino acid sequence of the intermediate protein of M, 74,000 and both the NH2- and COOH-terminal amino acid sequence of the light chain.

Several kinds of peroxidase are known in eukaryotic cells, for example, eosinophil peroxidase (7), macrophage peroxidase (48), cytochrome c peroxidase (49), etc. Southern hybridization analysis of human genomic DNA with the myeloperoxidase cDNA as a probe has indicated that there exist one or two genes for myeloperoxidase in the human haploid genome (Fig. 6b). This result suggests that other peroxidases are not closely related to myeloperoxidase. Actually, comparison of the structure of myeloperoxidase protein to those of horseradish peroxidase (50), yeast cytochrome c peroxidase (49), and bovine glutathione peroxidase (51) did not show an apparent homology. Furthermore, when the sequence of human myeloperoxidase was compared (52) with all sequences in the GenBank and NBRF protein database, no significant homology was found.

Northern hybridization analysis of RNA from human and mouse leukemia cells showed that myeloperoxidase is expressed in HL-60 and NFS-60 myeloid leukemia cells (Fig. 6a). Yamada and Kurahashi (15) and Koeffler et al. (16) have shown that HL-60 cells synthesize myeloperoxidase mRNA and, when HL-60 cells are induced to differentiate into granulocytes or macrophages by chemical inducers, the synthesis of myeloperoxidase ceases. Recently, we and others have isolated cDNA for human (27, 45) and murine (53) G-CSF and succeeded in producing G-CSF in a large quantity (45). Since G-CSF can induce differentiation of HL-60 cells or WEHI-3B D+ cells (51) but not NFS-60 cells (24), it will be interesting to study the effect of G-CSF on the expression of the myeloperoxidase gene in those cells. Furthermore, the isolation and structural analysis of the myeloperoxidase chromosomal gene will be useful to investigate the regulation of expression of the myeloperoxidase gene.

REFERENCES

Fig. 7. Model for the maturation process of human myeloperoxidase. The model was constructed from the structure of the myeloperoxidase precursor protein revealed from the cDNA (Fig. 4) and the data reported by Akin and Kinkade (20). CHO indicates the potential N-glycosylation site.
cDNA for Human Myeloperoxidase