

Molecular Cloning of cDNAs Encoding Bovine and Human Lactoperoxidase

THOMAS J. DULL, CAROL UYEDA, A. DONNY STROSBERG,
GLENN NEDWIN, and JEFFREY J. SEILHAMER

ABSTRACT

Peptide sequences obtained from cyanogen bromide fragments of bovine lactoperoxidase (bLPO) were used to design oligonucleotide probes for library screening. These probes were used to screen a cDNA library constructed from bovine mammary tissue. Three overlapping clones were obtained, the longest of which (T3) contained a reading frame of 712 amino acid residues. The encoded amino acid sequence was homologous to those recently reported for myelo-, thyro-, and eosinophil peroxidases. Two possible amino termini of the mature enzyme were identified, and the predicted mature protein matched previous molecular weight estimates of 78,500. Of eight bovine tissues tested, transcription of T3 sequences were detected in mammary tissue only. Using the bLPO cDNA as a probe, a single hybridizing clone was found in a human mammary gland cDNA library. This clone (M1) encoded the carboxy-terminal 324 residues of a peroxidase distinct from the other three known human peroxidases, and was closely related to bLPO. This result confirms the presence of at least one distinct lactoperoxidase in humans.

INTRODUCTION

LACTOPEROXIDASE (LPO; EC 1.11.1.7) has been characterized as a natural antimicrobial agent in bovine milk (Gotheffors and Marklund, 1975; Reiter, 1985; Sandholm *et al.*, 1988), a medium otherwise rich in its ability to support bacterial growth. In addition, it has been suggested that the presence of LPO may help protect the udder from infection (Reiter, 1985; Marshall *et al.*, 1986; Sandholm *et al.*, 1988). Of the two reaction substrates of LPO, one (thiocyanate) is normally present in milk. The other substrate, hydrogen peroxide, may be more limiting, perhaps due to the presence of catalase and superoxide dismutase (Reiter, 1985). Milk LPO levels vary among species, ranging from little or none in human beings to concentrations as high as 54,000 milliunits/ml in guinea pigs (Shindler *et al.*, 1976; Reiter, 1985). Human colostrum contains LPO activity (Gotheffors and Marklund, 1975; Langbakk and Flatmark, 1984; Tenuovo, 1985; Langbakk and Flatmark, 1989), however the levels drop rapidly within 1 week post partum. LPO has also been thought to play a role in the promotion of growth in newborn calves (Reiter *et al.*, 1981; Reiter, 1985).

The enzymatic properties of LPO have found usage in a variety of applications. Its antimicrobial activity has been used to preserve raw milk in warm climates, through the addition of hydrogen peroxide and thiocyanate (Reiter, 1985; Aparicio *et al.*, 1986). Other studies have targeted the cytotoxic effects of LPO to specific pathogenic cell types by chemically linking it to monoclonal antibodies (Ito *et al.*, 1989; Philpott *et al.*, 1973; Okuda *et al.*, 1980). Also, LPO itself has been used as an iodinating reagent and is considered to be less disruptive to protein structure than chloramine T (Okuda *et al.*, 1980; Redman and Tustanoff, 1984).

The biochemistry and enzymology of bovine LPO has been studied by several groups. First purified in 1943 (Theorell and Akesson, 1943), the purification method was refined in subsequent studies which analyzed the amino terminus, along with amino acid and carbohydrate composition (Rombauts *et al.*, 1967; Carlstrom, 1969a,b,c; Sievers, 1981; Paul and Ohlsson, 1985). Some of these studies (Rombauts *et al.*, 1967; Carlstrom, 1969a,b) suggested the enzyme consisted of multiple forms, probably reflecting heterogeneity in glycosylation, and examined the composition of the various forms. The most recent characteriza-

tions of LPO suggest that it is a single polypeptide of approximately 78,000 daltons and may contain leucine at its amino-terminus (Sievers, 1981; Paul and Ohlsson, 1985). The covalently bound heme group (protoheme IX) has been characterized in several studies (Sievers, 1979; Nichol

et al., 1987). Another recent study suggested LPO contains at least two domains differing in thermostability (Pfeil and Ohlsson, 1986). In addition, a peroxidase with very similar biochemical and immunological properties, which could originate from the same gene as LPO, has been found in

TABLE 1. CNBr FRAGMENTS OF BOVINE LACTOPEROXIDASE AND bLPO OLIGONUCLEOTIDE PROBES

A.

Fragment	Sequence	Position
1	(unintelligible)	
2	DLYKTPDNIDIWIGENAEP	599 – 617
3	DLYKTPDNIDIWIGENAEP	599 – 617
4	VTSELRNKLFQPTHK	530 – 544
5	VTSELRNKLFQPTHK	530 – 544
6	(unintelligible)	
7	?EYQDPDNPQD??G	(not found)
8	(unintelligible)	
9	PGYNSWRGF?GLSQPKT??G	564 – 583
10	PGYNSWRGF?GLSQPKT??G	564 – 583
11	QKWIP?YQGY?NSVDPRI SN	439 – 458
12	PGYNSWRGF?GLSQPKT??G	564 – 583
13	PGYNSWRGF?GLSQPKT??G	564 – 583

B.

CNBr Fragment 10:

Amino Acids	Pro	Gly	Tyr	Asn	Ser	Trp	Arg	Gly	Phe	(Cys)	Gly	Leu	Ser	Gln	Pro
mRNA	5-CCN	GGN	UAY	AA Y	AGY	UGG	AGR	GGN	UUY	UGY	GGN	UUR	AGY	CAR	CCN-3'
Probe 10bg	3-GGT	CCN	ATR	TTR	AGG	ACC	TCC	CCG	AAG	ACA	CCG	GAC	AGG	GTC	GG-5'
Mismatches	*											*	*		
cDNA	5-CCT	GGG	TAC	AAC	TCC	TGG	AGG	GGC	TTC	TGT	GGC	CTC	TCA	CAG	CC-3'

CNBr Fragment 11:

Amino Acids	(Pro)	Tyr	Gln	Gly	Tyr	(Asn)	Asn	Ser	Val	Asp	Pro	Arg	Ile	Ser	Asn
mRNA	5-CCN	UAY	CAR	GGN	UAY	NNN	AA Y	AGY	GUN	GAY	CCN	AGR	AUH	AGY	AA Y-3'
Probe 11bg	3-GGG	ATG	GTT	CCG	ATG	TTA	TTG	AGT	CAN	CTR	GGN	GCG	TAG	AGG	TTG-5'
Mismatches					*			*				*	*		*
cDNA	5-CCC	TAC	CAA	GGC	TAT	AAT	AAC	TCT	GTG	GAT	CCC	CGA	ATT	TCC	AAT-3'

A. CNBr fragments of bovine lactoperoxidase. The sequences obtained from each of 13 separate HPLC peaks obtained from CNBr digestion of bovine lactoperoxidase are shown, along with the corresponding amino acid residue numbers obtained from the sequence deduced from clone T3 (Fig. 2 below).

B. bLPO oligonucleotide probes. The sequences obtained for two CNBr fragments (11 and 12) of bLPO are shown, along with the designed oligonucleotide probes and the corresponding DNA sequence from clone T3 (below). Mismatched bases relative to T3 are indicated (*), and three amino acid residues were guesses made based upon homology to human MPO (parentheses). Degeneracy codes used are: N = A, G, C, or T; R = A or G, Y = C or T; and H = A, C, or T.

human saliva (Gothefors and Marklund 1975; Tenuovo, 1981; Mansson-Rahemtulla, 1988).

In the last few years, gene sequences encoding several other peroxidases, including myeloperoxidase (MPO, Weil *et al.*, 1987; Johnson *et al.*, 1987; Morishita *et al.*, 1987), thyroperoxidase (TPO, Kimura *et al.*, 1987, 1989), and eosinophil peroxidase (EPO, Sakamaki *et al.*, 1989; Ten *et al.*, 1989) have been published. Although both MPO and EPO contain two distinct polypeptide chains, their sequences show distinct homology with each other and with TPO, suggesting that these enzymes are members of a family of peroxidase enzymes with a possible common evolutionary origin. However, much less is currently known about the primary structure of LPO, which is distinguished from the other enzymes by its inability to catalyze the oxidation of chloride. We have now obtained sufficient internal amino acid sequence to permit the cloning of a cDNA encoding full-length bovine LPO. Subsequently, a homologous clone was obtained which encoded the 3'-terminal portion of human LPO. The homology of these LPO clones to MPO, TPO, and EPO suggest they may all belong to the same peroxidase family.

MATERIALS AND METHODS

Bovine LPO amino acid determination

Ten milligrams of bovine LPO (Sigma Chemical Company, St. Louis, MO) was cleaved with cyanogen bromide (CNBr) and the resulting fragments were separated by HPLC on a Vydac C-4 column. Purified fragments were sequenced by Edman degradation on an Applied Biosystems 477A gas-phase protein sequencer.

Preparation of RNA

Total RNA was isolated from bovine mammary and salivary gland tissue by extraction with guanidinium thiocyanate (Chirgwin *et al.*, 1979). Poly(A)⁺mRNA was selected

by oligo(dT)-cellulose chromatography (Aviv and Leder, 1972). Bovine brain, heart, spleen, kidney, liver, and lung poly(A)⁺RNA was obtained from Clontech Laboratories, Inc. (Palo Alto, CA).

Construction of bovine cDNA library

Double-stranded cDNA was synthesized and *Eco* RI linkers were attached using a cDNA synthesis kit (Pharmacia Inc, Piscataway, NJ). The cDNA was cloned using the *Eco* RI site of Lambda ZapII (Stratagene Inc., San Diego, CA). Portions of the ligated mixtures were packaged and transformed into *Escherichia coli* strain XL-1 Blue (Stratagene) and propagated on LB plates.

Library screening and clone isolation

Oligonucleotide probes corresponding to two of the CNBr fragment sequences were synthesized (Pharmacia Gene Assembler) in pools of 16 44-mers and 24 45-mers and purified on OPC columns (Applied Biosystems, Foster City, CA). The oligonucleotides were labelled to a specific activity of $>10^8$ cpm (Cerenkov)/ μ g using [γ -³²P]ATP ($>7,000$ Ci/mmol; ICN Biomedicals Inc., Costa Mesa, CA) and polynucleotide kinase (New England Biolabs Inc., Beverly, MA).

DNA fragments were generated by endonuclease digestion, isolated by standard methods and labeled by random priming using DNA polymerase Klenow fragment (Promega Corporation, Madison, WI) in a reaction containing [α -³²P]dATP and dCTP (Amersham, Arlington Heights, IL). Specific activity was comparable to the oligonucleotide probes. Bacteriophage plaques from plated libraries were transferred in duplicate to Hybond-N nylon filters (Amersham) using standard techniques and bound to the filters by ultraviolet irradiation. The filters were prehybridized in a solution containing 20% formamide, $5\times$ Denhardt's solution, $6\times$ SSC, 50 mM NaPO₄, and 10 mg/ml sheared herring sperm DNA at 37°C. Hybridization was

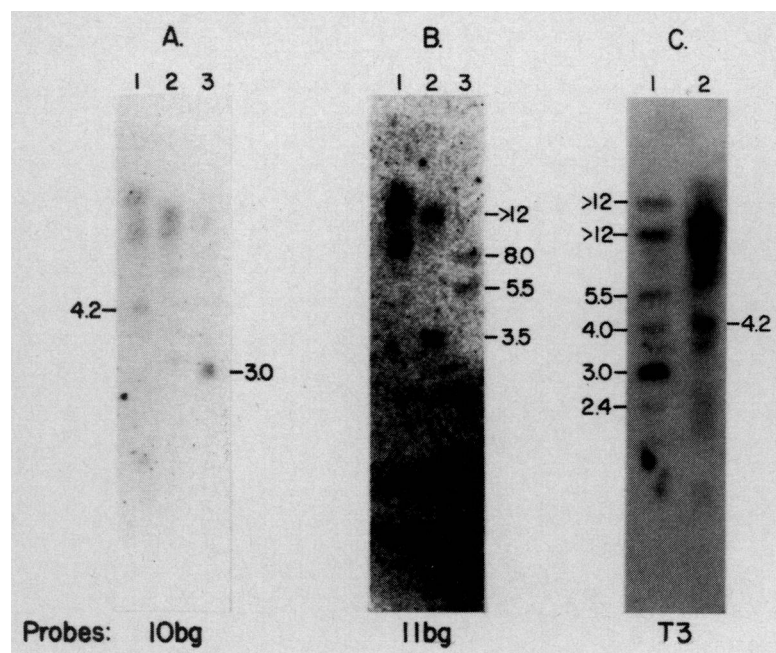


FIG. 1. Probe hybridization to bovine and human DNA. A and B. The indicated oligonucleotide probes were hybridized in 20% formamide mix at 37°C (see Materials and Methods) directly to gels containing *Bgl* II-digested human DNA (lanes 1), *Bgl* II-digested bovine DNA (lanes 2), and *Eco* RI-digested bovine DNA (lanes 3). C. Random-prime labeled cDNA insert DNA from clone T3 (see below) was hybridized to a Hybond-N blot of *Eco* RI-digested bovine (lane 1) and human (lane 2) DNA in 20% formamide mix at 42°C.

A	
CAGAATCTTTCCTTCTGGGCTTTTCTCTGAGATTAACCTCTGATAGACGGTATAAAAGGCCGGCTCCTCCAAGCAGAGAACTCCCTCT	89
GGCTGCCACAGGAGGCAAGGCACTCAATAGCGATGTGGTCTGTCTCCAACTTCCAGTCTTTTGGCTTCCGTGACCTTATTCGAGGTT	179
MetTrpValCysLeuGlnLeuProValPheLeuAlaSerValThrLeuPheGluVal	19
GCAGCATCTGACACAATTGCACAGCCGCGCAGCACCACCACCTCTCTGATGTGTAGTAAAGTCAAGATCCAGGTCAACAAGGCCTTC	269
AlaAlaSerAspThrIleAlaGlnAlaAlaSerThrThrThrIleSerAspAlaValSerLysValLysIleGlnValAsnLysAlaPhe	49
CTGGATTCCCGGACCGAGCTGAAGACGACCTTGAGCTCTGAGGCACCCACCCCAACAGCTCTCAGAGTACTTCAAGCAGCAAGGGC	359
LeuAspSerArgThrArgLeuLysThrThrLeuSerSerGluAlaProThrThrGlnGlnLeuSerGluTyrPheLysHisAlaLysGly	79
CGGACCCGACGGCCATTCCGAACGGGAGGTGTGGGAGGAGTCTTAAAGAGGCTGAGGCGGGACACAACCTGACCAACGTCACAGAC	449
ArgThrArgThrAlaIleArgAsnGlyGlnValTrpGluGluSerLeuLysArgLeuArgArgAspThrThrLeuThrAsnValThrAsp	109
CCTAGCCTGGACTTGACTGCACTCTCCTGGGAGGTGGGCTGCGGTGCCCGGTTCTCTGGTGAATGTGATGAAACAGCCCTTACCGC	539
ProSerLeuAspLeuThrAlaLeuSerTrpGluValGlyCysGlyAlaProValProLeuValLysCysAspGluAsnSerProTyrArg	139
ACCATCACGGGAGACTGTAATAACAGGAGGAGCCCGCACTGGGCGCGGCCAACAGGGCGCTGGCGCGCTGGCTGCCGGCGGAGTACGAG	629
ThrIleThrGlyAspCysAsnAsnArgArgSerProAlaLeuGlyAlaAlaAsnArgAlaLeuAlaArgTrpLeuProAlaGluTyrGlu	169
GACGGGCTCGCCCTGCCCTTCGGCTGGACGAGGAAGACGCGCAACGGCTTCCGCGTCCCGCTGGCCCGGGAGGTATCCAACAAAATT	719
AspGlyLeuAlaLeuProPheGlyTrpThrGlnArgLysThrArgAsnGlyPheArgValProLeuAlaArgGluValSerAsnLysIle	199
GTAGGCTACTGGACGAAGAGGGTGTCTGGACCAAAACAGGTCCCTGCTCTTCATGCAGTGGGGTCAGATTGTGGACACGACCTGGAC	809
ValGlyTyrLeuAspGluGluGlyValLeuAspGlnAsnArgSerLeuLeuPheMetGlnTrpGlyGlnIleValAspHisAspLeuAsp	229
TTCGCCCCGAAACGGAACCTGGGAGCAACGAGCACTCTAAAACCCAGTGTGAGGAGTACTGTATCCAGGGAGACAACCTGCTCCCCATC	899
PheAlaProGluThrGluLeuGlySerAsnGluHisSerLysThrGlnCysGluGluTyrCysIleGlnGlyAspAsnCysPheProIle	259
ATGTTCCCGAAAAATGATCCCAAGTTGAAGACTCAAGGAAATGCATGCCTTTCTTCCGAGCCGGGTTTGTCTGCCCACTCCACCTTAC	989
MetPheProLysAsnAspProLysLeuLysThrGlnGlyLysCysMetProPhePheArgAlaGlyPheValCysProThrProProTyr	289
CAGTCGTTGGCCCGAGAACAGATCAATGCTGTGACCTCCTTCTGGACGCGAGCTTAGTGTACGGCTCTGAGCCAGTCTGGCCAGCCGT	1079
GlnSerLeuAlaArgGluGlnIleAsnAlaValThrSerPheLeuAspAlaSerLeuValTyrGlySerGluProSerLeuAlaSerArg	319
CTCCGGAACCTCAGCAGCCGCTGGGCTCATGGCTGTCAACGAAGCTGGGACACGGGCTGGCCCTACCTGCCCTTTAACAACAAG	1169
LeuArgAsnLeuSerSerProLeuGlyLeuMetAlaValAsnGlnGluAlaTrpAspHisGlyLeuAlaTyrLeuProPheAsnAsnLys	349
AAGCCGAGCCCTGTGAGTTCATCAACACCACCGCCCGGTGCCCTGTTTCTTGGCGGGAGATTTTCGAGCCTCAGAGCAGATTCTGCTG	1259
LysProSerProCysGluPheIleAsnThrThrAlaArgValProCysPheLeuAlaGlyAspPheArgAlaSerGluGlnIleLeuLeu	379
GCCACTGCCACACCTCCTTCTCCGGGAGCACAACCGGCTGGCCAGAGAACTAAAGAACTCAACCTCCTGGAATGGAGAGAAGCTC	1349
AlaThrAlaHisThrLeuLeuLeuArgGluHisAsnArgLeuAlaArgGluLeuLysLysLeuAsnProHisTrpAsnGlyGluLysLeu	409
TACCAGGAAGCCCGGAAAACTCTGGGAGCCTTCATACAGATCATCACCTTTAGGGACTACCTACCCATTGTGTAGGTAGTGAGATGCAG	1439
TyrGlnGluAlaArgLysIleLeuGlyAlaPheIleGlnIleIleThrPheArgAspTyrLeuProIleValLeuGlySerGluMetGln	439
AAGTGGATCCCGCCTACCAAGGTATATAAATCTGTGGATCCCCGAATTTCCAATGTCTTACCTTTGCCCTTCCGCTTTGGCCACATG	1529
LysTrpIleProProTyrGlnGlyTyrAsnAsnSerValAspProArgIleSerAsnValPheThrPheAlaPheArgPheGlyHisMet	469
GAGGTTCCCTCCACTGTGTCCCGCTGGATGAGAATTACCAGCCATGGGGTCCGGAAGCAGAGCTCCCCCTACACACCTCTTCTTCAAC	1619
GluValProSerThrValSerArgLeuAspGluAsnTyrGlnProTrpGlyProGluAlaGluLeuProLeuHisThrLeuPhePheAsn	499
ACCTGGAGATATAAAGACGGTGAATAGACCTCTGGTGGGGTCTGCTGGCCAAGAGTCCAACTGTGAATCAGGATAAAATG	1709
ThrTrpArgIleIleLysAspGlyGlyIleAspProLeuValArgGlyLeuLeuAlaLysLysSerLysLeuMetAsnGlnAspLysMet	529
GTGACGAGTGAGCTGCGCAACAAGCTTTCCAGCCCACTCACAAGATCCACGGCTTTGACCTGGCTGCTATCAACTTACAGCGTTGCCGA	1799
ValThrSerGluLeuArgAsnLysLeuPheGlnProThrHisLysIleHisGlyPheAspLeuAlaAlaIleAsnLeuGlnArgCysArg	559
GACCATGGGATGCTGGGTACAACCTCTGGAGGGGCTTGTGGCTCTCACAGCCCAAGACACTGAAGGGGCTGCAGACTGTGCTGAAG	1889
AspHisGlyMetProGlyTyrAsnSerTrpArgGlyPheCysGlyLeuSerGlnProLysThrLeuLysGlyLeuLeuThrValLeuLys	589
AACAAGATACTGGCTAAGAAGTTAATGGATCTCTATAAGACCCCGACAACATTGACATCTGGATTGGAGGCAACGCTGAGCCCATGGTA	1979
AsnLysIleLeuAlaLysLysLeuMetAspLeuTyrLysThrProAspAsnIleAspIleTrpIleGlyGlyAsnAlaGluProMetVal	619
GAAAGGGGCGGGTGGGGCACTCCTGGCTGCCTCCTAGGGAGGCAATTCAGCAGATACGTGATGGGGACAGGTTCTGGTGGGAGAAC	2069
GluArgGlyArgValGlyProLeuLeuAlaCysLeuLeuGlyArgGlnPheGlnGlnIleArgAspGlyAspArgPheTrpTrpGluAsn	649
CCTGGGGTCTTCACTGAGAAGCAGCGGACTCTCTACAGAAAGTGTCTTCTCACGCCCTCATCTGTGACAACCCACATCACGAAGGTC	2159
ProGlyValPheThrGluLysGlnArgAspSerLeuGlnLysValSerPheSerArgLeuIleCysAspAsnThrHisIleThrLysVal	679
CCGCTGCATGCCTTCCAGGCCAACACTACCCACATGACTTTGTGGATTGCTCAACCGTTGATAAGCTGGATCTCTCACCTGGGCTCC	2249
ProLeuHisAlaPheGlnAlaAsnAsnTyrProHisAspPheValAspCysSerThrValAspLysLeuAspLeuSerProTrpAlaSer	709
AGGGAGAATTAGGGGCCGACTCCACAGCTCCACACTGTGCAGTAGAGCTCCCTTGGCCACGATGCCATTTCAAGTAGACTCAGTG	2339
ArgGluAsn	712
ACCTGGCCCTTTCGGGCTCCCTACCCCGCCAGGCTTCTGTCCAGCCGAGTCTCTCTGCACGCCCCCAAGCACACCTAGCTCAAGCC	2429
CAAGGCAGCCGCTTCCGCTCCAGCTCTTCCATTGAATCCACATCTCTCCGTCTTCCGAAACCTCCTTCTGTCAAGACTTACACC	2519
CTCCAAAATGCCCTTCTGATCTTGTGCTGCCAGATGTACCCATCCTTCCCAAAAAAGTCTTGGCTGAGGCTGTGCCCTTGACACCTGT	2609
ATCTCTCTCCCTTGAAGTAGATTGTAACTTCTTGAGCTTGGCCTTCAACCTGCTTCCAAGTGTGCCCTGGGGCCCCAGCGTAGTCC	2699
TTGGCACACAG	2710

FIG. 2. (See top of next page for rest of figure and legend.)

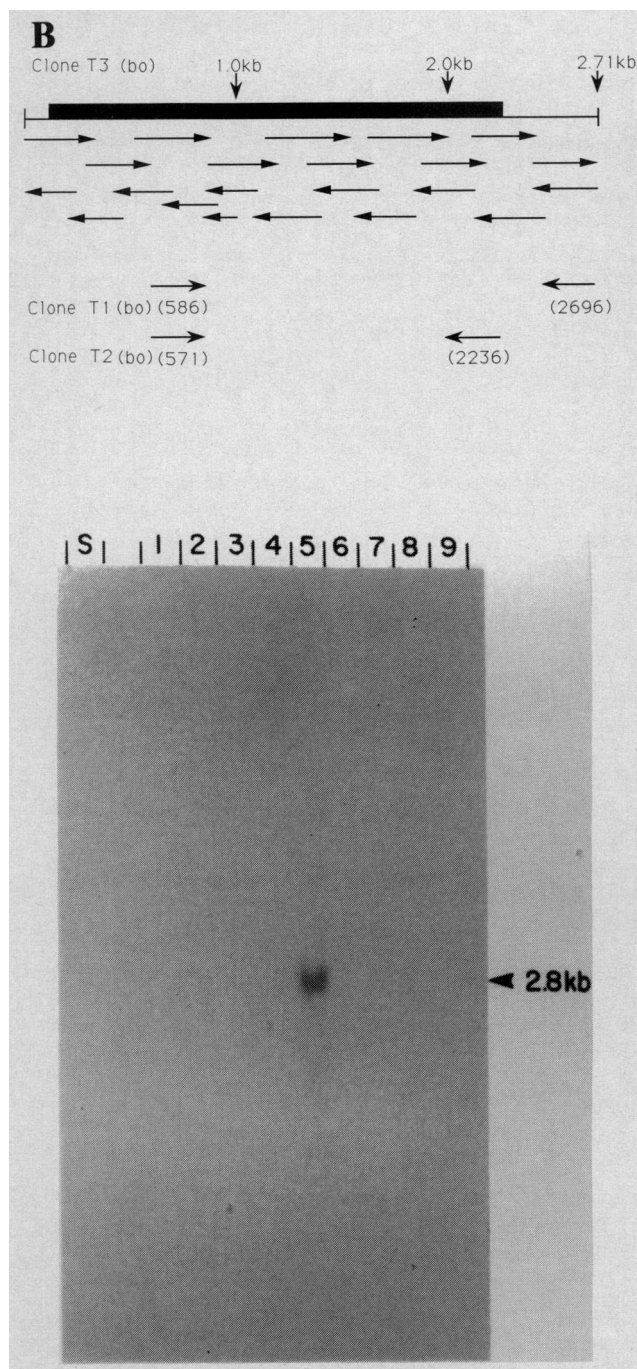


FIG. 3. Blot of RNA from various bovine tissues. Poly(A)⁺RNA from bovine heart (lane 1), brain (lane 2), salivary gland (lane 3), kidney (lane 4), mammary gland (lane 5), liver (lane 6), spleen (lane 7), lung (lane 8), and human HL60 (lane 9) were electrophoresed on a 1.0% agarose gel containing 5% formaldehyde, following denaturation at 65°C. The RNAs were blotted onto a Hybond-N filter and hybridized with a T4-polynucleotide kinase-labeled 51-base oligonucleotide probe (probe 81), comprising the inverse complement of bases 1,857–1,907 of the clone T3 sequence.

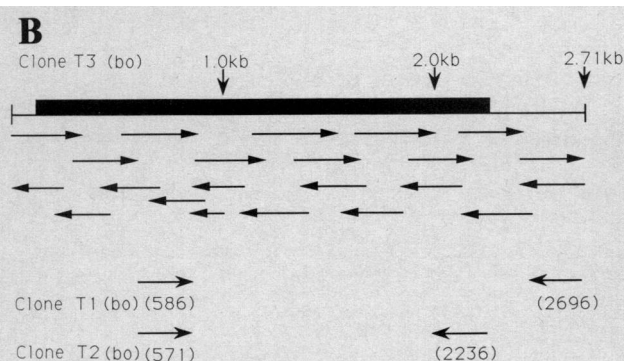


FIG. 2. Bovine LPO cDNA sequence. Above, the sequence of clone T3 is shown, along with deduced amino acids. Two potential signal peptidase cleavage sites (1 and 2), and two possible starting sites of the mature enzyme coding region (A and B), are indicated within the sequence. At left, the extents of the three hybridizing clones discussed in the text, and the sequencing strategy employed, are illustrated.

performed for 12–16 h in the same solution plus 10^6 cpm ^{32} P-labeled oligonucleotide probe per filter. Following hybridization, filters were washed briefly at room temperature in a solution containing $1\times$ SSC and 0.1% NaDodSO₄ followed by two 30-min washes in the same solution at 60°C. Filters were used to expose X-ray film for 6–48 h at -70°C with intensifying screens.

Recombinant bacteriophage were isolated from plaques corresponding to hybridization signals and were converted to plasmid Bluescript SK (Stratagene) by autoexcision. Bacteriophage DNA was purified from plate lysates using Lambda-trap (Clontech). *Bam* HI restriction fragments were cloned into plasmid Bluescript SK for further analysis. A human mammary gland cDNA library (Clontech) was screened essentially as above in 50% formamide at 42°C with a random-primed DNA probe.

Direct gel hybridization

Typically, 5.0 μg of restriction endonuclease-digested genomic DNA per lane was electrophoresed on 1.0% agarose gels. After electrophoresis, the DNA was denatured by soaking the gel in 0.5 M NaOH plus 0.15 M NaCl for 1 hr with several changes of solution, followed by neutralization in 0.5 M Tris-HCl pH 7.5 plus 0.15 M NaCl, also for 1 hr with changes. The gels were then dried onto filter paper (Whatman 3MM) at 60°C under vacuum. The dried gels were hybridized with oligonucleotide probes in the same manner as were the nylon filters described above. Washes were at 50 or 55°C in 0.2 or 0.5 $\times$ SSC plus 0.1% NaDodSO₄. Alternately, digested DNAs in agarose gels were blotted onto Hybond-N filters and hybridized in a similar fashion.

DNA sequencing

All DNA sequences were determined using the dideoxy chain-termination method with Sequenase (United States Biochemicals kit; Sanger *et al.*, 1977; Messing, 1983), and were confirmed from multiple readings of both DNA strands. Sequencing primers of 20–25 nucleotides were synthesized on an Applied Biosystem 380B DNA synthesizer and purified on OPC columns. Sequences were analyzed using the DNAsis/PROsis programs of LKB/Pharmacia.

GGAGCATAACCGGCTGGCCAGAGAACTAAAGAGACTCAACCCTCAGTGGGATGGAGAGAAGCTCTACCAGGAAGCCCGGAAAAATCCTG	88
GluHisAsnArgLeuAlaArgGluLeuLysArgLeuAsnProGlnTrpAspGlyGluLysLeuTyrGlnGluAlaArgLysIleLeu	29
GGAGCCTTTCATGCAGATTATCACCTTTAGGGACTACCTACCCATTTTGCTAGGTGACCACATGCAGAAGTGGATACCCCATATCAAGGC	178
GlyAlaPheMetGlnIleIleThrPheArgAspTyrLeuProIleLeuGlyAspHisMetGlnLysTrpIleProProTyrGlnGly	59
TACAGTGAATCTGTGGATCCAGAAATTTCAATGTCTTCACTTCGCCTTCGGCTTGGGCACTGGAGGTCCCCTCTAGTATGTTCCGC	268
TyrSerGluSerValAspProArgIleSerAsnValPheThrPheAlaPheArgPheGlyHisLeuGluValProSerSerMetPheArg	89
CTGGATGAGAATTATCAGCCATGGGGCCAGAACCAAGCTGATGAAACAGAATAAAATGATGACTGGAGAGCTGCCAACAAG	358
LeuAspGluAsnTyrGlnProTrpGlyProGluProGluLeuProLeuHisThrLeuPhePheAsnThrTrpArgMetValLysAspGly	119
GGAATTGATCTCTGGTGGGGCTGTGGCCAGAAATCCAAGCTGATGAAACAGAATAAAATGATGACTGGAGAGCTGCCAACAAG	448
GlyIleAspProLeuValArgGlyLeuLeuAlaLysLysSerLysLeuMetLysGlnAsnLysMetMetThrGlyGluLeuArgAsnLys	149
CTTTTCCAGCAACTCACAGGATCCATGGCTTGACCTGGCTGCCATCAACACACAGCGTTGCCGGGACCATGGGCAACCTGGGTACAAT	538
LeuPheGlnProThrHisArgIleHisGlyPheAspLeuAlaAlaIleAsnThrGlnArgCysArgAspHisGlyGlnProGlyTyrAsn	179
TCCTGGAGAGCCTTCTGTGACCTCTCACAGCCGAGACACTAGAGGAGTTGAACACAGTGCTGAAGAGCAAGATGCTGGCCAAGAAGTTA	628
SerTrpArgAlaPheCysaspLeuSerGlnProGlnThrLeuGluGluLeuAsnThrValLeuLysSerLysMetLeuAlaLysLysLeu	209
CTGGGTCTCTACGGGACCCCTGACAACATCGACATCTGGATAGGGGCCATTGCTGAGCCGCTGGTGGAAAGGGTTCGGGTGGGGCCTCTC	718
LeuGlyLeuTyrGlyThrProAspAsnIleAspIleTrpIleGlyAlaIleAlaGluProLeuValGluArgGlyArgValGlyProLeu	239
CTGGCCTGCCTCTTGGGCAAGCAGTTCAGCAGATCCGTGATGGAGACAGGTTCTGGTGGGAAAACCTGGGGTCTTACGAACAGCAG	808
LeuAlaCysLeuLeuGlyLysGlnPheGlnGlnIleArgAspGlyAspArgPheTrpTrpGluAsnProGlyValPheThrAsnGluGln	269
AAGGACTCTCTACAGAAATGTCTCTCTCAGCCTTGCTGTGACAACACCCGCATCACCAAGGTCCCACGGGACCCATTCTGGGCCAAC	898
LysAspSerLeuGlnLysMetSerPheSerArgLeuValCysAspAsnThrArgIleThrLysValProArgAspProPheTrpAlaAsn	299
AGCTACCCCTATGACTTCTGTGGATTGCTCAGCCATCGACAAGCTGGACCTGTACCCCTGGGCCTCAGTGAAGAATTAGGGGCCCGCGCTG	988
SerTyrProTyrAspPheValAspCysSerAlaIleAspLysLeuAspLeuSerProTrpAlaSerValLysAsn	324
CACAGGAAAGTCCCTTTGGTCCACAGGGCCATTTCAAGCAAGTTCAATGACCTGGTCCCTTAGAGCTCCATATCCCAGTCCCAGCCCTT	1078
CTTTGACGCTGGGCCTCTCTATACCCCTGGATGAACAGCTTGCTCAGGCCCCAGGGTGGCTGCCTCGGCCCTCCCAGCTCTTACACTCAG	1168
CTCCAGTGGCTTCTCCTTTCTGTCAAGACTTAGCCCGCTGAGATGCCCTTCTGTCTCCAGCTTGTGATGTTACCTGTCTCTTCCCTC	1258
CACAAGTCTTGGCCCTTAACCTTTATCTTCTTCTCTGCTCTCACCTAGATTGTAAGCTCCCTGGGCCAGGACTTCAGCTGCCTCCGA	1348
GGGTCCCTGTGGCACCTAGCATGGTGCACAGCACATTAGAAGTGCTCAAA	1399

Clone M1 (hu) 1399

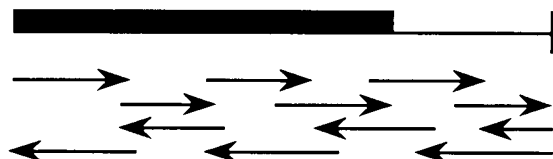


FIG. 4. Human LPO cDNA sequence. The sequence of clone M1 is shown, along with deduced amino acids. At left, the strategy for sequencing the plasmid insert is illustrated.

RESULTS

Commercial preparations of LPO from bovine milk were sufficiently pure to allow amino acid sequencing without further purification. However, such attempts with the native protein yielded little or no significant signals by Edman degradation, consistent with a blocked amino terminus. Upon digestion with CNBr, 13 distinct peaks were resolved by preparative HPLC, which were then sequenced separately. The resulting peptide sequences are listed in Table 1A. In all, five unique sequences were obtained, and three peaks yielded either multiple or otherwise nonreadable sequences. Two of the resulting sequences, shown in Table 1B, were of sufficient resolution, length, and degeneracy to permit the design of oligonucleotide probes to be used for library screening. The synthesized oligonucleotide pools were 44 (probe 10bg) and 45 (probe 11bg) bases in length, and were designed utilizing codons of mammalian usage preference (Lathé, 1985). In three cases where the se-

quences were ambiguous (see Table 1), reasonable predictions of amino acids were made based upon homology to human MPO.

To evaluate the relative abilities of the two probes to bind LPO sequences, each was hybridized to dried agarose gels containing digested bovine DNA at varying stringencies. As seen in Fig. 1, both probes 10bg and 11bg exhibited clear hybridization signals when washed at moderate stringency ($0.2 \times$ SSC, 55°C). Probe 10bg hybridized to a 3.0-kb *Eco* RI fragment (Fig. 1A), and probe 11bg bound to 5.5- and 8.0-kb *Eco* RI fragments (Fig. 1B). These two probes were used to screen the bovine milk gland cDNA library we constructed as described in Methods. The library of 375,000 individual members contained three clones (T1, T2, and T3) which hybridized to both probes when washed at 60°C in $1 \times$ SSC. Each clone was purified, converted to plasmid form *via* autoexcision, and analyzed by DNA sequencing of the plasmid DNA. The three clones contained overlapping sequences, the longest of which contained a

pTPO	MGARAVL	GUTLAUACAGAFFASIL	24
hTPO	MRALAVL	SUTLUMACTERAFFPFIS	24
hEPO	MHL	PALAGULATLULAQPCE	21
hMPO	MKL	LALAGLLAILATPQPSE	21
hLPO	MWUCL	QLPUFLASUTLFEU	19

pTPO	RRKDLLGGDTEASGUAGLUEASRL	LUDEAIHTTMRNLKR	--GIFSPSQLLSFSKL	PEPTSRTASRAA	91
hTPO	RGKELLWGKPEESRUSSULEESKRL	UDTAMYATMQNLKR	--GILSPAQLLSFSKL	PEPTSGUIAARA	91
hEPO	GTPASPGAVETSULRDCIAEAKLL	UDAA-YNWTQKSIKORL	SGSASPMDLLSYFKOPUAATRTU	UARA	90
hMPO	GAAPAVLGEUDTSLULSSMEERKOL	UDKA-YKERRESIKORL	SGSASPMELLSYFKOPUAATRTA	UARA	90
hLPO	AASDTIAQAASTTTISDAUSKUKI	QUNKA-FLDSRTLKTTLSSEAPT	TQQLSEYFKHAKGRTRTA	ANG	88

pTPO	EIMETAUQEUKRRUCRRARDTDQLP	TDULSEE-LLSTIANLSGCL	PHMLPPSCPHTC	-ANKYRLITGACN	159
hTPO	EIMETSIQAMKRKUNLKTQSQHPTD	ALSED-LLSIANMSGCL	PYMLPPKCPNTC	-ANKYRPIITGACN	159
hEPO	DYMHVALLLEEKLPQQRSGPFNUTD	ULTEP-QLRLLSQASGC	---ALRDQ-AERC	--SDKYRTITGACN	153
hMPO	DYLHVALLLERKLASLWARPFNUTD	ULTPA-QLNULSKSSGC	---AYQDU-GUTCPEQDKYRTI	ITGMCN	155
hLPO	Q-----UWEESLKRL-RRDTTL	INUTDPSLDLTALSWEVGC	---GAPUP-LUKCDENSPYRTI	ITGDCN	146

pTPO	NRDHPRWGASNTALARWLPPAYEDG	UTEPAGWNPHFLYNGLP	LPUREUTRQVIHUSNEAUTE	DGQYSDL	229
hTPO	NRDHPRWGASNTALARWLPPUYEDG	FSQPRGWNPGFLYNGFL	PPUREUTRHUIQUSNEUUT	DDRYSDL	229
hEPO	NKRRPILLGASNOALARWLPAEYEDG	LSLPFGWTPSRRANGFL	LPLURAHUSNQIURFPNER	LTSORGRALM	223
hMPO	NRRSPITLGASNRAFURWLPAEYEDG	SLPYGWTGUKRNGFP	UALARAHUSNEIURFPTD	QLTPQERSLM	225
hLPO	NRRSPALGAAALARWLPAEYEDG	LALPFGWTQRKTRNGF	RUPLAREUSNKIUGYLD	EGULDQNRSL	216

pTPO	LMAWGQYIDHDIAFTPOSTSKAA	FAGGADCOLTCENRSPCFPI	QLP-TNASGAAGATCLPFYR	SSAACGS	298
hTPO	LMAWGQYIDHDIAFTPOSTSKAA	FGGADCOMTCENQNPCFPI	QLP-EEARPAAGTACLPFYR	SSAACGT	298
hEPO	FMDWGQYIDHDIDFSPESPARUA	FTAGUDCERTCAQLPPCFPI	KIPPNDPRIKNQDCLPFYR	SPSC--	291
hMPO	FMDWGQLLDHDLDFTEPAARASE	UTGUNCETSCUQPPCFPLK	IPPNDPRIKNQDCLPFYR	SPAC--	293
hLPO	FMDWGQYIDHDIDFAPETELGS	NEHSKTQCEEYCIQGDNC	CFPMFPKNDPKLKTQGK	CMPPFRAGFUOPT	286

pTPO	GRQGALUGNLSWAAPRODMNGLT	SFLDASTUYGSSPAQEORLRN	WTSAEGLLRUNTRHRDAGRA	LPFAP	368
hTPO	GDQGALFGNLSANPRODMNGLT	SFLDASTUYGSSPALERLRN	WTSAEGLLRUHARLRDSGRA	LPFUP	368
hEPO	-PQ-----N--KNRURNOIN	ALTSFUDASMUYGSEUSLS	LRLNRATNYLGLLA	INORFQDNGRALLPF	-D 352
hMPO	-PG-----S--NITIRNOIN	ALTSFUDASMUYGSEUSLS	LRLNRATNYLGLLA	INORFQDNGRALLPF	-D 354
hLPO	-PPYQ---SL---AREQIN	AVTSFLDASUYGSEPSLAS	RLNLSSPLGLMAUNQEA	WDHGLAVLPF	-N 347

pTPO	PPAPPAACAPEPGTPA-APAPC	FLAGDSRASEUPGLTALHTL	WLREHNRLAARFKALNAH	USADTUYQEAR	437
hTPO	PRAPPAACAPEGIPGETAPC	FLAGDGRASEUPSLTALHTL	WLREHNRLAARFKALNAH	USADAVYQEAR	438
hEPO	NLHDDPCL--LTNRS-ARIP	CFLAGDTRSTETPKLAMHTL	FMREHNRLATELRRLN	PRUNGDKLYNEAR	419
hMPO	NLHDDPCL--LTNRS-ARIP	CFLAGDTRSEMPELTSMHT	LLREHNRLATELKS	LNPRADGERLYQEAR	421
hLPO			EHNRLARELKALNPQ	UDGEKLYQEAR	414
hLPO	NKKPSPC--EFINTT-ARUP	CFLAGDFRASEQILLATA	HTLLREHNRLARELKK	LNPHUNGKLYQEAR	414

pTPO	KUUGALHQIITLADYUPKILG	AEFGQHUGPYQGYDPAUPT	USNUFSTAFAFRGHAT	IHPLURRLDARF	507
hTPO	KUUGALHQIITLADYIPRI	LGPFAFQYUGPYEGYSTAN	PTUSNUFSTAFAFRGHAT	IHPLURRLDASF	508
hEPO	KIMGAMUQIITYRDELPLUL	GKARARLTGHYRGVCSNU	DPRUANUF-TLAFRFGHT	MLQPFMRDLSQY	488
hMPO	KIUGAMUQIITYRDYLPUL	GPTAMRKYLPTYSMNSUD	PRISNUF-TNAFRYGH	TLIOPFMRLDNRY	490
hLPO	KILGAFMQIITFRDYLPIL	LG-DHMQKWIPPYQGY	SESUDPRISNUF-TFAFR	FGHLEUPSSMRLDENY	482
hLPO	KILGAFIQIITFRDYLPIL	LG-SEMQKWIPPYQGY	NNSUDPRISNUF-TFAFR	FGHMEUPSTUSRLDENY	482

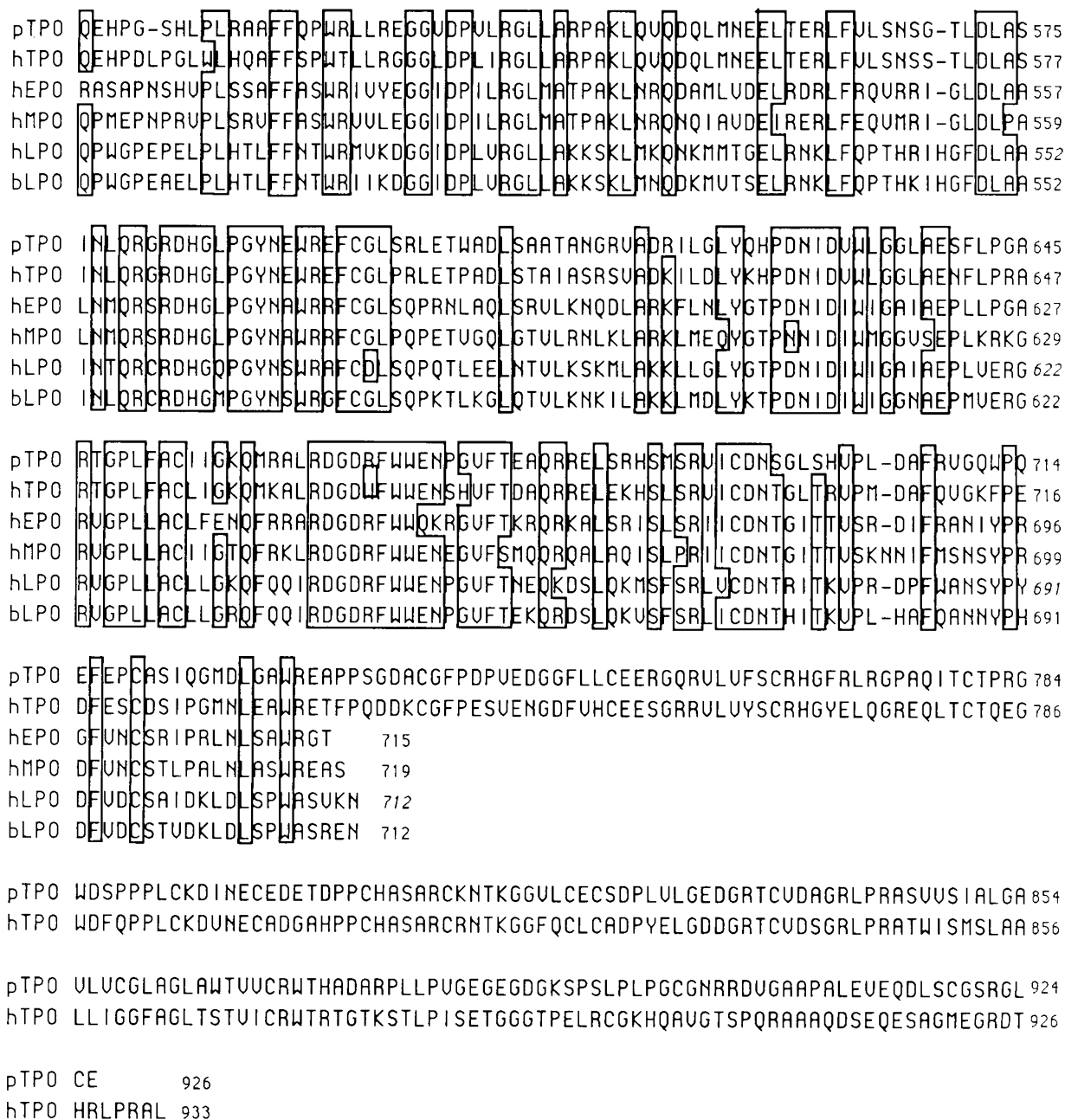


FIG. 5. Peroxidase sequence conservation. The amino acid sequences of porcine TPO (Magnusson *et al.*, 1987), human TPO (Kimura *et al.*, 1987, 1989), human EPO (Sakamaki *et al.*, 1989; Ten *et al.*, 1989), human MPO (Johnson *et al.*, 1987; Morishita *et al.*, 1987; Weil *et al.*, 1987), bovine LPO, and human LPO are shown mutually aligned. Gaps have been introduced so as to allow maximum homology, and residues common to all or all but one of the sequences have been boxed. The reported starts of light- (L) and heavy- (H) chain portions of EPO and MPO are indicated by horizontal arrows, and two possible cleavage positions for mature bLPO are noted (A and B).

2.7-kb insert. This clone, T3, contained a reading frame encoding 712 amino acids as shown in Fig. 2. Hybridization of isolated T3 insert DNA to blots of *Eco* RI-digested bovine DNA (Fig. 1C) yielded, among other bands, 3.0- and 5.5-kb bands of similar mobility as were obtained using the oligonucleotide probes (Fig. 1A and B, respec-

tively). The corresponding sequences of four of the five peptides isolated from cyanogen bromide cleaved material were present within the deduced amino acid sequence of T3, as seen in Table 1A. The sequence of one of the peptides, fragment 7, could not be located and was assumed to be either erroneous or of contaminant origin.

Hybridization of an oligonucleotide probe matching a 51-base region of T3, to a panel of RNAs from eight different bovine tissues, detected hybridization only in the mammary gland lane (Fig. 3). A single band of 2.8 kb was detected, corresponding exactly to the size of the T3 insert plus 1–200 bases of poly(A) tail. Hybridization to an identical panel using the insert DNA from T3 as a probe yielded identical results.

Both oligonucleotide probes 10bg and 11bg, as well as T3, also yielded clear signals when hybridized to human DNA (Fig. 1, lanes A1, B1, and C2). To obtain a cDNA clone encoding the putative human homolog of T3, the insert from T3 was used to screen a cDNA library from a lactation-competent human mammary gland. A single clone was obtained (M1), and the sequence as determined is shown in Fig. 4. The deduced amino acid sequence appeared to code for the carboxy-terminal 324 residues (or 45%) of a peroxidase enzyme very closely related to bLPO. Homology was 86.5% at the DNA level and 84% at the amino acid level. Re-screening of the same library yielded no additional clones, suggesting that a full-length clone was not contained in the library.

The deduced amino acid sequences from both T3 and M1 are compared with other recently published peroxidase sequences in Fig. 5. Alignment of the sequences was unambiguous over most of the molecule; however, gaps were introduced in some positions to maximize alignment. Two regions (residues 123–129 and 284–293 of bLPO) constituted hypervariable regions, for which alignment was ambiguous.

DISCUSSION

Three lines of evidence confirm that clone T3 encodes bLPO. First, the sequences of four of five CNBr fragments of bLPO were present in the deduced sequence. Second, assuming the amino terminus of mature LPO lies as indicated below, the deduced amino acid composition very closely matches the empirically determined composition (Theorell and Akeson, 1943; Carlstrom, 1969a). Finally, the deduced sequence exhibits obvious homology with other peroxidase sequences, as seen in Fig. 5.

The deduced sequence of T3 clearly contains both signal and propeptides, however there is currently some ambiguity in the location of the start of the mature LPO sequence. Amino-terminal analysis of purified LPO yielded leucine in one study (Carlstrom, 1969c), and partially blocked leucine in an earlier study (Theorell and Akeson, 1943). An examination of the alignment in Fig. 5 shows that the starts of the light chains of both MPO and EPO coincide exactly. It follows that Leu-129 of bLPO, occurring two residues upstream from the homologous position ("A" in Fig. 2), constitutes a likely bLPO amino terminus. Indeed, the encoded peptide sequence starting at Leu-129 reasonably matches the reported amino acid composition and molecular weight of 78,500, including carbohydrate residues (Theorell and Akeson, 1943; Carlstrom, 1969a). However, in recent work (Maillart, 1989), an amino-ter-

минаl sequence of "DTTLTNVTDPSL..." was obtained, suggesting that Asp-101 constituted the amino terminus. Several groups have reported heterogeneity in bLPO molecular weight. In particular, two major forms have been described: A and B, consisting of 76.5 and 78.5 kDa, respectively (Rombauts *et al.*, 1967; Carlstrom, 1969a,b; Sievers, 1979). Possibly the two forms have distinct amino termini, such as the two residues implicated above.

The amino-terminal region of the coding region contains a hydrophobic core resembling a signal sequence. Two potential signal peptidase cleavage sites of equal likelihood, as determined by similarity to consensus (Von Heine, 1983), are indicated in Fig. 2. Assuming one of these two sites is used, either a 26- or 28-residue signal peptide is present, suggesting that LPO is secreted by typical membrane translocation (Davis and Tai, 1980).

As seen in Fig. 5, throughout the enzyme coding region, bLPO shows 46%, 50%, and 52% homology at the amino acid level to human TPO, MPO, and EPO. The homologous residues are generally common to all of the sequences and clustered in regions. Several relatively long stretches (such as represented by residues 373–384, 457–468, and 640–654 of bLPO) are nearly 100% conserved in all of the forms. These regions probably constitute structurally or enzymatically important regions of the enzyme. There are five conserved His residues which lie in regions of strong conservation. One or more of these residues may constitute the binding site of the heme group, as has been demonstrated in the structurally and enzymatically related lignin peroxidases (Tien and Tu, 1987). With few exceptions, the pattern of Cys residues also is very strongly conserved. These exceptions include two extra Cys residues in EPO (residues 39 and 455), and single extra Cys residues in MPO (residue 290) and LPO (residue 557). In the case of LPO, this extra Cys residue may be available to form a disulfide bond with the heme group, as has been suggested to explain the unusually strong binding of the prosthetic group (Nichol *et al.*, 1987). The otherwise perfect conservation of the remaining 14 Cys residues suggests the four peroxidases may share a common overall structure.

From the segment of the coding sequence contained in M1, it is clear that the human form of the enzyme is quite homologous to its bovine counterpart. These carboxy-terminal 324 amino acids showed 84% conservation relative to bLPO. In spite of this similarity in structure, its physiological role appears to be different in humans since it is essentially absent from breast milk.

Human salivary peroxidase (SPO) and LPO are similar immunologically, enzymologically, in molecular weight, and in amino acid composition (Morrison *et al.*, 1965; Gothefors and Marklund, 1975; Tenuovo, 1981; Pfeil and Ohlsson, 1986). They appear to differ, however, in glycosylation as well as in sensitivity to azide inhibition and activity with certain substrates (Tenuovo, 1981). One possibility is that the two enzymes represent the same primary gene product and that the differences in enzymatic properties reflect differences in post-translational modifications such as glycosylation. If this is the case, it is surprising that we were unable to detect transcription of bLPO sequences in salivary gland RNA. Amino acid sequencing of human SPO should ultimately resolve this question.

ACKNOWLEDGMENTS

We thank J. Griffith for assistance with amino acid sequencing, and M. McGrogan for the gift of HL60 RNA.

REFERENCES

- APARICIO, M.A., PERALTA, L.M., and GARCIA, H.S. (1986). Potentialization of the lactoperoxidase system for preservation of raw milk in the tropics. *Archivos Latinoamericanos de Nutricion* **36**, 725-733.
- AVIV, H., and LEDER, P. (1972). Purification of biologically active globin messenger RNA by chromatography on oligothymidyllic acid-cellulose. *Proc. Natl. Acad. Sci. USA* **69**, 1408-1412.
- CARLSTROM, A. (1969a). Lactoperoxidase. Identification of multiple molecular forms and their interrelationships. *Acta Chem. Scand.* **23**, 171-184.
- CARLSTROM, A. (1969b). Physical and compositional investigations of the subfractions of lactoperoxidase. *Acta Chem. Scand.* **23**, 185-202.
- CARLSTROM, A. (1969c). Lactoperoxidase. Some spectral properties of a haemoprotein with a prosthetic group of unknown structure. *Acta Chem. Scand.* **23**, 203-213.
- CHIRGWIN, J., PRZYBYLA, A., MACDONALD, R., and RUTTER, W. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**, 5294-5299.
- DAVIS, B., and TAI, P.-C. (1980). The mechanism of protein secretion across membranes. *Nature* **283**, 433-438.
- GOTHEFORS, L., and MARKLUND, S. (1975). Lactoperoxidase activity in milk and in saliva of newborn infants. *Infect. Immun.* **11**, 1210-1215.
- ITO, H., MORIZET, J., COULOMBEL, L., GOAVEC, M., ROSSEAU, V., BERNARD, A., and STANISLAWSKI, M. (1989). An immunotoxin system intended for bone marrow purging composed of glucose oxidase and lactoperoxidase coupled to monoclonal antibody 097. *Bone Marrow Transpl.* **4**, 182-187.
- JOHNSON, K.R., NAUSEEF, W.M., CARE, A., WHEELLOCK, M.J., SHANE, S., HUDSON, S., KOEFFLER, H.P., SELSTED, M., MILLER, C., and ROVERA, G. (1987). Characterization of cDNA clones for human myeloperoxidase: Predicted amino acid sequence and evidence for multiple mRNA species. *Nucleic Acids Res.* **15**, 2013-2028.
- KIMURA, S., KOTANI, T., McBRIDE, O.W., UMEKI, K., HIRAI, K., NAKAYAMA, T., and OHTAKI, S. (1987). Human thyroid peroxidase: Complete cDNA and protein sequence, chromosome mapping, and identification of two alternately spliced mRNAs. *Proc. Natl. Acad. Sci. USA* **84**, 5555-5559.
- KIMURA, S., HONG, Y.-S., KOTANI, T., OHTAKI, S., and KIKKAWA, F. (1989). Structure of the human thyroid peroxidase gene: Comparison and relationship to the human myeloperoxidase gene. *Biochemistry* **28**, 4481-4489.
- LANGBAKK, B., and FLATMARK, T. (1984). Demonstration and partial purification of lactoperoxidase from human colostrum. *FEBS Lett.* **174**, 300-303.
- LANGBAKK, B., and FLATMARK, T. (1989). Lactoperoxidase from human colostrum. *Biochem. J.* **259**, 627-631.
- LATHE, R. (1985). Synthetic oligonucleotide probes deduced from amino acid sequence data. Theoretical and practical considerations. *J. Mol. Biol.* **183**, 1-12.
- MAGNUSSON, R., GESTAUTAS, J., TAUROG, A., and RAPOPORT, B. (1987). Molecular cloning of the structural gene for porcine thyroid peroxidase. *J. Biol. Chem.* **262**, 13885-13888.
- MANSSON-RAHEMTULLA, B., RAHEMTULLA, F., BALDONE, D.C., PRUITT, K.M., and HJERPE, A. (1988). Purification and characterization of human salivary lactoperoxidase. *Biochemistry* **27**, 233-239.
- MARSHALL, V.M., COLE, W.M., and BRAMLEY, A.J. (1986). Influence of the lactoperoxidase system on susceptibility of the udder to streptococcus uberis infection. *J. Dairy Res.* **53**, 507-514.
- MESSING, J. (1983). New M13 vectors for cloning. *Methods Enzymol.* **101**, 20-78.
- MAILLIART, P. (1989). "Contribution a la valorisation des proteines du lactoserum: de la purification au sequencage." Ph.D. Dissertation, University of Paris VII.
- MORISHITA, K., KUBOTA, N., ASANO, S., KAZIRON, Y., and NAGATA, S. (1987). Molecular cloning and characterization of cDNA for human myeloperoxidase. *J. Biol. Chem.* **262**, 15208-15213.
- MORRISON, M., ALLEN, P., BRIGHT, J., and JAYASINGHE, W. (1965). Lactoperoxidase V. Identification and isolation of lactoperoxidase from salivary gland. *Arch. Biochem. Biophys.* **111**, 126-133.
- NICHOL, A.W., ANGEL, L.A., MOON, T., and CLEZY, P.S. (1987). Lactoperoxidase haem, an iron-porphyrin thiol. *Biochem. J.* **247**, 147-150.
- OKUDA, K., ISHIWARA, K., NOGUCHI, Y., TAKAHASHI, T., and TAKOKORO, I. (1980). New type of antibody-enzyme conjugate which specifically kills *Candida albicans*. *Infect. Immun.* **27**, 690-692.
- PAUL, K.G., and OHLSSON, P.I. (1985). The chemical structure of lactoperoxidase. In *The Lactoperoxidase System, Immunology Series*, vol. 27. K.M. Pruitt and J.O. Tenovuo, eds. (Marcel Dekker, Inc., New York and Basel) pp. 15-29.
- PFEIL, W., and OHLSSON, P.I. (1986). Lactoperoxidase consists of domains: A scanning calorimetric study. *Biochim. Biophys. Acta* **872**, 72-75.
- PHILPOTT, G., SHEARER, W., BOWER, R., and PARKER, C. (1973). Selective cytotoxicity of hapten-substituted cells with an antibody-enzyme conjugate. *J. Immunol.* **111**, 921-929.
- REDMAN, L.W., and TUSTANOFF, E.R. (1984). Iodination of [Tyr8]-bradykinin—Comparison of chloramine-T and lactoperoxidase. *J. Immunoassay* **5**, 29-57.
- REITER, B. (1985). The lactoperoxidase system of bovine milk. In *The Lactoperoxidase System Immunology Series*, vol. 27. K.M. Pruitt and J.O. Tenovuo, eds. (Marcel Dekker, Inc., New York and Basel) pp. 123-141.
- REITER, B., FULFORD, R.J., MARSHALL, V.M.E., YARROW, N., DUCKER, M.J., and KNUTSSON, M. (1981). An evaluation of the growth promoting effect of the lactoperoxidase system in newborn calves. *Anim. Prod.* **32**, 297-306.
- ROMBAUTS, W.A., SCHROEDER, W.A., and MORRISON, M. (1967). Bovine lactoperoxidase. partial characterization of the further purified protein. *Biochemistry* **6**, 2965-2977.
- SAKAMAKI, K., TOMONAGA, M., TSUKUI, K., and NAGATA, S. (1989). Molecular cloning and characterization of a chromosomal gene for human eosinophil peroxidase. *J. Biol. Chem.* **264**, 16828-16836.
- SANDHOLM, M., ALI-VEHMAS, T., KAARTINEN, L., and JUNNILA, M. (1988). Glucose oxidase (GOD) as a source of hydrogen peroxide for the lactoperoxidase (LPO) system in milk: Antibacterial effect of the GOD-LPO system against mastitis pathogens. *J. Vet. Med. B* **35**, 346-352.
- SANGER, F., NICKLEN, S., and COULSEN, A.R. (1977).

- DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**, 5463-5469.
- SHINDLER, J.S., CHILDES, R.E., and BARDSLEY, W.J. (1976). Peroxidase from human cervical mucus: The isolation and characterization. *Eur. J. Biochem.* **65**, 325-331.
- SIEVERS, G. (1979). The prosthetic group of milk lactoperoxidase is protoheme IX. *Biochim. Biophys. Acta* **579**, 181-190.
- SIEVERS, G. (1981). Structure of milk lactoperoxidase. Evidence for a single polypeptide chain. *FEBS Lett.* **127**, 253-256.
- TEN, R.M., PEASE, L.R., McLEAN, D.J., BELL, M.P., and GLEICH, G.J. (1989). Molecular cloning of the human eosinophil peroxidase. *J. Exp. Med.* **169**, 1757-1769.
- TENUOVO, J. (1981). Different molecular forms of human salivary lactoperoxidase. *Archs. Oral Biol.* **26**, 1051-1055.
- TENUOVO, J.O. (1985). The peroxidase system in human secretions. In *The Peroxidase System, Immunology Series*, vol. 27. K.M. Pruitt and J.O. Tenovuo, eds. (Marcel Dekker, Inc., New York and Basel) pp. 101-123.
- THEORELL, H., and AKESON, A. (1943). Highly purified milk peroxidase. *Arkiv. Kemi. Mineral. Geol.* **17B**, No. 17.
- TIEN, M., and TU, C.-P. (1987). Cloning and sequencing of a cDNA for ligninase from *Phanerochaete chrysosporium*. *Nature* **326**, 520-523.
- VON HEIJNE, G. (1983). Patterns of amino acids near signal-sequence cleavage sites. *Eur. J. Biochem.* **133**, 17-21.
- WEIL, S.C., ROSNER, G.L., REID, M.S., CHISHOLM, R.L., FARBER, N.M., SPITZNAGEL, J.K., and SWANSON, M.S. (1987). cDNA cloning of human myeloperoxidase: Decrease in myeloperoxidase mRNA upon induction of HL-60 cells. *Proc. Natl. Acad. Sci. USA* **84**, 2057-2061.
- ZORAD, S., SVABOVA, E., KLIMES, I., and MACHO, L. (1985). Comparison of radiochemical purity and tissue binding of labelled insulin prepared by lactoperoxidase and chloramine T iodination. *Endocrinol. Exp.* **19**, 267-275.

Address reprint requests to:
Dr. Jeffrey J. Seilhamer
Ideon Corporation
515 Galveston Drive
Redwood City, CA 94063

Received for publication March 9, 1990, and in revised form June 14, 1990.

This article has been cited by:

1. Miryam A. Frago, Aliza Torbati, Nevis Fregien, Gregory E. Conner. 2009. Molecular heterogeneity and alternative splicing of human lactoperoxidase. *Archives of Biochemistry and Biophysics* **482**:1-2, 52-57. [[CrossRef](#)]
2. Tetsuya Tanaka, Xuenan Xuan, Asato Kojima, Ikuo Igarashi, Kozo Fujisaki, Kei-ichi Shimazaki. 2008. Expression and characterization of bovine lactoperoxidase by recombinant vaccinia virus. *Cytotechnology* **58**:3, 127-133. [[CrossRef](#)]
3. A MATSUSHITA, D SON, H SATSU, Y TAKANO, H KAWAKAMI, M TOTSUKA, M SHIMIZU. 2008. Inhibitory effect of lactoperoxidase on the secretion of proinflammatory cytokine interleukin-8 in human intestinal epithelial Caco-2 cells. *International Dairy Journal* **18**:9, 932-938. [[CrossRef](#)]
4. Richard E. Goodman, Steve L. Taylor, Junichi Yamamura, Toshiya Kobayashi, Hiroshi Kawakami, Claire L. Kruger, Gavin P. Thompson. 2007. Assessment of the potential allergenicity of a Milk Basic Protein fraction. *Food and Chemical Toxicology* **45**:10, 1787-1794. [[CrossRef](#)]
5. Tetsuya Tanaka, Shin Murakami, Haruto Kumura, Ikuo Igarashi, Kei-ichi Shimazaki. 2006. Parasiticidal activity of bovine lactoperoxidase against *Toxoplasma gondii*. *Biochemistry and Cell Biology* **84**:5, 774-779. [[CrossRef](#)]
6. Jean Ruf, Pierre Carayon. 2006. Structural and functional aspects of thyroid peroxidase. *Archives of Biochemistry and Biophysics* **445**:2, 269-277. [[CrossRef](#)]
7. M RUSSELL, L BOBEK, J BROCK, G HAJISHENGALLIS, J TENOVUO Innate Humoral Defense Factors 73-93. [[CrossRef](#)]
8. G NINO, M TURACCHIO, A DARCHIVIO, S LORA, B CORAIN, G ANTONINI. 2004. Catalytic activity of bovine lactoperoxidase supported on macroporous poly(2-hydroxyethyl methacrylate-glycidyl methacrylate). *Reactive and Functional Polymers* **61**:3, 411-419. [[CrossRef](#)]
9. Tomio Kotani, Kazumi Umeki, Jun-ichi Kawano, Tatsuo Suganuma, Akira Hishinuma, Tamio Ieiri, Shohei Harada. 2003. Partial iodide organification defect caused by a novel mutation of the thyroid peroxidase gene in three siblings. *Clinical Endocrinology* **59**:2, 198-206. [[CrossRef](#)]
10. Tetsuya TANAKA, Sanae SATO, Haruto KUMURA, Kei-ichi SHIMAZAKI. 2003. Expression and Characterization of Bovine Lactoperoxidase by Recombinant Baculovirus. *Bioscience, Biotechnology, and Biochemistry* **67**:10, 2254-2261. [[CrossRef](#)]
11. S Watanabe. 2000. Bovine Lactoperoxidase and Its Recombinant: Comparison of Structure and Some Biochemical Properties. *Biochemical and Biophysical Research Communications* **274**:3, 756-761. [[CrossRef](#)]
12. Anni R. Thomsen, Lars Sottrup-Jensen, Gerald J. Gleich, Claus Oxvig. 2000. The Status of Half-Cystine Residues and Locations of N-glycosylated Asparagine Residues in Human Eosinophil Peroxidase. *Archives of Biochemistry and Biophysics* **379**:1, 147-152. [[CrossRef](#)]
13. A Naidu Lactoperoxidase . [[CrossRef](#)]
14. K Shin. 2000. PCR Cloning and Baculovirus Expression of Human Lactoperoxidase and Myeloperoxidase. *Biochemical and Biophysical Research Communications* **271**:3, 831-836. [[CrossRef](#)]
15. Susan M. Wolf, Rosa P. Ferrari, Silvio Traversa, Klaus Biemann. 2000. Determination of the carbohydrate composition and the disulfide bond linkages of bovine lactoperoxidase by mass spectrometry. *Journal of Mass Spectrometry* **35**:2, 210-217. [[CrossRef](#)]
16. K Shin. 2000. Identification of lactoperoxidase in mature human milk. *The Journal of Nutritional Biochemistry* **11**:2, 94-102. [[CrossRef](#)]
17. H Chang. 1999. Inactivation of *Coprinus cinereus* peroxidase by 4-chloroaniline during turnover: comparison with horseradish peroxidase and bovine lactoperoxidase. *Chemico-Biological Interactions* **123**:3, 197-217. [[CrossRef](#)]
18. Cecilia L. S. Santos, Hennie Bikker, Katia G. M. Rego, Antonio C. Nascimento, Marcos Tambascia, Jan J. M. de Vijlder, Geraldo Medeiros-Neto. 1999. A novel mutation in the TPO gene in goitrous hypothyroid patients with iodide organification defect. *Clinical Endocrinology* **51**:2, 165-172. [[CrossRef](#)]
19. Shikiko Watanabe, Franca Varsalona, Yung-Choon Yoo, Jean-Paul Guillaume, Alex Bollen, Keiichi Shimazaki, Nicole Moguilevsky. 1998. Recombinant bovine lactoperoxidase as a tool to study the heme environment in mammalian peroxidases. *FEBS Letters* **441**:3, 476-479. [[CrossRef](#)]
20. R Ferrari. 1997. Spectroscopic and binding studies on the interaction of inorganic anions with lactoperoxidase. *Journal of Inorganic Biochemistry* **68**:1, 17-26. [[CrossRef](#)]

21. Tadashi Ueda, Kazuhiro Sakamaki, Tetsuo Kuroki, Ikuya Yano, Shigekazu Nagata. 1997. Molecular Cloning and Characterization of the Chromosomal Gene for Human Lactoperoxidase. *European Journal of Biochemistry* **243**:1-2, 32-41. [[CrossRef](#)]
22. C Kiser. 1996. Cloning and sequence analysis of the human salivary peroxidase-encoding cDNA. *Gene* **173**:2, 261-264. [[CrossRef](#)]
23. Koichi Sato, Hideyo Hasumi, Akihiro Tsukidate, Junji Sakurada, Shingo Nakamura, Toichiro Hosoya. 1995. Effects of mixed solvents on three elementary steps in the reactions of horseradish peroxidase and lactoperoxidase. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology* **1253**:1, 94-102. [[CrossRef](#)]
24. Hans-Dieter Zucht, Manfred Raida, Knut Adermann, Hans-Jürgen Mägert, Wolf-Georg Forssmann. 1995. Casocidin-I: a casein-#s2 derived peptide exhibits antibacterial activity. *FEBS Letters* **372**:2-3, 185-188. [[CrossRef](#)]
25. R Ferrari. 1995. Spectroscopic investigations on the highly purified lactoperoxidase Fe(III)-heme catalytic site. *Journal of Inorganic Biochemistry* **58**:2, 109-127. [[CrossRef](#)]
26. G Lukat. 1993. Magnetic resonance spectroscopy, calcium content, and anion coordination studies of bovine and goat lactoperoxidase. *Journal of Inorganic Biochemistry* **50**:3, 157-171. [[CrossRef](#)]
27. J ZENG. 1992. X-ray crystal structure of canine myeloperoxidase at 3 p resolution*1. *Journal of Molecular Biology* **226**:1, 185-207. [[CrossRef](#)]
28. Alvin Taurog, Martha L. Dorris. 1992. Myeloperoxidase-catalyzed iodination and coupling. *Archives of Biochemistry and Biophysics* **296**:1, 239-246. [[CrossRef](#)]
29. Prabir K. DE. 1992. Tissue distribution of constitutive and induced soluble peroxidase in rat. Purification and characterization from lacrimal gland. *European Journal of Biochemistry* **206**:1, 59-67. [[CrossRef](#)]
30. M.C.R. Franssen, H.C. van der PlasHaloperoxidases: Their Properties and Their Use in Organic Synthesis **37**, 41-99. [[CrossRef](#)]
31. Marie-Madeleine CALS, Pascal MAILLIART, Ghislaine BRIGNON, Patricia ANGLADE, Bruno Ribadeau DUMAS. 1991. Primary structure of bovine lactoperoxidase, a fourth member of a mammalian heme peroxidase family. *European Journal of Biochemistry* **198**:3, 733-739. [[CrossRef](#)]