Primary Structure, Tissue Distribution, and Chromosomal Localization of a Novel Isoform of Lysyl Hydroxylase (Lysyl Hydroxylase 3)*

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We report characterization of a novel isoform of lysyl hydroxylase (lysyl hydroxylase 3, LH3). The cDNA clones encode a polypeptide of 738 amino acids, including a signal peptide. The amino acid sequence has a high overall identity with LH1 and LH2, the isoforms characterized earlier. Conserved regions are present in the carboxyl-terminal portion of the isoforms and also in the central part of the molecules. Histidine and asparagine residues, which are conserved in the other isoforms and are known to be required for enzymatic activity, are also conserved in the novel isoform. The gene for LH3 (PLOD3) has been assigned to human chromosome 7q36 and rat chromosome 12. Gene expression of LH3 is highly regulated in adult human tissues. A strong hybridization signal, corresponding to an mRNA 2.75 kilobases in size, is obtained in heart, placenta and pancreas on multiple tissue RNA blots. Expression of the cDNA in vitro results in the synthesis of a protein that hydroxylates lysyl residues in collagenous sequences in a non-triple helical conformation.

Collagen is a group of structural proteins that are found essentially in all tissues. To date, 19 different collagen types have been identified that participate in the assembly of various kinds of polymers in the extracellular matrix (1–4). Collagens form the structural building blocks of tissues, and it also follows that they have important regulatory functions. Some collagens form scaffolds that keep cells in place within tissues, connect tissues within an organ, and facilitate attachment and migration of cells. Collagens can also form links between cells and matrices, and some regulate the assembly and properties of the scaffold-forming collagens (5). Recent reports indicate that collagen can directly serve as a ligand for receptor tyrosine kinases and, as a consequence of binding to the receptor, induces a cascade of phosphorylation in cells (6, 7).

The biosynthesis of collagens includes several posttranslational modifications, one of which is hydroxylation of lysyl residues. Hydroxylsine occurs in the Y position of the repeating X-Y-Gly triplets within the helical region of the collagen molecule. Hydroxylsine also occurs in the sequence of nonhelical telopeptide regions of some collagen molecules, when glycine is replaced by either serine or alanine (1, 2, 8). The amount of the hydroxylysyl residues varies considerably between different collagen types. Variation is also found within the same collagen type in different tissues and even within the same tissues in different physiological and pathological states (1, 2, 8). In addition to collagens, hydroxylsine residues are found in some other proteins, these include C1q complement, acetylcholinesterase, mannose-binding proteins, bovine conglutinin, and anglerfish somatostatin 28. It should be noted, however, that all these proteins, with the exception of somatostatin 28, contain collagenous domains in their structure (1).

Hydroxylysyl residues may be glycosylated to form galactosylhydroxylsyl or glucosylgalactosylhydroxylsine residues. This glycosylation is unique to collagenous structures, although the function of the glycosylation is unclear. There is evidence, however, that carbohydrates bound to collagen may play a role in collagen receptor recognition and activation (6).

Hydroxylsine residues have an important function in the formation of covalent cross-links in collagens. Two related pathways exist for cross-link formation in fibrillar collagens, one based on lysine aldehydes and the other on hydroxylysine aldehydes. Hydroxylsine derived cross-links predominate in bone, cartilage, ligament, most tendons, embryonic skin, and most major internal connective tissues of the body (2).

Lysyl hydroxylase (EC 1.14.11.14) catalyzes the hydroxylation of lysyl residues in collagens in a reaction that requires Fe²⁺, 2-oxoglutarate, O₂, and ascorbate (1). Two enzymes (lysyl hydroxylase 1, LH1; lysyl hydroxylase 2, LH2) with lysyl hydroxylase activity have been isolated and characterized in human tissues so far (1, 9). We report here a novel enzyme (lysyl hydroxylase 3, LH3), which is capable of hydroxylating lysyl residues of collagenous peptides. The gene for the enzyme has been localized to human chromosome 7q36, which is distinct from the locations of other lysyl hydroxylases (10–12), indicating that there are at least three different genes for lysyl hydroxylase in vivo.

MATERIALS AND METHODS

Search for Expressed Sequence Tags—The Blast program was used to find homologies between human LH1, LH2, and expressed sequence tags. Several sequences having homologies to lysyl hydroxylases were found in the search: AA250735, AA410941, AA10762, AA40561, AA353120, AA411291, AA313622, AA340606, AA077166, and W04357. Oligonucleotides were synthesized from the sequences. 1

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF046889.

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1 The abbreviations used are: LH, lysyl hydroxylase; PCR, polymerase chain reaction; kb, kilobase(s).
FIG. 1. Nucleotide sequence of cDNA encoding a novel human lysyl hydroxylase (LH3). First line, nucleotide sequence of the cDNA for LH3; second line, the deduced amino acid sequence of LH3; third line, amino acid sequence of human LH1 (10); fourth line, amino acid sequence of human LH2 (9). The translation termination codon is designated by an asterisk. The locations of oligonucleotides used in PCR are indicated by...
cDNA—Avian myeloblastosis virus reverse transcriptase (Marathon cDNA amplification kit, CLONTECH) and human kidney mRNA (CLONTECH) was used to prepare oligo(dT)-primed cDNA (13).

PCR—PCR amplifications of DNA templates were performed using Taq DNA polymerase (AmpliTaq Gold™, Perkin-Elmer) in the presence of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.001% (w/v) gelatin, 0.2 mM dNTPs. The following oligonucleotide pair and conditions were used in the amplification of the clone corresponding to the middle part of the novel cDNA: M1 (5'-GGGTGATGTGGCTCGAACA-3') from AA250735) and M15 (5'-CCTTCCTCGGGGAGGAGATCA-3') from AA340606), 95 °C, 15 min; 95 °C, 40 s; 59 °C, 40 s; 72 °C, 2 min; 30 cycles; 72 °C, 10 min.

5' Rapid Amplification of cDNA Ends—The 5' and 3' ends of the novel cDNA were obtained using a Marathon cDNA amplification kit (CLONTECH). The Marathon cDNA adaptor (containing the oligonucleotides AP1 or AP2) was ligated to the blunt-ended double-stranded cDNA prepared from oligo(dT)-primed human kidney poly(A) RNA. For cloning the 5' end of the molecule the following oligonucleotide pairs were used in amplification: M5 (5'-CGGATACGCACACACGGTTCCGA-3') and AP1 (5'-CCATCTCAAATCGACTAGTATAGGC-3'), 95 °C, 15 min; 95 °C, 40 s; 63 °C, 40 s; 72 °C, 1 min; 30 cycles; 72 °C, 10 min; M19 (5'-CAGTGATCACCAGCAGCTTC-3') and AP2 (5'-ACTCACTATAGGCTGAGCGGC-3') and M18 (5'-CCACCAACTGTTCGAGCCA-3') paired with AP2, 95 °C, 15 min; 95 °C, 40 s; 55 °C, 40 s; 72 °C, 1 min; 30 cycles; 72 °C, 10 min. For cloning the 3' end of the molecule the following oligonucleotide pairs were used: M20 (5'-CTGCTGCGGATATGTGGGCC-3') and AP1, M20 and AP2, 95 °C, 15 min; 95 °C, 40 s; 65 °C, 40 s; 72 °C, 1.5 min; 30 cycles; 72 °C, 10 min. All fragments were subcloned into pUC18-plasmid (SureClone, Amersham Pharmacia Biotech).

DNA Sequencing—Plasmid DNA or PCR products were used for sequencing. Plasmid DNA was isolated using a Qiagen plasmid kit (Qiagen). PCR products were extracted from the agarose gel by a QIAquick gel extraction kit (Qiagen). DNA sequencing was performed by dideoxynucleotide sequencing system using the ABI PRISM™ Dye Terminator, dRhodamine terminator, or Big Dye Terminator cycle sequencing ready reaction kits (Perkin-Elmer) or Thermo Sequenase kit (Amersham Pharmacia Biotech). Sequencing was carried out using an ABI Prism 377 DNA sequencer (Perkin-Elmer).

Northern Analysis—A human multiple Northern blot containing poly(A) RNA from different tissues (CLONTECH) was hybridized for 1 h in Express Hyb solution (CLONTECH) at 42 °C using radioactively labeled M1-M15 PCR product as a probe. The blot was washed in 2 x SSC, 0.1% SDS at room temperature for 40 min and then in 0.1 x SSC, 0.1% SDS at 50 °C for 30 min.

Expression of cDNA in Insect Cells—Expression of the novel cDNA was carried out by baculovirus transfer vector in the Bac-to-Bac™ expression system (Life Technologies, Inc.). PCR was used to generate a cDNA construct for the novel molecule using human kidney cDNA as a template in the amplification. The construct covered the nucleotides from 214 to 2447 of the cDNA. The nucleotides of the construct were confirmed by sequencing. Insect cells were harvested 48 or 72 h after infection as described previously (9).

Activity Assay—Lysyl hydroxylase activity was assayed in supernatants of transfected insect cell extracts. The activity was measured by a method based on the hydroxylation-coupled decarboxylation of 2-oxo-
RESULTS AND DISCUSSION

Isolation and Characterization of cDNA Clones—Human kidney mRNA was used as a starting material for the reverse transcriptase reaction to prepare an oligo(dT)-primed cDNA. The oligonucleotides M1 and M15 (Fig. 1) were used to synthesize the first cDNA clone, which was 1.9 kb in size. The M1-M15 clone was sequenced, and the sequence was used to develop primers (M5, M18, M19, and M20) for 5’ and 3’ rapid amplification of cDNA ends. The full-length cDNA (Fig. 1) was 2745 base pairs long and contained an open reading frame from nucleotide 217 to nucleotide 2433. The 5’-noncoding region of the clone was 216 base pairs in length and the 3’-noncoding region 312 base pairs in size. The 3’ end of the sequence contained a putative poly(A) signal, AATAAA, which precedes multiple A nucleotides ending the sequence.

cDNA Clones Encode a Polypeptide That Is Able to Hydroxylate Lysyl Residues in X-Lys-Gly Sequences—After infection of insect cells with a baculovirus containing a cDNA for the novel molecule, a significant amount of lysyl hydroxylase activity was present in the supernatant fraction of an extract of the infected cells (Fig. 2). The data indicate that the novel molecule, a lysyl hydroxylase subtype, which is now called LH3, belongs to a family of lysyl hydroxylases that are able to hydroxylate Lysyl Residues in X-Lys-Gly Sequences—

Chromosomal Localization of the Gene for Lysyl Hydroxylase 3 (PLOD3)—The PLOD3 gene was first assigned to human chromosome 7 by Southern blot analysis of 19 human X rodent hybrid clones (Ref. 15 and references therein), using a cloned cDNA (M1-M15 clone, Fig. 1). In SacI-digested human DNA, the probe detected two fragments, at 5.7 and 4.3 kb. These two fragments could be followed unambiguously in the hybrids. They segregated together and with human chromosome 7 (no discordant clone for chromosome 7, and at least four discordant clones for each of the other chromosomes) (data not shown). To further localize the gene, the regional position of the PLOD3 gene was determined by fluorescence in situ hybridization, as described elsewhere (16, 17), using the same probe. Double spots (two labeled sister chromatids) formed by the probe were found only on chromosome 7. The fluorescent signals were clearly located at the end of the 7q arm, corresponding to the terminal band, i.e. 7q36 (Fig. 3). The results indicate that the PLOD3 gene is not linked to other lysyl hydroxylases, because the gene for LH1 (PLOD1) has been localized to 1p36.2 (10, 11) and LH2 (PLOD2) to 3q23–24 (12).

The homologous rat gene (Pld3) was also localized using a panel of 13 somatic cell hybrids segregating rat chromosomes (15). The human probe detected two fragments in BamHI-digested rat DNA (15.0 and 9.0 kb). These fragments could easily be distinguished from the three homologous mouse fragments (sizes <5.0 kb). They segregated together and with rat chromosome 12 (no discordant clone for chromosome 12 and at least three discordant clones for each of the other chromosomes) (data not shown). The human probe did not generate specific signals on rat chromosomes, preventing the regional localization of the rat gene.

Human chromosome 7 and rat chromosome 12 are known to be partially homologous: they share seven genes, thus showing conserved synteny (18–20). Interestingly, six of these genes (tropoelastin, erythropoietin, glucuronidase β, mucin 3, and plasminogen activator inhibitor 1) map to a more proximal region than 7q36, i.e. between 7cen and 7q22 (the regional localization of the seventh gene, epimorphin, is not known). Thus it appears that PLOD3 defines a new region of homology between rat chromosome 12 and human chromosome 7, located near the telomeric region of 7q. The intermediate region (7q31.1 to 7q35) contains genes whose rat homologs map on chromosomes others than chromosome 12 (most are on rat chromosome 4) (18–20). These observations provide a new and clear example of a syntenic gap split into distinct conserved segments disrupted by a region of homology to a different chromosome (see, for instance, Ref. 21).

Expression of the Gene in Different Human Tissues—Expression of LH3 in various human tissues was determined by multiple tissue Northern blot (Fig. 4), where the probe for LH3 hybridized with an mRNA 2.75 kb in size. A strong hybridization signal was obtained in heart, placenta, and pancreas, whereas the signal was much fainter in brain, lung, liver, skeletal muscle, and kidney. The data furthermore indicate that the expression is highly regulated.

Comparison of the size of the LH3 mRNA with that of LH1 and LH2 (9, 10) indicates that the LH3 mRNA is smaller than the mRNA for LH1 (3.2 kb) or LH2 (4.2 kb). Comparison of expression levels of lysyl hydroxylases in different human tissues indicates that heart, placenta, and pancreas are the tis-
sues that have high expression levels for lysyl hydroxylase 2 (9)
and 3. The expression of LH1 differs from the others, the
constitutive expression of the gene in variety of tissues agrees
with the housekeeping gene-like structure of its 5′-flanking
region (22, 23).

Comparison of the Amino Acid Sequences of the Three Hu-
man Lysyl Hydroxylases—The amino acid sequence of the novel
human lysyl hydroxylase is compared with human LH1 and
human LH2 (Fig. 1). The length of the translated product of
LH3 is 738 amino acids, which is one amino acid longer than
the length of LH2. The overall identity in amino acid sequences
between LH3 and LH1 or between LH3 and LH2 was 59%. The
identity between all lysyl hydroxylases is 47%. The similarity
values between the isoforms were 83 and 85%, respectively.
Over 80% identity in amino acid sequences (more than 10
amino acids) between all three lysyl hydroxylases was found in
the sequence covering the amino acids 160–171, 217–233, 254–
264, 420–445, 448–460, 559–570, 577–588, 602–616, and
727–738 of lysyl hydroxylase 3. The data indicate that the
carboxy-terminal end of the molecule is highly conserved in all
three lysyl hydroxylases; and in addition, there are also regions
in the middle part of the molecules, which are identical in all of
them. Less identity is seen in the region of amino acids 271–356
(32% identity between all isoforms).

A search for conserved residues within the sequences of 2-
oxoglutarate dioxygenases and a related dioxygenase,
isopenicillin N synthase, suggested that two histidine-contain-
ing motifs in the carboxy-terminal end of the molecule may
function as ferrous binding ligands (24, 25). Site-directed mu-
tageneis of LH1 (26) confirms the importance of the histidines
for the function of the enzyme. It is interesting to find that
these histidines are conserved in all lysyl hydroxylase, His-
667, His-668, His-711, His-717, His-719, in the sequence of
LH3. Furthermore, two aspartate residues, Asp-669 and Asp-
685, causing dramatic reduction in enzyme activity in the LH1
mutational analysis (26), are also conserved in all three hy-
droxylases. Seven out of eight cysteine residues, conserved
between LH1 and LH2, are also conserved in the sequence of
LH3.

Glycosylation of asparagine residues is probably required for
the enzymatic activity of LH1 and LH2 (9, 26, 27). Comparison
of amino acids between three hydroxylases indicate that there
is no potential attachment site for asparagine-linked oligosac-
arides, which is conserved in all three enzymes. Mutation
analysis of LH1 indicated that Asn-209 and Asn-697 are re-
quired for LH1 activity (26); these amino acids have been
changed to serine and aspartate residues, respectively, in the
novel lysyl hydroxylase. Asn-63 may function as an attachment
site for mannose-rich oligosaccharides in LH2 and LH3; this
is not, however, a potential glycosylation site for LH1.

Conclusions—Characterization of a novel isoform for lysyl
hydroxylase (LH3) indicates that there are at least three genes
for lysyl hydroxylase activity in cells. The gene for the LH3
isoform (PLOD3) is located on human chromosome 7 (7q36) and
on rat chromosome 12. The isoform has a high identity with the
lystyl hydroxylases characterized earlier (9, 10) and all isoforms
hydroxylate lysyl residues in collagenous sequences in a non-
triple helical conformation. The expression patterns of LH2
and LH3 resemble each other but differ from that of LH1. The
discovery of multiple isoforms for lysyl hydroxylase is signifi-
cant because they provide tools for further investigation of
Ehlers-Danlos syndrome type VI, a disease associated with
gene defects in LH1 (28–33). Several questions remain unan-
swered, such as: why hydroxylation of collagenous lysyl resi-
dues is normal in cartilage of Ehlers-Danlos syndrome type VI
patients, although it is markedly reduced in skin (34); why
mRNA levels for LH1 do not correlate to the lysyl hydroxylase
activity measured in cells; and why removal of the most con-
served portion of LH1 does not totally abolish the enzyme
activity within cells (28). The comparison of these isoforms in
their substrate specificity, distribution in different tissues/
cells, activity at different developmental stages, and under
conditions in which the genes have been mutated or knocked
out will provide more information about the function of the
different isoforms in cells.

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REFERENCES

1. Kivirikko, K. I., Myllyla, R. & Pihlajaniemi, T. (1991) in Post-translational
Modifications of Proteins (Crabbe, M. J. C., and Harding, J., eds) pp. 1–51,
CRC Press, Boca Raton, FL
2. Kielty, C. M., Hopkinson, I. & Grant, M. E. (1993) in Connective Tissue and Its
Heritable Disorders (Royce, P. M., and Steinmann, B., eds) pp. 103–147,
Wiley-Liss, Inc., New York
3. Kivirikko, K. I. (1993) Ann. Med. 25, 113–126
4. Prockop, D. J. & Kivirikko, K. I. (1995) Annu. Rev. Biochem. 64, 405–434
5. Reichenberger, E. & Olsen, B. R. (1996) Cell Dev. Biol. 7, 631–638
6. Vogel, W., Gish, G. D., Alves, F. & Pawson, T. (1997) Mol. Cell 1, 13–23
7. Shirvastava, A., Radziejewski, C., Campbell, E., Kowac, L., McGlynn, M., Ryan, T. E., Davis, S., Goldfarb, M. P., Glass, D. J., Lemke, G. &
Vance, L. J. (1996) J. Biol. Chem. 271, 25–34
8. Barnes, M. J., Constable, B. J., Morton, L. F. & Royce, P. M. (1974) Biochem.
J. 139, 461–468
9. Valtavaara, M., Papponen, H., Perttila, A.-M., Hiltunen, K., Helander, H. &
Myllyla, R. (1997) J. Biol. Chem. 272, 6831–6834
10. Hautala, T., Byers, M. G., Eddy, L. R., Shows, T. B., Kivirikko, K. I. & Myllyla,
R. (1992) Genomics 13, 62–69
11. Van Roy, N., Laureys, G., Versteeg, R., Opdenakker, G. & Spelman, F. (1993)
Genomics 17, 71–78
12. Szpirer, C., Szpirer, J., Riviere, M., Vanvooren, P., Valtavaara, M. & Myllyla,
R. (1997) Mamm. Genome 8, 707–708
13. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Smith, J. A., Seidman,
J. G. & Struhl, K. (1989) Current Protocols in Molecular Biology, John Wiley
& Sons, New York
14. Kivirikko, K. I. & Myllyla, R. (1982) Methods Enzymol. 82, 245–304
15. Szpirer, C., Szpirer, J., Riviere, M., Hajnal, A., Kies, M., Scharm, B. &
Schafer, R. (1996) Mamm. Genome 7, 701–703
16. Pinkel, D., Landegent, J., Collins, C., Pucec, J., Segraves, R., Lucas, J. &
Gray, J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 9138–9142
17. Stephanoa, E., Tisarat, F., Dusserti, N., Lovanna, J., Szpirer, J. & Szpirer, C.
(1996) Cyanogen. Cell Genet. 72, 83–85
18. Szpirer, C., Szpirer, J., Klinga-Levan, K., Stahl, F. & Levan, G. (1996) Folia
Biol. (Praha) 42, 175–226
19. RATMAP (1997) The Rat Genome Data Base, University of Gothenburg,
Gothenburg, Sweden. World Wide Web. URL: http://ratmap.gen.gu.se
(December 1997)
20. GDB (1997) Genome Database (data base on line), Johns Hopkins University,
Baltimore, MD. World Wide Web. URL: http://gdbwww.gdb.org (December
1997)
21. Watkins-Chow, D. E., Buckwalter, M. S., Newhouse, M. M., Lassie, A. C.,
Brinkmeier, M. L. & Camper, S. A. (1997) Genomics 40, 114–122
22. Heikkinen, J., Hautala, T., Kivirikko, K. I. & Myllyla, R. (1994) Genomics 24,
464–471

2 J. Heikkinen, B. Pousi, and R. Myllyla, unpublished data.
23. Yeowell, H. N., Ha, V., Clark, L. C., Marshall, M. K. & Pinnell, S. R. (1994) J. Invest. Dermatol. **102**, 382–384
24. Myllyla¨, R., Günzler, V., Kivirikko, K. I. & Kaska, D. (1992) Biochem. J. **286**, 923–927
25. Roach, P. L., Clifton, I. J., Fulop, V., Harlos, K., Barton, G. J., Hadju, J., Andersson, I., Schutfield, C. J. & Baldwin, J. E. (1995) Nature **375**, 706–704
26. Pirskanen, A., Kaimio, A.-M., Myllyla, R. & Kivirikko, K. I. (1996) J. Biol. Chem. **271**, 9398–9402
27. Myllyla, R., Pajunen, L. & Kivirikko, K. I. (1988) Biochem. J. **253**, 489–496
28. Hyland, J., Ala-Kokko, L., Boyce, P., Steinmann, B., Kivirikko, K. I. & Myllyla, R. (1992) Nat. Genet. **2**, 228–231
29. Ha, V. T., Marshall, M. K., Elsas, L. J., Pinnell, S. R. & Yeowell, H. N. (1994) J. Clin. Invest. **93**, 1716–1721
30. Heikkinen, J., Toppinen, T., Yeowell, H., Krieg, T., Steinmann, B., Kivirikko, K. I. & Myllyla, R. (1997) Am. J. Hum. Genet. **60**, 48–56
31. Pousi, B., Hautala, T., Hyland, J. C., Schröter, J., Eekes, B., Kivirikko, K. I. & Myllyla, R. (1996) Hum. Mutat. **11**, 55–61
32. Yeowell, H. N. & Walker, L. (1997) Proc. Assoc. Am. Physicians **109**, 383–396
33. Pajunen, L., Suokas, M., Hautala, T., Kellokumpu, S., Tebbe, B., Kivirikko, K. I. & Myllyla, R. (1998) DNA Cell Biol. **17**, 117–123
34. Pinnell, S. R., Krane, S. M., Kenzora, J. E. & Glimcher, M. J. (1972) N. Engl. J. Med. **286**, 1013–1020