The proteolytic processing site of the precursor of lysyl oxidase

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The precise cleavage site of the N-terminal propeptide region of the precursor of lysyl oxidase has not yet been established, due to N-terminal blocking of the mature protein. Using a combination of peptide fragmentation, amino acid sequencing, time-of-flight m.s. and partial chemical unblocking procedures, it is shown that the mature form of lysyl oxidase begins at residue Asp-169 of the precursor protein (numbered according to the

human sequence). The cleavage site is 28 residues to the C-terminal side of the site previously suggested on the basis of apparent molecular mass by SDS/PAGE, with the consequence that the two putative, N-linked glycosylation sites and the position of the Arg/Gln sequence polymorphism are now all in the precursor region.

INTRODUCTION

Covalent cross-linking in collagens and elastin is essential for the biological functions of these extracellular matrix proteins (Reiser et al., 1992). The nature and extent of the cross-linking plays an important role in connective tissue turnover. All cross-links in elastin and most cross-links in collagens (Hulmes, 1992; van der Rest and Garrone, 1991) are formed spontaneously after oxidative deamination of specific lysine (or hydroxylysine) residues by lysyl oxidase (Kagan, 1986; Kagan and Trackman, 1991).

Lysyl oxidase (EC 1.4.3.13), has been characterized from a variety of species (Kagan, 1986; Kagan and Trackman, 1991; Shackleton and Hulmes, 1990; Cronshaw et al., 1993; Romero-Chapman et al., 1991). In most cases, the enzyme has a relative molecular mass (M_r), by SDS/PAGE, of approximately 32000. Lysyl oxidase is copper-dependent and also requires molecular oxygen and an unknown carbonyl cofactor, possibly topaquinone (Janes et al., 1990, 1992), for enzyme activity. Several variants of lysyl oxidase have been identified, but the molecular basis of this variation is not understood (Sullivan and Kagan, 1982). Recent studies have shown that the enzyme is synthesized in precursor form (Wakasaki and Ooshima, 1990; Romero-Chapman et al., 1991; Trackman et al., 1992), with an approx. M_r of 50000.

Direct protein sequencing has given only limited information on the amino acid sequence of lysyl oxidase. The N-terminus of the protein is chemically blocked (Sullivan and Kagan, 1982), though sequences for a number of internal peptide fragments are available (Trackman et al., 1990; Cronshaw et al., 1993). Complete sequence information has come from cDNA analysis, and sequences coding for the lysyl oxidase precursor in rat aorta (Trackman et al., 1990, 1991), human placenta (Hamalainen et al., 1991), chick aorta (Wu et al., 1992) and mouse (Mariani et al., 1992) have been described. The predicted sequence of the human lysyl oxidase precursor is 417 amino acid residues in length, with a theoretical M_r of 46944. Sequences derived from cDNA analysis provide no information on post-translational modifications (e.g. formation of topaquinone) or precursor proteolytic-processing sites. C-terminal peptide sequence data have shown that conversion of the precursor into the mature form of lysyl oxidase occurs by proteolytic removal of an N-terminal propeptide (Trackman et al., 1990, 1991). From estimates of the M_r of lysyl oxidase by SDS/PAGE, it has been suggested that the cleavage site is between residues 134 and 135 of the rat precursor sequence (Trackman et al., 1990, 1991), equivalent to residues 140 and 141 of the human precursor

(Hamalainen et al., 1991). Here we show, by amino acid sequencing and precise mass determinations of peptide fragments, that cleavage is most likely to occur at the Gly-Asp bond between residues 168 and 169 (numbered according to the human sequence).

MATERIALS AND METHODS

Reagents

CNBr and endoproteinase Asp-N (type XXXII from *Pseudomonas fragi* mutant) were obtained from Sigma, Poole, Dorset, U.K. Endoproteinase Lys-C (from *Lysobacter enzymogenes*) was purchased from Boehringer, Lewes, East Sussex, U.K. All other reagents were from BDH/Merck, Poole, Dorset, U.K.

Preparation of porcine lysyl oxidase and peptide fragments

Lysyl oxidase variant L3 was prepared from porcine skin, as described (Shackleton and Hulmes, 1990; Cronshaw et al., 1993). Peptide fragments were prepared using CNBr (Cronshaw et al., 1993), endoproteinase Asp-N, endoproteinase Lys-C and mild acid cleavage, as described below. All analyses were carried out on enzyme variant L3.

Endoproteinase Asp-N cleaves on the N-terminal side of aspartic acid (and sometimes glutamic acid) residues (Drapeau, 1980). The N-terminal CNBr peptide of lysyl oxidase (approx. 1.5 mg, 300 pmol) was dissolved in 250 μ l of 100 mM ammonium formate, pH 8.5. To this, 5 μ l of endoproteinase Asp-N (0.1 μ g) was added, followed by incubation at 37 °C for 2 h. After drying, the digest was redissolved in 250 μ l of PBU buffer (6 M urea containing 10 mM sodium phosphate, pH 7.8) and 200 μ l was loaded onto an Aquapore RP-300 C₈ reverse-phase cartridge (7 μ m particle size; 2.1 mm $\times$ 30 mm). The column was washed with aqueous 0.1 % trifluoroacetic acid and peptides were eluted, at 100 μ l/min, with a linear gradient of 0–70 % acetonitrile in 0.8 % trifluoroacetic acid (Cronshaw et al., 1993).

Lysyl oxidase was oxidized with performic acid (Hirs, 1967) as follows. The performic acid solution was prepared by adding 25 μ l of 30 % (v/v) H₂O₂ to 475 μ l of concentrated formic acid and allowing to stand for 2 h at 4 °C. The mixture was then incubated at room temperature for 2 h, followed by 30 min at 4 °C. Lysyl oxidase (approx. 500 pmol) was then dissolved in 50 μ l of concentrated formic acid, cooled to 4 °C and incubated with 100 μ l of ice-cold performic acid for 2 h at 4 °C. After

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dilution by the addition of 350 μ l of H₂O, followed by drying in a Savant Speed Vac vacuum concentrator, the undigested, oxidized lysyl oxidase was re-purified by reverse-phase chromatography, as above (Cronshaw et al., 1993).

The Asp-Pro bond is particularly susceptible to acid cleavage (Ambler et al., 1984). As the occurrence of this bond is relatively rare, proteins can be cleaved into a limited number of peptides. There are two Asp-Pro bonds in human and rat lysyl oxidase (Hamalainen et al., 1991; Trackman et al., 1991). Cleavage was achieved by incubation in 5% (v/v) formic acid at 60 °C for 16 h (Ambler et al., 1984).

Chemical unblocking of N-termini blocked with N-formyl groups

To examine the possibility that lysyl oxidase was blocked by N-terminal formylation, two unblocking procedures were used. In the first method, approximately 200 pmol of lysyl oxidase (variant L3) was dissolved in 1 ml of 0.5 M HCl in methanol and left at room temperature for 48 h (Sheehan and Yang, 1957). The sample was dried and redissolved in 0.1% (v/v) aqueous trifluoroacetic acid and loaded onto the sequencer. This procedure failed to unblock the sample. In the second method, approximately 200 pmol of L3 was loaded onto a glass-fibre filter and exposed to 3 M HCl at 100 °C in the vapour phase, in a borosilicate glass vial (27 mm $\times$ 70 mm), for 15 min (Tsong and Fraenkel-Conrat, 1965). This method also failed to unblock the sample.

Mass determination and amino acid sequencing

Precise mass determinations were made with a laser desorption ion-source coupled to a time-of-flight mass analyser (Lasermat, FinniganMAT Ltd., Hemel Hempstead, U.K.) as described (Cronshaw et al., 1993). All samples were dissolved in 0.1% (v/v) trifluoroacetic acid in acetonitrile. Mixtures of 0.1–0.2 μ l of sample (1–2 pmol of peptides; 100 pmol of intact protein) and 0.5 μ l of matrix (approx. 50 mM sinapinic acid in 30% (v/v) 0.1% trifluoroacetic acid/70% acetonitrile) were dried onto a stainless-steel target before introduction into the Lasermat instrument (Hillenkamp and Karas, 1990). Mass data for intact lysyl oxidase were calibrated using carbonic anhydrase (M_r = 29024) as external standard. Substance P (synthetic porcine; M_r = 1347.7) and renin substrate (porcine; M_r = 1759) were used as external standards to measure the masses of lysyl oxidase CNBr and endoproteinase Asp-N (AN) peptides. Each mass determination was the result of multiple (usually 11) firings of the laser. The experimental error specified by the manufacturer is $\pm 0.1\%$. The mass of the peptide CN3AN3 (M_r 1272) was determined experimentally for seven such multiple firings, and the S.D. was found to be 1.2 (0.094%).

Purified peptides were sequenced on an Applied Biosystems 477A pulsed-liquid microsequencer (Hayes et al., 1989).

RESULTS

Porcine lysyl oxidase was found to be N-terminally blocked, as also reported elsewhere for bovine lysyl oxidase (Sullivan and Kagan, 1982). Precise mass determination of variant L3, using the Lasermat mass analyser (Figure 1a), gave an M_r of 29377, compared with M_r values in the range 32000–34000 determined by SDS/PAGE (Kagan, 1986; Shackleton and Hulmes, 1990). The minor peak in Figure 1(a) corresponds to the doubly charged form of lysyl oxidase.

Lysyl oxidase was treated with CNBr (CN) and the resulting CNBr peptides were separated by reverse-phase h.p.l.c. Three peaks were obtained (CN1, CN2 and CN3) as described by

Figure 1 Spectra from the Lasermat mass analyser

(a) Approx. 100 pmol of intact lysyl oxidase variant L3, showing singly and doubly charged forms of the protein. The MH^+ ion gives the strongest signal with this instrument. (b) Approx. 1 pmol of L3 CNBr digest. Peaks are identified by the first three residues of the corresponding peptide sequence. Unlabelled peaks correspond to multiply charged forms of the peptides. (c) Approx. 1 pmol of L3 CNBr peak CN3, showing some contamination by peak CN2 (peptide YNL). (d) Approx. 1 pmol of peak CN3AN3 from the Asp-N digest of CN3 (Figure 2), showing slight contamination of the major, blocked N-terminal peptide (M_r = 1272) with peptide DPY (M_r = 1679). Indicated M_s are for the MH^+ ion; observed M_r values in Tables 1 and 2 have been reduced by 1 to take this into account.

Table 1 Mass analysis of porcine lysyl oxidase variant L3 and its CNBr peptides

Sequence position	Peak*	Theoretical $M_r^\dagger$	Lasermat $M_r^\ddagger$
169–417	—	29034.6§	29377
169–231	CN3	7563.0§	7622
232–233	n/o	206.2	n/o
234–298	CN2	7840.6	7821
299–417	CN1	13388.6	13742

* Peaks correspond to those described by Cronshaw et al. (1993).

† Theoretical M_s are based on porcine sequence data but identity with the human sequence (Hamalainen et al., 1991) has been assumed where the porcine sequence is not known (see Figure 4). Average M_r data were used. The mass loss due to conversion of methionine to homoserine during CNBr cleavage has been taken into account.

‡ Errors are $\pm 0.1\%$ (see Materials and methods section).

§ A putative N-terminal blocking group is not included in the theoretical M_r .

|| n/o = Not observed (M_r too small to be retained by reverse-phase h.p.l.c. or detected on the Lasermat mass analyser).

Cronshaw et al. (1993). Mass analyses of a crude CNBr digest and of h.p.l.c.-purified peak CN3, are shown in Figures 1(b) and 1(c) respectively. Peak CN3 appeared as a doublet with M_r values of 7622 and 7821. The mass of the larger peptide was identical

Figure 2 Reverse-phase h.p.l.c. of peptides released by endoproteinase Asp-N sub-digestion of CNBr peptide 3

The peaks (1–6) were identified (first three residues shown) as follows: (CN3AN1) peptide DDN (initial yield = 75 pmol, repetitive yield = 94% based on tyrosine); (CN3AN2) peptide DPY (initial yield = 40 pmol, repetitive yield = 88% based on tyrosine); (CN3AN3) peptide DPY (initial yield = 10 pmol, repetitive yield = 92% based on proline) and N-terminally blocked peptide; (CN3AN4) peptide ERP (initial yield = 79 pmol, repetitive yield = 94% based on tyrosine); (CN3AN5) endoproteinase Asp-N; (CN3AN6) peptide CN3 undigested by endoproteinase Asp-N. The arrow indicates the point of sample injection.

with that found for h.p.l.c.-purified peak CN2, which gave a 56 residue sequence beginning YNL. No other sequence was given by peak CN3, and hence we conclude that peak CN3 was incompletely resolved from peak CN2 and that the peptide with M_r 7622 contained the blocked N-terminus. Peak CN1 gave an M_r of 13742 and began with a 28 residue sequence beginning DEF. Peptides beginning YNL and DEF were uniquely identified by comparison with cDNA-derived sequence data (Figure 4). Observed and predicted M_r values are compared in Table 1. The M_r values are consistent (within 60) with the N-terminal sequence beginning at residue 169 of the precursor (numbered according to the human sequence).

In order to gain further sequence information, the blocked, N-terminal CNBr peptide (peak CN3) was sub-digested with endoproteinase Asp-N (AN). Peptides were purified (Figure 2; peaks CN3AN1, CN3AN2, etc.), sequenced (where possible) and identified by comparison with cDNA-derived sequences (Figure 4) and by mass analysis (Table 2). Four large peptides were expected and identified (first three residues shown) as follows: (CN3AN1) peptide DDN, (CN3AN2) peptide DPY, (CN3AN3) N-terminal peptide and (CN3AN4) peptide ERP. The peptides DTY and DLVP/A (Figure 4) were not found, probably as they were insufficiently hydrophobic to be retained by the reverse-phase column. The initial yield for the DPY peptide (peak CN3AN2, Figure 2) was 40 pmol. Peak CN3AN3 (Figure 2) also gave a sequence beginning with DPY but with an initial yield of only 10 pmol, which was sequenced through to completion. We conclude that the major component of peak CN3AN3 was N-terminally blocked, but there was some contamination with peak CN3AN2. After complete sequencing of the minor peptide (DPY) in peak CN3AN3, the glass-fibre filter with the retained blocked peptide was removed from the sequencer, exposed to 3 M HCl vapour at 100 °C for 15 min (Tsung and Fraenkel-Conrat, 1965) and re-sequenced. A new sequence was observed (initial yield approx. 5 pmol) beginning DDP, which we deduce to be the N-

Table 2 Mass analysis of the N-terminal CNBr fragment after sub-digestion with endoproteinase Asp-N

Sequence position	Peak*	Theoretical M_r †	Laserstat M_r ‡
169–178	CN3AN3	1261.3§	1272
179–187	CN3AN1	1226.2	1245
188–190	n/o	397.4	—
191–213	CN3AN4	2674.9	2675
214–217	n/o	416.5	—
218–231	CN3AN2	1676.9	1678

* From Figure 2.

† Theoretical masses are based on porcine sequence data but identity with the human sequence (Hamalainen et al., 1991) has been assumed where the porcine sequence is not known (see Figure 4). Average M_r data were used.

‡ Errors are $\pm 0.1\%$ (see Materials and methods section).

§ A putative N-terminal blocking group is not included in the theoretical M_r .

|| n/o = Not observed.

Figure 3 Reverse-phase h.p.l.c. of peptides released by mild acid cleavage of lysyl oxidase variant L3

The peaks were identified (first three residues shown) as follows: (MA1) unknown; (MA2) peptide PYN (initial yield = 48 pmol, repetitive yield = 87% based on tyrosine); (MA3) peptide PYY (initial yield = 25 pmol, repetitive yield = 90% based on tyrosine); and (MA4) intact lysyl oxidase. The arrow indicates the point of sample injection.

terminal sequence of the previously blocked peptide (Figure 4). Mass analysis of peak CN3AN3 (Figure 1d) confirmed that the major peptide (M_r 1272) was contaminated by a peptide of M_r 1678 (DPY). Both the sequence data and the M_r value (Table 2) suggest that the sequence of the major peptide in peak CN3AN3, and hence the N-terminus of mature lysyl oxidase, begins at aspartate residue 169 (numbered according to the human lysyl oxidase precursor sequence; Figure 4).

As the blocked N-terminal peptide was sensitive to HCl vapour we decided to see if the blocking group could be removed by treatment of the intact enzyme with performic acid. The results showed the same N-terminal sequence beginning DDP (initial yield = 31 pmol) when 1 nmol of performic acid-oxidized lysyl oxidase variant L3 was applied to the sequencer (Figure 4).

Finally, to confirm and extend the above sequence analysis, the peptides generated by mild acid (MA) hydrolysis (see

Figure 4 Comparison of cDNA-derived data with direct protein sequencing of porcine lysyl oxidase

cDNA-derived data for the entire C-terminal region of the chick, rat, mouse (*ras* recision gene), and human lysyl oxidase precursors are compared with protein sequencing data for porcine lysyl oxidase and its peptide fragments. Residues are numbered according to the sequence of the human lysyl oxidase precursor (Hamalainen et al., 1991). The putative cleavage site suggested by Trackman et al. (1990, 1991) is indicated by a small arrowhead, while the large arrowhead shows the cleavage site indicated by the present study. Key: rrg = *ras* recision gene; - = identical to the chick sequence; * = gap; ? = unidentified residue; + = sequence continues; ! = non-identity between the human and porcine enzymes.

Materials and methods section) of lysyl oxidase variant L3 were separated by reverse-phase h.p.l.c. (Figure 3; peaks MA1, MA2, etc.) and sequenced (Figure 4). With the exception of the sequences 188–190, 214–217 and 286–298 (Figure 4), protein sequencing has revealed the N-terminal 184 residues of mature porcine lysyl oxidase.

DISCUSSION

Tables 1 and 2 compare the observed M_r data for porcine lysyl oxidase and its peptide fragments with information from cDNA sequencing of the human lysyl oxidase precursor (Hamalainen et al., 1991; Mariani et al., 1992). The calculation of the theoretical masses assumes: (i) inter-species identity and (ii) lack of mass differences due to post-translational modifications. The first assumption is supported by the data in Figure 4, which shows only two amino acid differences (at positions 197 and 310) between the cDNA derived data for human lysyl oxidase (residues 169–417 of the precursor) and 159 residues of the N-terminal region of the porcine sequence determined by direct protein

sequencing. From comparison of cDNA-derived data, the C-terminal region is also highly conserved between the human, rat and mouse enzymes (Mariani et al., 1992). Comparison of the observed and theoretical masses for the mature enzyme (based on a precursor processing-site at residues 168–169) shows a difference in M_r of 342 (Table 1). This discrepancy is likely to reside in the C-terminal region of the protein, as a similar difference in M_r (353) was found for the C-terminal CNBr peptide (residues 299–417), a region for which relatively little protein sequencing information is available. The difference in M_r of 59 for the blocked N-terminal CNBr peptide may be partially accounted for by the additional M_r of the blocking group (11, see below) and possible sequence differences in residues 188–190 or 214–217. Residue 217 is Ala in the human sequence and Pro in other species (M_r difference 26; Figure 4). In addition, there may be a post-translational modification in the AspN peptide beginning DDN (residues 179–187; M_r difference 19) that was not detected by amino-acid sequencing. Similarly, sequence differences and modifications may account for the mass difference with CNBr peptide CN2 (residues 234–298), for which only 54 residues have been determined by protein sequencing (Table 1 and Figure 4).

It has been suggested that the N-terminal proteolytic cleavage site is at the Arg-134–Arg-135 bond in the rat precursor sequence (Trackman et al., 1990, 1991), to comply with the observed M_r of 32000 derived from SDS/PAGE. The human sequence has a six residue insertion shortly after the putative signal peptide cleavage site (Hamalainen et al., 1991; Mariani et al., 1992), so the equivalent bond in the human lysyl oxidase precursor is Ser-140–Arg-141. If precursor processing does occur at this site, then peptides beginning RAA (141–166; M_r = 2303) and VGD (167–231; M_r = 7689) would be expected in the CNBr digest. The smaller peptide was not observed and the M_r of the N-terminally blocked peptide was less than 7689. These observations, and the failure to expose an N-terminal peptide beginning VGD by CNBr cleavage, suggests that Met-166 is in the precursor region.

Table 2 summarizes M_r and sequence data for the endoproteinase Asp-N sub-digestion of the N-terminal CNBr peptide. The M_r data for DDP are consistent (within 11) with the N-terminal sequence beginning at Asp-169 (numbered according to the human precursor sequence). An N-terminus at Gly-168 or Val-167 would give additional M_r s of 57 or 156 respectively. Furthermore, while initially the N-terminal endoproteinase Asp-N peptide was blocked, brief exposure to 3 M HCl vapour revealed the sequence beginning DDP at residue 169. The same sequence was revealed after oxidation of intact lysyl oxidase with performic acid, and the sequence of the entire peptide was identical to its human, cDNA-derived counterpart. We conclude that the blocking group had been removed by these procedures and therefore that the N-terminus of the mature form of lysyl oxidase begins at Asp-169. We ascribe the apparent M_r of lysyl oxidase from SDS/PAGE (32000–34000) to anomalous migration; the accurate M_r is close to 29000 (Figure 1a).

It has previously been reported, using dansyl chloride to label the α -NH₂ group, that the N-terminus of bovine lysyl oxidase begins with an aspartate residue (Sullivan and Kagan, 1982). The N-terminus is usually blocked however (Trackman et al., 1990), so it is difficult to see how the α -NH₂ group can be dansylated, unless the blocking group is labile. Our results also indicate that the blocking group is relatively labile, since brief exposure to 3 M HCl vapour, or performic acid treatment, was sufficient to expose Asp-169.

The nature of the blocking group is unknown. Of the known blocking groups with additional M_r of less than 100, acetylation accounts for 80–90% of blocked proteins (Aitken, 1990; Arfin

Figure 5 Inter-species variability in the lysyl oxidase precursor

Residues are numbered from the N-terminus of the precursor of human lysyl oxidase, with sequences aligned for maximum identity. Variability is indicated by the number of different residues at each residue position, based on cDNA data from chick aorta (Wu et al., 1992), human placenta (Hamalainen et al., 1991), rat aorta (Trackman et al., 1990, 1991) and the murine *ras* recision gene (Mariani et al., 1992). Sequence deletions are counted as residue changes. A five point smoothing factor has been applied to the data. (A) First N-linked glycosylation site; (B) site of first chick insertion sequence; (C) site of second chick insertion sequence; (D) processing site (140–141) predicted by Trackman et al. (1990, 1991); (E) second N-linked glycosylation site; (F) restriction fragment length polymorphism (R/Q) site (Csiszar et al., 1993); and (G) proposed N-terminus of lysyl oxidase, i.e. residue D169 (Figure 4).

and Bradshaw, 1988; Driessen et al., 1985) but the additional M_r of an acetyl group (43) is too large. An additional possibility is carbamylation of the α -amino group (also $M_r = 43$; Means and Feeney, 1971), due to long-term storage in urea-containing buffers, but again the mass difference is too great. Two methods for unblocking proteins with N-formyl groups (additional M_r of 29) were used (Sheehan and Yang, 1957; Tsung and Fraenkel-Conrat, 1965). On the intact enzyme, both methods failed to unblock the N-terminus. On the Asp-N subdigested N-terminal CNBr peptide, however, the second method (brief exposure to HCl vapour) exposed the sequence beginning DDP. This suggests that the N-terminus may be blocked by N-formylation, though the observed M_r difference (11) is too small. Apart from acetyl or formyl groups at the N-terminus, the occurrence of cyclized glutamine residues (pyroglutamic acid) is one of the most common post-translational modifications (Podell and Abraham, 1978), but there are no glutamine residues in the region of the N-terminus. Finally, a wide range of proteins of different function and distribution are methylated at their N-termini (Stock et al., 1987). Monomethylated (additional M_r of 14; Stock et al., 1987), dimethylated ($M_r = 28$; Pettigrew and Smith, 1977) and trimethylated ($M_r = 42$; Henry et al., 1982) N-termini have been reported, but methylated amino acids are resistant to acid hydrolysis.

Though inter-species identity is strong in the region C-terminal to Asp-169, the precursor sequence N-terminal to this is relatively variable (Figure 5). There is an abrupt change in variability between N-terminal and C-terminal sequences, and unlike the previously suggested precursor processing site (Trackman et al., 1990, 1991), the change coincides with Asp-169. It appears that there has been strong evolutionary pressure to conserve the sequence of the mature form of lysyl oxidase. The processing site is unrelated to the exon-intron structure of the lysyl oxidase gene, as exon 1 codes for the first 210 amino acids of the precursor (Csiszar et al., 1993).

It is noteworthy that the N-terminal nine residues of lysyl oxidase (DDPYNPYKY) show 78% identity with a tyrosine-

rich region in bovine fibromodulin (residues 40–48; DDPYDPYPY; Oldberg et al., 1989). As both lysyl oxidase and fibromodulin interact with fibrillar collagens, this sequence may be involved in collagen binding. In fibromodulin the residue N-terminal to this sequence is tyrosine, unlike glycine at the cleavage site of the lysyl oxidase precursor.

There are two important consequences of the N-terminal sequence starting at Asp-169. First, the putative N-linked glycosylation sites (Asn-97 and Asn-144 in the human sequence, Trackman et al., 1992) are now both in the precursor region. This is consistent with the observed lack of N-linked glycosylation in the mature enzyme. Secondly, the recently described Arg/Gln polymorphism at residue 158 (Csiszar et al., 1993) is also in the precursor region, so the origin of the multiple ionic variants of lysyl oxidase (Kagan, 1986) remains unknown.

The nature of the proteinase that cleaves the lysyl oxidase precursor is unknown. An identical sequence (Gly–Asp) is cleaved during removal of the C-propeptides from procollagen III by procollagen C-proteinase (Kessler et al., 1986), which also cleaves a similar sequence (Ala–Asp) in procollagens I and II (Hojima et al., 1985). This suggests the possibility that the same enzyme may be involved in processing of both lysyl oxidase and collagen precursors.

We thank Dr. E. Kessler for discussions. This work was supported by the Arthritis and Rheumatism Council (project grant to D.J.S.H. and L.A.G.) and the Wellcome Trust (Research Leave Fellowship to D.J.S.H.). The Edinburgh WelMet protein characterization facility was established with grants from the Wellcome Trust, the Salvesen Trust, Heriot-Watt University and the University of Edinburgh.

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Received 4 July 1994/27 September 1994; accepted 3 October 1994