Expression of active, human lysyl oxidase in *Escherichia coli*

M. Ouzzine[a], A. Boyd[a], D.J.S. Hulmes[a,b,*]

[a]*Department of Biochemistry, University of Edinburgh, George Square, Edinburgh EH8 9XD, UK*
[b]*Institut de Biologie et Chimie des Protéines, 7 Passage du Vercors, 69367 Lyon cedex 07, France*

Received 25 September 1996; revised version received 28 October 1996

Abstract Lysyl oxidase (LO) is a copper amine oxidase of the extracellular matrix which initiates covalent cross-linking in collagens and elastin. Human LO was expressed in *Escherichia coli*. At 37°C, large amounts of protein were obtained, but in the form of insoluble aggregates. Lowering the growth temperature, and reducing the amount of inducer, resulted in the production of soluble LO, which was active on a [^{3}H]lysine-labeled elastin substrate. LO was also targeted to the periplasm as a fusion protein with the pelb signal peptide. The periplasmic enzyme was soluble, active and inhibited by β-aminopropionitrile. Production of the carbonyl co-factor is therefore not a limitation in the expression of active LO in bacteria.

Key words: Lysyl oxidase; Copper amine oxidase; Protein expression; Bacteria

1. Introduction

The formation of lysine- (or hydroxylysine-) derived covalent cross-links in collagens and elastin is essential for the structural integrity and function of connective tissues [1,2]. The enzyme that initiates cross-linking, lysyl oxidase (EC 1.4.3.13), is a copper-dependent amine oxidase of the extracellular matrix that oxidatively de-aminates the ε-amino groups of specific lysine (and hydroxylysine) residues, leading to the spontaneous formation of a number of bi-functional, tri-functional and tetra-functional crosslinks [3]. As the terminal enzymatic step in the biosynthetic pathway of collagens and elastin, lysyl oxidase (LO) is a potential target in the control of fibrotic disease. LO has also been implicated in tumor suppression, as the product of the *ras* recision gene after oncogenic transformation of mouse fibroblasts [4]. In addition, the enzyme has been shown to be a potent chemotactic agent for unstimulated human peripheral blood monocytes [5].

Lysyl oxidase (29 kDa) is secreted in precursor form (50 kDa), and conversion to the mature form of the enzyme occurs by proteolytic removal of an *N*-terminal propeptide [6]. We have recently identified [7] the propeptide cleavage site in porcine pro-LO as gly–asp, which corresponds to residues 168–169 in the human LO precursor. This sequence also corresponds to the *C*-terminal processing site of fibrillar collagen precursors by procollagen *C*-proteinase, the enzyme that has recently been shown to be the same as bone morphogenetic protein-1 (BMP-1) [8,9]. The sequence data suggest that BMP-1 may be required for processing of both pro-LO and the precursor forms of its collagen substrates [7], a suggestion that has recently received experimental support [10].

The nature of the carbonyl co-factor in lysyl oxidase has been the subject of much controversy [11]. Trihydroxyphenylalanine (topa) quinone has been shown to be the co-factor in a number of copper-dependent amine oxidases from *E. coli*, yeast, plants and mammals [11–13], where the presence of the Asn-Tyr-Asp consensus sequence appears to be sufficient for the spontaneous conversion of polypeptide bound tyrosine to topaquinone, in the presence of copper and oxygen [12]. This consensus sequence is not present in LO. Very recently, it has been demonstrated [14] that LO contains a novel quinone co-factor, designated lysine tyrosylquinone (LTQ), which is derived from the cross-linking of the ε-amino group of a peptidyl lysine with the modified side chain of a tyrosine residue.

Structure–function studies on LO using physical techniques (e.g. X-ray crystallography) have been hampered by the difficulty in obtaining adequate amounts of enzyme. The small amounts of enzyme that can be purified from tissues require the presence of denaturing agents (4–6 M urea) for efficient extraction. Enzyme activity is recovered after dilution/dialysis of tissue-derived enzyme into physiological buffers, but this also results in the formation of high molecular mass aggregates [15]. It is not known if this aggregation is physiologically significant or merely the result of partial, irreversible denaturation by urea. Modest amounts of enzymatically active, recombinant rat LO have recently been expressed in mammalian cells [16], but not in the amounts that would be required for crystallisation trials. Here we report the efficient expression of milligram amounts of active, human LO in *E. coli*, which paves the way for 3-dimensional structure–function studies of the enzyme and its interactions with collagen and elastin substrates. In addition, these results show that formation of the LTQ co-factor is not a limitation in the bacterial expression of this enzyme.

2. Materials and methods

2.1. Bacterial strains and plasmid construction

The *E. coli* strain JM109 (Promega) was used for transformation and propagation of plasmids, while the *E. coli* strain BL21(F$^-$ *ompT* r^-_B m^-_B; DE3; Novagen) was used for recombinant expression of LO. Both pET3d and pET20b expression vectors were from Novagen.

The cDNA encoding the mature form of LO was amplified by the polymerase chain reaction, using Vent DNA polymerase (New England Biolabs), from a human placenta cDNA library (Clontech). PCR primers (5′-CCATGGACGACCCTTACAACCCCTAC-3′ and 5′-GGATCCTCAATACGGTGAAATTGTGCAGCCT-3′) were designed to introduce unique *Nco*I and *Bam*HI restriction sites at the 5′ and 3′ ends of the gene, respectively. The amplified fragment (761 bp) was subcloned into the *Sma*I site of pGEM 3Z (Promega) to create the plasmid pGEM-LOm. Plasmid constructions were performed using established techniques [17] and cloning steps were verified by DNA sequencing [18].

For cytoplasmic expression of LO, the sequence encoding the mature form of the enzyme was isolated as a 761 bp *Nco*I–*Bam*HI fragment from pGEM-LOm and ligated into the *Nco*I–*Bam*HI sites of the

*Corresponding author. Fax: (33) 72-72-26-02.
E-mail: hulmes@ibcp.fr

PII S0014-5793(96)01323-3

pET3d expression vector [19] to create the construct pET3-LOm. For periplasmic expression of LO, the *NcoI–BamHI* fragment was ligated into the *NcoI–BamHI* opened pET20b expression vector, in frame with the sequence coding for the pelb signal peptide, to create the construct pET20-LO. In both expression constructs, the *LO* gene was under the control of the *T7* gene 10 promoter and ribosome binding site (Fig. 1).

2.2. Expression and preparation of recombinant proteins

E. coli BL21(DE3), transformed with the vector pET3-LOm (for intracellular expression) or pET20-LO (for periplasmic expression), was grown in 100 ml of LB medium containing ampicillin (50 μg/ml), at 37°C or 22°C, until an OD_{600} of 0.5–0.6 was reached. Isopropyl-β-D-thiogalactopyranoside (IPTG; 0.4 mM or 0.04 mM) was added and the incubation was continued for 3 h. Cells were harvested by centrifugation at 5000×*g* for 15 min at 4°C.

Prior to extraction, cells were resuspended in ice-cold 50 mM Tris-HCl, 1 mM phenylmethylsulfonyl fluoride, pH 8.0 (lysis buffer). Cell disruption was accomplished by sonication at 60% efficiency with a micro-tip-type sonicator (Sanyo). Lysates were then centrifuged at 5000×*g* for 10 min to remove cell debris and supernatants were further centrifuged at 12000×*g* for 20 min to precipitate inclusion bodies [20]. The inclusion bodies were homogenised in 6 ml of 50 mM Tris-HCl, 0.5% (v/v) Triton-X100, pH 8.0 (Triton wash) and incubated for 10 min at room temperature. The solution was then centrifuged at 12000×*g* for 15 min. The pellet (Triton-washed inclusion bodies) was solubilised in 10 ml of 20 mM Tris-HCl, 250 mM NaCl, 0.01% (v/v) Tween 80, 8 M urea, pH 8.5, and incubated at room temperature for 1 h, with gentle stirring. The solution was then diluted by addition of 9 volumes of 20 mM Tris-HCl, 250 mM NaCl, 0.01% (v/v) Tween 80, pH 8.5, and further incubated at room temperature for 2 h. Finally the solution was dialysed extensively against phosphate-buffered saline (PBS: 0.15 M NaCl, 0.1 M sodium phosphate, pH 7.2). Under these conditions LO precipitated selectively out of solution and could be recovered by centrifugation for 15 min at 12000×*g*. Preparation of periplasmic proteins was achieved by osmotic shock [21], followed by dialysis into PBS.

2.3. Immunological procedures and protein analysis

Rabbits were injected subcutaneously at multiple sites with a total of 100 μg of purified protein mixed 1:1 (v/v) with complete Freund's adjuvant (Sigma). Three booster injections were given at 4-week intervals with the same quantity of protein in incomplete Freund's adjuvant. Production of antibodies was checked by immunoblotting 8 days after each injection. The IgG fraction was isolated using caprilic acid and ammonium sulfate precipitation [22].

Protein concentrations were measured [23] using bovine serum albumin as standard. SDS-PAGE was performed essentially as described [24], followed by staining with Coomassie Brilliant Blue. Immunoblotting was carried out on Immobilon-P membrane (Millipore) by semi-dry electrotransfer (Pharmacia). Immunological detection was accomplished using anti-LO IgG and immunopurified anti-rabbit IgG alkaline phosphatase conjugates (Pierce).

2.4. Enzyme assay and purification of porcine lysyl oxidase

Enzyme activity was assayed by ultrafiltration [25] using an elastin substrate prepared as described [26] from chick aorta cultured for 24 h in the presence of L-[4,5-^{3}H]lysine. For each assay, 100 μl of [^{3}H]elastin (300000 dpm) was incubated for 16 h at 35°C with 700 μl of assay buffer (0.1 M $Na_2B_4O_7$, 0.15 M NaCl, pH 8.0) and 100 μl of enzyme or buffer control. The reaction was stopped by the addition of 100 μl of 50% (w/v) trichloroacetic acid, followed by centrifugation (5 min at 12000×*g*) and ultrafiltration of the supernatant [25]. Assays were also carried out in the presence of 50 μg/ml β-aminopropionitrile (BAPN), a specific inhibitor of LO, after pre-incubation with the enzyme for 2 h prior to the addition of [^{3}H]elastin substrate. Results are expressed (Table 1) per milliliter of assay mixture, after subtraction of background activity (i.e. substrate and assay buffer alone).

Lysyl oxidase was purified from urea extracts of piglet skin [25,27] using a procedure originally devised for bovine aorta [28], modified by the use of a Superdex 75 gel permeation column in place of Sephacryl S-200 in the final purification step. The purified enzyme (specific activity: 1730 cpm/ml/μg protein), which appeared as a single band at 32 kDa by SDS-PAGE and Coomassie Blue staining, was stored at −20°C in 6 M urea, 16 mM sodium phosphate buffer, pH 7.7.

3. Results

The cDNA coding for the mature form of LO was amplified by PCR from a human placental cDNA library and cloned into the pGEM3Z plasmid. Sequencing of the DNA confirmed the data previously reported for human placental LO [29] with the exception of two nucleotides (TG to GT) at position 943–944 in the sequence encoding the full-length LO precursor, which resulted in a Trp to Val replacement at residue 315 in the protein sequence. Valine is also found in the corresponding position in the cDNA-derived, predicted amino acid sequences of human skin fibroblast [30], rat aorta [31], chick aorta [32] and mouse [33] LO. In addition, human genomic LO has also been reported to code for valine at residue 315 in the protein sequence [34].

The human LO cDNA (starting at Asp^{169}) was ligated into the pET3d expression vector under the control of the bacteriophage T7 promoter (Fig. 1). When cultured at 37°C, induction by 0.4 mM IPTG led to high-level expression of recombinant LO protein (Fig. 2), though essentially all in the form of insoluble inclusion bodies. Extracting the pellet with 0.5% (v/v) Triton X-100 solubilised small amounts of recombinant LO, though efficient extraction required the use of 8 M urea. When the urea extract of the Triton-washed inclusion bodies was dialysed against PBS, recombinant LO precipitated selectively out of solution, and could be recovered in essentially pure form in the pellet (Fig. 2).

The selective precipitation of recombinant protein from urea extracts of inclusion bodies proved to be a convenient method for the preparation of LO antigen for production of polyclonal antiserum in rabbits. As shown by Western blotting (Fig. 3), antibodies raised against the recombinant human protein specifically recognised tissue-derived porcine LO, where both proteins co-migrated with an apparent molecular mass of 32 kDa. LO was also the sole protein to be recognised in Western blots of crude, urea extracts from porcine skin (not shown).

Urea extracts of recombinant LO from inclusion bodies were devoid of LO activity (after dilution to lower the urea concentration) when assayed on a [4,5-^{3}H]lysine labeled elastin substrate. To avoid possible irreversible denaturation by

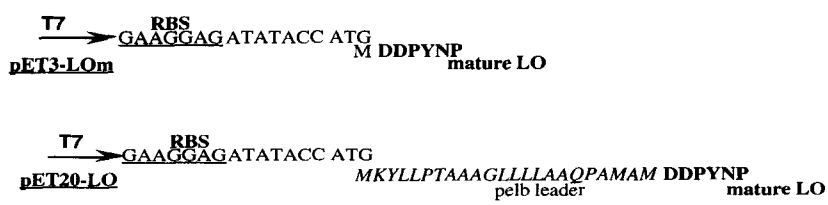

Fig. 1. Schematic representation of the expression cassette contained in the pET3-LOm and pET20-LO constructs, designed for expression of human LO in the cytoplasmic and periplasmic space, respectively. The ribosome binding site (RBS) sequence is underlined.

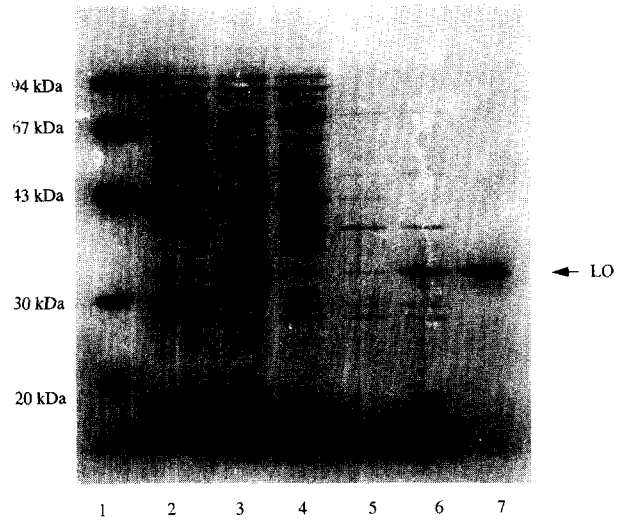

Fig. 2. Expression of recombinant LO at 37°C. Analysis by SDS-PAGE, followed by staining with Coomassie Blue. Lane 1: molecular weight markers. Lane 2: cell lysate before induction (100 μg protein). Lane 3: cell lysate after induction (100 μg protein). Lane 4: supernatant after high-speed centrifugation of the cell lysate shown in lane 2 (100 μg protein) showing that the recombinant LO is mostly present in inclusion bodies. Lane 5: supernatant after Triton washing of inclusion bodies (30 μg protein). Lane 6: Triton washed inclusion bodies (30 μg protein). Lane 7: recombinant LO purified from inclusion bodies by urea extraction and subsequent removal of urea by dialysis (20 μg protein).

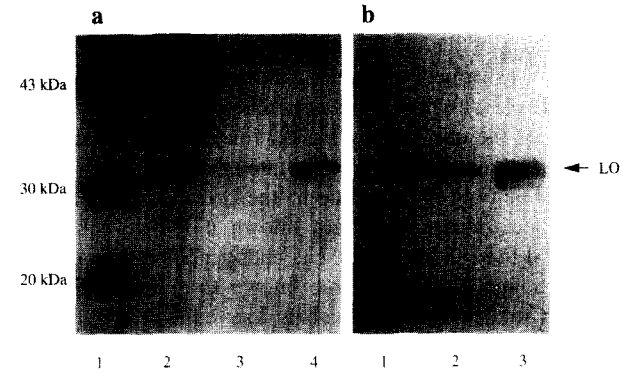

Fig. 3. Recognition of porcine skin LO by antibodies raised against recombinant human LO. (a) SDS-PAGE, Coomassie Blue staining. Lane 1: molecular weight markers. Lane 2: partially purified porcine skin LO (active eluate from the Cibacron Blue column; 70 μg). Lane 3: purified porcine LO (5 μg). Lane 4: recombinant human LO (10 μg). (b) Immunoblot, probed with antibodies to human recombinant LO. Lanes 1–3 are duplicate samples to those analysed in lanes 2–4 of part (a), respectively.

urea extraction, attempts were made to increase the yield of soluble, recombinant enzyme. It was found that lowering the incubation temperature during bacterial culture from 37°C to 22°C led to a marked increase in the amount of soluble LO (Fig. 4). A further, small increase in solubility was found after lowering the concentration of IPTG to 0.04 mM. In these conditions, up to 20% of total recombinant LO was recovered in the soluble fraction, which showed LO activity (Table 1). Activity was reduced to control levels (i.e. using extracts from cells transformed with non-recombinant vector) when pre-incubated (for 2 h) and assayed in the presence of 50 μg/ml BAPN. The maximum amount of soluble LO produced in this way was approximately 5 mg/l of culture.

In order to investigate the production of active, recombinant LO in a less reducing environment, an additional expression construct was created (pET20-LO; Fig. 1), in which the LO cDNA was ligated downstream of the pelb signal peptide coding region, in order to target the enzyme to the *E. coli* periplasmic space. Western blotting showed that the pelb–LO fusion protein was successfully expressed, translocated to the periplasm and processed to mature LO (Fig. 5). By densitometry, approximately 1% of total soluble periplasmic protein was recombinant LO, and the total yield of periplasmic, recombinant LO was approximately 0.3 mg/l of culture. Compared to the soluble intracellular expression systems (above), the periplasm extract showed increased specific LO activity on elastin (Table 1). Again, enzyme activity was reduced to background levels in the presence of BAPN.

4. Discussion

Using the cytoplasmic expression construct (pET-LO) we found that essentially all the recombinant LO was present in the form of inclusion bodies. However, when cultures were grown at 22°C and induced with reduced amounts of IPTG, both solubility and activity of recombinant LO increased. A similar temperature dependance of solubility has been reported for the P22 tailspike protein [35], diphtheria toxin [36], basic fibroblast growth factor [37] and human UDP-glucuronosyltransferase [38].

By SDS-PAGE, recombinant human LO co-migrates with purified porcine LO with an apparent molecular mass of 32 kDa, in agreement with the apparent molecular mass of tissue-derived LO purified from several species [1,27,39]. The predicted molecular mass of the mature form of human LO, based on cDNA data, is 29 034 Da. By mass spectrometry, the molecular mass of the porcine enzyme (29 377 Da) is similar, and both human and porcine LO are known to be highly homologous [7]. Therefore the anomalous migration of both

Table 1
Activity of recombinant human LO on a ^{3}H[lysine]-labeled elastin substrate

Plasmid	Net cpm/ml/100 μg protein (−BAPN)	Net cpm/ml/100 μg protein (+BAPN)
Intracellular expression		
pET3-LOm (construct)	1450 ± 143 (7)	727 ± 183 (7)
pET3d (vector alone)	727 ± 157 (3)	733 ± 177 (3)
Periplasmic expression		
pET20-LO (construct)	2210 ± 170 (7)	613 ± 223 (7)
pET20b (vector alone)	547 ± 130 (3)	487 ± 157 (3)

Activity was assayed in the presence and absence of 50 μg/ml BAPN. Errors are ± SEM (with number of assays shown in parentheses). Background activity (i.e. substrate and assay buffer alone) has been subtracted from the data.

recombinant and tissue-derived LO by SDS-PAGE is an intrinsic feature of the amino acid sequence of the protein.

E. coli transformed with vector alone showed low levels of activity (Table 1) which were not inhibitable by BAPN. The specificity of the ultrafiltration assay for LO [26] is dependent on successful inhibition by the specific inhibitor BAPN. Non-inhibitable activity may be due to the presence of an endogenous amine oxidase. It is not known if the *E. coli* strain BL21(DE3) used in this work has endogenous amine oxidase activity, though *E. coli* K12 are known to express a copper-dependent amine oxidase which is located in the periplasm and induced by phenylethylamine [40]. Successful expression of active, human LO in the experiments reported here is demonstrated by the observation of BAPN-inhibitable activity only in extracts of cells transformed with the LO encoding constructs.

The specific activity of the isolated, recombinant, periplasmic LO can be estimated from the data shown in Fig. 5 and Table 1. Assuming that 100 μg of crude extract contains 1 μg LO, the specific activity of the recombinant enzyme (after substraction of endogenous activity) is approximately 1660 cpm/ml/μg. This is similar to the specific activity of the tissue-derived enzyme from porcine skin (1730 cpm/ml/μg).

It is well established that purification of LO from various sources requires use of high concentration of urea for the extraction of the protein from the extracellular matrix [1]. After removal of urea, the enzyme polymerises [14], which suggests that this is the native form of the enzyme in physiological buffers. In the work presented here, we have demonstrated that human LO can be expressed in soluble form in *E. coli*. It is possible therefore that the aggregation of tissue-derived LO could be a consequence of partial denaturation by exposure to high concentrations of urea.

A number of copper-dependent amine oxidases have now been identified or overexpressed in *E. coli*. These include a monoamine oxidase of the *E. coli* strain K-12, whose 3-dimensional structure has recently been determined by X-ray crystallography [41], and both phenethylamine oxidase [42] and histamine oxidase [12] from *Arthrobacter globiformis*. Overexpression of the *A. globiformis* enzymes in the absence of copper, and subsequent activation by added copper, has demonstrated that, at least in vitro, production of the topaquinone co-factor is not dependent on an additional enzymatic step [43]. In LO, the organic co-factor has recently been shown to be lysine tyrosylquinone (LTQ) [14]. The successful expression of active human LO in *E. coli* reported here shows that formation of LTQ is not a limitation of using a bacterial expression system, and suggests that this modification may also occur in the absence of an additional enzyme. Production of milligram amounts of recombinant, human LO now paves the way for detailed, 3-dimensional structural analysis of this enzyme.

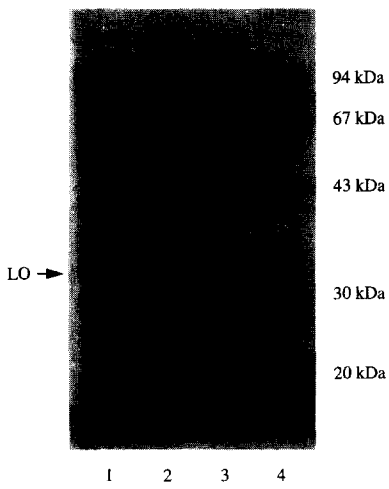

Fig. 4. Intracellular expression of recombinant human LO. Analysis by SDS-PAGE, followed by staining with Coomassie Blue. Lanes 1–3: supernatants after high-speed centrifugation of cell lysates (100 μg protein), after culture at (1) 37°C, induction with 0.4 mM IPTG (2) 22°C, induction with 0.4 mM IPTG (3) 22°C, induction with 0.04 mM IPTG. Lane d: molecular weight markers.

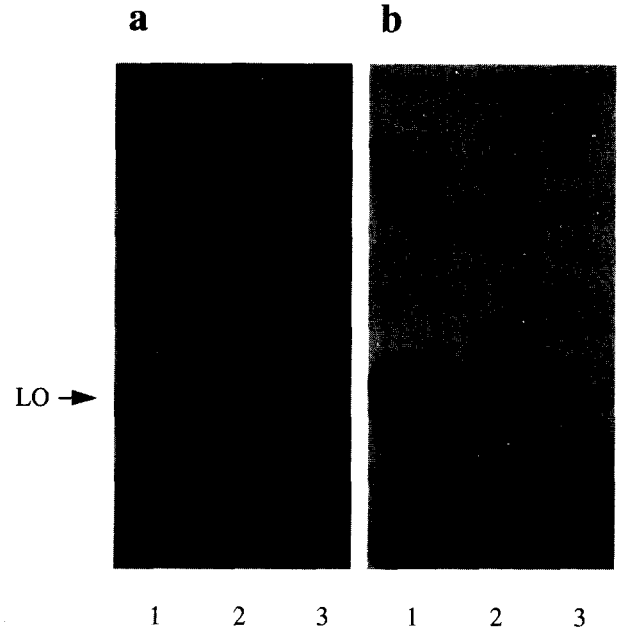

Fig. 5. Expression of recombinant, human LO in the bacterial periplasm. (a) SDS-PAGE, Coomassie Blue staining. Lane 1: recombinant LO purified from inclusion bodies produced in cells harbouring pET3-LOm (20 μg protein). Lane 2: periplasmic fraction of cells transformed with pET20-LOm, cultured at 22°C after induction with 0.04 mM IPTG (100 μg protein). Lane 3: periplasmic fraction before induction (100 μg protein). (b) Immunoblot. Lane 1: recombinant LO from inclusion bodies (20 μg protein). Lane 2: periplasmic fraction after induction (100 μg protein). Lane 3: perisplasmic fraction before induction (100 μg protein). Recombinant LO undergoes limited degradation during storage (lane 1).

Acknowledgements: We thank Euan Forbes for his contribution to the early part of this project and Bernard Kazimierczak for expert assistance. The work was funded by the Wellcome Trust.

References

[1] Kagan, H.M. (1986) in: Biology of the Extracellular Matrix (Mecham. R.P., ed.), Vol. I, Academic Press, Orlando, FL, pp. 321–398.
[2] Kagan, H.M. and Trackman, P.C. (1991) Am. J. Respir. Cell. Mol. Biol. 5, 206–210.
[3] Reiser, K., Mccormick, R.J. and Rucker, R.B. (1992) FASEB J. 6, 2439–2449.
[4] Kenyon, K., Contente, S., Trackman, P.C., Tang, J., Kagan, H.M. and Friedman, R.F. (1991) Science 253, 802.
[5] Lazarus, H.M., Cruikshank, W.W., Narasimhan, N., Kagan, H.M. and Center, D.M. (1994) Matrix Biol. 14, 727–731.

[6] Trackman, P.C., Bedell-Hogan, D., Tang, J. and Kagan, H.M. (1992) J. Biol. Chem. 267, 8666–8671.
[7] Cronshaw, A.D., Fothergill-Gilmore, L.A. and Hulmes, D.J.S. (1995) Biochem. J. 306, 279–284.
[8] Kessler, E., Takahara, K., Biniaminov, L., Brusel, M. and Greenspan, D.S. (1996) Science 271, 360–362.
[9] Li, S.W., Sieron, A.L., Fertala, A., Hojima, Y., Arnold, W.V. and Prockop, D.J. (1996) Proc. Natl. Acad. Sci. USA 93, 5127–5130.
[10] Panchenko, M.V., Stetler-Stevenson, W.G., Trubetskoy, O.V., Gacheru, S.N. and Kaga, H.M. (1996) J. Biol. Chem. 271, 7113–7119.
[11] Klinman, I.P. and Mu, D. (1994) Annu. Rev. Biochem. 63, 299–344.
[12] Choi, Y., Matsuzaki, R., Fukui, T., Shimizu, E., Yorifuji, T., Sato, H., Ozaki, Y. and Tanizawa, K. (1995) J. Biol. Chem. 270, 4712–4720.
[13] Cai, D. and Klinman, J.P. (1994) Biochemistry 33, 7647–7653.
[14] Wang, S.X., Mure, M., Medzihradszky, K.F., Burlingame, A.L., Brown, D.E., Dooley, D.M., Smith, A.J., Kagan, H.M. and Klinman, J.P. (1996) Science 273, 1078–1084.
[15] Kagan, H.M., Sullivan, K.A., Olsson, T.A. and Cronlund, A. (1979) Biochem. J. 177, 203–214.
[16] Kagan, H.M., Reddy, V.B., Panchenko, M.V., Nagan, N., Boak, A.M., Gacheru, S.N. and Thomas K.M. (1995) J. Cell. Biochem. 59, 329–338.
[17] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1987) in: Molecular Cloning, A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press.
[18] Sanger, F., Niklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA. 74, 5463–5467.
[19] Studier, F.W., Rosenberg, A.H., Dunn, J.J. and Dubendorf, J.W. (1990) Methods Enzymol. 185, 60–89.
[20] Marston, F.A.O. (1987) in: DNA Cloning, Vol. III. Practical Approach Series, pp. 59–88 (D. Rickwood and D.D. Hames, eds.).
[21] Randall, L.L. and Hardy, S.J.S. (1986) Cell 46, 921–928.
[22] Harlow, Ed. and Lane, D. (1988) in: Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press.
[23] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[24] Laemmli, U.K. (1970) Nature 227, 680–685.
[25] Cronshaw, A.D., Macbeath, J.R.E., Shackleton, D.R., Collins, J.F., Fothergill-Gilmore, L.A. and Hulmes, D.J.S. (1993) Matrix 13, 255–266.
[26] Shackleton, D.R. and Hulmes, D.J.S. (1990) Anal. Biochem. 185, 359–362.
[27] Shackleton, D.R. and Hulmes, D.J.S. (1990) Biochem. J. 266, 917–919.
[28] Williams, M.A. and Kagan, H.M. (1985) Anal. Biochem. 149, 430–437.
[29] Hamalainen, E.R., Jones, T.A., Sheer, D., Taskinen, K., Pihlajaniemi, T. and Kivirikko, K.I. (1991) Genomics 11, 508–516.
[30] Mariani, T.J., Trackman, P.C., Kagan, H.M., Eddy, R.L., Shows, T.B., Boyd, C.D. and Deak, S.B. (1992) Matrix 12, 242–248.
[31] Trackman, P.C., Pratt, A.M., Wolanski, A., Tang, S., Offner, G.D., Troxler, R.F. and Kagan, H.M. (1990) Biochemistry 29, 4863–4870.
[32] Wu, Y., Rich, C.B., Lincecum, J., Trackman, P.C., Kagan, H.M. and Foster, J.A. (1992) J. Biol. Chem. 267, 24199–24206.
[33] Contente, S., Kenyon, K., Rimoldi, D. and Friedman, R.M. (1990) Science 249, 796–798.
[34] Csiszar, K., Mariani, T.J., Gosin, J.S., Deak, S.B. and Boyd, C.D. (1993) Genomics 16, 401–406.
[35] Sturtevant, J.M., Yu, M.H., Hasse-Pettingell, C.A. and King, J. (1989) J. Biol. Chem. 264, 10693–10698.
[36] Bishai, W.R., Rappuoli, R. and Murphy, J.R. (1987) J. Bacteriol. 169, 5140–5151.
[37] Takagi, H., Morinaga, Y., Tsuchiya, M., Ikemura, H. and Inouye, M. (1988) Bio/Technology 6, 948–950.
[38] Ouzzine, M., Fournel-Gigleux, S., Pillot, T., Burchell, B., Siest, G. and Magdalou, J. (1994) FEBS Lett. 339, 195–199.
[39] Romero-Chapman, N., Lee, J., Tinker, D., Uriu-Hare, J.Y., Keen, C.L. and Rucker, R.R. (1991) Biochem. J. 275, 657–662.
[40] Cooper, R.A., Knowles, P.F., Brown, D.E., McGuirl, M.A. and Dooley, D.M. (1992) Biochem. J. 288, 337–340.
[41] Parsons, M.R., Convery, M.A., Wilmot, C.M., Yadav, K.D.S., Blakeley, V., Corner, A.S., Phillips, S.E.V., McPherson, M.J. and Knowles, P.F. (1995) Structure 3, 1172–1184.
[42] Tanizawa, K., Matsuzaki, R., Shimuzu, E., Yorifuji, T. and Fukui, T. (1994) Biochem. Biophys. Res. Commun. 199, 1096–1102.
[43] Matsuzaki, R., Fukui, T., Sato, H., Ozaki, Y. and Tanizawa, K. (1994) FEBS Lett. 351, 360–364.