Revised nucleotide sequence of the *lasA* gene from *Pseudomonas aeruginosa* PAO1

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It has been shown that a 21 kDa active fragment of the LasA protein enhances the elastolytic activity of *Pseudomonas aeruginosa* elastase by possibly modifying the elastin substrate rendering it more susceptible to proteolytic degradation by elastase (1). N-terminal sequence analysis of the 21 kDa LasA active fragment revealed that it is encoded within the published *lasA* sequence (2). However, a comparison of the purified LasA active fragment with the published sequence of *lasA* revealed a significant inconsistency with the predicted size and isoelectric point (pI) of the active fragment.

To address this discrepancy, a 1.7 kb *Sma*I-*Hin*dIII DNA fragment harboring the entire *lasA* gene was cloned from the *P. aeruginosa* PAO1 chromosome using an oligonucleotide probe and its nucleotide sequence was determined by the dideoxy chain termination method (3). Juxtaposition of these sequences revealed differences in 10 nucleotide base pairs. Amino acid changes as a result of nucleotide changes in the revised *lasA* coding sequence are boxed. Discrepant nucleotides outside the coding region are denoted with lower case letters. The N-terminus of the LasA active fragment is denoted with a thick arrow. The most significant change is the absence of a translation termination codon at position 1276 which increases the *lasA* reading frame by an additional 41 amino acids. Immediately following the new termination codon at position 1399 is a 40 bp region (thin arrows) containing a possible transcription termination signal. This revised sequence predicts an active LasA fragment size of 20 kDa which closely approximates the SDS-PAGE estimated weight of 21 kDa. In addition, the predicted pI shifts from 7.48 to 9.24, a value which also corresponds closely with the pI of the purified active fragment. Since expression of the *lasA* gene in *E. coli* results in the production of a 40 kDa polypeptide (2), a new putative translational start to accommodate these changes is shown at position 289 (TTG) along with a Shine-Dalgarno (SD) sequence located about 12 bp upstream.

REFERENCES

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2. Schad,P.A. and Iglewski,B.H. (1988) *J. Bacteriol.* **170**, 2784–2789.
3. Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.

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CCCGGGTGACGGCGTTGCACGGTCCTGCGGCGTGCAACCCGGCCTGGGCGAAATACCGGCGGAGCGGACCAGGCTATCCCGTCGGAGGCGATGTCGCCGG 100
GCTGCTGGCTTTCAAGGTTTCCCTTCGATGACCAGGAGCTACCCATGCAGCACAAAAGATCCCGCGCGATGGCGAGTCCGCGCTCGCCGTTCCTCTTCGT 200
CTTGCTGGCGCTCGCCGTGGGCGGTACGGCCAATGCCCATGACGATGGCCTGCCGGCCTTCCGCTATTCGGCGGAGTTACTCGGCCAGTTGCAACTGCCC 300
                                                                SD              MetGlnLeuPro
AGCGTGGCCCTGCCGCTGAATGACGACCTGTTCCTCTACGGTCGCGACGCCGAGGCGTTCGACCTCGAGGCCTACCTGGCCTTGAACGCGCCGGCGCTG  399
SerValAlaLeuProLeuAsnAspAspLeuPheLeuTyrGlyArgAspAlaGluAlaPheAspLeuGluAlaTyrLeuAlaLeuAsnAlaProAlaLeu
CGCGACAAGAGCGAATACCTGGAGCACTGGAGCGGCTACTACAGCATCAACCCGAAAGTGTTGCTGACCCTGATGGTCATGCAATCCGGGCCGTTGGGG  498
ArgAspLysSerGluTyrLeuGluHisTrpSerGlyTyrTyrSerIleAsnProLysValLeuLeuThrLeuMetValMetGlnSerGlyProLeuGly
GCGCCGGACGAGCGCGCCTTGGCGGCGCCGCTGGGGCGGCTGTCGGCGAAACGCGGCTTCGATGCCCAGGTACGCGACGTGTTGCAGCAGTTGTCGCGG  597
AlaProAspGluArgAlaLeuAlaAlaProLeuGlyArgLeuSerAlaLysArgGlyPheAspAlaGlnValArgAspValLeuGlnGlnLeuSerArg
CGCTACTACGGTTTCGAGGAATACCAGTTGCGCCAGGCGGCTGCGCGCAAGGCCGTCGGCGAGGACGGCCTGAACGCGGCATCGGCCGCGCTGCTCGGT  696
ArgTyrTyrGlyPheGluGluTyrGlnLeuArgGlnAlaAlaAlaArgLysAlaValGlyGluAspGlyLeuAsnAlaAlaSerAlaAlaLeuLeuGly
CTGTTGCGAGAGGGGGCGAAGGTCTCCGCCGTGCAAGGCGGCAATCCGCTCGGCGCCTACGCGCAGACCTTCCAGCGCCTGTTCGGCACCCCGGCCGCC  795
LeuLeuArgGluGlyAlaLysValSerAlaValGlnGlyGlyAsnProLeuGlyAlaTyrAlaGlnThrPheGlnArgLeuPheGlyThrProAlaAla
GAACTCCTGCAGCCGAGCAACCGCGTGGCCCGGCAACTCCAGGCGAAGGCCGCGCTGGCGCCGCCATCCAACCTGATGCAATTGCCCTGGCGCCAGGGC  894
GluLeuLeuGlnProSerAsnArgValAlaArgGlnLeuGlnAlaLysAlaAlaLeuAlaProProSerAsnLeuMetGlnLeuProTrpArgGlnGly
TATTCCTGGCAGCCCAACGGAGCGCATTCGAACACCGGCTCGGGCTATCCGTACTCGTCCTTCGATGCGTCCTACGACTGGCCGCGCTGGGGCAGTGCG  993
TyrSerTrpGlnProAsnGlyAlaHisSerAsnThrGlySerGlyTyrProTyrSerSerPheAspAlaSerTyrAspTrpProArgTrpGlySerAla
ACCTACAGCGTGGTCGCCGCCCACGCCGGTACGGTACGGGTGCTGTCGCGCTGCCAGGTACGGGTGACCCACCCCAGCGGCTGGGCGACCAACTACTAC 1092
ThrTyrSerValValAlaAlaHisAlaGlyThrValArgValLeuSerArgCysGlnValArgValThrHisProSerGlyTrpAlaThrAsnTyrTyr
CATATGGACCAGATCCAGGTGAGCAACGGCCAGCAGGTCAGCGCCGACACCAAGCTCGGCGTCTATGCCGGCAACATCAACACCGCGCTCTGCGAGGGT 1191
HisMetAspGlnIleGlnValSerAsnGlyGlnGlnValSerAlaAspThrLysLeuGlyValTyrAlaGlyAsnIleAsnThrAlaLeuCysGluGly
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GlySerSerThrGlyProHisLeuHisPheSerLeuLeuTyrAsnGlyAlaPheValSerLeuGlnGlyAlaSerPheGlyProTyrArgIleAsnVal
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GlyThrSerAsnTyrAspAsnAspCysArgArgTyrTyrPheTyrAsnGlnSerAlaGlyThrThrHisCysAlaPheArgProLeuTyrAsnProGly
CTGGCGCTCTGAGTCGGCGCGGGGCCCGGCTCCAGCCGGgCTCCGCCGCGAGCGAAACGGCGCTGAACAGCTGGCGCGTCGCTGCCGCCGCGCCCGGCG 1488
LeuAlaLeu***
CCAGGCTGACCGCGTGGGCCGGCATGCTCAGCCGCGCCGGGGCAGCAGGGCGTAGGCGTCGCGAACCACATCCTTGCCGTAGTGgCGTTCCAGGCGGCG 1587
GACGACGAAGTGACCGCGCGCCATGGCCTGGAAATGGTTCATGAACACCGTGTTGATCGCCGCGCCGCCGAGGGCACCGACGATGGGCACGATCTGCGC 1686
CGCAAGCTT 1695
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