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Short Sequence-Paper

Listeriolysin genes: complete sequence of *ilo* from *Listeria ivanovii* and of *lso* from *Listeria seeligeri*

Albert Haas [1], Martina Dumbsky and Jürgen Kreft

Institut für Genetik und Mikrobiologie, Universität Würzburg, Würzburg (Germany)

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The complete DNA sequences coding for the thiol-activated cytolysins from *Listeria ivanovii*, ivanolysin O (ILO) and for seeligerolysin O (LSO) from *Listeria seeligeri* have been determined. The deduced amino acid sequences revealed that: (i) the primary translation products comprise 528 (ILO) and 530 (LSO) amino acids, respectively, (ii) ILO contains two cysteines, LSO has a substitution in the conserved cysteine motif.

The thiol-activated cytolysins are a family of pore-forming toxins from diverse genera of Gram-positive bacteria [16], most of which are secreted into the extracellular medium. These toxins include the species-specific types of listeriolysin O produced by the three *Listeria* species which are hemolytic on blood agar: *Listeria monocytogenes* (pathogenic for man and animals), *Listeria ivanovii* (animal pathogen) and *Listeria seeligeri* (apathogenic). Their listeriolysins have previously been characterized biochemically [6,10],and it has also been shown that they vary in cytolytic activity. The gene for listeriolysin O (*hly*) from *L. monocytogenes* has been cloned and sequenced [4,13] and homologous DNA sequences have been detected in the chromosome of the two other hemolytic *Listeria* species [11]. It has been firmly established, that listeriolysin O (LLO) is an essential virulence factor of *L. monocytogenes* (reviewed in Refs. 2 and 3), which enables this facultative intracellular parasite [2,5] to escape from the phagosome of the invaded mammalian cell, e.g., macrophages or other phagocytes. *L. ivanovii* has also been shown to replicate intracellularly, whereas the avirulent *L. seeligeri* does not [5,8].

We have established genomic DNA libraries of *L. seeligeri* (SLCC3379) and *L. ivanovii* (ATCC19119) by ligating chromosomal DNA fragments from partial *Sau*3A digests (*L. ivanovii*) or size-fractionated *Hin*dIII digests (*L. seeligeri*) into the plasmid vector pTZ18R [12]. Transformed recombinant *E. coli* DH5-α clones were screened by colony hybridization for homology to a *hly*-specific gene probe (651 bp *Hin*dIII fragment from pLM47, Ref. 11). One of the positive clones yielded plasmid pAHA9, which by DNA sequencing with the dideoxy chain termination method was shown to contain the complete determinant (*lso*) for listeriolysin O from *L. seeligeri*, termed seeligerolysin O (LSO) (Fig. 1). Among more than 15 000 recombinants from the *L. ivanovii* library, three positive clones were identified which contained overlapping inserts of different sizes, but which were too small to span the entire listeriolysin gene. The DNA sequence from all three recombinants was determined. The largest insert (from pAHA10) included 1029 base pairs from the carboxy-terminal moiety of the gene (*ilo*) for listeriolysin O from *L. ivanovii*, ivanolysin O (ILO). The other two independent inserts contained shorter segments from the identical *ilo* sequence. For both pAHA9 and pAHA10 the listerial origin of the inserted DNA was confirmed by Southern hybridization. Several attempts to detect the complete *ilo* gene in the gene bank failed, but by the polymerase chain-reaction (PCR) we could isolate, clone into pTZ18R and sequence the complete determinant (Fig. 1). The PCR-primer for the 5′ region was deduced from a previously determined upstream sequence (Kreft and Weber, unpublished). In addition the complete *lso* gene was

The sequence data reported in this paper have been submitted to the EMBL/Genbank Data Libraries under the accession numbers X60461 (*ilo*) and X60462 (*lso*).

[1] Present address: UCLA, Molecular Biology Institute, 405 Hilgard Ave., Los Angeles, CA 90024-1570, USA.

Correspondence: J. Kreft, (from 16 March 1992 on) Biozentrum der Universität Würzburg, Lehrstuhl Mikrobiologie, Am Hubland, W-8700 Würzburg, Germany.

reisolated by the same method in order to confirm the sequence data from the library.

The DNA sequence homologies between *ilo/hly*, *lso/hly* and *ilo/lso* were 78%, 77% and 76%, respectively. The deduced amino acid sequences (Fig. 1) showed that the primary translation product of *ilo* is a 528 amino acid protein of 58 541 Da, that of *lso* comprises 530 amino acids (59 181 Da). We have previously determined the N-terminal amino acid sequence of mature ILO [10], therefore the signal peptide cleavage site for ILO was placed after Ala-24. The N-termini of mature LSO and LLO are not known, but signal peptides are preferentially cleaved after an alanine, which is also found in both LSO and LLO at position 24.

The deduced amino acid sequences were compared (Fig. 2) to the deduced sequence for LLO from *L. monocytogenes* [4,13]. ILO, LSO and LLO are highly homologous: identity ILO/LLO 80%, LSO/LLO 82%, ILO/LSO 76%; similarity 91%, 90% and 86%, respectively, with rather heterogeneous N-termini. ILO shows one deletion at position 25 and LSO the insertion of one serine at position 33. The analysis of the predicted signal peptide of LSO revealed that it differs significantly from the corresponding ILO and LLO sequences: LSO lacks one positive charge at position 3 (Ile versus Lys) and has one hydrophobic amino acid less in the core region. Therefore, the LSO signal peptide might be less effective as an export-directing sequence.

The most interesting findings from the analysis of the deduced protein sequences were: (i) that LSO has a non-conservative amino acid substitution (Phe versus

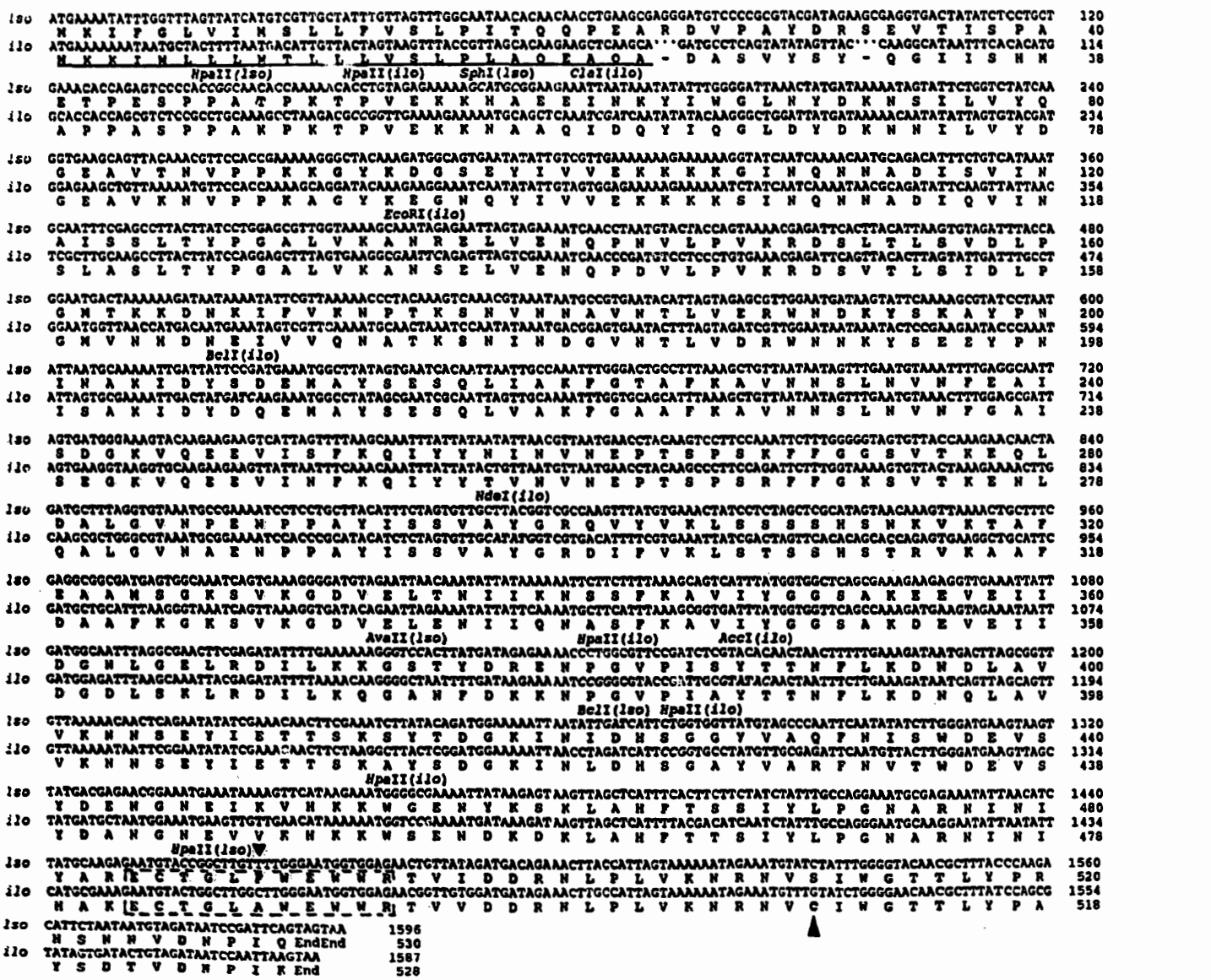

Fig. 1. Nucleotide and deduced amino acid sequences of *lso* (34.6% G+C) and *ilo* (35.5% G+C). A few restriction sites, serving as landmarks, are indicated above the DNA sequence. The presumptive signal peptide is underlined, the conserved undecapeptide is boxed with a broken line. Phe-489 in LSO and Cys-509 in ILO are indicated by arrows.

Ala) at position 489. Ala-489 is the sixth amino acid residue in the undecapeptide believed to be absolutely conserved among all thiol-activated cytolysins sequenced so far [7,9,17,18], including LLO from *L. monocytogenes* [4,13]. By site-directed mutagenesis it has been demonstrated by others that the integrity of this domain, and in particular the presence of certain tryptophane residues, is crucial for the hemolytic activity of these toxins [1]; this is also true for LLO from *L. monocytogenes* [14]. Although the normally conserved alanine is replaced in LSO by another hydrophobic, nonpolar, uncharged amino acid, the bulky aromatic ring of phenylalanine might have some detrimental effect on the hemolytic activity of LSO, compared to LLO. This notion is supported by the fact that the phenylalanine is directly adjacent to the critical tryptophane residues. To ensure that the observed amino acid substitution in LSO did not result from a cloning artifact during the construction of pAHA9, the relevant chromosomal region of *L. seeligeri* was amplified by the polymerase chain reaction (PCR). The DNA sequence determined from the PCR product was identical to the one found for pAHA9. (ii) The conserved undecapeptide mentioned above is termed the 'cysteine motif', as it contains the single cysteine residue in all thiol-activated cytolysins analyzed so far. It has been reported that the presence of this cysteine is not absolutely required for the hemolytic activity of pneu-

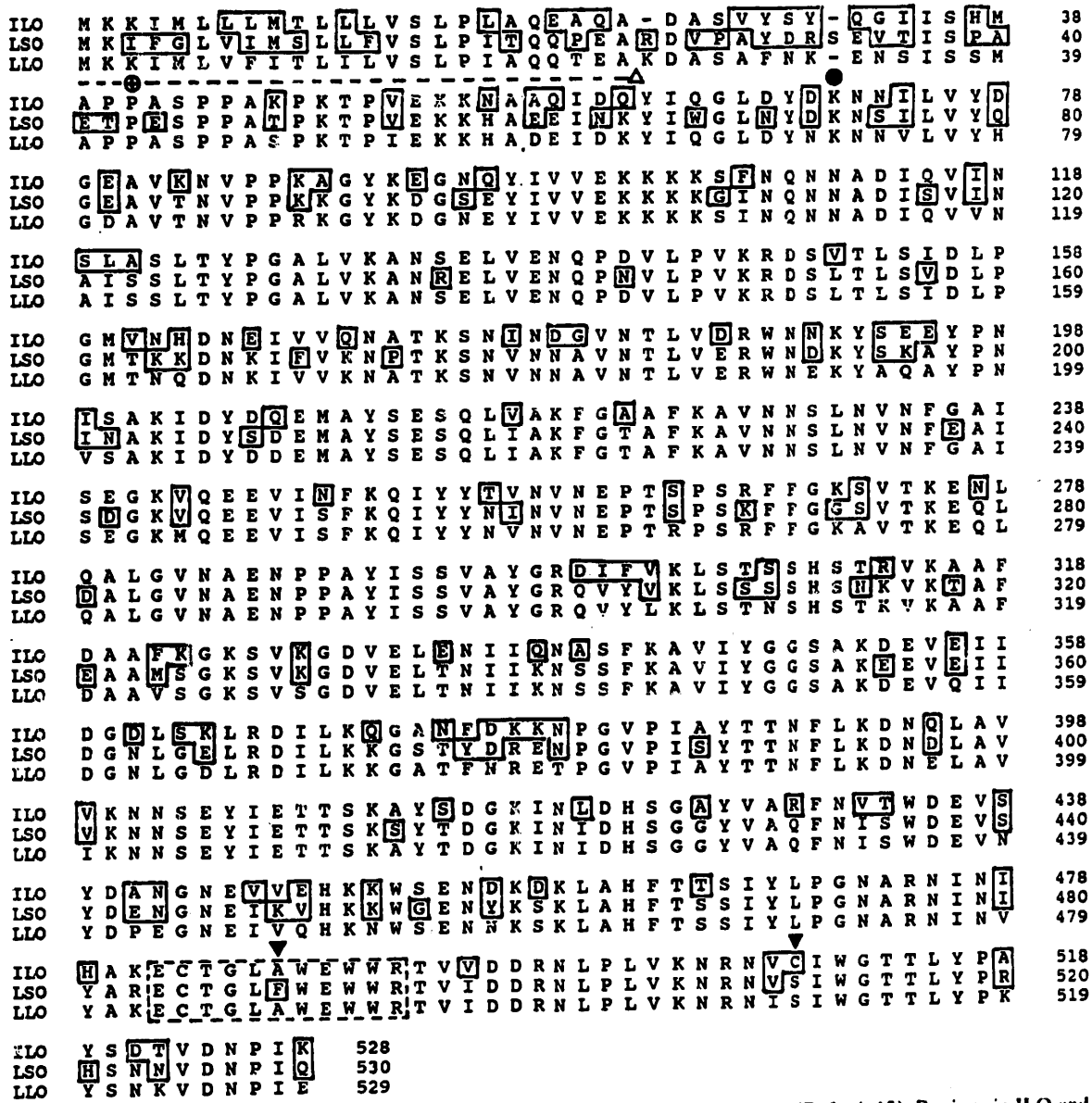

Fig. 2. Comparison of the deduced amino acid sequences for ILO and LSO with the LLO sequence (Refs. 4, 13). Regions in ILO and/or LSO which are not identical to LLO are boxed. The presumptive signal peptide is indicated by a broken line, the position where LSO lacks one positive charge is marked. The additional Ser-33 in LSO is indicated by a black dot below the sequence. Phe-489 in LSO and Cys-509 in ILO by arrows above.

molysin [1], streptolysin O [15] and also listeriolysin O (LLO) from *L. monocytogenes* [14]. Our results show that ILO contains two cysteine residues, one in the conserved region and another one 26 amino acids distal from there (position 509 in Figs. 1 and 2). Although all members of this group of toxins are oxygen-labile and thiol-activated, only ILO might be able to form intramolecular disulfide bonds upon oxidation. Further studies with the purified protein and with the isolated *lso* and *ilo* genes will show the significance of the differences described here to LLO and to the other toxins in this group.

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