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NUCLEOTIDE SEQUENCE OF A GENE ENCODING A NEW GENUS SPECIFIC PROTEIN OF *CHLAMYDIA PSITTACI*

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SUMMARY

DNA fragment No. 13 from the *C. psittaci* pigeon strain, has been cloned in the plasmid pUC19. Hybridization analysis revealed that the fragment maintained a chlamydial common sequence. Furthermore, nucleotide seqencing identified two partial open reading frames (ORF), 675b. p. and 530b. p.. Expression of ORF_S revealed that the second ORF encoded 25KD polypeptide, whereas the first ORF did not produce any antigenic product. The 25KD β-galactosidase fusion protein reacted strongly with chlamydia-specific antibodies elicited against a number of different chlamydial strains.

Gene Bank analysis showed that this cloned gene is not highly homologous with *chlamydia* or other organisms for which nucleotide sequences have already been published. This 25KD polypeptide may be an additional genus-specific antigen of Chlamydiae.

Key words: *Chlamydia psittaci*

INTRODUCTION

The genus *Chlamydia* consists of three species, *C. trachomatis*, *C. psittaci* and *C. pneumoniae*[1)]. *C. trachomatis*, known only to infect humans and mice, is composed of three biotypes on the basis of natural hosts, disease signs and biological properties[3)14)]. *C. pneumoniae*, previously known as TWAR strain, is emerging as an important human respiratory pathogen[4)]. *C. psittaci* comprises a diverse group of strains that have broad host range and pathogenic potential[15)16)].

At present, many chlamydial antigenic epitopes with genus-, species- or type-specificities have been characterized on different molecules[2)17)18)]. Among them, genus-specific epitopes were known to locate on chlamydial LPS which has also

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cross-reactivity with other Gram-negative bacteria[10]. Recently, another genus-specific antigen was identified and characterized. This epitope was located on the 57KD polypeptide that was one of the heat shock protein families of the genus *Chlamydia* and widely conserved in both eukaryotes and prokaryotes[8)9)].

In this report, DNA fragment No. 13 encoding 25KD β-galactosidase fusion protein, was cloned from a *C. psittaci* pigeon strain was characterized. The expressed protein had genus-specific epitopes as revealed by the cross-reaction with various antisera against *C. psittaci* and *C. trachomatis* strains. To define further the properties of the newly recognized genus-specific antigen, nucleotide sequence analysis and characteristics of the cloned gene are described.

MATERIALS AND METHODS

Enzymes and radiolabeled compounds: Restriction endonucleases were purchased from TAKARA Inc. or NIPPON GENE Inc.. The radio-labeled nucleotide triphosphate [α-32P] dCTP was obtained from AMERSHAM JAPAN Inc.. All of the enzymes were used according to the instructions provided by the manufacturers (7).

DNA hybridization: The DNA fragment was cut with *Acc*I and divided into two pieces of ca. 820b. p. and 520b. p. in length. The digested pieces were isolated from agarose gel by electroelution, then each of the fragments was labeled with a digoxygenine DNA labeling kit (BOEHRINGER MANNHEIM) and used as a probe. Dot hybridization assay was followed as described previously[5)11)]. DNA samples were spotted on the nitrocellulose filter, denatured with alkali solution and fixed. Hybridization reaction was performed at 68°C for 16h in a solution containing 6XSSC (IXSSC is 0.15M NaCl plus 0.015M sodium citrate) and 0.5% sodium dodecyl sulfate (SDS). Subsequently, the nitrocellulose filter was washed twice with 2XSSC and 0.1% SDS at room temperature for 15min and then reacted with 5-bromo-4-chloro-3-indolyl and nitroblue tetrazolium salt.

DNA sequencing and sequence analysis: Both strands of the DNA were sequenced by the primer extention dideoxy-chain termination procedure by Sanger et al.[12)]. Various DNA fragments generated by the restriction endonuclease digestion method or the DNasel/Shotgun cloning method were cloned into appropriate restriction sites of the vectors pUC19 and pUC18 [6)19)]. Sequence analysis was done by using the DNASIS programs (HITACHI Software Engineering Co., Ltd.), and searching of sequence homology was done by GenBank and EMBL nucleic acid sequence data bank.

Expression of deletion fragments: Each of the deletion fragments generated by DNasel/Shotgun cloning methods was subcloned into pUC19 in multiple cloning sites. The products of recombinant plasmids were further tested for antigenic properties. Characterization of these proteins was carried out by dot-ELISA as previously described[7)].

Results and discussion

Genomic recombinants in the plasmid pUC19, containing *C. psittaci* DNA fragment No. 13 at approximately 1.3Kb. p., were digested with several restriction enzymes and the insert was mapped as previously described[13]. The fragment had a single cleavage site for *Acc*I, *Bgl*II, *Hin*dIII, *Pvu*II and *Xho*I, and two cleavage sites for *Eco*RV and *Hin*cII. The restriction map of the chlamydial gene is shown in Fig. 1.

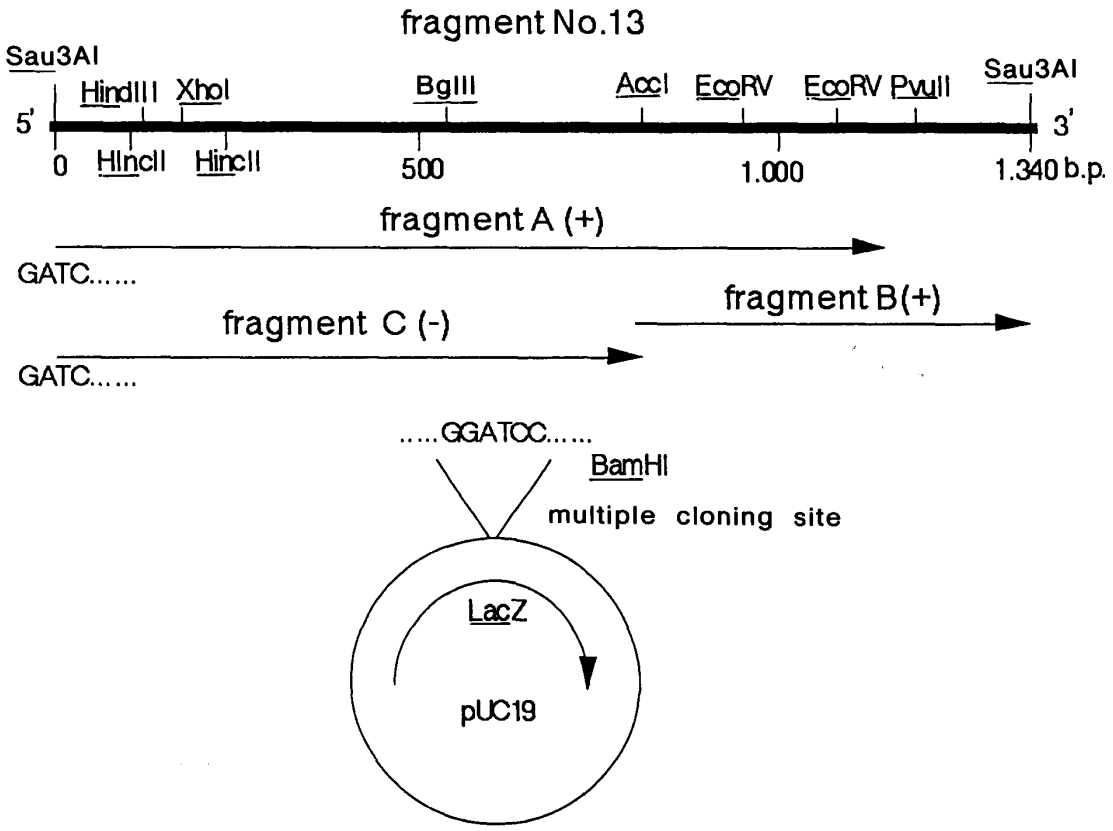

Fig. 1 Restriction map of *C. psittaci* DNA fragment No. 13 and antigenic expression of the deleted fragment.

Fragment A and C were generated from deleted 3'end of fragment No. 13, whereas fragment B was generated from deleted 5'end. Each fragment was ligated to pUC19 and expressed in *E. coli*, and the products were tested with antiserum. Deleted fragments encoded immunoreactive polypeptide (+) or non-immunoreactive polypeptide (−).

The nucleotide sequence and deduced amino acid sequence for *C. psittaci* DNA fragment are shown in Fig. 2. Two partial open reading frames (ORFs) were identified, one spanned for ca. 675b. p. and the other spanned for ca. 530b. p. The second ORF started from 130 bases far downstream of the first ORF. In this section,

```
5'  1 TTGCTAACCAAATTTCAGAACTTACACAGTTGCATGAGAAGTTATCTGATTTCGGCAATAAACTTGAAACA
        A  N  Q  I  S  E  L  T  Q  L  H  E  K  L  S  D  F  G  N  K  L  E  T
   72 TGTACGGGAGAATTTGATGAGCTTATCTCAGAGTTTAAGGTTAACTTGGAAGCTTTCAAATCTGTAGGTACT
        C  T  G  E  F  D  E  L  I  S  E  F  K  V  N  L  E  A  F  K  S  V  G  T
  144 CGAGTTGAGACTATAATCTCTCCTTTTGAGCGTTTAGCGGAATCGTTAACAGGAGTGTTCTCTAAAGACGCT
        R  V  E  T  I  I  S  P  F  E  R  L  A  E  S  L  T  G  V  F  S  K  D  A
  216 GTTAAGAACATGGTGGATGCTGTGAGCGCCTTGAGAGTGGAAATGGAGACATTAAAACAACATGTGGAGGAA
        V  K  N  M  V  D  A  V  S  A  L  R  V  E  M  E  T  L  K  Q  H  V  E  E
  288 AGTCGCGCTGTTCTACAACAGCTACAATCTGATGCTCAACTAAGAGAACAGCATTTACTTTACTTAGAACAG
        S  R  A  V  L  Q  Q  L  Q  S  D  A  Q  L  R  E  Q  H  L  L  Y  L  E  Q
  360 CGTAAGCAAGAGTTAGAAGCCGTTTGTTCGACTCTATCAGCTTCAATTGAACAATTACGTAGTTCTACTTCT
        R  K  Q  E  L  E  A  V  C  S  T  L  S  A  S  I  E  Q  L  R  S  S  T  S
  432 AACTTACAAGCTGTTGAGAGCCGGATTGTGAGTGCTGTAAGTGAGGACACGAGGCGTGTATCATTGACCTCT
        N  L  Q  A  V  E  S  R  I  V  S  A  V  S  E  D  T  R  R  V  S  L  T  S
  504 ACCACAGAAACGGCAGATCAGGGAGATCTGAGAGATCCAAGTGGGGACCGCTATGGTGGATGGGGTGCGCAA
        T  T  E  T  A  D  Q  G  D  L  R  D  P  S  G  D  R  Y  G  G  W  G  A  Q
  576 AGCTCCTATAGACTTTCTCCATCAGTAACCATGAGCCGGTTAGAACAACGAGGCAGAAGAATCATAGGAACC
        S  S  Y  R  L  S  P  S  V  T  M  S  R  L  E  Q  R  G  R  R  I  I  G  T
  648 GATGATCAAGGCTTCCCAATATATGAACAGTAAT-CCCAGTCATCGCATGATTAAAAAAAGGCAGAGAGCTT
        D  D  K  A  S  P  I  Y  E  Q  *
  719 CTTCTTGCCTTTTTTTATGTCTCTGAGTAAATTCTCTCTCTCATTGTCGCGACACTTTTCCAATAAAAAGCA
  791 CCGTTCTTATTCTAAGAAAG-ATGATAACCAAGTCTACATCTTGGGAGCCCATAGCTGAAGATTATCATAAG
                            M  I  T  K  S  T  S  W  E  P  I  A  E  D  Y  H  K
  862 ATCGTTCAGAGTGACGGGCATTACTATCATAGGGAAGTCATCCTGCCTAAATTACTTCCTTTACTCGATTTA
        I  V  Q  S  D  G  H  Y  Y  H  R  E  V  I  L  P  K  L  L  P  L  L  D  L
  934 AAATCAAAAGATCGTTTGGTAGATATCGGTTGTGGTCAGGGGGTTTTAGAACGGATGCTCCCTAAGGAATGT
        K  S  K  D  R  L  V  D  I  G  C  G  Q  G  V  L  E  R  M  L  P  K  E  C
 1006 GGATATTTAGGTATAGATATCTCCCCGAGTTTGATTTCCATCGCAAGAAAATTGCGAAAATCTCGATCTCAT
        G  Y  L  G  I  D  I  S  P  S  L  I  S  I  A  R  K  L  R  K  S  R  S  H
 1078 GAATTTCTTGTCTCTGATCTTACGAAAAAGGTGAAGATAGAGAGTTCCCTCTCTTTTTCCACAGCTGTGGCG
        E  F  L  V  S  D  L  T  K  K  V  K  I  E  S  S  L  S  F  S  T  A  V  A
 1150 ATTTTATCTCTGCAAAATATGGAGGCTCCAGATCAGGCAATTGATAATGTTGCCAGGCTTCTTGGTGATCAG
        I  L  S  L  Q  N  M  E  A  P  D  Q  A  I  D  N  V  A  R  L  L  G  D  Q
 1222 GGAAGATTCTTTATCGTGTTAAATCATCCTTGTTTTCGTATTCCTAGAGTCTCTTCATGGCATTATGATGAA
        G  R  F  F  I  V  L  N  H  P  C  F  R  I  P  R  V  S  S  W  H  Y  D  E
 1294 GATAAAAAGCTATTTTCAAGAAAGATCCCCGGGTACCGAGCTAATTCA  3'
        D  K  K  L  F  S  R  K  I  P  G  Y  R  A  N  S
```

Fig. 2 Nucleotide sequence and deduced amino acid sequence of *C. psittaci* DNA fragment No. 13. The first base of the 5,end of the DNA fragment is numbered as 1. Asterisk indicates the stop codon of the putative first ORF, and underline indicates the intiation codon of the second ORF.

there was no putative termination structure or putative promoter site.

To identify the chlamydial antigen coding region, deletion fragments generated for sequencing were expressed in *E. coli* and products were tested for antigenic properties. The dot-ELISA revealed that both fragment A (5' end of the DNA fragment No. 13 to ca. 1,150 base) and fragment B (3' end to ca. 520 base), lacking the first ORF region, expressed an immunoreactive product, whereas fragment C (5' end to ca. 820 base), lacking the second ORF region, produced no immuno-reactive polypeptide.

Previous study showed that the fragment No. 13 expressed about 25 KD polypeptide[13]. Similar molecular mass was obtained when the fragment B products were used for western blotting analysis (data not shown). The deduced amino acid sequence of 201 residues from this second ORF was predicted approximately as a 20. 1KD polypeptide. From these results, it was suggested that the immuno-reactive polypeptide had been encoded only in the second ORF. Additionally, this antigenic product was cross-reactive with all heterologous chlamydial antisera against the 10 strains of *C. psittaci* and 3 strains of *C. trachomatis* employed[13]. These data indicate the 25KD immunoreactive polypeptide contained genus-specific epitopes. On the other hand, the first ORF with unknown function was not related to the conformation of genus-specific epitopes.

To determine the homology of *C. psittaci* DNA fragment with other chlamydial strains, the fragment was cut into two pieces at the *Acc*I site, each containing a different ORF. Under highly stringent conditions (68°C for 15h), these two fragments were hybridized with all chlamydial strains including the 10 of *C. psittaci* and 4 of *C. trachomatis* employed (data not shown). This result suggests that the DNA fragment has maintained a genus-common sequence.

After the searching of nucleotide homology on the data bank, however, the DNA fragment did not show any appreciable homology with chlamydial genes, such as MOMP, LPS, 57KD heat-shock protein and other genes, for which sequences have been published; futhermore, no similarities between the nucleotide sequence of *C. psittaci* DNA fragment and that of other organisms were found. Therefore, the DNA fragment seems to encode an additional genus-specific polypeptide distinct from other characterized antigens of Chlamydiae.

Since it was known that *C. psittaci* and *C. trachomatis* shared less than 10% homology in their genomes, this conserved sequence may be an essential region for the biological function of the genus *Chlamydia*.

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