Brief Notes

Amino acids of the cholera toxin from *Vibrio cholerae* O37 strain S7 which differ from those of strain O1

(DNA sequence; NAG; probe; cloning; oligomer formation)

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**SUMMARY**

Unique differences in amino acid (aa) residues were found in the deduced aa sequence of cholera toxin (CT) from *Vibrio cholerae* non-O1 strain S7, some of which are suggested to be important sites for the unusual oligomer formation of subunit B.

The isolates of *Vibrio cholerae* (Vc) non-O1 from the Sudanese outbreak in 1968 produce an enterotoxin similar to cholera toxin (CT) of Vc O1. However, we have observed that the subunit B of CT purified from a Sudanese isolate (strain S7) was a larger oligomer than that of Vc O1 (Yamamoto et al., 1983). This larger oligomer formation was suggested to be due to difference in the subunit B from CT of Vc O1 (Yamamoto et al., 1983).

To clarify the possible aa change(s) in S7 CT, we cloned the structural genes (ctxAB) of S7 and determined the nt sequence of the gene.

Vc non-O1 serovar 037 strain S7 (Yamamoto et al. 1983) was used for gene analysis. A ctxA probe-positive clone (pKY310) isolated from a gene library of whole bacterial DNA of S7 cloned in pUC18 was used for nt sequencing. The oligodeoxynucleotide primers for sequencing were selected from the reported nt sequence of ctxAB of Vc O1 strain 2125 (Mekalanos et al., 1983).

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**Abbreviations:** aa, amino acid(s); bp, base pair(s); CT, cholera toxin; ctxAB, gene encoding CT; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); subunits A and B, products of genes ctxA and ctxB; Vc, *V. cholerae*.

The ctxA and ctxB genes of S7 had the same numbers of nt as those of Vc O1: 774 bp for ctxA and 372 bp for ctxB with a 4-bp overlap at the end of ctxA and the start codon of ctxB. In S7, however, 137th nt in ctxA and four nt (115, 138, 165, and 203) in ctxB differed from that of Vc O1 biotype El Tor (Lockman et al., 1984, Lockman and Kaper, 1983; Olsvik et al., 1993) (Table I) resulting in different aa residues.

Protein chemistry of CT has been extensively studied mainly with a prototype CT (569B CT) purified from a classical-biotype strain, 569B (Finkelstein and LoSpalluto, 1972). The CT is composed of one 27-kDa subunit A and five 11.6-kDa subunits B forming a B-subunit oligomer (Lai, 1976). Subunit A functions as ADP-ribosyltransferase, which activates adenylate cyclase on target cells, and subunit B has binding activity to the receptor, G_{M1}-ganglioside. Our previous physicochemical analysis suggests that the B subunit oligomer of S7 CT, unlike the 569B CT, comprises more than five B subunits (Yamamoto et al., 1983). Fragment A2 (Met$^{194}$ to Leu$^{246}$) of subunit A has been considered to be an anchor between fragment A1 (Asn$^4$ to Arg$^{192}$) and B oligomer and may affect formation of the subunit B oligomer (Jobling and Holms, 1992). However, there were no differences in the CT fragments A2 of S7, 569B (Dams et al., 1991) and El Tor biotype strains (Lockman et al.,...
<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>ctxA (subunit A)</th>
<th>ctxB (subunit B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nt113</td>
<td>nt115</td>
<td>nt128</td>
</tr>
<tr>
<td></td>
<td>(aa18)</td>
<td>(aa18)</td>
<td>(aa28)</td>
</tr>
<tr>
<td>Vc O37 S7</td>
<td>Novel</td>
<td>A (Asn)</td>
<td>C (His)</td>
</tr>
<tr>
<td>Vc O1 Classical-biotype</td>
<td>Genotype 1</td>
<td>G (Ser)</td>
<td>C (His)</td>
</tr>
<tr>
<td>El Tor-biotype (Australian)</td>
<td>Genotype 2</td>
<td>G (Ser)</td>
<td>C (His)</td>
</tr>
<tr>
<td>El Tor-biotype (7th pandemic)</td>
<td>Genotype 3</td>
<td>G (Ser)</td>
<td>T (Tyr)</td>
</tr>
</tbody>
</table>

* The nt numbering of ctxA (subunit A) and ctxB (subunit B) is from the A of the start codon ATG. In the parenthesis the number of aa from the N-terminal aa of mature polypeptides is indicated.

* Data for strain S7 is from the present study. The whole nt sequence data will appear in the GSDB, DDBJ, EMBL and NCBI nt sequence database with the accession number D30052. Other presented data are from Olsvik et al. (1993).

REFERENCES