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# Antigenicity of the *Campylobacter coli* CjaA Protein Produced by *Escherichia coli*

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Abstract

Immunogenic *C. coli* CjaA protein is a candidate for a chicken anti-*Campylobacter* subunit vaccine. In order to enhance its immunogenicity an antigenic determinant of the CjaA protein was identified. Thereafter, two copies of the antigenic epitope were cloned in tandem directly or with a flexible hinge between them. All experiments documented that the CjaA protein contained not only linear antigenic epitope/s but also conformational ones.

Key words: Immunogenic protein, *Campylobacter coli*, subunit vaccine

*Campylobacter* spp., gram-negative bacteria, is now the most commonly isolated human enteropathogen. Human *Campylobacter* infection predominantly manifests as acute, often bloody diarrhoea accompanied by a fever and abdominal pain (Ketley, 1997). Although the illness usually lasts for just a few days, it may sometimes, especially in immunocompromised patients, lead to bacteraemia and a variety of systemic infections. The most serious complication of the infection is the acute inflammatory polyneuropathy, known as Guillain-Barré syndrome (GBS) (Schwerer, 2002). Contaminated water, milk and mainly undercooked poultry meat are considered as major sources of human infection. *Campylobacter* is a commensal micro-organism for chickens. The reported level of *Campylobacter* organisms in the chicken intestine varies between $10^5$–$10^{10}$ CFU per g of cecael contents. Due to extremely high *Campylobacter* contamination of the chicken carcasses present on the market as well as relatively low human infection dose, improperly prepared poultry meat accounts for the majority of sporadic cases of campylobacteriosis. Effective immunisation of birds, which would result in decreasing of the level of chicken intestinal track colonisation by the pathogen, is considered the promising strategy to lower the number of human infections. Therefore, we undertook an attempt to construct a chicken anti-*Campylobacter* vaccine using avirulent *Salmonella enterica* strain as a carrier for *Campylobacter* antigens (Lis *et al.*, 2003)

The main goal of the presented paper was to evaluate the immunogenic structure of the CjaA antigen – a potential candidate for a chicken anti-*Campylobacter* vaccine. The *cjaA* gene, encoding for a 33 kDa immunopositive protein, was isolated from a genomic library of *C. coli* 72Dz/92 (previously described as *C. jejuni*) constructed in λgt11. *C. coli* 72Dz/92 is the most common serotype in Poland (Lior71). The immunological screening of the library was done with *E. coli* absorbed rabbit antiserum generated against whole *C. coli* cells. CjaA protein, ortholog of the product of *C. jejuni* NCTC 11168 *cj0982* gene, belongs to family 3 solute binding proteins, component of the ABC transport system (Boos and Lucht, 1994). The presence and conservation of the *cjaA* gene among different *C. coli* and *C. jejuni* clinical isolates, as well as among chicken derived strains, were determined by hybridisation and Western blot analysis (Pawelec *et al.*, 2000). The gene has been cloned and successfully expressed in avirulent *Salmonella enterica* sv. Typhimurium – a strain commonly used as a carrier of foreign antigens (Chatfield and Dougan, 1997; Dietrich *et al.*, 2003).

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In order to enhance the CjaA immunogenicity we decided to identify its antigenic determinant/s and clone appropriate DNA fragment under the control of strong *lacZ* promoter.

*E. coli* strains and plasmids used in this study are listed in Table I. All *E. coli* strains were grown on LB supplemented with appropriate antibiotic (ampicillin or kanamycin at the final concentration 50 μg/ml). PCR product purification and plasmid's DNA isolation were done with appropriate kits (A&A Biotechnology). Two fragments of the *cjaA* gene lacking the DNA region that specifies the signal sequence were cloned into pBGS18 to create a *lacZ-cjaA* gene. DNA fragments carrying part of the *cjaA* gene were generated through PCR amplifications. All primers, ordered from Ark-Sigma, are presented in Tab. II. They were employed to amplify fragments of the *C. coli cjaA* gene on the chromosomal DNA isolated according to procedure described by Nachampkin *et al.*, 1993. There were two *lacZ-cjaA* fusions constructed. The first one was a 617 bp fragment of the *cjaA* gene amplified by PCR using primers 306–1 – 327–3 and cloned into the pGEM-T Vector. Next the insert was removed from the recombinant plasmid after cleavage with *Bam*HI and *Pst*I and after purification was inserted into pBGS18 cleaved with the same restriction enzymes, resulting in pUWM287. The second fusion was constructed analogously, using primers 306–1 – 373, resulting in pUWM288. Both constructs were verified by sequencing analysis. To determine the antigenicity of fused proteins whole-cell extracts of *E. coli*/pUWM287 and *E. coli*/pUWM288 were examined by Western immunoblots. Rabbit antiserum raised against rCjaA failed to react with any proteins present in both extracts. Furthermore, an *in vitro* transcription/translation assay using pUWM287 and pUWM288 DNA as templates failed to produce expected 24 kDa and 14 kDa molecular weight proteins, respectively (data not shown). Examination of the amino-acid sequence of CjaA revealed the presence in the 5' end of the gene of a large number of codons for Arg (AGG, AGA), Ile (AUA), Gly (GGA) and Leu (CUA) which are rarely used in highly expressed *E. coli* genes (Kane, 1995). They add up to more than 7% of the codons present in the *Campylobacter* DNA cloned into pUWM287 and up to 10% in pUWM288, and furthermore, three of these codons are in tandem as amino acids 20 to 22 of the mature protein. This observation suggests that the lack of fused-protein synthesis in the pUWM287 and pUWM288 – dependent *in vitro* transcription/translation assay may be related to overexpression of the *cjaA* gene. The high level of the *cjaA*-specific messenger

Table I
Bacterial strains and plasmids used in this study

| Bacterial strains and plasmids | Phenotypic or genotypic features | Origin |
|---|---|---|
| **Strains** | | |
| *E. coli* XL1-Blue | *supE*44 *hsdR*17 *recA*1 *endA*1 *gyrA*96 *thi*1 *relA*1 lac⁻ F'[*proAB*⁺ *lacI*$^{q}$ZDM15 Tn*10* (*tet*$^{r}$)] | Stratagene |
| *C. coli* 72Dz/92 | Isolated from a patient with an acute bloody diarrhea (serotype Lior71, biotype 1) | Child Health Centre, Warsaw, Poland |
| **Plasmids** | | |
| pBGS18 | Km$^{R}$, LacZα | (Spratt *et al.*, 1986) |
| pBluescript II KS | Ap$^{R}$, LacZα | Stratagene |
| pGEM-T Vector | Ap$^{R}$, LacZα | Promega |
| pUWM287 | pBGS18 derivative containing a fragment of the *cjaA* gene amplified with starters: 306–1 – 327–3 | This study |
| pUWM288 | pBGS18 derivative containing a fragment of the *cjaA* gene amplified with starters: 306–1 – 373 | This study |
| pUWM418 | pBluescript II KS derivative containing a fragment of the *cjaA* gene amplified with starters: H2B – H3X | This study |
| pUWM425 | pUWM418 derivative containing duplicated fragment of the *cjaA* gene amplified with starters: Tan-L – Tan-R | This study |
| pUWM430 | pUWM418 derivative containing duplicated fragment of the *cjaA* gene with a short hinge type 1 amplified with starters: ZAW-1 – TAN-R | This study |
| pUWM467 | pUWM418 derivative containing duplicated fragment of the *cjaA* gene with a short hinge type 2 amplified with starters: ZAW-2 – TAN-R | This study |

Table II
Oligonucleotides used for the *cjaA* gene PCR amplifications

| Name | Priming sequence* (5' to 3') | Orientation | Restriction enzyme |
|---|---|---|---|
| **306–1** | ACAGGATCCG**AATTCAGATTCTGGTGCTTC** | forward | *Bam*HI |
| **327–3** | **TTAACAGCAGGAGCAATTAC** | reversed | ∅ |
| **373** | **TCTTGAGGCACAGCCACACC** | reversed | ∅ |
| **H2B** | GTCTAGGATCCTA**GTTAAGACGCTCCTCATATT** | forward | *Bam*HI |
| **H3X** | ATCTGTCTAGAGC**AATCCACTTGCTCTGCTCTT** | reversed | *Xba*I |
| **Tan-L** | ACATCTAGA**AATTCAGATTCTGGTGCTTC** | forward | *Xba*I |
| **Tan-R** | ATCTGGAGCTC**AATCCACTTGCTCTGCTCTT** | reversed | *Sac*I |
| **ZAW-1** | ACATGTCTAGAaactacgcgccggtggat**AATTCAGATTCTGGTGCTTC** | forward | *Xba*I |
| **ZAW-2** | ACATGTCTAGAgggcccgggcccgcgccg**AATTCAGATTCTGGTGCTTC** | forward | *Xba*I |

* capital, bold letters indicate *C. jejuni* sequence, small letters indicate a hinge sequence, restriction recognition sequences introduced for cloning purposes are underlined

RNA probably causes fast depletion of the t-RNA specific for the rarely used *E. coli* codons. That was the reason to repeat the experiment but this time by cloning the *cjaA* fragment together with its own promoter sequences. When the 5' fragment of the *cjaA* gene was expressed in *E. coli* from the native *C. coli* promoter (pUWM418), the expected 14 kDa protein was detected by anti-rCjaA antibodies in the whole-cell extract. This experiment demonstrated that a 294 bp 5' fragment of the *cjaA* present in pUWM418 (amplified using primers: H2B – H3X) encodes for the antigenic determinant of the protein. Then, in order to magnify its antigenicity we attempted to clone this part of the *cjaA* gene in tandem. The strategy was successfully used for several bacterial antigens (Redman *et al.*, 1996; Zhang *et al.*, 2002). Two kinds of the recombinant plasmids were constructed containing tandem repeats of the 294 bp fragment of the *cjaA*. N-terminal parts of the CjaA were fused either directly (encoded by pUWM425) or alternatively by incorporating a short flexible hinge regions between them (encoded by pUWM430 and pUWM467). The construction of the recombinant plasmid carrying direct duplication of the *cjaA* antigenic determinant (pUWM425) was achieved by amplification of the 5' part of the *cjaA* using primers TAN-L – TAN-R and cloning it into *Sac*I and *Xba*I treated pUWM418. pUWM430 and pUWM467 were generated in the same way, except that the forward primers ZAW-1 (used for pUWM430 construction) and ZAW-2 (used for pUWM467 construction) allow to insert short oligonucleotides between two *cjaA* gene fragments. The latter constructs were specially designed to facilitate the proper folding of both components of the chimeric protein (Chatfield and Dougan, 1997; Jagusztyn-Krynicka *et al.*, 1993). Fig. 1 presents data on the antigenic properties of the proteins specified by pUWM418, pUWM425 and pUWM430 (30 µg of whole-cell proteins were resolved by 12% SDS-PAGE, transferred to nitrocelulose and reacted with anti-rCjaA serum). Both the N-terminal part of the CjaA and the duplication fragments showed significantly weaker immunoreactivity than the whole CjaA protein (Fig. 1, Lanes 2, 3, 4). Moreover, insertion of the 6 amino-acid linker between the two tandem components of the chimera did not enhance its antigenicity. Modification of the amino-acid sequence of the linker (AsnTyrAlaProValAsp encoded by pUWM430 into GlyProGlyProAlaPro encoded by pUWM467) also did not augment the antigenicity of the chimera (data not shown).

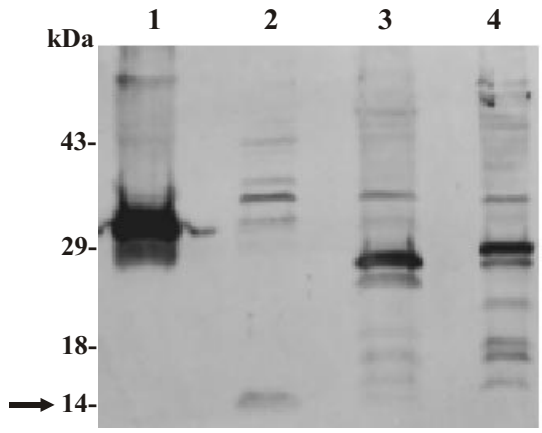


Fig. 1. Western blot analysis of antigenic properties of proteins specified by pUWM418, pUWM425 and pUWM430.

Lines: *Escherichia coli* XL1-Blue containing 1. pUWM60 (whole CjaA- control); 2. pUWM418 (arrow indicates the position of the antigenic determinant); 3. pUWM425; 4. pUWM430

Then, we concluded that CjaA epitopes are conformational ones, made up of amino acid residues that aggregate from various part of folded protein. The presented work also showed that, probably due to the differences in

codon usage between *Escherichia* spp. and *Campylobacter* spp., the achievement of high-level expression of *Campylobacter* proteins, such as CjaA, in *Escherichia* strains will not be an easy goal. To overcome the difficulty one has to manipulate specific *Campylobacter* codons. The alternative approach, which probably would conquer the translational problem, is to clone *Campylobacter* genes in a carrier strain containing extra genes encoding t-RNA for host rarely used codons (Brinkmann *et al.*, 1989).

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