Chinese Science Bulletin 2004 Vol. 49 No. 7 668—671

Cytotoxicity of HIV-*gp41* segments expressed in *E. coli*

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Abstract **The failed attempt to express HIV-*gp41* in *E. coli* led to the investigation of HIV-*gp41* segments, which is responsible for the toxicity to *E. coli* cells. A series of deletion mutants containing different regions of *gp41* gene were constructed and expressed in *E. coli* BL21(DE3) strain. After IPTG induction, the high mortality of host bacteria was observed in host bacteria carrying the deletion mutants of *gp41* gene except for those transformed with pET-H*N2*; coordinately, the mRNA transcripts of the *gp41* was rapidly decreased; and the release of [^{3}H]uridine increased upon induction. All these data suggested that GP41 protein has a cytotoxic effect on *E. coli*, and it is the cytotoxicity of the *gp41* gene product that contributes to the high mortality when expressed in *E. coli*.**

Keywords: HIV, GP41, cytotoxicity, *E. coli*.

DOI: 10.1360/03wc0460

GP41 transmembrane protein is an important examination maker for HIV infection confirmation due to its relatively conserved antigen determinants. However, the HIV-1 *gp41* gene cannot be expressed effectively in *E. coli* expression systems. It has been suggested that the cytotoxicity of GP41 protein to *E. coli* is the main factor hindering *gp41* expression[1].

The special topological structure of the viral transmembrane protein may attribute to the cytotoxicity and dominate the fate of the proteins expressed in *E. coli*. For example, Semliki Forest virus 6 K protein, protein 3AB of hepatitis A virus, and protein E of SARS[2—4] could be expressed effectively in *E. coli*, but Hepatitis C virus E1 protein, NS2B protein of Japanese Encephalitis Virus, and rotavirus NSP4 protein[5—7] could not. The transmembrane proteins function via their hydrophobicity and formation of α-helix. The transmembrane helix is inserted into the hydrophobic core of the lipid bilayers by hydrophobic force from Hydrophobic amino acids, this insertion raises a perturbation and makes the lipid bilayers irregularly rearranged to complete the transmembrane procedure. Our previous study[1)] showed that GP41 contains two transmembrane α-helices, the first one is formed by amino-acid residues 4—16 and the second one by 167—189 resudues. Segments N1 and N3 of GP41, each containing one of the trasmembrane α-helices, could not be expressed effectively in *E. coli*, where as segment N2, which contains no transmembrane α-helices, could be successfully produced. By investigating the expression level of different *gp41* segments, as well as their cytotoxicity in *E. coli,* we have determined the cause for the failed *gp41* expression in *E. coli*. This paper establishes the base for the further exploration of the effectors of GP41 protein in HIV pathology.

1 Materials and methods

(i) Materials. Plasmids pET-H*gp41*, pET-H*N1*, pET-H*N2*, pET-H*N3*, and pET-H*C* were constructed as described previously[9] 1). The loci of segments *N1*, *N2*, *N3*, *C* in *gp41* gene are shown in Fig. 1. [^{3}H]Uridine was purchased from China Institute of Atom Energy, and [α-^{32}P]dATP/dCTP (370 MBq/mL) from Beijing Furui Company; All the other reagents were AR grade and produced in China.

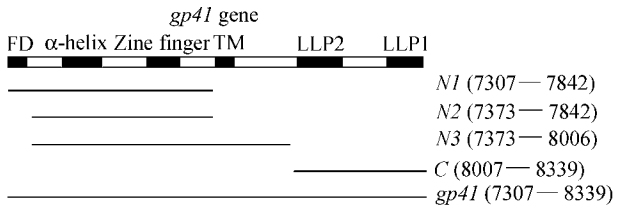

Fig. 1. Construction of truncated *gp41* gene segments.

(ii) Gene manipulation. Plasmids transformation, RNA extraction, probe hybridization were performed as described in ref. [10].

(iii) Preparation of DNA probe. Segments *N1* and *C* of *gp41* gene were labelled by [α-^{32}P]dATP/dCTP as probe *N1* and *C*.

(iv) Growth curve of bacteria. pET-H*N3*, pET-H*C* were transformed into *E. coli* BL21(DE3), single clones of BL21(DE3) cells containing the indicated plasmid were grown overnight at 37℃ in LB medium in the presence of ampicillin at 100 mg/mL. Then, the cells were diluted 100-fold in LB medium supplemented with the antibiotics and grown at 37℃ respectively. Once the cultures reached an A_{600} of about 0.8, they were induced by the addition of 1 mmol/L of isopropylthiogalactopyranoside (IPTG) and plotted against A_{600} at given times.

(v) Comparison of the survival rate of bacteria. Cells were grown as described above and once A_{600} = 0.5, they were induced by adding 1 mmol/L IPTG and diluted aliquots of cultures were spread on LB solid plate

1) Yuan, Y. H., BI, C. H., LI, J. et al., Finding two amino-acid sequences of expression affecting HIV-*gp41* N-terminal 1/2 segment in *E. coli*, Acta Scientiarum Naturalium Universitatis Nankaiensis (in Chinese), 2004, 37(1).

at given times and the clones were counted after incubation for 18 h at 37℃.

(vi) Uridine loading of *E. coli* cells. Cells were grown as described above, incubated with 2 mCi of [^{3}H] uridine for 1 h before induction. The cells were sedimented and washed twice with the uridine-free, prewarmed growth medium. Then, the cells were resuspended in the initial volume of the growth medium and incubated at 37℃. Fifteen minutes later, the cells were induced with IPTG. Samples are taken at the given times, and 0.2-mL supernatant is used to quantify the radioactivity released to the medium. The results represent the mean of triplicated experiments, CV<15%.

(vii) RNA dot blotting. The overnight culture of bacteria containing pET-H*gp41*, pET-H*N1*, pET-H*N2*, pET-H*N3*, pET-H*C* were diluted 100-fold and continued to grow at 37℃ in LB medium supplemented with the antibiotics. Once the cultures reached an A_{600} of about 0.5, they were induced by adding 1 mmol/L IPTG. 3-mL aliquots of cultures were collected at 0.5, 1, and 1.5 h, respectively, the total RNA was extracted from each sample. 20 μg total RNA were dotted on a nitrocellulase membrane, pre-hybridized for 2 h, then hybridized with probe *N1* and *C* overnight at 68℃, respectively. After drying, membranes were exposed to X-ray films (Kodak) at −70℃ overnight.

2 Results

(i) Expression of *gp41* segments affects the growth of host bacterium. In order to understand the effect of expression of different segments of *gp41* gene on bacterial growing, A_{600} of the host bacteria at given times was measured and plotted (Fig. 2). *E. coli* BI21 (DE3) with pET-H and pET-H*N2* had an increase in A_{600}, but the other clones had almost no change up to 4 h.

(ii) Expression of *gp41* of some segments is lethal to the host. Counting the survival rate of bacterium induced showed that bacterium with pET-H*gp41*, pET-H*N1*, pET-H*N3* and pET-H*C* has a high death rate, as the counts decrease from about 10^8 to 10^5 mL during 4 h. But bacteriums carrying pET-H*N2* were relatively viable (Fig. 3).

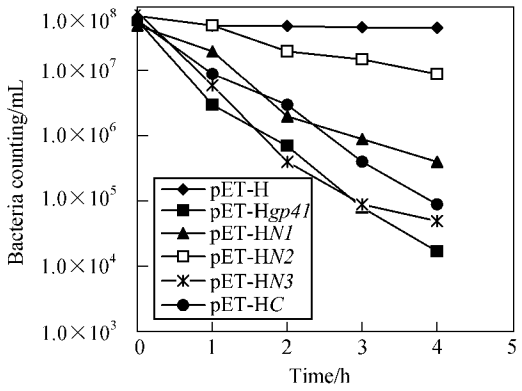

Fig. 3. Postinduction survival counting of *E. coli* BI21 (DE3) expressing different segments of *gp41* gene.

(iii) Segments of GP41 permeabilize BL21(DE3) cells membrane. In order to understand the reason for host bacteria death caused by expression of *gp41* segments, [^{3}H]uridine release assay was performed to measure the cytotoxicity of GP41 protein to host bacteria cells. In Fig. 4, expression of segments of *gp41* led to [^{3}H] uridine release from the host bacterium more or less (cpm from 67 to 163 in 5 h). The data showed that the expression of the segments permeabilizes host bacteria cells' membrane and leads to cytotoxicity. From Fig. 4, GP41 showed the highest permeability (163 cpm), while N2, though expressed well, also permeablizes host membrane

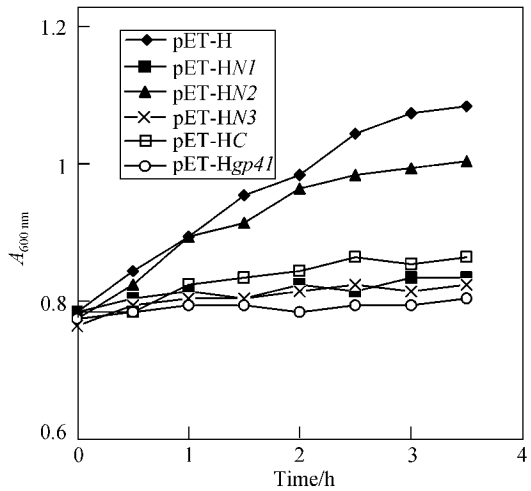

Fig. 2. Growth of *E. coli* BI21 (DE3) expressing different segments of *gp41* gene.

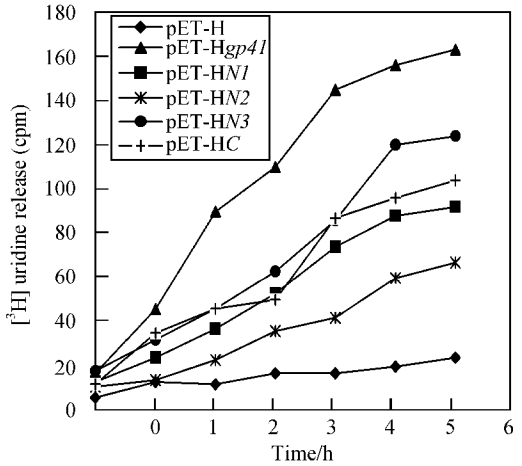

Fig. 4. [^{3}H]uridine release in *E. coli* BI21 (DE3) expressing different segments of *gp41* gene.

(67 cpm).

(iv) Transcript of some *gp41* mutants decreases rapidly after induction. RNA dot blotting assay was used to measure transcript of segments of *gp41* gene. In Fig. 5, mRNA transcript of the segments except for *N2* decreased rapidly following IPTG induction, especially the full-length *gp41* gene. The results suggested that the expression of various segments causes different levels of permeability to cell membrane, and the rise of permeability leads to bacteria death and a rapid mRNA transcript decrease.

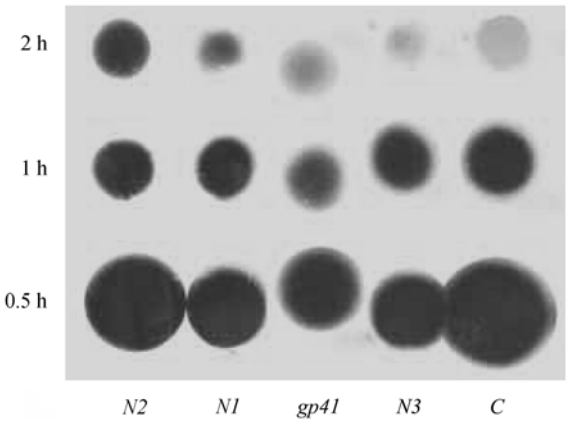

Fig. 5. RNA Dot Blotting of *E. coli* BI21 (DE3) expressing different segments of *gp41* gene.

In brief, the cytotoxicity of GP41 protein to bacteria cell membrane results in the low survival rate of host bacteria in which the GP41 is expressed.

3 Discussion

Because GP41 protein plays an important role in clinical diagnosis of HIV infection, it is useful to express large scale GP41 protein in *E. coli* at a lower cost. Some experiments[1] found that it is difficult for *gp41* gene to be expressed in *E. coli*. But the definite reason is not reported in previously literature. Our experiments demonstrated that different regions of GP41 have different levels of cytotoxicity to host bacteria due to the specific topology structure of GP41 protein. The release assay of [^{3}H] uridine showed that the segments of GP41 permeabilize its host bacteria membrane and lead to death of bacterium and decrease of mRNA transcription.

Analyses of N-terminal segments of GP41 reveal that segment N2 contains a leucine zipper structure and an α-helix region, which may also permeabilize cells membrane[11]. However, because the hydrophobicity of N2 is lower than that of N1 and N3, its cytotoxicity is lower, therefore, it can be expressed effectively. 26 residues at N-terminal of segment N1 are fusion peptide of GP41 protein and are mainly composed of hydrophobic residues. Its function is to induce fusion of HIV envelop with host cell membrane; segment N3 is mainly composed of hydrophobic amino acid too and its function is to anchor GP41 protein on HIV envelop and host cell membrane. Hydrophobic amino acids bring a perturbation effect on hydrophobic core of lipid bilayers when embedded in membrane, thus causing cytotoxicity on cells[8]. Because segments N1 and N3 have higher hydrophobicity than N2 does, they may have stronger cytotoxicity. Therefore, they are expressed less effectively in host bacteria. There are two lentivirus lysis peptide (LLP), LLP1 and LLP2, in segment C. LLP can be folded into the amphipathic α-helix structure, which contains abundant Arginine and the hydrophilic Arginine may change cell membrane's structure and function. It is speculated that the Arginines in segment C are the main reason for cytotoxicity to cell. Edward[7] found that the expression of the rotavirus nonstructural glycoprotein NSP4 in *E. coli* leads to a decrease in optical density of the culture and release of [^{3}H] uridine into the medium, and the NSP4 contains abundant Lysines in the membrane-spanning-proximal region. Ciccaglione[5] found that Hepatitis C virus E1 protein membrane-spanning hydrophobic region and Arginine-339 and Lysine-370 of membrane-spanning-proximal region have cytotoxicity effect on *E. coli*. Chang[6] found that the deletion of 6 residues (LKTTKR) of NS2B C-terminal protein in Japanese encephalitis virus may alleviate its cytotoxicity. Cole[12] have studied antibacterial activity of peptides derived from envelop glycoproteins of HIV-1, and found that membrane-spanning domain of GP41 protein and two 15-residue peptide derived from LLP1 have antibacterial activity. Though the antibacterial action occurred *in vitro*, it is likely that there would be a common mechanism involved in GP41 protein's cytotoxicity *in vivo* in our experiment.

Using different assays we have found that GP41 protein permeabilizes cell membrane and kills most bacteria, this may attribute to the difficulty of its expression in *E. coli*. In addition, among all the *gp41* gene segments, only segment *N2* can be expressed effectively in *E. coli*, with a few cells dead. This reported data suggest that the hydrophobicity of protein and positively charged Arg may be the major factor for their cytotoxicity.

Acknowledgements This work was supported by the Doctoral Fund in High School (Grant No. 2000005512)

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(Received January 9, 2004; accepted February 19, 2004)

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