KMITL Sci. Tech. J. Vol. 8 No. 1 Jan. - Jun. 2008

OPTIMIZATION OF THE HEXON GENE FROM HUMAN ADENOVIRUS TYPE 3 EXPRESSION IN E.COLI

Liming Yang[1] and Zhangyi Qu[2*]

[1]Department of Life Science and Engineering, Harbin Institute of Technology, Harbin, P R China
[2]Department of Microbiology, Harbin Medical University, Harbin, P R China

ABSTRACT

A study of the optimization of hexon gene expression in *E. coli* M15/ pQE31-hexon was undertaken. The Hexon gene was directionally cloned into expression vector pQE31 and the resulting recombinant plasmid pQE31 Hexon was transformed into *E.coli* M15 and induced by IPTG to express the hexon protein. The four factors, including the initial concentration of M15/pQE31-Hexon, concentration of IPTG, incubation temperature and induction time, were optimized during the induction of target protein. To detect the expression efficiency under different induction conditions, the total protein in the samples were subjected to SDS-PAGE. The Hexon protein was successfully expressed by *E.coli* M15/pQE31-Hexon. The optimal conditions for Hexon expression were: initial concentration of *E.coli* M15/pQE31-Hexon at OD_{600nm} =0.5, IPTG concentration of 0.125 mmol/L, incubation at 37℃ and incubation for four hours. This study established a good foundation for producing adenovirus genetic engineering vaccine in future.

KEYWORDS: hexon protein; expression; optimization; adenovirus type 3

1. INTRODUCTION

Adenovirus is DNA virus and one of the main causes of human respiratory tract and digestive tract infections. Besides, adenovirus can cause childhood pneumonia especially from six-months to two-years old and the major types are type 3, 5 and 7. In recent years there has been a large epidemic outbreak in north China with serious consequences to children's health and life. Hexon is the major structural protein of adenovirus [1-3]. It stimulates the body to produce anti-bodies which inhibit conformational shift of the virus and further act to neutralize the viron. So in this study we have expressed the hexon protein and studied the optimization of the expression conditions in *E.coli* for development of genetically engineered vaccine against adenovirus [4].

* Corresponding author:Tel: 86 451 86412952 Fax: 86 451 86412952
E-mail: cooperationyang@yahoo.com.cn

2. MATERIALS AND METHODS

2.1 Materials

E. coli M15/ pQE31-hexon was constructed by YANG Li-Ming, Department of Microbiology, Harbin Medical University. Vector pQE31 and *E. coli* M15 were stored at Department of Microbiology, Harbin Medical University, P.R. China.

2.2 Induced expression at different incubation periods

2 ml of overnight culture of *E. coli* M15 harboring the pQE31-hexon recombinant plasmid was inoculated into 50 ml LB containing 50 μg/ml kanamycin. The culture was incubated at 37 by shaking for 2 to 3 h, until the OD_{600nm} was about 0.5. After taking 1 ml sample as uninduced control, isopropyl O-D-thiogalactopyranoside (IPTG) was added to the culture to a final concentration of 0.1mmol/L. The culture was incubated at 37℃ with sampling of 1 ml after 2.0 min, 12.0 min, 30.0 min, 1.0 h, 2.0 h, 4.0 h and 8.0 h induction. The cells were collected by centrifugation and stored at -20℃. The culture samples obtained at different induction times were lysed in 2×SDS sample buffer, and SDS-PAGE was employed to detect the hexon protein.

2.3 Induced expression at different IPTG concentrations

2 ml of overnight culture of *E. coli* M15 harboring the pQE31-hexon recombinant plasmid was inoculated into 50ml LB containing 50μg/ml kanamycin. The culture was incubated at 37℃ by shaking for 2 to 3 h, until the OD_{600nm} was about 0.5. After taking 1 ml sample as uninduced control, the culture was incubated at 37℃ for 4 h with sampling 1 ml after 2 mM, 1 mM, 0.5 mM, 0.25 mM, 0.125 mM, 0.0625 mM, 0.032 mM and 0.016 mM IPTG induction. The samples dealt were the same as the above.

2.4 Induced expression at different initial concentrations of MIS/pQE 31-Hexon (OD_{600nm})

2 ml of overnight culture of *E. coli* M15 harboring the pQE31-Hexon recombinant plasmid was inoculated into 50ml LB containing 50 μg/ml kanamycin. The culture was incubated at 37℃ by shaking for 2 to 3 h. After taking 1ml sample as uninduced control, IPTG was added to the culture to a final concentration of 0.1 mmol/L.The culture was incubated at 37℃ for 4 h with sampling 1 ml after OD_{600nm} 0.0280，0.284，0.382，0.530，0.786，1.270，1.556 induction. The samples dealt were the same as the above.

2.5 Induced expression at different incubation temperatures

2 ml of overnight culture of *E. coli* M15 harboring the pQE31-hexon recombinant plasmid was inoculated into 50 ml LB containing 50 μg/ml kanamyacin. The culture was incubated at 37℃ by shaking for 2 to 3 h. After taking 1ml sample as uninduced control, IPTG was added to the culture to a final concentration of 0.1 mmol/L.The cultures were incubated at 28℃,30℃,34℃, 37℃ and 39℃ for 4 h with sampling of 1 ml.

3. RESULTS AND DISCUSSION

3.1 Induced expression at different incubation periods

A specific band of protein at the position about 52 kD was detected by SDS-PAGE (Figure1), which was similar to that predicted. The amount of expression was significantly increased with extension of induction time, and it reached the peak after 4 h induction.

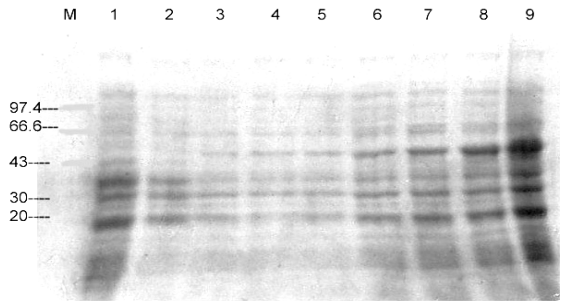

Figure 1 Analysis of induced expression at different incubation periods M: Protein Markers; Lane1: *E.coli* M15; Lane2: Uninduced control (pQE31-Hexon /M15); Lane3~9: pQE31-Hexon /M15 induced for 2 min, 12 min, 30 min, 1 h, 2 h, 4 h, 8 h respectively

3.2 Induced expression at different IPTG concentrations

A specific band of protein at the position about 52 kD was detected by SDS-PAGE (Figure2), which was similar to that predicted. It reached the peak at IPTG concentration of 0.125 mM.

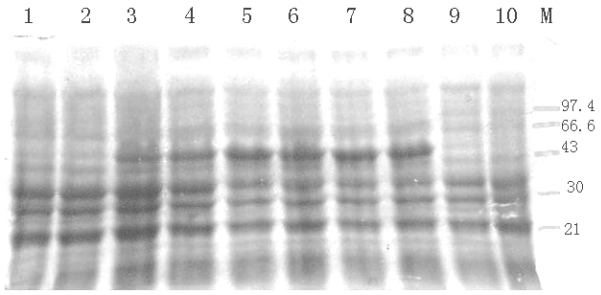

Figure 2 Analysis of induced expression at different IPTG concdntrations M: Protein Markers; Lane1: E.coli M15; Lane2: Uninduced control (pQE31-Hexon /M15); Lane3~10: pQE31-Hexon /M15 induced at different IPTG concentration [2.0mM, 1.0mM, 0.5 mM, 0.25 mM 0.125 mM, 0.0625 mM, 0.032 mM, 0.016 mM]

3.3 Induced expression at different initial concentration of MIS/pQE 31-Hexon

A specific band of protein at the position about 52 kD was detected by SDS-PAGE (Figure 3), which was similar to that predicted. It reached the peak at initial concentration of MIS/pQE 31-Hexon (OD_{600nm} 0.582).

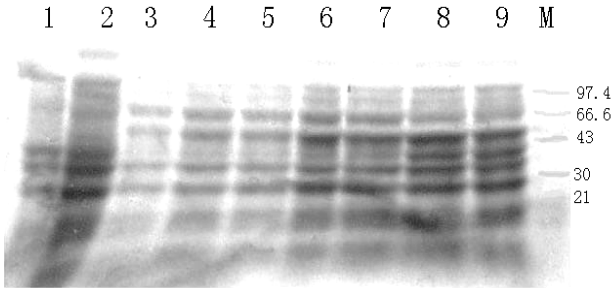

Figure3 Analysis of induced expression at different initial incubation concentrations of MIS/pQE 31-Hexon M: Protein Markers; Lane1: *E.coli* M15; Lane2: Uninduced control (pQE31-Hexon /M15); Lane3~9: pQE31-Hexon /M15 induced at different initial Concentration [OD600＝0.28 ， 0.284，0.382，0.530，0.786, 1.270, 1.556]

3.4 Induced expression at different incubation temperatures

A specific band of protein at the position about 52kD was detected by SDS-PAGE (Figure 4), which was similar to that predicted. It reached the peak at 37℃.

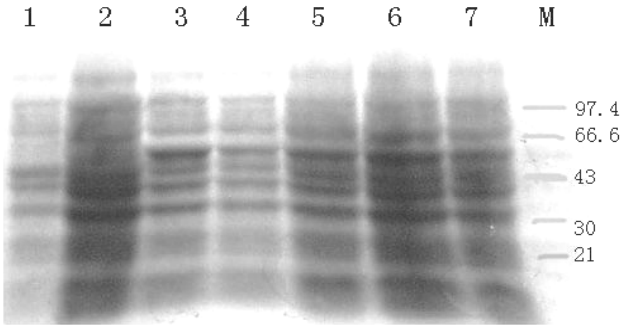

Figure 4 Analysis of induced expression at different incubation temperatures M: Protein Markers; Lane1: E.coli M15; Lane2: Uninduced control (pQE31-Hexon /M15); Lane3~7: pQE31-Hexon /M15 induced at 28, 30, 34, 37, 39℃

3.5 General discussion

Adenovirus infection is very widespread and until now there are no effective preventive measures. So the manufacture and development of genetically engineered vaccine have broadly prospect.

We have studied the optimal expression conditions of the Ad3 hexon gene protein in *E. coli*. The optimal conditions were OD_{600nm} 0.5, IPTG 0.125 mmol/L, incubation at 37℃ and incubation for 4 hours. This provided a good foundation for producing adenovirus genetic engineering vaccine in future research [5].

4. CONCLUSIONS

The optimum of Hexon expression protein conditions was studied. This provided the foundation for purifying the protein and studying the protein activity in future research.

REFERENCES

[1] Soumitra R., Pamelas S., Alan M. and Michael, K. **1998** Circumvention of immunity to the adenovirus major coat protein hexon, *Journal of Virology*, *72*(8), 6875-6879.

[2] John J. R., Paula R. K. and Roger M. B. **2003** Structurel and phylogenetic analysis of adenovirus Hexons by use of high-resolution X-ray crystallographic, molecular modeling and sequence-based methods, *Journal of Virology*, *77*(17), 9553-9566.

[3] Yasuo, I., Shuhei, K., Masatoshi, O., Kunihiko, K. and Shozo S.**2001** Identification of the Hexon region of an adenovirus involved in a new outbreak of keraticonjunctioitis, *Journal of Clinical Microbiology*, *39*, 2975-2977.

[4] Hou, Y, Liu J., Guo, C. L., Jin, Y. X. and Qu, Z. Y. **2003** Cloning and characterization of Hexon gene of human adenovirus type 3, *Chinese Journal of Endemiology*, *22*(3), 245-247.

[5] Yang, L. M., QU, Z. Y., HOU, Y., Liu, J. and Guo C. L. **2004** Construction and expressing of hexon gene of human adenovirus type 3, *Journal of Harbin Medical University*, *38*(3, 218-220.