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STUDY ON THE NUCLEOTIDE MUTATION IN –35 BOX SEQUENCE OF GENE PROMOTER BY MEANS OF MICROCALORIMETRIC METHOD

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The important thermochemistry research method–microcalorimetry was successfully applied to study the effect of different nucleotide mutations in –35 box sequence on the gene promoter activity in *Escherichia coli*. The metabolic thermogenic curves of different *Escherichia coli* strains were obtained by microcalorimetric measurement using the TAM air Isothermal Microcalorimeter. By analyzing the data such as metabolic rate constant (k_m), inhibitory ratio (I) and half-inhibitory concentration of antibiotic (IC_{50}) obtained by the microcalorimetric method, it was found that the structure of –35 box sequence was very important for the gene promoter activity in *Escherichia coli*. Different nucleotide mutations in –35 box sequence had different effect on the gene promoter activity.

Keywords: *microcalorimetric method, nucleotide mutation, promoter activity*

Introduction

Promoter is a specific DNA sequence recognized and bound by RNA polymerase in the gene transcription initiation process. It is an important functional element necessary for gene transcription and expression [1].

The microcalorimetric method is an important technique for thermochemistry and thermodynamic study. Microcalorimetric study on bacterial growth can provide a continuous measurement of heat production in the microbial metabolic process and supply the thermogenic curves which can describe the growth process [2, 3]. In our previous work the microcalorimetric method has been successfully applied to study the effect of transcription start site mutagenesis on RM07 promoter activity in *E. coli*. It revealed that the nucleotide component of transcription start site was very critical for the promoter strength and gene transcription efficiency [4]. In this paper the microcalorimetric method based on the bacterial heat output in the growth process was further used to study the effect of specific nucleotide mutation in –35 box sequence on the RM07 promoter activity in *E. coli*.

Experimental

Bacterial strains and culture conditions

The bacterial strains, *E. coli* HB101, *E. coli* HB101/pKK232-07-3, *E. coli* HB101/pKK232-07-35-2 and *E. coli* HB101/pKK232-07-35-4 were grown in Luria–Bertani (LB) complex medium supplemented with antibiotics when necessary [5].

General molecular biological techniques

General molecular biological techniques (plasmid extraction, DNA fragment isolation, ligation, *E. coli* transformation and PCR) were performed as described [5].

DNA site-directed mutagenesis

The polymerase chain reaction (PCR) was used to perform the DNA site-directed mutagenesis according to the method described previously [6]. The presence of the desired nucleotide mutation in RM07 fragment (Fig. 1) was confirmed by DNA sequencing.

Calorimeter and microcalorimetric measurements

A TAM air Isothermal Microcalorimeter, manufactured by Thermometric AB company of Sweden, was used to measure heat output of the metabolism of different *E. coli* strains as in our previous paper was described [4]. For details of the performance and structure of the instruments, please see the Instruction Manual of TAM air Isothermal Microcalorimeter and [7]. Microcalorimetric measurement was performed as described in our previous paper [4].

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Calculation of the metabolic rate constant (k_m), inhibitory ratio (I) and half inhibitory concentration of antibiotic (IC_{50})

The values of metabolic rate constant (k_m), inhibitory ratio (I) and half inhibitory concentration of antibiotic (IC_{50}) were calculated according to the method described in our previous paper [4].

Results

Site-directed mutagenesis of the –35 box sequence

In the previous work of our laboratory, the 492 bp RM07 DNA fragment having gene promoter activity in *E. coli* was isolated from the chromosomal DNA of halophilic Archaea. DNA sequence analysis revealed that the RM07 fragment contained the typical –35 and –10 box sequences of *E. coli* gene promoter [8, 9]. In this work, the unmodified RM07 DNA fragment (355 bp–492 bp, relative to the first nucleotide of RM07 fragment) was inserted upstream of the promoter-less chloramphenicol acetyltransferase gene (chloramphenicol resistance gene, *cat*) in the promoter probe vector pKK232-8 [10], generating plasmid pKK232-07-3 (Fig. 1). The second nucleotide T in the –35 box sequence of RM07 (TTGTCA) was changed to G by means of DNA site-directed mutagenesis technique, then the mutated RM07 fragment (355 bp–492 bp) was inserted upstream of the promoter-less *cat* gene in pKK232-8, generating plasmid pKK232-07-35-2 (Fig. 1). Using the similar method, the fourth nucleotide T in the –35 box sequence of RM07 (TTGTCA) was changed to A, generating plasmid pKK232-07-35-4 (Fig. 1). The presence of the desired nucleotide mutations (shown in Fig. 1) was confirmed by DNA sequencing. Then above plasmids were transformed into *E. coli* HB101, respectively.

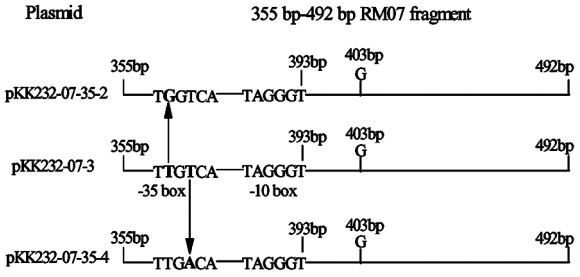

Fig. 1 Site-directed mutagenesis of the –35 box sequence of RM07 DNA fragment. In pKK232-07-35-2, the second nucleotide T in the –35 box sequence of RM07 was changed to G. In pKK232-07-35-4, the fourth nucleotide T in the –35 box sequence of RM07 was changed to A

Studying the effect of nucleotide mutations in –35 box sequence on promoter activity by means of microcalorimetric method

The metabolic thermogenic curves of different transformants growing in LB medium containing various

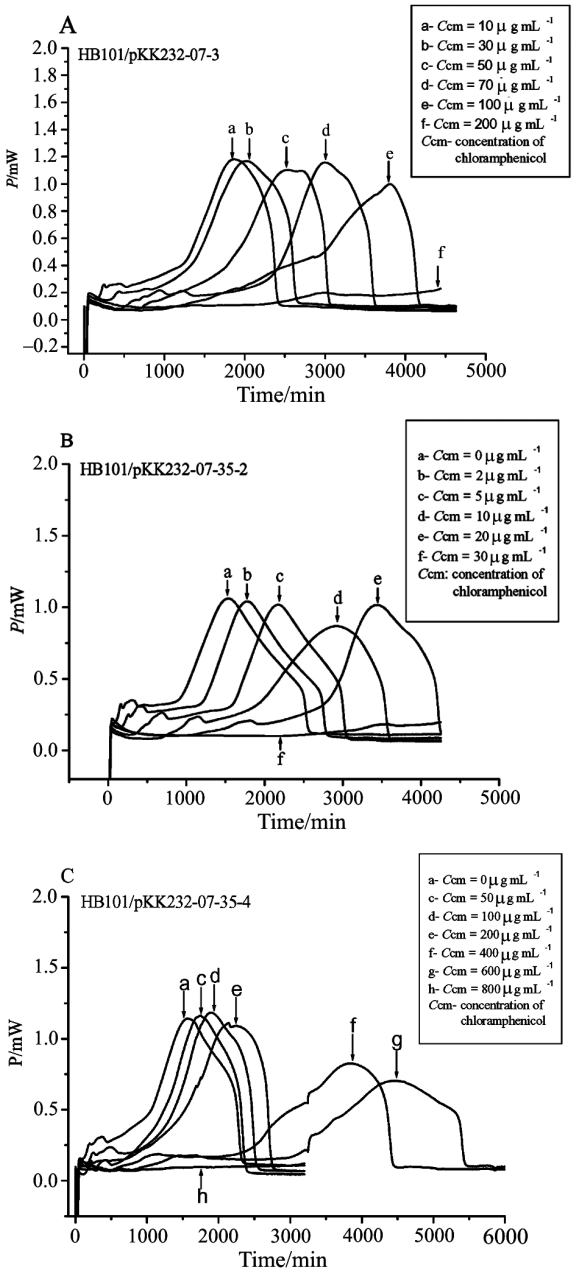

Fig. 2 The metabolic power-time curves of *E. coli* transformants growing in LB medium containing various concentrations of chloramphenicol. A: *E. coli* HB101/pKK232-07-3 (the concentration of chloramphenicol is 10, 30, 50, 70, 100 and 200 μg mL^{-1}); B: *E. coli* HB101/pKK232-07-35-2 (the concentration of chloramphenicol is 0, 2, 5, 10, 20 and 30 μg mL^{-1}); C: *E. coli* HB101/pKK232-07-35-4 (the concentration of chloramphenicol is 0, 50, 100, 200, 400, 600 and 800 μg mL^{-1}). C_{cm}: concentration of chloramphenicol

Table 1 Data about the *E. coli* growth in LB medium containing ampicillin[a]

E. coli	C_{amp}/μg mL^{-1}	k_m/min^{-1}
HB101	100	0
HB101/pKK232-07-3	100	0.01572
HB101/pKK232-07-35-2	100	0.01790
HB101/pKK232-07-35-4	100	0.02217

[a] C_{amp}: concentration of ampicillin

concentrations of chloramphenicol were obtained by microcalorimetric measurement and shown in Fig. 2. The data about the growth of *E. coli* HB101, *E. coli* HB101/pKK232-07-3, *E. coli* HB101/pKK232-07-35-2 and *E. coli* HB101/pKK232-07-35-4 in LB medium containing ampicillin and chloramphenicol, such as the metabolic rate constant (k_m), inhibitory ratio (I) and half inhibitory concentration of antibiotic (IC_{50}), were calculated according to the metabolic thermogenic curves and listed in Tables 1 and 2.

The following results could be acquired by analyzing these data: (*i*) *E. coli* HB101 was sensitive to ampicillin. When the concentration of ampicillin reached 100 μg mL^{-1}, *E. coli* HB101 could not grow (k_m=0) (Table 1). The other transformants: *E. coli* HB101/pKK232-07-3, *E. coli* HB101/pKK232-07-35-2 and *E. coli* HB101/pKK232-07-35-4 were all resistant to ampicillin. When the concentration of ampicillin reached 100 μg mL^{-1}, they all could grow (Table 1). The plasmids pKK232-07-3, pKK232-07-35-2 and pKK232-07-35-4 all contained the ampicillin resistance gene, so the *E. coli* transformants containing these plasmids were resistant to ampicillin. (*ii*) *E. coli* HB101/pKK232-07-3 was resistant to chloramphenicol. IC_{50} for chloramphenicol was 37.64 μg mL^{-1} (Table 2). (*iii*) The level of resistance to chloramphenicol of *E. coli* HB101/pKK232-07-35-4 was increased greatly compared with *E. coli* HB101/pKK232-07-3. IC_{50} for chloramphenicol was 248.73 μg mL^{-1}. When the concentration of chloramphenicol reached 600 μg mL^{-1}, *E. coli* HB101/pKK232-07-35-4 could still grow (Table 2). Compared with the unmodified RM07 fragment in pKK232-07-3, the fourth nucleotide T in the –35 box sequence of RM07 was mutated to A in pKK232-07-35-4 (Fig. 1). Thus this nucleotide mutation resulted in the increase of chloramphenicol resistance level and improvement of RM07 promoter activity. (*iv*) The level of resistance to chloramphenicol of *E. coli* HB101/pKK232-07-35-2 was decreased compared with *E. coli* HB101/pKK232-07-3. IC_{50} for chloramphenicol was only 6.24 μg mL^{-1}. When the concentration of chloramphenicol reached 30 μg mL^{-1}, *E. coli* HB101/pKK232-07-35-2 could not grow

Table 2 Data about the growth of *E. coli* transformants in LB medium containing various concentrations of chloramphenicol[a]

E. coli	C_{cm}/μg mL^{-1}	k_m/min^{-1}	I/%	IC_{50}/μg mL^{-1}
HB101/pKK232-07-3	0	0.01648	–	
	10	0.01279	22.4	
	30	0.00925	43.9	
	50	0.00607	63.2	37.64
	70	0.00233	85.9	
	100	0.00169	89.7	
	200	0	100	
HB101/pKK232-07-35-2	0	0.01476	–	
	2	0.01101	25.4	
	5	0.00786	46.7	6.24
	10	0.00417	71.7	
	20	0.00244	83.4	
	30	0	100	
HB101/pKK232-07-35-4	0	0.01749	–	
	50	0.01504	14.0	
	100	0.01375	21.4	
	200	0.01019	41.7	248.73
	400	0.00361	79.4	
	600	0.00338	80.1	
	800	0	100	

[a] C_{cm}: concentration of chloramphenicol

(k_m=0) (Table 2). Compared with the unmodified RM07 fragment in pKK232-07-3, the second nucleotide T in the –35 box sequence of RM07 was changed to G in pKK232-07-35-2 (Fig.1). Thus this nucleotide mutation resulted in the decrease of chloramphenicol resistance level and RM07 promoter activity.

Discussion

In this work, the effect of specific nucleotide mutations in –35 box sequence on gene promoter activity in *E. coli* was studied by means of microcalorimetric method. Through analyzing the values of metabolic rate constant (k_m), inhibitory ratio (I) and half-inhibitory concentration of antibiotic (IC_{50}) calculated from the metabolic thermogenic curves, it was found that different nucleotide mutations in –35 box sequence had different effect on the promoter activity. The results obtained by the microcalorimetric method also suggested that the structure of the –35 box sequence was very important to the gene promoter function in *E. coli*. Previous research have revealed that the –35 box sequence is the core promoter element. It is the RNA polymerase binding site necessary for the gene transcription initiation, so its structure is very critical for the promoter activity and gene transcription efficiency [1]. Our research results are consistent with it.

The microcalorimetric method is a very sensitive analytic method which has some advantages compared with other technique. It is particularly valuable for monitoring a variety of processes as it is non-destructive and non-invasive, that it does not interfere with the normal reaction system [11]. Previous work have shown that the microcalorimetric method can be successfully used to study the microbial growth and metabolism [2, 12, 13, 14]. In this paper, our work further suggest that the microcalorimetric method is useful and applicable for studying gene promoter combined with other molecular biological method.

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References

1 R. F. Weaver, Molecular Biology, McGraw-Hill Companies, Inc. 1999.
2 C. L. Xie, H. K. Tang and S. S. Qu, Thermochim. Acta, 123 (1988) 33.
3 A. Abderrahmane, L. Yi, G. Wen-Ying, S. Ping and Q. S. Sheng, J. Therm. Anal. Cal., 68 (2002) 909.
4 Y. Yang, Y. Liu, J. Zhu and P. Shen, J. Therm. Anal. Cal., 75 (2004) 293.
5 J. Sambrook, E. F. Fritsch and T. Maniatis, Molecular Cloning: A Laboratory Manual, 2nd Edn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 1989.
6 C. W. Dieffenbach and G. S. Dveksler, PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 1995.
7 I. Wadsö, Thermochim. Acta, 394 (2002) 305.
8 Z. H. Duan, Master thesis, Wuhan University, Wuhan, China 2000.
9 Y. P. Huang, Ph.D. thesis, Wuhan University, Wuhan, China 2001.
10 J. Brosius, Gene, 27 (1984) 151.
11 I. Wadsö, Thermochim. Acta, 269/270 (1995) 337.
12 L. Ruan, Y. Liu, Z. Gao, P. Shen and S. S. Qu, J. Therm. Anal. Cal., 70 (2002) 521.
13 Y. P. Huang, Y. Liu, P. Shen and S. S. Qu, J. Therm. Anal. Cal., 74 (2003) 163.
14 X. Li, Y. Liu, J. Wu, H. G. Liang and S. S. Qu, J. Therm. Anal. Cal., 67 (2002) 589.

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