Investigation of dimethylglycine oxidase operon transcription

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Analysis of the transcription of dimethylglycine oxidase encoding operon from *Arthrobacter globiformis* showed that all five genes encoding 10-formyltetrahydrofolate deformylase (*purU*), dimethylglycine oxidase (*dmg*), 5-formiminotetrahydrofolate cyclodeaminase (*fic*), 5,10-methylentetrahydrofolate dehydrogenase (*folD*) and formate dehydrogenase (*fdh*) are transcriptionally coupled. A stem-loop structure localized between the *dmg* and *fic* genes has been found to influence the transcription of *fic*, *folD* and *fdh* genes. Additional sequences, which are recognized by *E. coli* as a promoter, were detected between this hairpin and the 3' end of the operon.

Key words: dimethylglycine oxidase operon, *Arthrobacter globiformis*, transcription

INTRODUCTION

One of the mechanism that bacteria have evolved to cope up with osmotic stress in the environment is the intracellular accumulation of osmoprotectants. Glycine betaine is the preferred osmoprotectant in most eubacterial species [1–4]. In contrast to the biosynthesis of this compound, the catabolism of glycine betaine in microorganisms is very scarcely investigated. Some bacterial strains capable of using glycine betaine as a single carbon source have been isolated, but the enzymes participating in the catabolism have not been purified or characterized [5, 6]. Glycine betaine can be progressively demethylated to glycine with dimethylglycine and sarcosine as intermediates. The conversion of betaine to dimethylglycine is catalyzed by betaine–homocysteine transmethylase [7]. The enzymatic formation of sarcosine from dimethylglycine is poorly studied. Only *N*,*N*-dimethylglycine oxidases (DMGO) from the fungus *Cylindrocarpon didymum* M-1 [8] and *Arthrobacter globiformis* [9] have been purified to homogeneity and *N*,*N*-dimethylglycine dehydrogenases from *Pseudomonas putida* [10] and *Rhizobium meliloti* [11] were briefly mentioned. The fate of methyl groups from dimethylglycine remains uncertain. It is only known that oxidation of the methyl groups of glycine betaine in the presence of tetrahydrofolate and dimethylglycine or sarcosine dehydrogenases from *Arthrobacter* sp. P1 and *Corynebacterium* sp. P-1 generates 5,10-methylentetrahydrofolate [12–14].

The *N*,*N*-dimethylglycine oxidase encoding (*dmg*) operon containing four complete ORFs and one truncated ORF were previously cloned from *Arthrobacter globiformis* [9]. It was proposed that there are promoter-like sequences upstream these genes. The aim of the current study was to investigate the transcription of the *dmg* operon in *E. coli* cells and to elucidate whether all of ORFs are transcribed from single or multiple promoters.

MATERIALS AND METHODS

Bacterial strains, plasmids and culture conditions. The pEH1 plasmid containing the entire *dmg* operon has been described previously [9]. The promoter cloning vectors pNM480–482 were used for all cloning experiments [15]. *Escherichia coli* DH5α strains were cultured at 37 °C on NA agar or in BHI broth supplemented with ampicillin (Ap, 50 μg/ml) and X-gal (0.004%) (when required).

DNA isolation and manipulation. Restriction endonucleases, DNA isolation kit, Klenow polymerase and T4 DNA ligase were purchased from MBI Fermentas (Lithuania) and used according to the manufacturer's instructions. Competent cells were prepared according to the method of Hanahan [16].

Promoter cloning. After pEH1 digestion with appropriate restriction endonucleases the DNA fragments containing promoter sequences were isolated from aga-

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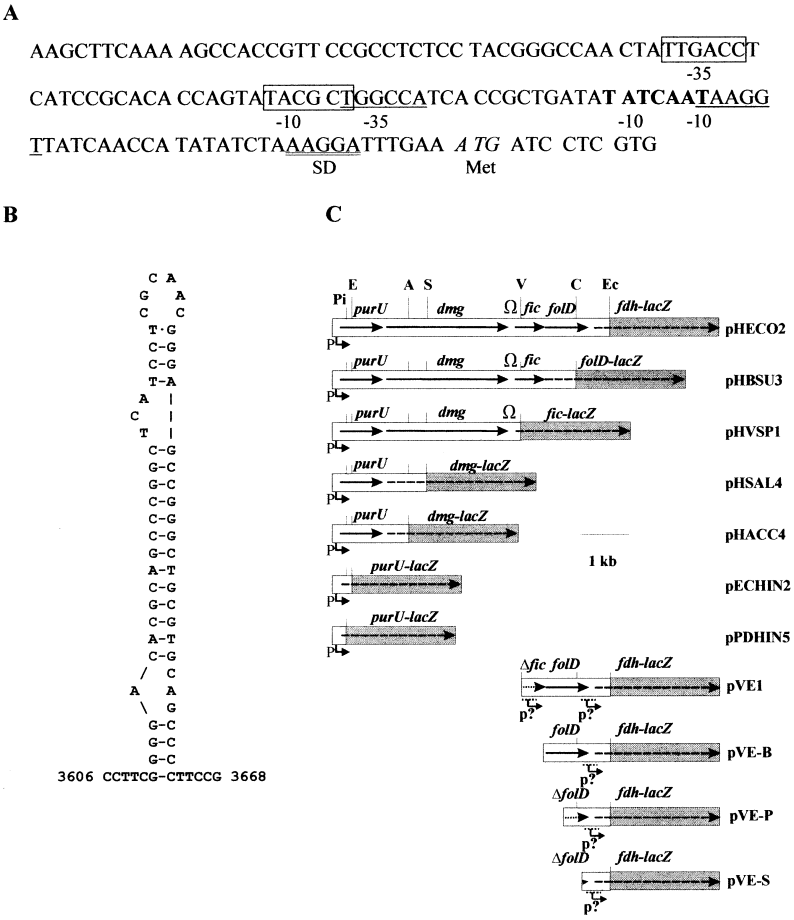
rose gel, treated with Klenow polymerase and ligated with the linearized pNM480–482 plasmids. *E. coli* DH5α was transformed with the ligation mixture, and the *lacZ*-positive transformants were selected on NA agar plates containing ampicillin and X-gal.

β-galactosidase assay. *E. coli* carrying various plasmids were grown in BHI broth overnight. A 0.5-ml sample of each culture was transferred into the fresh BHI broth supplemented with Ap. The bacterial cultures were further incubated for additional four or 24 hours. The β-galactosidase activity was measured as described by Miller [17].

RESULTS AND DISCUSSION

Determination of promoter. The *dmg* operon from *A. globiformis* contains five ORFs clustered in the same orientation suggesting the transcriptional coupling. In addition, the G+C content of the region located upstream of the first ORF is much lower as compared to G+C content of the entire fragment. Moreover, putative promoter sequences can be traced in this A+T reach region (Figure, A). The promoter(s) probably functions in the *E. coli* cells since the production of Dmg protein by *E. coli* harboring pEH1 plasmid does not need any induction with IPTG. To test this idea more directly, the DNA fragments from pEH1 containing a putative promoter and a part of each ORF from the *dmg* operon were fused in-frame with a promoterless *lacZ* gene in pNM480–482 vectors resulting in plasmids pHECO2, pHBSU3, pHVSP1, pHSAL4, pHACC4, pECHIN2 and pPDHIN5 (Fig. 1, C). *E. coli* strains containing these recombinant plasmids produced β-galactosidase according to the blue colonies in the presence of X-gal, however, the intensity of color was different. The weakest color was found in the case of the pHVSP1 plasmid. The reason for such a low expression of β-galactosidase might be a theoretically predicted putative hairpin structure localized in front of the *fic* gene (Fig. 1, B), which can influence the transcription level of the fused β-galactosidase gene. However, the biosynthesis of fused proteins was not so markedly affected in the cells olduring pHECO2 or pHBSU3 plasmid containing the same putative hairpin structure. This discrepancy can be explained, if an additional promoter exists downstream the secondary structure. To detect this promoter, the *Vsp*I-*Eco*RI fragment was cut off and fused with the promotorless *lacZ* gene in the pNM481 vector resulting in the pVE1 plasmid (Fig. 1, C). Cells carrying this plasmid were found to form blue colonies. These results confirmed the presence of an additional promoter in this region. To localize this promoter, various DNA fragments from the 3'-end of the *dmg* operon were ligated to *lacZ* gene (Fig. 1, C). All those recombinant plasmids determined synthesis of β-galactosidase. So, it can be concluded that at least one additional promoter is localized downstream the hairpin structure.



A. Nucleotide sequence of pEH1 upstream the *purU* encoding region. Two putative promoters containing both –35 and –10 sites are in box and underlined. The additional –10 region is in bold style. A putative ribosomal-binding site is double-underlined. The first four *purU* codons are in italic type

B. The structure of putative secondary structure between *dmg* and *fic* genes

C. Construction of fusions between dimethylglycine oxidase operon and *lacZ*. *purU* – 10-formyltetrahydrofolate deformylase, *dmg* – dimethylglycine oxidase, *fic* – 5-formiminotetrahydrofolate cyclodeaminase, *folD* – 5,10-methylentetrahydrofolate dehydrogenase and *fdh* – formate dehydrogenase. P – promoter; filled boxes – *lacZ* gene; Ω – the stem-loop structure; A – *Acc*65I; E – *Eco*47III; Ec – *Eco*RI; C – *Bsu*15I; Pi – *Pdi*I; S – *Sal*I; V – *Vsp*I. Numbering in the figure is according to [9]

Analysis of β-galactosidase expression level. The power of the putative promoters was determined assaying the activity of fused β-galactosidase in *E. coli* cells harvested both in exponential and stationary phase. Results are shown in Table. These data confirmed that

Table. **Dependence of β-galactosidase activity on recombinant plasmid and growth phase. Results of two independent experiments are averaged**

Plasmid	β-galactosidase activity; Miller units/ml of culture	
	Exponential growth phase	Stationary growth phase
pHECO2	101.0	64.9
pHBSU3	148.7	176.3
pHVSP1	0	5.2
pHSAL4	1620.0	1164.9
pHACC4	1502.7	362.8
pECHIN2	521.4	66.9
pPDHIN5	1218.7	729.7
pVE1	9.72	23.9
pVE-B	0.95	74.2
pVE-P	2.76	27.4
pVE-S	3.78	19.2

the level of β-galactosidase activity was considerably lower in the presence of hairpin structure (pHECO2, pHBSU3, and pHVSP1) than when this structure was lost (pHSAL4, pHACC4, pECHIN2 and pPDHIN5). It is important to note that the power of the promoter measured using the described method is under the influence of a great number of various parameters, such as the activity of the promoter itself, the stability of the fused protein, the presence of an appropriate RNA polymerase capable to recognize the promoter, etc. This is seen if we compare the β-galactosidase production levels determined by pECHIN2 and pPDHIN5 plasmids or the dependence of the activity of different promoters (for example, pHACC4 and pVE-B) on the growth phase (Table).

The results of this study allow the following conclusions: *i)* all five genes in the *dmg* operon are transcripted from the promoter sequences found upstream the *purU* gene, *ii)* the stem-loop structure localized between the *dmg* and *fic* genes influences the transcription of *fic*, *fold* and *fdh* genes, *iii)* additional promoter(s) exists between the stem-loop structure and the 3' end of the operon.

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DIMETILGLICINO OKSIDAZĖS OPERONO TRANSKRIPCIJOS TYRIMAS

S a n t r a u k a

Dimetilglicino oksidazės operono iš *Arthrobacter globiformis* transkripcijos tyrimas parodė, kad visi penki genai (10-formiltetrahidrofolato deformilazės (*purU*), dimetilglicino oksidazės (*dmg*), 5-formiminotetrahidrofolato ciklodeaminazės (*fic*), 5,10-metilentetrahidrofolato dehidrogenazės (*folD*) ir formato dehidrogenazės (*fdh*)) nuskaitomi nuo promotoriaus, esančio prieš pirmąjį operono geną *purU*. Nustatyta, kad tarp *dmg* ir *fic* genų esanti antrinė struktūra turi įtakos *fic*, *folD* ir *fdh* genų transkripcijai, kad bent viena papildoma promotorinė seka yra tarp šios antrinės struktūros ir operono 3' galo.