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NOTE

The N-Terminal Domain of *Aliivibrio fischeri* LuxR Is a Target of the GroEL Chaperonin▽

Ilya V. Manukhov,[1]* Ol'ga E. Melkina,[1] Ignatii I. Goryanin,[1]
Ancha V. Baranova,[1,2]* and Gennadii B. Zavilgelsky[1]

State Research Institute of Genetics and Selection of Industrial Microorganisms (Genetika), 1st Dorozhnii pr. 1, 117545 Moscow, Russia,[1] and Department of Molecular Biology and Microbiology, David King Hall, MSN 3E1, George Mason University, Fairfax, Virginia 22003[2]

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Here we show that the C-terminal domain of LuxR activates the transcription of *Aliivibrio fischeri luxICDABEG* in *Escherichia coli* SKB178 *gro*$^+$ and *E. coli* OFB1111 *groEL673* strains to the same level. Using affinity chromatography, we showed that GroEL binds to the N-terminal domain of LuxR, pointing to a GroEL/GroES requirement for the folding of the N-terminal domain of LuxR.

In the marine bacterium *Aliivibrio fischeri*, the expression of *lux* genes is regulated by the LuxI-LuxR system, which determines the intensity of the bioluminescence of growing cells depending on the density of the cell population (quorum sensing) (8, 9, 17). LuxR is a quorum-sensing transcriptional regulator of the *luxICDABEG* operon (8, 9, 13). Upon binding with an autoinducer (AI), the LuxR protein acquires the ability to form a complex with the *lux* box and to activate *luxICDABEG* operon transcription (6, 7, 21, 22). It was demonstrated that *rpoH* and *groE* mutants with the entire *lux* system of *A. fischeri* show a significant decrease in bioluminescence; it was proposed that the GroEL/GroES chaperonins participate in the folding of LuxR (1, 5). The GroEL/GroES chaperonin-folding chamber is an encapsulated space with a hydrophilic wall where many cellular proteins acquire their native state (12).

During extraction using affinity chromatography, the glutathione *S*-transferase (GST)–LuxR fusion protein and chaperonin GroEL coelute (15). Interestingly, both GroEL and cochaperonin GroES were previously shown to enhance the accumulation of the soluble LuxR-like protein TraR, a quorum-sensing transcription factor from *Agrobacterium tumefaciens* (3).

The LuxR protein consists of two domains: the 88-amino-acid (aa) C-terminal domain (CTD), which is responsible for contacts of the protein with the *lux* box in DNA (the DNA-binding domain), and the 162-aa N-terminal domain (NTD), which determines its binding with the AI (4, 11, 20). Here we tested the hypothesis that GroEL/GroES chaperonins are required for the folding of the NTD of LuxR.

The *Escherichia coli* strains and plasmids used in this study are listed in Table 1.

In order to determine the effect of the GroEL chaperonin on LuxR and CTD activities, we transformed pLuxR or pLuxRΔN in *E. coli* SKB178 *gro*$^+$ and OFB1111 *groEL673* strains containing pOM plasmid. The pLuxR plasmid contains the entire *luxR* gene; the pLuxRΔN plasmid contains a 5′-region-deleted *luxR* gene encoding only the CTD of LuxR; both genes are transcribed from the *lac* promoter. In *E. coli* SKB178 *gro*$^+$(pOM) cells, the addition of the pLuxR or pLuxRΔN plasmid leads to a rise in the intensity of bioluminescence due to activation of the P_r promoter of the *luxICDABEG* operon (Fig. 1).

Single colonies of cells grown overnight on solid medium at 37°C were transferred to Tris buffer and washed twice by centrifugation. Then, the cells were resuspended in 200 μl of L broth (20) and grown at 22°C without agitation.

Bioluminescence of cells was measured at room temperature in 200 μl of cell suspension by an LMA01 luminometer (Beckman). The intensity of the bioluminescence dramatically decreased in *E. coli* OFB1111 *groEL673* cells containing full-length LuxR. However, in *E. coli* SKB178 *gro*$^+$ or OFB1111 *groEL673* cells with the pLuxRΔN plasmid, the *groEL673* mutation had no influence on the level of bioluminescence. Thus, chaperonin GroEL is likely to be necessary for the folding of the active full-length LuxR but not for the folding of its CTD.

Plasmid pVFR1 contains a 1-kb DNA fragment of *A. fischeri* including *luxR* and P_l/P_r promoters along with a *luxCDABE* cassette of *Photorhabdus luminescens*. In this model, the transcription of *luxCDABE* from P_r is initiated after the addition of AI to the medium. Overnight bacterial inoculates with an optical density (OD) equal to 0.01 were grown in LB media in the presence of ampicillin (100 μg/ml) with aeration at 28°C until the OD reached 0.4 to 0.5. AI was added after 30 min of incubation at 42°C, which allowed for inactivation of any endogenous LuxR. After AI addition, cells were incubated at 22°C with no mixing. After 1 h,

* Corresponding author. Mailing address: GosNIIGenetika, 1st Dorozhnii pr. 1, 117545 Moscow, Russia. Phone for I. V. Manukhov: (903)790-59-19. Fax: (7) (495)315-05-01. E-mail: manukhov@genetika.ru. Phone for A. V. Baranova: (703)993-42-93. Fax: (7) (495)315-05-01. E-mail: abaranov@gmu.edu.

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TABLE 1. *E. coli* strains and plasmids used

Strain or plasmid	Relevant characteristics[a]	Reference(s)
Strains		
SKB178	F$^-$ *galE sup gro*$^+$	25
OFB1111	*groE673* Gly173Asp Gly337Asp (others markers the same as SKB178)	25
TG-1	*thi-1 supE44 hsd*Δ*5* Δ(*lac-proAB*) [F′ *traD36 proAB*$^+$ *lacI*^{q} *lacZ*ΔM15]	20
Plasmids		
pF1	pBR322 with a 16-kb BamHI fragment (*luxR luxICDABE*) of *A. fischeri* strain MJ-1 (AF170104); Ap^r	24
pGEX-KG	Plasmid vector for the synthesis of the GST fusions, ColE1 replicon; Ap^r	10
pGEX-LuxR	pGEX-KG vector containing the *gst-luxR* fusion gene; Ap^r	10, 16
pDEW201	The replication origin of pBR322; promoterless *P. luminescens luxCDABE* genes; Ap^r	23
pVFR1	pDEW201 with a 1-kb fragment of *A. fischeri* DNA (*luxR* and P_l and P_r promoters), Ap^r; the *luxCDABE* cassette of *P. luminescens* controlled by P_r promoter	16
pLuxR	pUC19 with *luxR* under the *lac* promoter; Ap^r	This study
pLuxRΔN	pTZ57R with a 5′-region-deleted *luxR* gene encoding the LuxR CTD (88 aa), under *lac* promoter; Ap^r	This study
pGEX-LuxRΔN	pGEX-KG with a 5′-region-deleted fragment of *luxR* gene fused with *gst*; Ap^r	This study
pGEX-LuxRΔC	pGEX-KG with a 3′-region-deleted fragment of the *gst-luxR* fusion; Ap^r	This study
pOM	pACYC184 with a BamHI/NruI fragment of *A. fischeri* DNA from pF1 (*luxICDABEG* under the P_r promoter and *lux*-regulatory DNA between *luxR* and *luxI* [without *luxR*]); Cm^r	This study

[a] Ap^r, ampicillin resistant; Cm^r, chloramphenicol resistant.

samples were collected for the measurement of bioluminescence. As shown in Fig. 2, in *E. coli* OFB1111 *groEL673* cells, the defect of GroEL may be compensated by an increase of the AI level in the medium. In the SKB178 *gro*$^+$ strain, bioluminescence is initiated at 10^{-9} M AI, while OFB1111 *groEL673* cells require at least 10^{-7} M AI.

We constructed the *gst*-fused variants of *luxR* and 5′-region-deleted *luxR* genes. The resulting fusion proteins, GST-LuxR and GST CTD, maintained their ability to activate *in vivo* the *lux* operon of *A. fischeri*. However, only GST-LuxR retained its ability to activate *lux* operon expression in the presence of AI (data not shown).

E. coli TG-1 cells were transformed with pGEX-LuxR, pGEX-LuxRΔN, or pGEX- LuxRΔC and grown in LB medium supplemented with ampicillin until the mid-exponential phase (OD, 0.5 to 0.6). The promoter (P_{lac}) was induced with 1 mM IPTG (isopropyl β-D-thiogalactopyranoside). Cells were incubated for 2 h at 37°C and then 12 h at 18°C. Cells were collected by centrifugation, disrupted by sonication in 1× phosphate-buffered saline (PBS; 140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.3) with supplemental 0.5% Triton X-100, and centrifuged to obtain cell extract. GST-containing proteins were purified by affinity chromatography on glutathione Sepharose (Novagen) by using a reduced glutathione solution as an eluent (10, 15).

Electrophoresis of proteins in 12% polyacrylamide gel (PAG) was performed under denaturing conditions (with SDS) according to the method of Laemmli (14).

Proteins isolated from the SDS-PAGE gels were identified by mass spectrometry. Samples for matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometry (MS) were prepared as described previously (19). MS

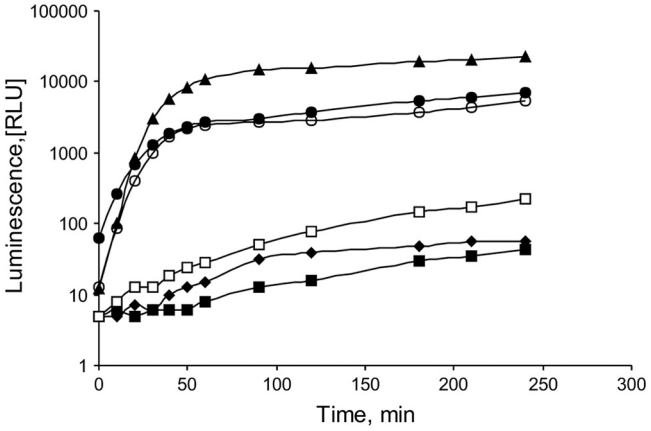

FIG. 1. Effect of *groEL* mutation on activities of the full-length LuxR and its CTD. RLU, relative luminescence units. The OD was controlled before and after each experiment. All strains contained pOM. Data for SKB178 *gro*$^+$(pUC19) (filled diamonds), OFB1111 *groEL673*(pUC19) (filled squares), SKB178 *gro*$^+$(pLuxR) (filled triangles), OFB1111 *groEL673*(pLuxR) (open squares), SKB178 *gro*$^+$(pLuxRΔN) (open circles), and OFB1111 *groEL673*(pLuxRΔN) (filled circles) are shown.

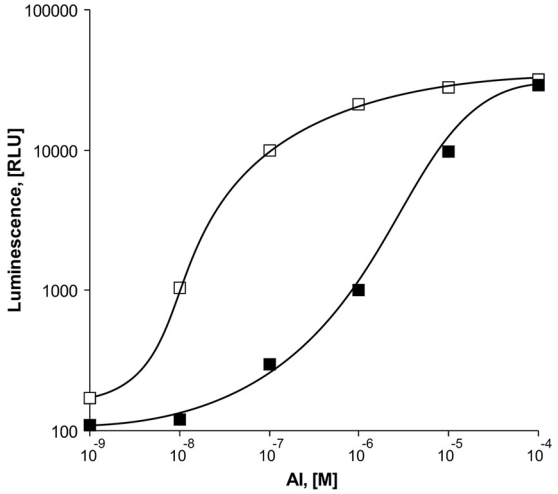

FIG. 2. Effects of AI concentrations in the media on the peak bioluminescence intensities of SKB178 *gro*$^+$(pVFR1) (open squares) and OFB1111 *groEL673*(pVFR1) (filled squares).

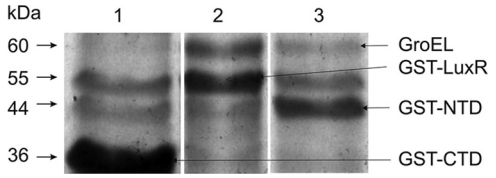

FIG. 3. SDS electrophoresis, in 12% gel, of the protein fractions obtained by affinity chromatography on a column with glutathione Sepharose. Proteins were isolated from *E. coli* TG-1 containing pGEX-LuxRΔN (lane 1), pGEX-LuxR (lane 2), or pGEX-LuxRΔC (lane 3). The bands of glycerol kinase (56.2 kDa; lanes 1 and 3) and of elongation factor EF-Tu (43.3 kDa; lanes 1 and 2) are present also (according to mass-spectrometry analysis, these bands correspond to the glycerol kinase and EF-Tu). These proteins accompanied the GST protein purified using gentle washing conditions.

was carried out after trypsin digestion in a Reflex III unit (Bruker) at the Orekhovich Institute of Biomedical Chemistry (Moscow, Russia). The resulting mass fingerprints were identified by using the Swiss-Prot database (2).

The GST, GST-LuxR, GST-NTD, and GST-CTD proteins were extracted by affinity chromatography using glutathione Sepharose columns. The molecular masses of the proteins were estimated by SDS electrophoresis in 12% gel (Fig. 3). In addition to the bands corresponding to GST-LuxR (54 kDa) and GST-NTD (45 kDa), one 60-kDa band was detected (Fig. 3, lanes 2 and 3). The 60-kDa protein was analyzed by mass spectrometry. According to the distribution and compositions of peptide fingerprints and comparison with the Swiss-Prot database (2), the 60-kDa protein was identified as chaperonin GroEL. Meanwhile, during affinity extraction of the deletion mutant, the GST-CTD protein was not accompanied by GroEL (Fig. 3, lane 1).

Our results provide evidence for the notion that the GroEL/GroES chaperonins participate in the folding of the LuxR protein through binding to the NTD, while the CTD of LuxR does not require GroEL/GroES for its folding. Interestingly, at high concentrations of AI (10^{-5} M and higher), the *groEL673* defect is completely compensated (Fig. 2). As AI forms a complex with the N-terminal domain of LuxR, one can assume that the formation of soluble LuxR takes place either simultaneously with the process of its synthesis on the ribosome after the binding of its N-terminal domain to already available AI or later, after the binding of the GroEL/GroES to the same domain.

Here we showed that the NTD of LuxR is a target for the GroEL/GroES chaperonins participating in the folding of the LuxR protein. In our previous study, we showed that the NTD of LuxR is targeted by Lon protease (18). Taken together, these data indicate that the NTD of LuxR may play a role both in quorum sensing and in the recognition of the stress signals.

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