Intragenic suppression of a luxR mutation: Characterization of an autoinducer-independent LuxR

Kristi A. Poellinger, Jean Pyo Lee, Juan V. Parales Jr., E.P. Greenberg *

Department of Microbiology and Program in Molecular Biology, The University of Iowa, Iowa City, IA 52242, USA

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Abstract

The Vibrio fischeri luminescence genes are activated by the LuxR protein and a diffusible signal termed the autoinducer. LuxR consists of two domains, a C-terminal transcriptional activator domain, and an N-terminal autoinducer-binding domain, which serves to regulate the function of the C-terminal domain. We have isolated and characterized an intragenic suppressor of a mutation that maps to the N-terminal domain and blocks autoinducer binding. The suppressor changes an alanine residue at position-221 in the C-terminal domain to a valine. In Escherichia coli, the suppressor allows partial activation of the V. fischeri luminescence genes although E. coli containing this protein remains unable to bind autoinducer.

To further analyze the influence of the second-site mutation on luxR function, we constructed a luxR gene that coded for a protein with a wild-type N terminal domain and with the ala-221 to val substitution in the C-terminal domain. This protein activated the luminescence genes in the presence or absence of autoinducer, and it bound autoinducer at levels comparable to the wild-type LuxR protein. Apparently, the alanine to valine substitution at position-221 allows activity of the C-terminal domain in a fashion independent of whether autoinducer is bound to the N-terminal domain.

Keywords: Autoinduction; LuxR; Quorum sensing; Transcriptional activator; Vibrio fischeri

1. Introduction

The Vibrio fischeri LuxR protein is the best-studied member of a family of transcription factors found in a number of genera of Gram-negative bacteria. LuxR family members are involved in a phenomenon termed quorum sensing and response [1]. In quorum sensing the bacteria produce an N-acetyl homoserine lactone, the autoinducer. The V. fischeri autoinducer, N-(3-oxohexanoyl) homoserine lactone [2], is a diffusible molecule that accumulates in the culture medium during growth [3]. When autoinducer reaches a sufficient concentration, LuxR activates transcription of the luminescence (lux) genes [4]. Thus LuxR and autoinducer are components of a system that allows V. fischeri to monitor its own population density and activate lux gene transcription only at sufficiently high cell densities, only when a quorum has been attained.

Previous studies indicate that LuxR is a 250-amino acid polypeptide consisting of two domains. The N-terminal domain (approximately two-thirds of the polypeptide) can bind autoinducer and regulate the activity of the C-terminal domain, which is responsible for DNA binding and activation of the lux genes.
In the absence of autoinducer, the N-terminal domain blocks the function of the C-terminal domain [5]. Point mutations mapping to the autoinducer binding region have been isolated [6,7], and we were interested in whether an analysis of intragenic suppressors of such mutations might enrich our understanding of interactions between the two domains of LuxR. We describe a second-site mutation that partially suppresses an autoinducer-binding mutation. Our analysis of this second-site mutation shows that it alters the DNA-binding domain and serves to protect this domain from the inhibitory effects of the N-terminal autoinducer-binding domain.

2. Materials and methods

2.1. Bacterial strains, plasmids, and culture conditions

The E. coli strains and plasmids used are described in Table 1. As described previously [8,9], E. coli was grown in L-broth or plated on L-agar containing the appropriate antibiotics for plasmid maintenance. Isopropyl-β-D-thiogalactoside (IPTG, 1 mM) and V. fischeri autoinducer (200 nM) were added as indicated. Incubation temperature was 30–32°C. Because the temperature-sensitive luxI on pJR551 is active below 30°C, extra care was taken to ensure that cultures of E. coli containing pJR551 were not exposed even briefly to permissive temperatures.

2.2. Measurements of culture luminescence and autoinducer binding

The luminescence of E. coli containing lux plasmids was measured when cultures reached an optical density of 0.5 at 660 nM as described elsewhere [10]. The light-measuring equipment and a calibration standard described by Hastings and Weber [11] were used. Autoinducer binding to E. coli expressing mutant LuxR proteins was measured by using the procedure of Hanzelka and Greenberg [12]. For autoinducer binding assays we used E. coli XL 1-Blue (pGroESL) containing luxR plasmids as indicated.

2.3. Western immunoblotting

Western immunoblotting with preadsorbed LuxR antiserum was by previously described techniques [7].

2.4. Plasmid purification, transformation, and DNA sequencing

Plasmids were purified by the method of Kraft et al. [13]. Manipulations of plasmid DNA were according to the methods of Sambrook et al. [14]. Transformations were by the procedure of Hanahan [15]. The sequence of luxR DNA in pKP700 and pKP700.1 was determined by using the chain-termination method. Primers described previously [7] were used to obtain the complete sequence for each DNA strand.

Table 1

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristics</th>
<th>Source [reference]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JM109</td>
<td>laq^R recA1 supE44</td>
<td>[17]</td>
</tr>
<tr>
<td>XL1-Blue</td>
<td>laq^R recA1 supE44</td>
<td>[18]</td>
</tr>
<tr>
<td>Plasmids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pDV751</td>
<td>pHK724 with a luxR point mutation coding for a gly-121 to arg substitution in LuxR</td>
<td>[7]</td>
</tr>
<tr>
<td>pKP700</td>
<td>pDV751 with second-site mutation coding for LuxR ala-221 to val</td>
<td>This study</td>
</tr>
<tr>
<td>pKP700.1</td>
<td>pHK724 with a point mutation coding for an ala-221 to val substitution in LuxR</td>
<td>This study</td>
</tr>
<tr>
<td>pHK555</td>
<td>luxR^- luxCDABE, P15A, Cm'</td>
<td>[19]</td>
</tr>
<tr>
<td>pJR551</td>
<td>luxR^- lux^15CDABE, P15A, Cm'</td>
<td>[20]</td>
</tr>
<tr>
<td>pGroESL</td>
<td>p lac groE</td>
<td>[21]</td>
</tr>
</tbody>
</table>
2.5. Mutagenesis of pDV751 and construction of pKP700.1

Mutagenesis of pDV751 in vitro was carried out using hydroxylamine as described elsewhere [7,16]. E. coli JM109 (pHK55.5) was transformed with pools of mutagenized plasmids. Transformants were selected on L-agar containing ampicillin, chloramphenicol, and IPTG. Over 10,000 colonies were screened for production of visible levels of luminescence after 1–2 days at 30°C. Two luminous colonies were observed and selected for further study. To construct pKP700.1, a PstI restriction fragment containing the 3'-146 bp of luxR was excised from pHK724 and replaced with the 146-bp PstI restriction fragment from pKP700 using standard recombinant DNA technology [14].

3. Results and discussion

3.1. An intragenic suppressor of luxR gly-121 to arg in pDV751

As described in the Materials and methods, we isolated two weakly luminescent transformants containing pHK555 and a mutagenized pDV751 derivative. The luxR genes in each of the two derivative plasmids were sequenced, and both had an identical missense mutation coding for an ala to val substitution at residue-221. Apparently, this substitution serves to partially compensate for the gly to arg substitution at position-121 in the LuxR polypeptide.

3.2. The mechanism of gly-121 to arg suppression by ala-221 to val

To gain information on how ala 221 to val was suppressing gly-121 to arg, the activity of the double mutant was compared to the activity of three other LuxR polypeptides: the wild-type polypeptide, the gly-121 to arg single substitution polypeptide, and a polypeptide containing the wild-type gly-121 and the suppressor substitution at position 221. The LuxR proteins were examined for their ability to activate the lux operon and to bind autoinducer in E. coli. To assess activation of the lux operon, we measured luminescence of E. coli containing pJR551 and a luxR expression vector as indicated in Table 2. Because pJR551 does not contain luxR, E. coli containing this plasmid but no luxR expression vector are weakly luminescent. With pHK724, which encodes a wild-type LuxR protein, culture luminescence is much higher, and under the conditions of our experiments, autoinducer increased the level of luminescence over 100-fold. With pDV751, which codes for a mutant LuxR with a gly-121 to arg substitution, the level of luminescence was about 0.001% of the induced level with the wild-type protein. Consistent with the notion that gly-121 resides within the autoinducer binding region, addition of autoinducer did not stimulate the activity of the gly-121 to arg LuxR. With pKP700, which codes for a LuxR containing not only the gly-121 to arg substitution but also the ala-221 to val suppressor, the level of cellular luminescence was intermediate between the level with the wild-type protein and the level with the protein.

Table 2

<table>
<thead>
<tr>
<th>luxR plasmids</th>
<th>Amino acid substitutions in the luxR product</th>
<th>Luminescence units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>– autoinducer</td>
</tr>
<tr>
<td>pHK724</td>
<td>None</td>
<td>600</td>
</tr>
<tr>
<td>pDV751</td>
<td>gly121arg</td>
<td>0.8</td>
</tr>
<tr>
<td>pKP700</td>
<td>gly121arg, ala221val</td>
<td>18</td>
</tr>
<tr>
<td>pKP700.1</td>
<td>ala221val</td>
<td>52,000</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>3.5</td>
</tr>
</tbody>
</table>

*a As judged by a Western immunoblot analysis, LuxR levels were roughly equivalent in these experiments with E. coli containing any of the luxR plasmids.

*b Luminescence units are given as quanta s⁻¹ ml⁻¹ 10⁻⁵. The numbers are the averages of at least three independent experiments.
containing only the substitution at position-121. Addition of autoinducer to cells with pKP700 had no appreciable effect on luminescence. The suppressor mutation seemed to restore the autoinducer-independent level of activity to the protein. However, the level of activity was lower than the autoinducer-independent activity of the wild-type protein coded on pHK724. Autoinducer-binding experiments showed that whereas E. coli containing the wild type LuxR bound significant amounts of autoinducer, E. coli with the gly-121 to arg LuxR or the LuxR containing both the gly-121 to arg substitution and the suppressor failed to bind autoinducer at levels above background (Table 3). These data are consistent with the conclusions that gly-121 plays an important role in autoinducer binding, and that the suppressor serves to restore some autoinducer-independent activity to LuxR. The suppressor does not seem to restore autoinducer-binding activity to the mutant LuxR.

Is the suppressor specific for the gly-121 to arg substitution or does it interfere with the interaction between the two domains in a more general fashion? One way to address this question is to place the ala-221 to val suppressor in the context of a LuxR with the wild-type gly at position-121. In the presence of autoinducer, activation of the lux operon by this LuxR protein was comparable to activation by the wild-type LuxR. Furthermore, the substantial activity of the LuxR containing the single substitution at position-221 was autoinducer independent (Table 2). Finally, the substitution at position-221 in the DNA-binding domain did not interfere with autoinducer binding in E. coli (Table 3). Apparently, the ala-221 to val substitution generally interferes with the ability of the N-terminal regulatory domain to inhibit the activity of the C-terminal domain.

In summary, replacement of gly-121 with arg appears to block autoinducer binding and it interferes with LuxR activity even in the absence of autoinducer. The ala-221 to val suppressor does not restore autoinducer binding activity, but it does dampen the ability of gly-121 to arg to interfere with autoinducer-independent lux gene activation. One interpretation of these results is that gly-121 plays an important role in both autoinducer binding and in the physical interaction between the two domains of LuxR. In the context of an otherwise wild-type LuxR, ala-221 to val results in high-level of autoinducer-independent activity (Table 2). This suggests a role for ala-221 in the interaction between the two domains that enables the N-terminal domain to impair the activity of the C-terminal domain. It will be of interest to determine the effects of substitutions other than val at position 221 on LuxR activity.

Acknowledgements

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References


