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The *Vibrio fischeri* Luminescence Gene Activator LuxR Is a Membrane-Associated Protein

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The *Vibrio fischeri* luminescence (*lux*) genes are activated at sufficiently high culture densities by the transcriptional activator LuxR in combination with a diffusible signal compound termed autoinducer. We have used antibodies directed against LuxR in immunoprecipitation experiments to study the subcellular location of this transcription factor. The LuxR polypeptide was detected in membranes and not in the soluble pool of cytoplasmic proteins from *V. fischeri*. LuxR was not released from the membranes by 0.6 M KCl or by the nonionic detergents Nonidet P-40, *N*-octyl-β-D-glucopyranoside, and Triton X-100. LuxR and a number of other *V. fischeri* proteins were released from the membranes by EDTA. The autoinducer had no detectable influence on the subcellular location of LuxR. In spheroplasts, neither the abundance nor the molecular mass of the LuxR antigen was influenced by treatment with proteinase K. Together with other information, these results indicate that LuxR is an amphipathic protein that is associated with the cytoplasmic membrane of *V. fischeri*.

The *Vibrio fischeri* LuxR protein is the best-studied member of a family of transcriptional activators required for a phenomenon termed autoinduction. Autoinduction controls transcription of luminescence genes in *V. fischeri* and certain other marine bacteria. More recently, autoinduction has been shown to control genes coding for extracellular proteases in *Pseudomonas aeruginosa* (38), conjugal transfer genes in *Agrobacterium tumefaciens* (41, 55), and antibiotic synthesis genes in *Erwinia carotovora* (2). In each case the cells produce a substance termed autoinducer. In *V. fischeri* the autoinducer is *N*-(3-oxohexanoyl)homoserine lactone (14). Because cells are freely permeable to this autoinducer it accumulates in the medium during growth (27). When the autoinducer reaches a sufficient concentration, it triggers synthesis of specific luminescence enzymes. Thus, autoinduction provides communication between *V. fischeri* cells, allowing them to sense their own population density. At low cell densities, the autoinducer will diffuse out of cells; at high cell densities, the autoinducer can accumulate and reach a critical concentration (5 to 10 nM) required for activation of luminescence gene transcription (13, 15, 36). *V. fischeri* can be isolated from seawater, and it also occurs as the specific bacterial symbiont in the light organs of certain marine fishes and squids (for recent reviews, see references 11 and 43). At the high cell densities achieved in the light organs (10^{10} to 10^{11} cells per ml), autoinducer can accumulate, and luminescence is induced. In seawater, where *V. fischeri* exists at less than 10^2 cells per ml, autoinduction of luminescence should not occur (42, 44, 45).

LuxR activity has not yet been demonstrated in vitro, nor has the activity of any of the more recently discovered homologs of LuxR. Nevertheless, molecular genetic analyses of LuxR function, most of which have been carried out using *Escherichia coli*, have enabled the development of a general view of LuxR structure and function. These studies were made possible by the cloning of a 9-kb fragment of *V. fischeri* DNA that encodes the functions necessary for autoinducible luminescence in *E. coli* (15). This *V. fischeri* DNA contains seven *lux* genes organized as two divergent transcriptional units. One unit contains *luxR*, the gene coding for the LuxR protein. The other unit, which is activated by the LuxR protein in the presence of autoinducer, is the *luxICDABE* operon. The *luxA* and *luxB* genes encode the α and β subunits of luciferase, respectively; *luxC*, *luxD*, and *luxE* code for polypeptides involved in synthesis of the aldehyde substrate for luciferase; and *luxI* is the only *V. fischeri* gene required for synthesis of autoinducer by *E. coli* (15, 16).

Based on a mutational analysis, it appears that a 20-bp inverted repeat centered at position −40 from the *luxI* transcriptional start site is a LuxR-binding sequence, a *lux* box, required for *lux* gene regulation (9). The LuxR polypeptide contains 250 amino acid residues (8, 17, 28). The available evidence indicates that LuxR is a two-domain polypeptide. There is a C-terminal domain extending from around residue 160 to residue 250 (3). This domain is involved in DNA binding and transcriptional activation of *luxICDABE* (3, 4). The proposed N-terminal domain is believed to regulate the DNA binding of the C-terminal domain (3). Apparently, in the absence of autoinducer, the N-terminal domain (roughly the N-terminal 162 amino acids) interacts with the C-terminal domain to block DNA binding. Interaction of the LuxR N-terminal domain with autoinducer allows binding of the C-terminal domain to the *lux* box (3).

Although the subcellular location of LuxR has not been determined experimentally, there are no hydrophobic, α-helical regions characteristic of membrane-spanning regions in the predicted LuxR sequence that would indicate a membrane association of this protein. A number of recent reports show that several other transcriptional activators are membrane proteins (6, 7, 22, 35, 47, 51, 52). Some of these activators possess hydrophobic sequences typical of membrane-spanning regions (7, 35, 52), but others do not possess such regions (22, 47, 51). The latter cases indicate that an analysis of the predicted LuxR sequence is not sufficient to conclude that this transcription factor is localized in the cytoplasmic compartment; the cellular location of LuxR must be determined experimentally. In this report we show that the LuxR protein in

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its native environment, *V. fischeri* cells, is associated with membranes.

MATERIALS AND METHODS

Bacterial strains and plasmids. We used the luminous parental strain *V. fischeri* MJ1 (44) and an MJ1 derivative, MJ141, which contains a *lacZ* insertion in *luxI* (12) for protein localization studies. *E. coli* JM109 containing pHK724, a plasmid with *luxR* under control of the *tac* promoter (28), was used to overexpress the LuxR protein in the form of insoluble inclusion bodies.

Production of anti-LuxR antibodies. The procedure described by Kaplan and Greenberg (28) was employed to obtain LuxR protein for use as an antigen in the production of anti-LuxR.

Anti-LuxR serum was produced by the Berkeley-Antibody Company (Richmond, Calif.). As determined by a quantitative enzyme-linked immunosorbent assay using purified LuxR as the primary antigen, the antibody titer was 1:300,000. The immunoglobulin G fraction was purified by protein A affinity chromatography and stored at −25°C at a concentration of 4 mg of protein per ml in phosphate-buffered saline with 0.02% sodium azide.

Conditions for culturing and labeling *V. fischeri*. *V. fischeri* cultures were grown in the minimal medium described by Friedrich and Greenberg (19) with glycerol (40 mM) as the carbon and energy source. For one experiment, 200 nM autoinducer synthesized by Kaplan et al. (26) was included in the culture medium. One hundred-milliliter cultures contained in 250-ml Erlenmeyer flasks were incubated at 26°C with shaking. When the cultures reached the mid-logarithmic phase of growth (an optical density at 660 nm of 0.1 to 0.2), 5- to 20-ml samples were removed and the cells were labeled for 5 min with 40 μCi of [^{35}S]methionine per ml (specific activity, >1,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). Labeled cells were harvested by centrifugation at 3,500 × *g* for 5 min at 23 to 25°C and washed once in cold minimal medium before subsequent use.

Cell fractionation. [^{35}S]methionine-labeled *V. fischeri* cells or cells of *E. coli* containing pHK724 were resuspended in 10 mM Tris buffer (pH 7.4 at 5°C) containing 1 mM $MgCl_2$, and 1 μg of DNAse I per ml (unless otherwise specified). The final volume was 20% of the volume of the culture sample used for labeling. Lysozyme (50 mg/ml in 0.1 M EDTA [pH 8]) was added to a final concentration of 50 μg/ml. After 30 min on ice, the cells were broken by sonication. After removal of remaining whole cells by low-speed microcentrifugation (2 min at 1,000 × *g* at 4°C), the membranes and soluble components were separated by ultracentrifugation in a Beckman TLA 100.3 rotor at 100,000 × *g* for 45 min at 4°C.

Sucrose density gradient centrifugation of [^{35}S]methionine-labeled *V. fischeri* membranes was done by the procedure of Osborn et al. (37) with the following exceptions. EDTA was omitted from all buffers, and to limit protease digestion, a mixture of protease inhibitors was added throughout (1 mM phenylmethylsulfonyl fluoride, 2 mM leupeptin, 1.5 mM pepstatin A, and 1 μg of chymostatin per ml).

Spheroplast preparation. Spheroplasts of *V. fischeri* were made by using a modification of a method described elsewhere (33) for making spheroplasts of *E. coli*. Cells in a 20-ml volume of culture were labeled with [^{35}S]methionine as described above and suspended in 2 ml of a sucrose-salt solution containing 0.8 M sucrose, 0.3 M NaCl, 0.05 M $MgSO_4$, 0.01 M $CaCl_2$, and 0.01 M KCl. The following solutions were then added in order: 150 μl of 1 M Tris buffer (pH 8), 120 μl of a 5-mg/ml solution of lysozyme, and 30 μl of a 5-mg/ml solution of DNAse I. This mixture was incubated at approximately 25°C for 30 min to hydrolyze the peptidoglycan and form spheroplasts. Spheroplast formation was assessed by examining the osmotic sensitivity of the cells: when diluted 1:10 in deionized water, enzyme-treated preparations lost turbidity within minutes, whereas suspensions of cells that had not been treated with enzymes remained turbid for at least 30 min. When diluted in the sucrose-salt solution both enzyme-treated and untreated suspensions remained turbid for at least 30 min.

Immunoprecipitation procedure. Antibody precipitations were done by the procedure of Ito et al. (25). Samples were treated with 1% sodium dodecyl sulfate (SDS) in a boiling water bath for 2 min and diluted in Triton buffer. Nonspecific precipitates were removed by centrifugation, and the supernatant fluid was mixed with an antibody preparation (20 μl of anti-LuxR immunoglobulin G prepared as described above or 20 μl of antiluciferase rabbit serum provided by Thomas Baldwin). To this mixture, *Staphylococcus* protein A (Sigma Chemical Company, St. Louis, Mo.) was added (500 μg). After incubation on ice for 20 min the antibody-protein A complexes were pelleted by microcentrifugation for 2 min at 4°C. The antibody-protein A complexes were washed twice in 50 mM Tris buffer (pH 8.0) containing 1 M NaCl and 1% Triton X-100 and then once in 10 mM Tris buffer (pH 8.0). The washed pellets were dissolved in SDS sample buffer by heating in a boiling water bath for 5 min. After a 5-min microcentrifugation the proteins in the SDS sample buffer were separated by SDS-polyacrylamide gel electrophoresis, and the proteins labeled with [^{35}S]methionine were visualized by fluorography as described below.

NADH oxidase assays. NADH oxidase activity was measured by using a modification of the procedure described by Osborn et al. (37). Reactions were started by the addition of 100 μl of cell extracts, soluble cell proteins, or membranes (suspended in 50 mM Tris [pH 7.5 at 25°C]–200 mM NaCl–0.2 mM dithiothreitol) to 900 μl of assay buffer (Tris [pH 7.5 at 25°C], 200 mM NaCl, 0.2 mM dithiothreitol plus 0.12 mM NADH). The rate of decrease in A_{340} was used as a measure of NADH oxidation.

Gel electrophoresis. Proteins were separated by SDS-polyacrylamide gel electrophoresis with a 12.5% resolving gel (31). Gels were either stained with Coomassie blue or, for fluorography, fixed in an aqueous solution of 30% methanol–10% acetic acid, treated with Fluoro-hance according to the manufacturer's instructions (Research Products International Corp., Mount Prospect, Ill.), and exposed to Kodak XAR-5 film at −70°C. The molecular weight standards were from Bio-Rad Laboratories, Hercules, Calif. (prestained SDS-PAGE standards, low range).

RESULTS

Using our previously described Western immunoblotting procedure (49), we could not detect LuxR in *V. fischeri*. Based on the sensitivity of this procedure with purified LuxR, it appears that LuxR is present at less than 500 polypeptide molecules per cell of *V. fischeri* MJ1 (30). To determine the cellular location of LuxR, we have adapted the technique of immunoprecipitation to detect this transcription factor in subcellular fractions of *V. fischeri* MJ1. The LuxR polypeptide was detected in cell extracts of *V. fischeri*. This protein was also detected in membranes isolated from these extracts by ultracentrifugation; however, it was not detected in the pool of soluble proteins remaining in the supernatant fluid after ultracentrifugation (Fig. 1). As a control, identical cell frac-

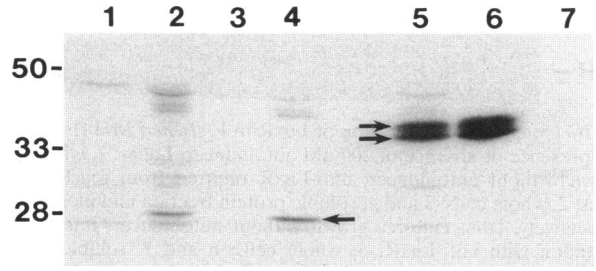

FIG. 1. Subcellular localization of LuxR and luciferase in *V. fischeri* MJ1. [^{35}S]methionine-labeled cells were fractionated and immunoprecipitated. The immunoprecipitates were subjected to SDS-polyacrylamide gel electrophoresis, and the immunoprecipitated proteins were visualized by autoradiography (see Materials and Methods for details). Lane 1, control, no antibodies were used to form immunoprecipitates. Lanes 2, 3, and 4, whole-cell extract, soluble proteins, and membrane-associated proteins, respectively, immunoprecipitated with anti-LuxR. Lanes 5, 6, and 7, whole-cell extract, soluble proteins, and membrane-associated proteins, respectively, immunoprecipitated with antiluciferase serum. Locations of protein molecular size standards are indicated on the left in kilodaltons. The arrow pointing to the left indicates the location of the 28-kDa LuxR polypeptide, and the arrows pointing to the right indicate the locations of the 40- and 37-kDa α and β subunits of luciferase.

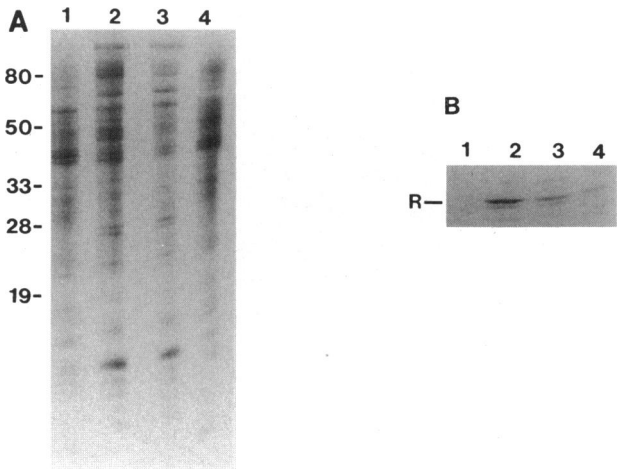

FIG. 2. Effects of Nonidet P-40 treatment and EDTA treatment on total membrane proteins and LuxR from *V. fischeri* MJ1. (A) Autoradiogram of total membrane proteins separated by SDS-polyacrylamide gel electrophoresis. Locations of protein molecular size standards are indicated on the left in kilodaltons. (B) Autoradiogram of LuxR polypeptide recovered by immunoprecipitation. Lanes: 1, membrane polypeptides solubilized by Nonidet P-40; 2, polypeptides remaining with the total membrane fraction after treatment with Nonidet P-40; 3, membrane polypeptides solubilized by EDTA; 4, polypeptides remaining with the total membrane fraction after EDTA treatment. R indicates the location of the LuxR polypeptide.

tions were immunoprecipitated with antiluciferase serum rather than anti-LuxR. Luciferase is a cytoplasmic, heterodimeric protein consisting of an α and a β subunit (18, 21). Both subunits were found in cell extracts and in the soluble fraction but were not found in the membrane fraction (Fig. 1). Also, NADH oxidase activity was monitored. NADH oxidase is a cytoplasmic membrane protein in other bacteria (37). In *V. fischeri*, NADH oxidase was detected in the membrane pellet (specific activity, 383 nmol of NADH oxidized per min per mg of protein), but it was not found in the soluble protein fraction. These controls confirm that soluble and membrane proteins of *V. fischeri* were separated by ultracentrifugation. Thus, they support the conclusion that LuxR is associated with *V. fischeri* membranes and is not a cytoplasmic polypeptide.

We attempted to separate the cytoplasmic membrane and the outer membrane of *V. fischeri* by sucrose density gradient centrifugation, but this proved unsuccessful. Centrifugation to equilibrium resulted in the appearance of a single, broad membrane band with a buoyant density of approximately 1.16 to 1.2 g/cm^3. We presume that this was a mixture of both cytoplasmic and outer membranes. Our inability to separate cytoplasmic membranes from outer membranes was not wholly unexpected. This is because it has been shown previously that outer membranes of marine vibrios do not have typical lipopolysaccharides (24, 53), and lipopolysaccharide contributes to the unique buoyant density of outer membranes from other bacteria (37). Nevertheless, it is important that, as detected by immunoprecipitation, LuxR was primarily in the membrane band resulting from sucrose density gradient centrifugation. Although this does not provide insight concerning which of the two types of membranes contains LuxR, it supports the conclusion that LuxR is a membrane-associated protein; LuxR does not appear to be in a particle that pellets together with membranes upon ultracentrifugation. Rather it bands in sucrose density gradient centrifugation at a buoyant density indicative of an association with lipid-containing membranes.

Because it has been shown that LuxR has a tendency to form aggregates in *E. coli* (28), we remained concerned that the majority of LuxR even in *V. fischeri* was in the form of submicroscopic aggregates. It seemed unlikely that such aggregates would be found in the membrane band resulting from centrifugation in a sucrose density gradient; however, we sought additional evidence that LuxR was a membrane protein and not an aggregated cytoplasmic protein. To this end, [^{35}S]methionine-labeled membranes isolated by ultracentrifugation were treated with agents we suspected might solubilize membrane-associated proteins but not solubilize protein aggregates. Two treatments were of particular interest, EDTA treatment (1 mM EDTA for 1 h at 4°C) and treatment with nonionic detergents such as Nonidet P-40 (2% for 1 h at 4°C). EDTA treatment resulted in the solubilization of a subset of *V. fischeri* membrane proteins (Fig. 2A). We found that Nonidet P-40 treatment resulted in the solubilization of a different set of *V. fischeri* membrane proteins (Fig. 2A). Other nonionic detergents (Triton X-100 and *N*-octyl-β-D-glucopyranoside, both at 2% for 1 h at 4°C) had lesser effects on membrane-associated proteins and did not solubilize LuxR. The ionic detergent sodium deoxycholate (2% for 1 h at 4°C) solubilized all proteins in the membrane preparations, including LuxR.

Does either the EDTA treatment or the Nonidet P-40 treatment solubilize membrane-associated LuxR? To address this question, proteins remaining in the membrane pellet and proteins solubilized by EDTA treatment and Nonidet P-40 treatment were subjected to immunoprecipitation with anti-LuxR. Whereas the majority of LuxR was solubilized by treatment with EDTA, this polypeptide was not solubilized by Nonidet P-40 (Fig. 2B).

To determine whether EDTA or Nonidet P-40 solubilized aggregates of LuxR, the following experiment was performed. LuxR was overexpressed as insoluble inclusion bodies in *E. coli* containing pHK724 (28; see Materials and Methods). The cells were then broken and fractionated by ultracentrifugation. This procedure should yield a pellet of *E. coli* membranes and LuxR inclusion bodies. In fact, LuxR was found together with a

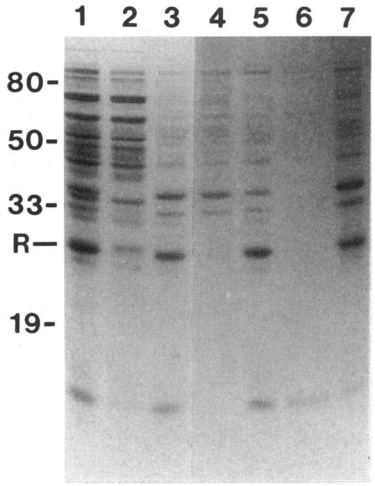

FIG. 3. Effects of Nonidet P-40 treatment and EDTA treatment on LuxR inclusion bodies from *E. coli* containing pHK724. SDS-polyacrylamide gel stained with Coomassie blue. Lanes: 1, whole cells; 2, soluble polypeptides after ultracentrifugation (100,000 × *g* for 45 min at 4°C); 3, polypeptides in the pellet resulting from ultracentrifugation; 4 to 7, pellets treated with 2% Nonidet P-40 (1 h at 4°C) or 1 mM EDTA (1 h at 4°C) followed by ultracentrifugation. Lanes 4 and 5, polypeptides in the soluble supernatant fluid and in the pellet, respectively, after Nonidet P-40 treatment; lanes 6 and 7, polypeptides in the soluble supernatant fluid and in the pellet, respectively, after EDTA treatment. Locations of protein molecular size standards are indicated on the left in kilodaltons. R indicates the location of the LuxR polypeptide.

number of other proteins in the pellet (Fig. 3). Neither EDTA treatment nor detergent treatment resulted in detectable solubilization of the LuxR inclusion bodies (Fig. 3). In this form at least, aggregates of LuxR are not solubilized by EDTA.

The finding that LuxR remains in association with *V. fischeri* membranes in the presence of the nonionic detergents suggests that this luminescence gene activator is tightly bound to the membranes. Furthermore, the presence of a high concentration of potassium chloride during collection and processing of membranes did not affect the localization of LuxR (Fig. 4). This indicates that the association of LuxR with membranes is not due to electrostatic interactions. On the other hand solubilization of LuxR by EDTA (Fig. 2) suggests that the LuxR-membrane association might involve a protein-protein interaction or be dependent on a metal. An alternative explanation is that chelation of metals or cations by EDTA resulted in the enzymatic degradation of the lipid bilayer. We favor this second explanation because a number of *V. fischeri* polypeptides were released from the membranes by EDTA treatment

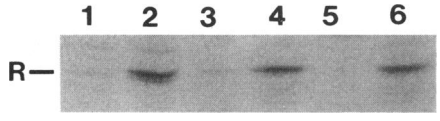

FIG. 4. Subcellular location of LuxR in the presence of high salt. Lane 1, control, whole-cell extract, no antibodies were used prior to addition of protein A. Lane 2, whole-cell extract immunoprecipitated with anti-LuxR. Lanes 3 and 4, soluble proteins and membrane-associated proteins, respectively, precipitated with anti-LuxR, no added salt. Lanes 5 and 6, membranes were collected and processed in the presence of 0.6 M KCl, and soluble proteins (lane 5) and membrane-associated proteins (lane 6) were immunoprecipitated with anti-LuxR. R indicates the location of the LuxR polypeptide.

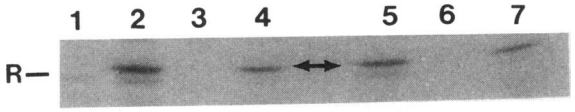

FIG. 5. Subcellular location of LuxR in *V. fischeri* MJ141 grown in the presence or absence of 200 nM autoinducer. Lanes: 1, whole cells grown without autoinducer, anti-LuxR omitted from reaction mixtures; 2, whole cells; 3 and 4, soluble protein fraction and membranes, respectively, from cultures grown without autoinducer, immunoprecipitation with anti-LuxR; 5, whole cells; 6 and 7, soluble protein fraction and membranes, respectively, from cultures grown in the presence of 200 nM autoinducer, immunoprecipitation with anti-LuxR. The R indicates the location of the LuxR polypeptide.

(Fig. 2A), whereas no polypeptides were released from *E. coli* membranes (Fig. 3).

We also wanted to determine whether autoinducer influenced the subcellular location of LuxR. Absolute levels of autoinducer in cultures of *V. fischeri* MJ1 cannot be controlled precisely because this strain produces an autoinducer. Thus, we used strain MJ141, a LuxI$^-$ mutant derived from strain MJ1 by insertion mutagenesis (12). Because MJ141 is incapable of producing autoinducer, the level of autoinducer in cultures of MJ141 is determined by the amount of this compound added exogenously. LuxR was associated with the membranes of *V. fischeri* MJ141 grown in the presence or the absence of 200 nM autoinducer (Fig. 5). This concentration of autoinducer is sufficient for maximal induction of luminescence in *V. fischeri* (27). Apparently, autoinducer, which is somewhat lipophilic, was not required for the association of LuxR with membranes, nor did it release LuxR from membranes.

To gain further insight regarding the topology of LuxR in *V. fischeri* membranes, we treated spheroplasts with proteinase K (100 μg/ml). The effect of proteinase K treatment was assessed by immunoprecipitation with anti-LuxR. This approach has been used to demonstrate that specific cytoplasmic membrane proteins of other bacteria possess periplasmic loops (1, 29, 54). If there are periplasmic loops exposed to and digested by proteinase K in spheroplasts, then there should be a loss of full-length antigen with the concominant appearance of lower-molecular-weight antigenic fragments of the protein. Proteinase K treatment of *V. fischeri* spheroplasts did not alter the LuxR polypeptide detected by immunoprecipitation (Fig. 6). This suggests that membrane-bound LuxR does not possess periplasmic loops exposed to proteinase K in spheroplasts. Because there is a very limited knowledge of membrane proteins in *V. fischeri* and a lack of specific antibodies to likely membrane-spanning polypeptides in this organism, these results must be interpreted with caution, and we have not studied the topology of LuxR in *V. fischeri* membranes further.

DISCUSSION

On the basis of the evidence presented here, we conclude that LuxR, the activator of luminescence gene transcription, is a membrane-associated protein in *V. fischeri* (Fig. 1 and 2). Because LuxR remains in association with membranes in the presence of a number of detergents, in the presence of 0.6 M KCl, and in the presence of autoinducer (Fig. 2, 4, and 5), it seems unlikely that LuxR is loosely associated with the membrane through a protein-protein interaction as is, for example, PutA, a transcription factor in *Salmonella typhimurium* which dissociates with the membrane in response to ligand binding (32).

We were not successful in attempts to separate the cytoplas-

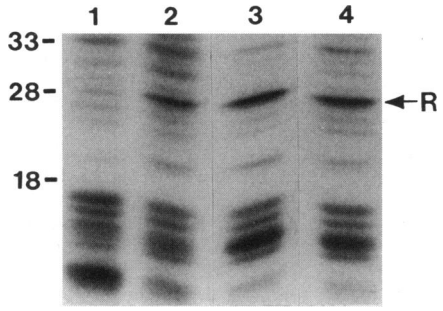

FIG. 6. Immunoprecipitation analysis of LuxR in *V. fischeri* spheroplasts treated with proteinase K. Lanes: 1, whole-cell extract, anti-LuxR omitted from the reaction mixture; 2, whole-cell extract immunoprecipitated with anti-LuxR; 3 and 4, untreated and proteinase K-treated spheroplasts, respectively, immunoprecipitated with anti-LuxR. The arrow indicates the location of LuxR (R), and the locations of protein molecular size standards are indicated on the left in kilodaltons. The film was overexposed in an effort to detect potential low-molecular-weight fragments of LuxR.

mic membranes and outer membranes of *V. fischeri* from each other by sucrose density gradient centrifugation. Nevertheless, we propose that LuxR is associated specifically with the cytoplasmic membrane. Only then is a membrane-associated LuxR consistent with the function of this protein as a transcription factor that binds *lux* boxes in the DNA of *V. fischeri* (9, 15, 16, 48, 49). A further point supporting the proposal that LuxR is associated with the cytoplasmic membrane comes from studies of *luxR* deletion mutations in *E. coli*. When expressed in *E. coli*, the majority of LuxR forms cytoplasmic inclusion bodies (28), and we have not even attempted to determine the subcellular location of the active fraction of LuxR in *E. coli*. However, a LuxR protein deleted for residues 2 to 162 is a soluble, cytoplasmic protein (50) which retains function as a transcriptional activator of luminescence genes. The deletion protein, unlike the full-length protein, is not dependent on autoinducer (3). This leads to the suggestion that the N-terminal regulatory domain of LuxR is associated with the cytoplasmic membrane and the C-terminal, DNA-binding or transcription activator domain, which by itself is a cytoplasmic protein in *E. coli*, extends into the cytoplasm of the cell.

Several hydrophobic regions, all in the N-terminal regulatory module, can be distinguished in analyses of the primary sequence of LuxR (for example, see references 5 and 8). However, none of these sequences have the characteristics of a typical transmembrane α-helix. This, together with the fact that in spheroplasts LuxR is resistant to protease treatment (Fig. 6), indicates a lack of periplasmic loops in membrane-bound LuxR. Rather, we suggest that LuxR is an amphipathic protein that is inserted in the inner leaflet of the cytoplasmic membrane. The transcriptional activator NodD in *Rhizobium leguminosarum* has been proposed by Schlaman et al. (47) to be an amphipathic protein, inserted in the inner leaflet of the cytoplasmic membrane. Like LuxR, NodD does not possess any predicted transmembrane α-helical regions.

Recently, a number of other bacteria have been shown to possess transcriptional activators which are homologs of LuxR (20, 23, 40). For transcriptional activation, these homologs of LuxR require a diffusible signal molecule homologous in structure to the *V. fischeri* autoinducer (2, 39, 55). For example, the TraR protein of *A. tumefaciens* activates transcription of conjugal transfer genes in the presence of the autoinducer *N*-(3-oxooctanoyl)homoserine lactone (41, 55), whereas LuxR activates *V. fischeri* luminescence genes in conjunction with *N*-(3-oxohexanoyl)homoserine lactone. For other bacteria in which a LuxR homolog and an autoinducer homolog are involved in cell-density-dependent gene expression, it is of interest to determine whether the LuxR homolog is associated with the cytoplasmic membrane.

Finally, this report, indicating that LuxR is associated with membranes of *V. fischeri*, adds another transcriptional activator to a growing list of membrane-associated transcription factors in prokaryotes (6, 7, 22, 32, 35, 47, 51, 52). These membrane-associated transcription factors do not form a group based on amino acid sequence alignments. However, it should be pointed out that many of these transcription factors respond to environmental signals related to an association with an animal or plant host. For example, LuxR is responsive to cell density as mediated by autoinducer, and this allows expression of luminescence in a specific light organ symbiosis; NodD in *R. leguminosarum* activates genes involved in root nodule formation in response to its interaction with flavonoid compounds produced by the plant host (for a recent review see reference 46); and ToxR in *Vibrio cholerae* is a transmembrane protein that activates the gene encoding cholera toxin in response to an environmental signal (10, 34).

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