# A Luminescence-Based Mercury Biosensor

**ARTICLE** *in* ANALYTICAL CHEMISTRY · FEBRUARY 1995

Impact Factor: 5.64 · DOI: 10.1021/ac00099a027



**3 AUTHORS:**

Marko Virta
University of Helsinki
**86** PUBLICATIONS **2,266** CITATIONS
SEE PROFILE

Jorma Lampinen
Thermo Fisher Scientific
**8** PUBLICATIONS **228** CITATIONS
SEE PROFILE

Matti Karp
Tampere University of Technology
**193** PUBLICATIONS **4,247** CITATIONS
SEE PROFILE

*Anal. Chem.* **1995,** *67,* 667–669

# A Luminescence-Based Mercury Biosensor

**Marko Virta,*,† Jorma Lampinen,† and Matti Karp‡**

*Department of Biochemistry and Pharmacy, Abo Akademi University, P.O. Box 66, FIN-20521 Turku, Finland, and Department of Biotechnology, University of Turku, Tykistokatu 6, FIN-20520 Turku, Finland*

**A new biosensor for the determination of bioavailable mercury is presented. It utilizes firefly luciferase gene as a reporter under the control of the mercury-inducible *mer* promoter from transposon *Tn*21 and *Escherichia coli* MC1061 as a host organism. The luminescence-based sensor was evaluated for the selectivity and sensitivity of the detection of mercury. The lowest detectable concentration of mercury was 0.1 fM. Cadmium, zinc, cobalt, copper and manganese ions did not interfere with the measurement even at millionfold concentrations compared to mercury. The results were in agreement with the results obtained by a mercury-independent luciferase expressing strain which acted as a control strain measuring total toxicity.**

The measurement of the bioavailability of metals is quite difficult with traditional analytical methods. The bioavailability of a metal is a very important factor in the determination of a metal toxicity,[1] and therefore, the detection of bioavailable metals is of great interest. Biosensors provide a promising way to assess bioavailability of different substances in the environment.[2]

Microbes offer a cornucopia for the construction of biosensors for monitoring the status of the environment. Numerous unspecific sensors that react to any kind of toxic substance have been developed.[2] A novel approach for a microbial biosensor is to connect a strictly regulated promoter to a sensitive reporter gene. The most interesting promoters for environmental analysis are found in bacteria that survive in environments contaminated by, for example, heavy metals or organic compounds. The ability of the bacteria to survive in a contaminated environment is usually based on a genetically encoded resistance system, the expression of which is regulated very precisely.[3] Some biosensors using the promoter–reporter gene concept have been developed, for example, for the detection of mercury[4,5] and xenobiotic compounds.[6,7] However, these approaches have been limited by the lack of sensitivity (parts per trillion, pptr) required for a biosensor that is intended to be used as an early warning system.

We report here a luminescence-based biosensor with the required sensitivity. It is based on a very sensitive reporter gene, firefly luciferase, and a very selective and sensitive promoter, the promoter from the *mer* operon from transposon *Tn*21.[8] The *mer* operon encodes for resistance to mercury, which is a nonessential and toxic metal for bacteria. The firefly luciferase from *Photinus pyralis* (EC 1.13.12.7) catalyses the reaction

$$\text{ATP} + \text{O}_2 + \text{D-luciferin} \rightarrow \text{AMP} + \text{PP}_i + \text{CO}_2 + \text{oxyluciferin} + \text{light (560 nm)}$$

The firefly luciferase gene (*lucFF*) was cloned[9] a decade ago, and it has since become a widely used reporter gene in prokaryotic as well as in eukaryotic systems because it provides sensitive and simple detection of the gene regulation. The quantification of the luminescence is possible with a liquid scintillation counter, a luminometer, or even X-ray film.

## EXPERIMENTAL SECTION

**Materials.** Tryptone, yeast extract, and casamino acids were from Difco. D-Luciferin was from BioTools Oy (Turku, Finland). $HgCl_2$, $CdCl_2$ $ZnCl_2$, $ZnSO_4$, $CuSO_4$, $CoCl_2$, and $MnCl_2$ were analytical grade from Riedel-de Haën. DNA-modifying enzymes were either from Boehringer-Mannheim or from New England Biolabs. Vent DNA polymerase was from New England Biolabs. All other chemicals were of analytical grade.

**Construction of Plasmid pTOO11.** The plasmid pTOO11 is a p602/22[10]-based shuttle vector, in which the *lucFF* expression is controlled by the *mer* operon from transposon *Tn*21 (Figure 1). Standard recombinant-DNA techniques were used for the construction of the pTOO11. The *merR* gene and promoter/operator of the *mer* operon was isolated, and *Bam*HI and *Xho*I restriction sites were generated by the polymerase chain reaction (PCR)[11] using the following primers: 5′-TTAAGGATCCCCTCATAGTTAATTTCTCCTCTTTTGAATTTGGATTGGATAG-3′ at the 5′ end of the *merR* gene and 5′-ATATCTCGAGCTAAGGCATAGCTGACCT-3′ at the 3′ end of *merR* gene. Plasmid R100 isolated from *Escherichia coli* UB5201 (NCTC50278) was used as a template of the PCR reaction. The promoter at 5′ end of the *merR* included 12 bases from the beginning of the *lucFF* because the *Bam*HI site was originally engineered inside the luciferase gene. The PCR product was purified by using the Gene Clean procedure

* Fax: +358-21-654 745. E-mail: Marko.Virta@abo.fi.
† Abo Akademi University.
‡ University of Turku.

(1) Farrell, R. E.; Germida, J. J.; Huang, P. M. *Appl. Environ. Microbiol.* **1993,** *59,* 1507–1514.
(2) Karube, I.; Nakanishi, K. *Curr. Opin. Biotechnol.* **1994,** *5,* 54–59.
(3) Summers, A. O. *J. Bacteriol.* **1992,** *174,* 3097–3101.
(4) Tescione, L.; Belfort, G. *Biotechnol. Bioeng.* **1993,** *42,* 945–952.
(5) Selifonova, O.; Burlage, R.; Barkay, T. *Appl. Environ. Microbiol.* **1993,** *59,* 3083–3090.
(6) Ikariyama, Y.; Nishiguchi, S.; Kobatake, E.; Aizava, M.; Tsuda, M.; Nakazawa, T. In *Bioluminescence and Chemiluminescence—Status Report*; (Salazy, A. A., Kricka, L. J., Stanley, P., Eds.; Wiley: Chichester, UK, 1993; pp 420–424.
(7) King, J. M. H.; DiGrazia, P. M.; Applegate, B.; Burlage, R.; Sanseverino, J.; Dunbar, P.; Larimer, F.; Sayer, G. S. *Science* **1990,** *249,* 778–781.
(8) Misra, T. K.; Brown, N. L.; Fritzinger, D.; Pridmore, R.; Barnes, W.; Silver, S. *Proc. Natl. Acad. Sci. U.S.A.* **1984,** *84,* 5975–5979.
(9) deWet, J. R.; Wood, K. W.; De Luca, M. *Proc. Natl. Acad. Sci. U.S.A.* **1985,** *80,* 7870–7873.
(10) Pesche, U.; Beuck, V.; Bujard, H.; Gentz, R.; LeGrice, S. *J. Mol. Biol.* **1985,** *186,* 547–555.
(11) Saiki, R. K.; Gelfand, D. H.; Stoffel, S.; Scharf, S. J.; Higuchi, R.; Horn, G. T.; Mullis, K. B.; Erlich, H. A. *Science* **1985,** *230,* 487–491.

0003-2700/95/0367-0667$9.00/0 

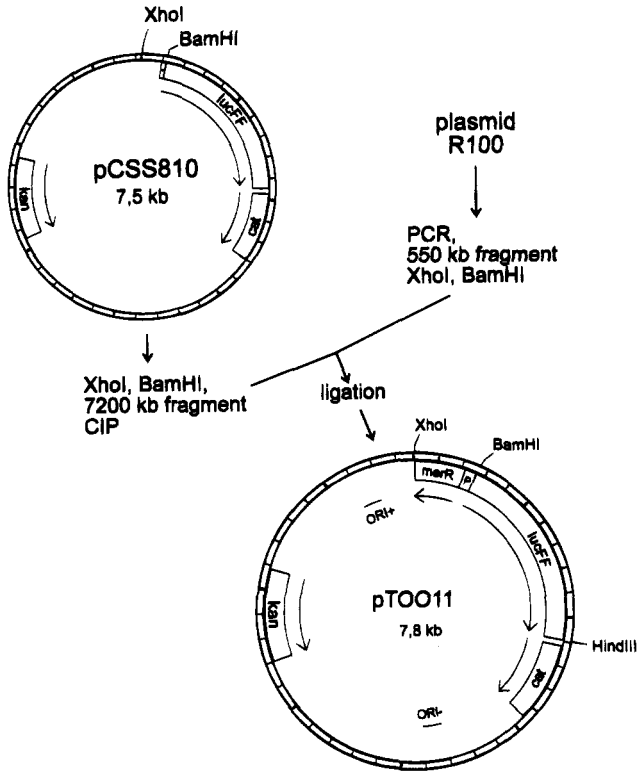


**Figure 1.** Construction of the plasmid pTOO11. Plasmid pCSS810 was digested with *Bam*HI and *Xho*I; the resulting 7.2 kb fragment was isolated and treated with calf intestine phosphatase (CIP). A 550 kb fragment was amplified from plasmid R100 by polymerase chain reaction (PCR) with the primers described in the Experimental Section. The fragment was purified, cut with *Bam*HI and *Xho*I, repurified, and ligated with a 7.2 kb fragment from pCSS810. Abbreviations used: *kan*, gene encoding kanamycin resistance; *cat*, gene encoding chloramphenicol resistance; *mer*R gene encoding repressor/activator of the *mer* promoter; P, *mer* promoter; *lucFF* gene encoding firefly luciferase.

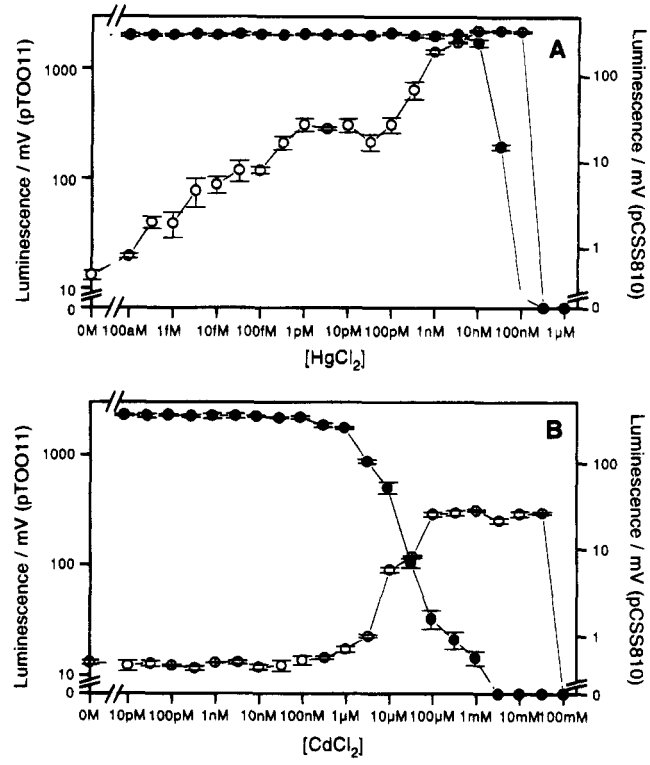


**Figure 2.** Luminescence produced by mercury-specific strain *E. coli* MC1061/pTOO11 (○) and by stable light producing strain *E. coli* MC1061/pCSS810 (●) when incubated with $HgCl_2$ (A) or with $CdCl_2$ (B). Data represent mean ± standard deviation for three determinations.

(Bio 101 Inc., San Diego, CA) and digested with *Bam*HI and *Xho*I. The resulting 550 bp fragment was purified from an agarose gel by Gene Clean. The fragment was ligated with *Bam*HI and *Xho*I digested and phosphatase treated pCSS810[12] and transformed into *E. coli* MC1061[13] cells by electroporation.[14] *E. coli* MC1061 strain harboring the parental plasmid pCSS810 was used as a control strain since in pCSS810 the expression of the *lucFF* is controlled by the *lac* operator and thus independent of the metal concentration.

**Cultivation of the Bacteria.** Bacteria were cultivated in LB medium (10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter, pH 7.0) supplemented with 30 μg/mL kanamycin in a shaker at 30 °C. The bacteria were harvested at $OD_{600}$ of 2.0 by centrifugation and washed twice with M9 medium[15] supplemented with 0.1% casamino acids. Bacteria were suspended and diluted to 1:100 with the same media before the measurements. About $2 \times 10^6$ cells were used in one measurement.

**Luminescence Measurements.** A 100 μL sample of the diluted stock of the metal salt solution in water was pipeted into wells of a 96-well black microtiter plate. The bacterial dilution was added with a dispenser, and the plate was incubated 60 min at 30 °C. After that the plate was moved to a Labsystems Luminoskan (Labsystems, Helsinki, Finland) luminometer, 100 μL of luciferase substrate (1 mM D-luciferin in sodium citrate buffer, pH 5.0) was added automatically through the dispenser, and luminescence peak values were measured immediately. All measurements were done in triplicate.

## RESULTS

**Construction of the Plasmid pTOO11.** The original *lac* operator/T5 promoter of pCSS810 was replaced by the mercury-responsive regulation unit (Figure 1). The cloned regulation unit contains the *merR* gene and two divergent promoters, one from which *merR* is transcribed and the second one from which the structural *mer* genes of *Tn*21 are transcribed.[8] In pTOO11, the *lucFF* gene was placed under transcriptional control of this latter promoter. In the absence of Hg, the expression of the luciferase was strongly repressed. In addition, the expression of chloramphenicolacetyltransferase (*cat*) gene, which is located downstream from luciferase gene, was repressed and thus *E. coli* MC1061/pTOO11 cells were not able to grow in the presence of 15 μg/mL chloramphenicol in contrast to the *E. coli* MC1061/pCSS810 strain (data not shown).

**Induction of *E. coli* MC1061/pTOO11 with Different Metals.** Hg(II) induced light production at very low concentrations (Figure 2A). The lowest concentration that caused a noticeable (background + 2 × standard deviation) induction was 0.1 fM. The luminescence increased with increasing $HgCl_2$ concentration in a linear manner to concentration of 0.1 μM, after

(12) Lampinen, J.; Koivisto, L.; Wahlsten, M.; Mäntsälä, P.; Karp, M. *Mol. Gen. Genet.* **1992**, *232*, 498–504.
(13) Casabadan, M. J.; Cohen, S. N. *J. Mol. Biol.* **1978**, *138*, 179–207.
(14) Dower, W. J.; Miller, J. F.; Ragsdale, C. W. *Nucleic Acids Res.* **1988**, *16*, 6126–6144.
(15) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, **1989**.

which the luminescence very rapidly fell to zero. Cd(II) induced remarkable luminescence too (Figure 2B); however, the concentration needed for induction was almost $10^7$-fold that of Hg(II). The maximum induction coefficient was 480 with $HgCl_2$ and 80 with $CdCl_2$. $ZnCl_2$, $ZnSO_4$, $CuSO_4$, $MnCl_2$, and $CoCl_2$ did not cause significant luminescence even at millimolar concentrations (data not shown).

**Effect of Metals on the Constitutive Light Producing Strain *E. coli* MC1061/pCSS810.** The luminescence was stable at low Hg concentrations (Figure 2A). At Hg(II) concentration of 0.1 $\mu$M, the luminescence began to fall rapidly, and it reached zero at a concentration of 0.5 $\mu$M. This kind of response for Hg and other nonessential and toxic heavy metals is typical for microbial biosensors in contrast to metals to which bacteria show homeostasis.[16] Cd(II) caused a similar but less drastic decrease in luminescence in the concentration range from 0.5 $\mu$M to 0.1 mM (Figure 2B).

## DISCUSSION

A new biosensor constructed by a gene fusion between a regulatory region of the *mer* operon and firefly luciferase is described. The construct is shown to provide very sensitive and selective detection of mercury. The luminescence correlates well with $HgCl_2$ concentrations in the range of 100 aM to 1 pM. In the concentration range from 1 pM to 100 pM, the luminescence is stable. After that, the sensitivity resumes with an apparently steeper slope from about 100 pM to 10 nM. At a concentration of 500 nM $HgCl_2$ the luminescence rapidly falls to zero, obviously due to the toxic effect of the Hg ions. The toxic effect was confirmed by using a constitutive light producing *E. coli* MC1061 harboring pCSS810 plasmid as a control strain during the induction measurements. It acted like a traditional toxicity test strain: light production was stable until the toxic effect of the metals caused reduction in light production. We believe that this kind of setup in which a constitutive light producing strain is used in addition to a specific, inducible strain helps considerably to separate differences in light production caused by the toxic effect of a metal or other toxic substances in the sample from an inductive effect of a metal. Other ions did not noticeably affect to the measurement; the only ion that was observed to interfere with the measurement was cadmium. However, for cadmium, both the induction coefficient and especially the concentration needed for induction differed remarkably from those for mercury. In addition to system described here, it is possible to immobilize the luminescent sensing cells in order to construct an on-line measuring system. We have done preliminary experiments about the immobilizing the sensing cells to calcium alginate as described before,[17] and the sensitivity of the immobilized system was close to the soluble system described here.

The sensitivity obtained here exceeded earlier biosensors described for mercury by Selifonova et al.[5] and by Tescione and Belfort.[4] The detection limit in those studies was about 0.1 nM or higher, which is almost a millionfold greater than reported here. One probable explanation is the difference in the sensitivity between firefly luciferase and bacterial luciferase (used in constructions by Selifonova et al.[5] and Tescione and Belfort[4]), which is caused by different quantum yields of the enzymes (about 90% and about 5%, respectively). An alternative reason might be a difference in turnover rate, which is limited for bacterial luciferase.[18] In addition, our genetic construction is designed to obtain the maximal sensitivity the *mer* promoter can provide. We have recently shown that luciferase from *Pyrophorus plagiophthalamus*,[19] which is closely related to firefly luciferase, expressed in similar construction[12] results in a more sensitive detection of the metal toxicity than the bacterial luciferase.[20] The sensitivity obtained here is comparable to or even greater than the sensitivity of the direct chemical methods such as inductively coupled plasma spectrometry or a recently reported electrochemical method.[21] Of course, one has to keep in mind that the chemical methods measure total amount of Hg ions in the sample in contrast to biosensors which measure the amount of Hg that is biologically available. Tests to verify the applicability of the reported biosensor to environmental monitoring are in progress.


(16) van der Lelie, D.; Corbisier, P.; Baeyens, W.; Wuertz, S.; Dies, L.; Mergeay, M. *Res. Microbiol.* **1994**, *145*, 67–74.
(17) Åkerman, K.; Kukkonen, J.; Karp, M. In *Physiology of the Immobilized Cells* de Bont, J. A. M., Visser, J., Mattiasson, B., Tramper, J., Eds.; Elsevier: Amsterdam, The Netherlands, 1990; pp 173–180.
(18) Li, Z.; Meighen, E. A. *J. Biol. Chem.* **1994**, *269*, 6640–6644.
(19) Wood, K. V.; Lam, Y. A.; Seliger, H. H.; McElroy, W. D. *Science* **1989**, *244*, 700–702.
(20) Lampinen, J.; Virta, M.; Karp, M. *Environ. Toxicol. Water Qual.*, in press.
(21) Turyan, I.; Mandler, D. *Nature* **1993**, *362*, 703–704.



## ACKNOWLEDGMENT

The work was supported by the Maj and Tor Nessling Foundation and Neste Oy Foundation. Dr. Mary Metzler is thanked for proofreading the text.

Received for review July 6, 1994. Accepted November 15, 1994.[⊗]

AC940676A

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1994.