REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	10/16/00	Final Report 6/1/97-5/31/00

4. TITLE AND SUBTITLE
Genetically engineered pores sensing metal ions

5. FUNDING NUMBERS
N-00014-97-1-0754

6. AUTHOR(S)
BAYLEY, Hagan

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Medical Biochemistry & Genetics
440 Reynolds Medical Bldg.
1114 TAMU
College Station, TX 77843-1114

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
800 N. Quincy St.
Arlington, VA 22217-5000

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Stochastic sensing with pore-forming proteins has been established as a means for sensing metal ions by using engineered forms of alpha-hemolysin as the sensor elements. The alpha-hemolysin pore is a heptamer, which has disadvantages for certain manipulations in protein engineering. Therefore a single-chain protein pore was sought. In this work, the *Escherichia coli* outer membrane protein, OmpG, was shown to be a monomeric, single-chain molecule. The availability of milligram amounts of this porin will be useful for membrane protein engineering studies that will yield stochastic sensing elements more diverse than those previously accessible. Analyte-responsive pores are an inherently powerful technology for the real-time quantification of nanomolar levels of essential or toxic metal ions. Genetically engineered pores can be tailored for sensitivity, selectivity, resistance to fouling and other important characteristics. For many metal ions, the response time is limited only by analyte diffusion.

14. SUBJECT TERMS	15. NUMBER OF PAGES
biosensor/ sensor/ stochastic sensing/single molecule detection/channel/pore/porin/protein engineering	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

FINAL REPORT

GRANT #: N00014-97-1-0754

PRINCIPAL INVESTIGATOR: Hagan Bayley, PhD; AASERT student: Sean Conlan

INSTITUTION: The Texas A&M University System Health Science Center

GRANT TITLE: The student was be engaged in research under the ONR grant N00014-93-1-0962: Genetically engineered pores for sensing metal ions.

AWARD PERIOD: 1 June 1997 - 31 May 2000

OBJECTIVE: Stochastic sensing with pore-forming proteins has been established as a means for sensing metal ions by using engineered forms of alpha-hemolysin as the sensor elements. The alpha-hemolysin pore is a heptamer, which has disadvantages for certain manipulations in protein engineering. Therefore a single-chain protein pore was sought.

APPROACH: The Escherichia coli outer membrane porins form pores that are formed from single polypeptide chains. Unfortunately, most porins are trimeric, which would add another set of complications were they to be used in stochastic sensing. However, a report of a monomeric, single-chain porin, OmpG, appeared in 1998. The evidence for a monomer was weak and therefore a definitive evaluation of the situation was undertaken.

ACCOMPLISHMENTS: A recombinant form of the porin OmpG, OmpGm, lacking the signal sequence, was expressed in *Escherichia coli*. After purification under denaturing conditions, the protein was refolded in the detergent Genapol X-080, where it gained a structure rich in beta sheet as evidenced by a CD spectrum similar to that of the native form. Electrophoretic analysis and limited proteolysis experiments suggested that refolded OmpGm exists in at least three forms. Nevertheless, the recombinant protein formed uniform channels in planar bilayers with a conductance of 0.81 nS (1 M NaCl, pH 7.5). Bilayer recordings substantiated the proposal that OmpG is a trimer; voltage-induced closures occurred consistently in a single step and channel block by Gd^{3+} lacked the cooperativity seen with the trimeric porin OmpF.

CONCLUSIONS: The availability of milligram amounts of a monomeric porin will be useful for membrane protein engineering studies that will yield more diverse stochastic sensing elements.

SIGNIFICANCE: Analyte-responsive pores are an inherently powerful technology for the real-time quantification of nanomolar levels of essential or toxic metal ions in the ocean, waste water and biological fluids. Genetically engineered pores can be tailored for sensitivity, selectivity, resistance to fouling and other important characteristics. For many metal ions, the response time is limited only by analyte diffusion.

PATENT INFORMATION: Gu, L., Braha, O., and Bayley, H. Stochastic sensing mediated by carrier molecules (November 1998, provisional patent filed; November 1999, full patent filed)

AWARD INFORMATION: none

PUBLICATIONS:

Gu, L., Braha, O., Conlan, S. Cheley, S. and Bayley, H. (1999) Stochastic sensing of organic analytes by a pore-forming protein containing a molecular adapter, Nature 398, 686-690.

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