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TEXAS MEDICAL CENTER NASA/JOHNSON SPACE CENTER

COOPERATIVE AGREEMENT PROGRAM NCC 9-36, ROUND II

COVER SHEET FOR FINAL REPORT

Name of Subcontractor: Magnus Hook, Ph.D.

Title: Director, Center for Extracellular Matrix Biology

Institution: Albert B. Alkek Institute of Biosciences and Technology, Texas A & M University

Name of Project: The Structure and Function of Non-Collagenous Bone Proteins

Amount of Grant: [redacted]

* Amount Spent, if Different from Amount Granted: [redacted]

Date Project Was Completed: November 1997

Grants Officer: Matthew Shaunty

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Report on NASA Project: Structure and Function of Non-Collagenous Bone Proteins

1. Description of Research

The long-term goal for this program is to determine the structural and functional relationships of bone proteins and proteins that interact with bone. This information will used to design useful pharmacological compounds that will have a beneficial effect in osteoporotic patients and in the osteoporotic-like effects experienced on long duration space missions. The first phase of this program, funded under a cooperative research agreement with NASA through the Texas Medical Center, aimed to develop powerful recombinant expression systems and purification methods for production of large amounts of target proteins. Proteins expressed in sufficient amount and purity would be characterized by a variety of structural methods, and made available for crystallization studies. In order to increase the likelihood of crystallization and subsequent high resolution solution of structures, we undertook to develop expression of normal and mutant forms of proteins by bacterial and mammalian cells. In addition to the main goals of this program, we would also be able to provide reagents for other related studies, including development of anti-fibrotic and anti-metastatic therapeutics.

2. Research Achievements and Outcomes

Over the course of the current program we have set in place all the technologies required for the high level expression, purification, and characterization of the complex proteins and glycoproteins that comprise the mineralized matrix of mineralized tissues. Specifically, we have focused attention on a complex glycoconjugate termed decorin, which has been expressed using different systems (1, 2, 9), depending on particular requirements. Decorin represents a prototype for our approach to study a broad range of bone proteins, and our success is encouraging. Briefly decorin has been expressed in large quantity (>30 mg per batch) by use of recombinant vaccinia viridae as both a proteoglycan and core protein form; however, the core protein is variably substituted with 2-3 N-linked oligosaccharides. We have generated site-specific mutations in the core protein so that carbohydrate attachment sites have been deleted; in addition, expression of chemical amounts of homogeneous core protein by CHO mutant cell lines has increased the spectrum of molecules produced and therefore increased the likelihood of crystallization. Decorin and the related biglycan possess extensive secondary structure (8), and this project represents the first time that these molecules have been produced in significant amount with maintenance of putative functional domains. Other structural and functional studies have identified new activities, including a zinc binding motif (9), and ability to modulate tumor cell growth by interaction with cell surface receptors (6, 7). These studies have formed the basis of two new programs submitted to the NIH for funding in the next cycle (see below). It is anticipated that similar approaches to the other bone proteins will yield significant new information. The versatility of the mammalian expression system has been greatly expanded (3, 5) by the development of a panel of expression constructs that have been made available to several investigators. Recombinant vaccinia viridae encoding other bone proteins have been generated and are now ready for large scale production.

In collaboration with the Center for Macromolecular Crystallography at the University of Alabama at Birmingham, we are analyzing the structure of several bone protein-binding bacterial proteins. Four of these proteins have been crystallized and the structure of a collagen binding protein from *S. aureus* has been solved at a resolution of 1.9Å (4).

Publications:

1. Hocking, A.M., Strugnell, R.A., Ramamurthy, P., and McQuillan, D.J. (1996) Eukaryotic Expression of Recombinant Biglycan: post-translational processing, and the importance of secondary structure for biological activity. *J.Biol. Chem.* **271**, 19571-19577
2. Ramamurthy, P., Hocking, A.M., and McQuillan, D.J. (1996) Recombinant Decorin Glycoforms: purification and structure. *J. Biol. Chem.* **271**, 19578-19584
3. Chan, D., Weng, Y.M., Hocking, A.M., Golub, S., McQuillan, D.J., and Bateman, J.F. (1996) Site-directed Mutagenesis of Human Type X Collagen: expression of α1(X) NC1,

NC2 and helical domain mutations *in vitro* and in transfected cells. *J. Biol. Chem.* **271**, 13566-13572
4. Symersky, J., Patti, J.M., Carson, M., House-Pompeo, K., Teale, M., Moore, D., Jin, L., Schneider, A., De Lucas, L.J., Hook, M., Narayana, S.V.L. (1997) Structure of the collagen-binding domain from a Staphylococcus aureus adhesin. *Nature Struct. Biol.* **4**, 833-838
5. Chan, D., Lamande, S.R., McQuillan, D.J., and Bateman, J.F. (*in press*) In vitro expression analysis of collagen biosynthesis and assembly. *Biochem. Biophys. Meth.*
6. Moscatello, D.K., Santra, M., Mann, D.M., McQuillan, D.J., Wong, A.J., and Iozzo, R.V. (*in press*) Decorin supresses tumor cell growth by activating the epidermal growth factor receptor. *J. Clin. Invest.*
7. Patel, S., Santra, M., McQuillan, D.J., Iozzo, R.V., and Thomas, A.P. (*in press*) Decorin activates the EGF receptor and elevates cytosolic Ca^{2+} in A431 carcinoma cells. *J. Biol. Chem.*
8. Krishnan, P., Hocking, A.M., Scholtz, M., Pace, C.N., and McQuillan, D.J. (*submitted*) Comparative structural analysis of biglycan and decorin.
9. Yang, W-C., LaBrenz, S.R., Rosenberg, L.C., Höök, M. (*submitted*) Decorin: a zinc binding protein

Grants submitted:

1. McQuillan, D.J. (P.I.) Biosynthesis, structure, and function of decorin. Submitted to the NIH, RO1GM58009
2. Border, W.A., Noble, N.A., McQuillan, D.J. (co-P.I.s) Decorin: mechanisms of anti-fibrotic effects. Submitted to the NIH (assignment number pending)

3. Future Goals

In the event that continued funding is available, we would anticipate pursuing second stage objectives with proteins already produced in large amounts i.e. crystallization trials with decorin, biglycan, cell surface integrin receptors, and COMP. We have in place expression constructs for most of the other major bone proteins, and would proceed with large scale expression, purification, and characterization prior to crystallization trials. It would be anticipated that in the next two years we would have available high resolution structures for a number of bone proteins and related structures. This would then set the stage for defining in detail the interactions in the matrix, the role in mineral deposition and mineral loss, and initiate a rational drug design strategy to treat disorders related to bone composition.

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Ms. Mary Schiflett
Program Administrator
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Dear Ms. Schiflett:

Enclosed please find two manuscripts entitled, "Distinct Secondary Structures of the Leucine-Rich Repeat Proteoglycans Decorin and Biglycan: glycosylation-dependent conformation stability" and "Decorin is a Zn^{2+} metalloprotein", respectively. These manuscripts present studies partially supported by the NASA/JSC Cooperative Program as indicated in the acknowledgements. The manuscripts have been submitted to the Journal of Biological Chemistry. Before we proceed with the publication process, we request approval from NASA/Johnson Space Center.

Sincerely yours,

Magnus Höök, Ph.D.

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Enclosures