Physical Properties of a Calmodulin Hydrogel as a Scaffold for Protein Immobilization

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INTRODUCTION

Protein immobilization is the attachment of proteins to surfaces resulting in reduction or loss of mobility, ideally without a loss of activity or function. Immobilized proteins are easier to work with compared to their soluble counterparts because immobilization provides convenient handling, facile separation, efficient recovery and reuse, and enhanced stability both in storage and in operation. These benefits facilitate the application of immobilized proteins, making them useful in microarrays, continuous flow reactor systems, enzymology, proteomics, drug screening, nanotechnology, and as biosensors.

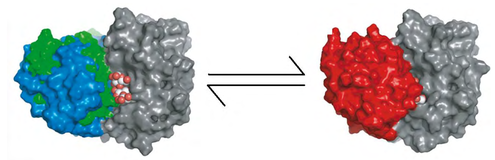
Current approaches in studying protein function following immobilization on solid supports do not address how the properties of the support material affect protein structure and dynamics. In order to more efficiently create appropriate immobilization systems, general principles regarding the interaction between the material properties and broad classes of proteins need to be understood. Our goal is to gain some insight as to what material properties are optimal in the functional immobilization of proteins through the study of maltose binding protein (MBP) immobilized in a calmodulin (CaM) hydrogel. Maltose binding protein undergoes a ~35˚ rigid body domain reorientation upon binding of sugar that can be detected by solution NMR. Through various NMR experiments, we plan on confirming MBP's function and analyzing the interactions between MBP and the hydrogel through a range of pHs.

OBJECTIVES

- To understand the physical properties of a material that are important in the immobilization of proteins through the:
- Functional immobilization of MBP in a CaM hydrogel
- Examination the interactions between the CaM hydrogel and the MBP

The authors would like to thank John Cort and Garry Buchko for all their help and the Defense Threat Reduction Agency for supporting this work. A portion of the research was performed using EMSL, a national scientific user facility sponsored by the Department of Energy's Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory.

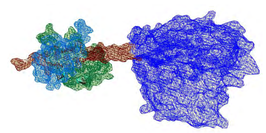
MBP STRUCTURE



Molecular surface representation of MBP bound to maltose: open (blue) and partly closed (green) N-terminal domain of the apo species of MBP and closed/holo MBP (red)

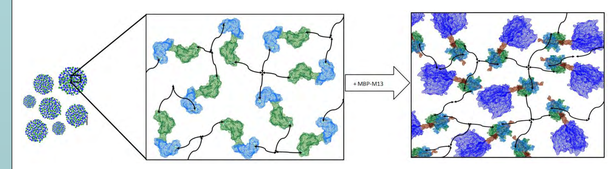
Structures obtained from Tang, Schwieters, Clore paper

CaM/MBP-M13 STRUCTURE



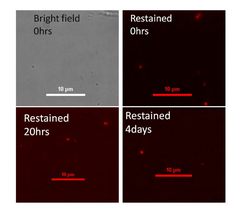
CaM (light blue and green) bound to M13 (brown) attached to MBP (dark blue)

HYDROGEL STRUCTURE



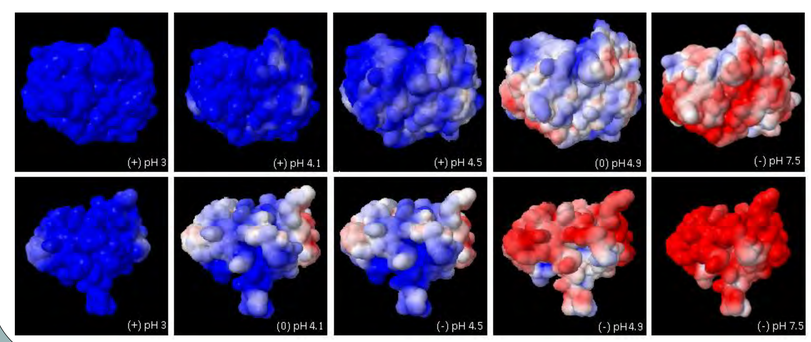
Schematic representation of CaM hydrogel microspheres: without and with MBP-M13

HYDROGEL MICROSPHERES

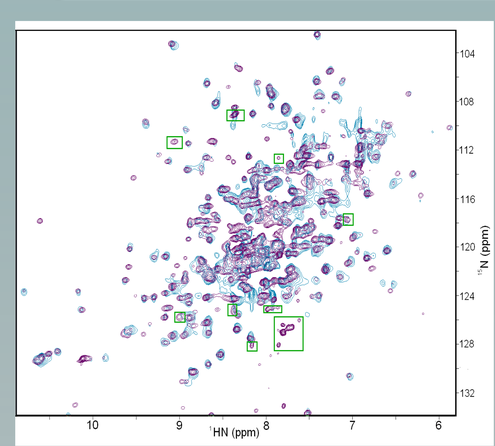


MBP-M13 was immobilized in the microspheres and then washed out with EGTA (not shown). Then it was restained and visualized at multiple time points.

SURFACE CHARGE COMPARISONS

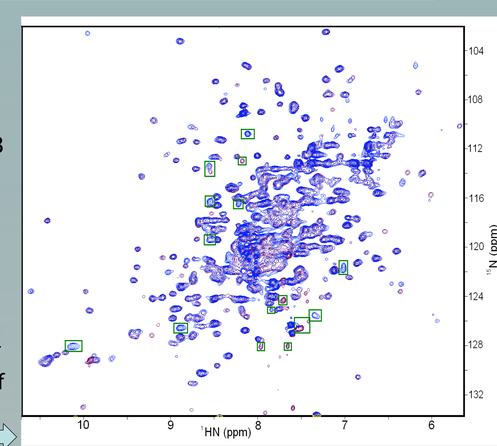


The top row is MBP and the bottom row is CaM bound to M13. Each column is a representation of the surface charges at successive pHs (3, 4.1, 4.5, 4.9, 7.5) We will investigate the interactions between the MBP and the CaM hydrogel at each pH via NMR.



MBP without the M13 linker (light blue) and MBP-M13 (purple). Peaks associated with M13 are boxed in green.

MBP-M13 with CaM (blue) and without CaM (purple). Differences in the spectra are boxed in green, indications of structural changes of M13 upon binding to CaM.



METHODS

- Purified Calmodulin (CaM)
- Purified Maltose Binding Protein with CaM target attached: M13 (MBP-M13)
- Formed CaM hydrogel microspheres using PEGDA
- Immobilized MBP-M13 in the CaM hydrogel
- Visualized MBP-M13 in CaM hydrogel via fluorescence
- Visualized MBP-M13 in CaM hydrogel via Nuclear Magnetic Resonance (NMR) spectroscopy

CONCLUSIONS

CaM hydrogel appears to be a satisfactory immobilization support for MBP

We were able to get well resolved NMR spectra of the large MBP-M13 complex using HSQC-TROSY at 600MHz

FUTURE WORK

- Obtain spectra of MBP-M13 in CaM hydrogel in different pH environments
- Purify MBP-M13 without maltose
- Test the functionality of MBP in the gel (by adding maltose)
- Test the functionality of MBP with regards to different pHs

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