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Engineering the Maltose Binding Protein for Metal Ions Sensing

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Introduction

This work describes the development of an array-based sensor for the detection of metal ions, in particular Zn^{2+}, using *in vivo* biotinylated variants of the maltose binding protein (MBP) as the biorecognition element. MBP is a member of the periplasmic binding proteins that typically adopt two conformations: an open and closed form upon ligand binding. This ligand-mediated conformational change forms the basis of the sensing system.

Figure 1. Perspective view of the superimposed backbone structure of unliganded and maltose bound MBP (looking into the binding cleft from the side) with bound maltose (ball-and-stick model). The conformational change is driven by the hinge bending motion by 35˚ flip with 8˚ twist of one domain relative to the other (Sharff et al. 1992).

Pioneering work by Gilardi et al. (1994) on the development of the MBP for reagentless fluorescence sensing involved covalently attaching an environmentally sensitive fluorophore, IANBD, to a unique thiol group of cysteine. MBP has no native cysteine residues and therefore a single cysteine was introduced by site-directed mutagenesis to replace a serine residue at a position within the cleft (S337) where maltose binding occurred. Marvin and co-workers (1997) have argued that positioning the fluorophore at the cleft can be accompanied by unfavourable steric interactions between the fluorophore and the ligand, which lowers the ligand affinity. To address this issue, Marvin's group located the fluorophore at a position opposite of the maltose-binding cleft (D95) to ensure total steric independence. Dissociation constants (K_d) for IANBD labeled D95C and S337C mutants were determined to be 1.4 µM and 62 µM respectively.

The rational, structure-based redesign of substrate binding specificity remains a major challenge in protein chemistry. A zinc biosensor was recently created by modifying the ligand binding site of non-tagged MBP to coordinate zinc (Marvin and Hellinga, 2001). Idealised tetrahedral zinc primary coordination sites comprising of (1) three histidines and a water molecule and (2) two histidines and two glutamates were engineered to replace the native maltose binding residues. The optimised coordination sphere (H63, H66, E155, E340) gave the best observed affinity for zinc (K_d = 5.1 µM).

Figure 2. Example of Zinc Centers in Proteins

Carbonate dehydratase : Zinc (orange ball) firmly bound to 3 histidine nitrogen atoms (blue balls) and 1 oxygen (red ball) from a water molecule.

Carboxypeptidase A : Zinc bound to 2 histidine nitrogen atoms, 2 glutamate oxygen atoms, and water.

Experiment

Construction of an Expression System for MBP & Its Cysteine Mutants

An AviTag or biotin tag (GLNDIFEAQKIEWHE) contained within the pAC6 vector (Avidity Inc. USA) was introduced to the C-terminus of the cytoplasmic form of MBP. Two cysteine mutants of the MBP (D95C & S337C) were made by site-directed mutagenesis and cloned into pAC6. The protein fusion constructs were transformed into *E.coli* AVB101 (Avidity Inc. USA) for protein expression and *in vivo* biotinylation. AVB101 contains a plasmid (pBirAcm) with an IPTG-inducible *birA* gene to overexpress biotin protein ligase.

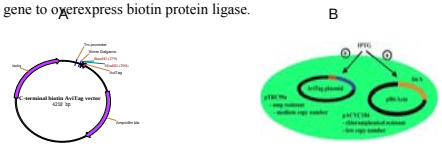


Figure 3. (A) pAC6 vector for making C-terminal AviTag fusions to proteins. (B) Transformed AVB101 cells in which expression of both biotin protein ligase and the AviTag protein are induced with IPTG (1.5mM). Biotin was added at the time of induction to a concentration of 50 µM. The pAC vector requires ampicillin 100 µg/ml for maintenance.

Construction of Tetrahedral Zn-binding sites in MBP

Three zinc-binding site designs described in literature (Marvin and Hellinga, 2001) were constructed in place of the maltose-binding residues using site-directed mutagenesis. The tetrahedral Zn coordination sphere comprised of (Figure 4) :

- M1 (A63H, R66H, W340H)
- M2 (A63H, R66H, Y155H)
- M3 (A63H, R66H, Y155E, W340E)

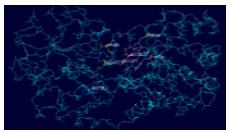


Figure 4. Backbone structure of the maltose-bound form of the MBP showing the maltose molecule (GLC371 & GLC372), the position of D95 and side chains of the residues mutated to form the Zn-binding site designs.

Labeling of Proteins

Labeling of proteins with IANBD were carried out as previously described (Gilardi et al. 1994) but with modifications. Metal binding conjugates were pretreated with Chelex resin to remove adventitious metal prior to fluorescence measurements.

Fluorescence Measurements in Solution

Emission spectra were obtained using a Perkin Elmer LS-50B spectrofluorimeter (λ_{ex}=480nm for IANBD). Ligand binding titrations were performed using a SpectraMax Gemini XS multi-well plate reader by measuring fluorescence at a single wavelength. Each well contained 1µM labeled protein with various concentrations of ligand in a total volume of 100 µl metal-free MOPS buffer.

Protein Microarray Fabrication & Ligand Binding Assay

0.5 nl spot volumes of *in vivo* biotinylated IANBD labeled D95C metal-binding proteins were printed onto streptavidin coated slides (Xenopore) using a MicroSys SQ series 4000/4100 microarrayer (Cartesian Technologies). Slides were washed with metal-free MOPS buffer before incubating with increasing concentrations of the ligand. Slides were imaged using an aQuire microarray scanner and data analysed with QScan 1.1 software (Genetix).

Results & Discussion

Reagentless Sensing System for Maltose

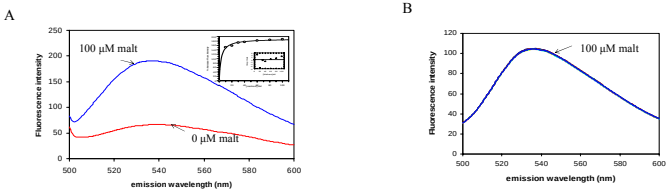
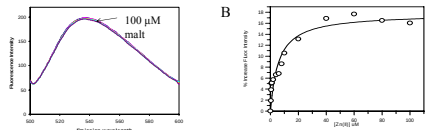


Figure 5. Fluorescence emission spectra from (A) biotinylated IANBD labeled D95C in the absence, and presence of 100 µM maltose. (*Inset*) The data fit to a single site hyperbolic binding isotherm with an apparent K_d maltose of 2.90 ± 0.40 µM;
(B) biotinylated IANBD labeled S337C showing a complete lack of response of the label to maltose. The lack of fluorescence response of labeled S337C is thought to be due to steric interference from the C-terminal biotinylated Avitag which could block the fluorophore.

Affinity of D95C Metal-Binding Proteins (M1, M2, M3) to Maltose and Zinc

- No change in fluorescence intensity observed for IANBD labeled M1, M2 and M3 in the presence of up to 100 µM maltose implying that all metal-binding variants of D95C MBP have lost the ability to bind its native ligand (Figure 6).
- M1, M2 and M3 all have acquired the ability to bind Zn^{2+} with similar dissociation constants (K_d = 4-6 µ M). This is in disagreement with the findings of Marvin's group (2001) in that the His_2Glu_2 design gave a tighter binding for Zn^{2+} compared to the His_3 design. The similar K_d's obtained from this study suggests that the coordination sphere for His_3 is more similar to His_2Glu_2 in that an additional metal coordination could come from an amino acid residue of the AviTag (eg. glutamate).



Mutants	K_d Malt (µM)	K_d Zn^{2+} (µM)
M1	nb	6.20 ± 1.70
M2	nb	4.10 ± 1.20
M3	nb	5.90 ± 1.30
D95C	2.90 ± 0.40	nb

Figure 6. (A) Fluorescence emission spectra of IANBD labeled D95C metal-binding protein M1 showing no change in fluorescence intensity with increasing amounts of maltose up to 100 µM. Similar response was observed for M2 and M3. (B) Zn^{2+} binding curve shown for M3, although all three metal-binding proteins show similar trend. Each data point represents the average of three replicate measurements.

Protein Microarray for Metal Ions Sensing

It was found that when immobilised onto streptavidin coated slides, M1, M2 and M3 did not show similar fluorescence response to Zn^{2+} in solution format. This could be due to (1) the proteins being attached in a random fashion which may alter the proteins native conformation thereby reducing the proteins activity (2) some proteins being denatured during handling or processing (3) proteins rearranging themselves in a way that would make them inaccessible to Zn^{2+}.

Figure 7. Arraying of 5 µM IANBD conjugates of biotinylated MBP(D95C) metal variants M1, M2 and M3 (3x10 array) on streptavidin coated slides. Picture on the left was imaged after arraying whilst that on the right was imaged after washing with buffer.

References

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