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\(^\circ\) 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 third globule 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 unfavorable 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). Idealized 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

Construction of an Expression System for MBP & Its Cysteine Mutants

An AviTag or biotin tag (GLNDIFEA/QKEIKIEWHE) contained within the pAC6 vector (Avicidin Inc. USA) was introduced to the C-terminus of the cysteamic 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 (Avicidin Inc. USA) for protein expression and in vivo biotinylation. AVB101 contains a plasmid (pBlrAcm) with an IPTG-inducible lacI gene to express biotin protein ligase.

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):

- M2 (A63H, R66H, V155E)

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

Figure 4. Backbone structure of the maltose-bound form of the MBP showing the maltose molecule (GLND77 & GLND75), the position of D95 and side chains of C-terminal biotinylated AviTag residues involved in 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 preincubated 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 spectrophotometer (Gh.488nm for IANBD). Ligand binding titrations were performed using a SpectraMax Gemini XS multi-well plate reader by measuring fluorescence at a single excitation wavelength of 480 nm for IANBD. Each well contained 3 µl 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 µl spot volumes of in vivo biotinylated IANBD labeled D95C metal-binding proteins were printed onto streptavidin coated slides (Xenopore) using a Microsys model. Slides were washed with metal-free MOPS buffer before incubating with increasing concentrations of the ligand. Slides were imaged using an Axioris microarray scanner and data analysed with GImage 1.1 software (Genetix).

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 hypochromic binding isotherm with an apparent K_d maltose of 2.9 ± 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 Zine

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 µM). This is in disagreement with the findings of Marvin's group (2001) that in the His6His6 design gave a tighter binding for Zn\(^{2+}\) compared to the His6 design. The similar K_d's obtained from this study suggests that the coordination sphere for His6 is more similar to His6His6 in that additional metal coordination could come from an amino acid residue of the AviTag (eg glutamate).

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 on 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\(^{2+}\).

Results & Discussion

Reagentless Sensing System for Maltose

Table 1. 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 hypochromic binding isotherm with an apparent K_d maltose of 2.9 ± 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.

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References


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