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ORIGINAL PAPER

# Selective deuteration of tryptophan and methionine residues in maltose binding protein: a model system for neutron scattering

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**Abstract** We describe methods that have been developed within the ILL-EMBL Deuteration Laboratory for the production of maltose binding protein (MBP) that has been selectively labelled either with deuterated tryptophan or deuterated methionine (single labelling), or both (double labelling). MBP is used as an important model system for biophysical studies, and selective labelling can be helpful in the analysis of small-angle neutron scattering (SANS) data, neutron reflection (NR) data, and high-resolution neutron diffraction data. The selective labelling was carried out in *E. coli* high-cell density cultures using auxotrophic mutants in minimal medium containing the required deuterated precursors. Five types of sample were prepared and studied: (1) unmodified hydrogenated MBP (H-MBP), (2) perdeuterated MBP (D-MBP), (3) singly labelled MBP with the tryptophan residues deuterated (D-*trp* MBP), (4) singly labelled MBP with methionine residues deuterated (D-*met* MBP) and (5) doubly labelled MBP with both tryptophan and methionine residues deuterated (D-*trp*/*met* MBP). Labelled samples were characterised by size exclusion chromatography, gel electrophoresis, light scattering and mass spectroscopy. Preliminary small-angle neutron scattering (SANS) experiments have also been carried out and show measurable differences between the SANS data recorded for the various labelled analogues. More detailed SANS experiments using these labelled MBP analogues are planned; the degree to which such data could enhance structure determination by SANS is discussed.

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## Introduction

### Selective labelling for neutron scattering

The ability to deuterate or selectively deuterate biological macromolecules adds a powerful dimension to neutron scattering experiments and can provide information that is outside the scope of X-ray methods. For low resolution studies, the strength and uniqueness of this approach relies on the application of the contrast variation methods originally developed by Stuhrman (1974), and has subsequently been exploited in numerous small-angle neutron scattering (SANS) and low-resolution crystallography studies (Jacrot 1976; Pardon et al. 1977; Kuzmanovic et al. 2003; Pebay-Peyroula et al. 1995). In the absence of any specific isotope labelling, the large range of contrasts available through the use of $D_2O/H_2O$ solvent mixtures allows SANS methods to distinguish between protein, nucleic acid and lipid components of a multi-component system—all of which have markedly different scattering length densities (Timmins and Zaccai 1988). However, the only way to distinguish between the components of a system in which the scattering length densities are all similar (e.g. a multi-subunit protein system)

is through the use of selective labelling of specific components—normally with deuterium. This technique was first exploited in the classic experiments on the 30S and 50S subunits of the ribosome (Capel et al. 1987; May et al. 1991) where labelling was exploited to determine by triangulation the distances between the component protein sub-units. Two more recent examples in which labelling of selected subunits has allowed quaternary structure to be determined have been published by King et al. (2005), and by Callow et al. (2007). In the former case, selective deuteration of a full set of ternary complexes of troponin has been used to study the large structural change that occurs upon calcium binding and to identify the sub-unit in which this change principally takes place. In the latter example, the deuteration of specific protein sub-units was used in conjunction with the ab initio modelling methods of Svergun et al. (1999) to study the subunits within a type I restriction modification system. These studies demonstrate the major opportunities available to SANS work by selective labelling of particular domains/subunits. Svergun et al. (2000) have proposed that if deuterium labelling can be carried out for specific amino acids within a protein, SANS contrast variation data can assist in determination of the tertiary structure.

At higher resolution, the ability to distinguish H/D or solvent molecules in crystalline or fibrous samples may provide unique information and insight into hydration, catalysis, and protein–ligand interactions. Furthermore, perdeuteration of macromolecular systems provides a major advantage through the elimination of hydrogen incoherent scattering from both solvent and macromolecule, relieving severe limitations on sample size and data collection times (Hazemann et al. 2005; Liu et al. 2007). The use of amino-acid-specific labelling has also been proposed for the development of new direct methods in protein structure determination by neutron crystallography (Hauptman 2003). For high resolution neutron diffraction work on fibrous polymers, it is possible to use selective residue deuteration to label polymers such as DNA (Parrot et al. 2006, 2008), cellulose (Langan et al. 1999; Nishiyama et al. 2002, 2003; Wada et al. 2004), and synthetic polymers (Gardner et al. 2004), providing critically important information in model building and structure determination. In neutron reflection experiments, selective labelling can be used to distinguish between various components of a complex system (Lu et al. 2000; Grage et al. 2008). Specific *hydrogenation* of deuterated proteins has also been powerful in elastic incoherent neutron scattering experiments for the of study protein dynamics. Here the dynamic information is contained in the incoherent scattering from hydrogen. Experiments on isotope labelled bacteriorhodopsin have provided evidence for inhomogeneous dynamics in this membrane protein, related to biological function (Réat et al. 1998; Tehei et al. 2007). More recently, perdeuterated maltose binding protein (MBP) produced at the ILL-EMBL Deuteration Laboratory (Forsyth et al. 2001; Haertlein et al. 2008, http://www.ill.eu/deuteration) has been used to study water dynamics around the protein (Wood et al. 2008; Paciaroni et al. 2008).

## Amino acid selective labelling of maltose binding protein

MBP is a well-studied model protein that plays an important role in the metabolism of *E. coli* (Sharff et al. 1992). It is essential for the energy-dependent translocation of maltose and maltodextrins through the cytoplasmic membrane. It is a binding protein specific for maltose and maltodextrins with a $K_D$ around 1 μM. There is one binding site per maltose monomer (Schwartz et al. 1976), and upon binding of the substrate, the protein undergoes a conformational change that can be monitored by fluorescence techniques (Zukin 1979). MBP is constructed of two globular domains connected by a three-stranded hinge, with the ligand-binding site located in the cleft between the two domains. Wild-type MBP has been crystallised in two conformations: a ligand-bound "closed" form (Spurlino et al. 1991) and a ligand-free "open" form (Sharff et al. 1992); these differ primarily by a rigid-body rotation of one domain relative to the other, resulting in opening or closing of the ligand-binding cleft.

MBP was chosen for this development for a number of reasons. In the context of biophysical studies, MBP is a widely used model system for the study of the behaviour of proteins in a variety of contexts. It is highly soluble and relatively free of aggregation, even at high concentrations. *E. coli* MBP is frequently used as an affinity tag to facilitate the purification of recombinant proteins (Maina et al. 1988). An important additional attribute of MBP is its remarkable ability to enhance the solubility of its fusion partners (Fox and Waugh 2003). Furthermore, methods for stereospecific isotope labelling of MBP have been developed for solution NMR studies (Kainosho et al. 2006).

Depending on the required application, specific amino acid labelling can be achieved either by deuterating specific amino acids within the protein, or by selectively hydrogenating particular amino acids within a protein that is otherwise perdeuterated (so-called *reverse labelling*). In the former case, the required deuterated amino acid is added to a culture growing in an otherwise hydrogenated minimal medium, whereas in the latter, a hydrogenated amino acid is added to an otherwise deuterated minimal medium. For both, two concerns arise when the label is to be restricted to specific types of amino acids: dilution of the label by endogenous amino acid biosynthesis, and scrambling of the label to other types of residues by specific metabolic conversion. Both concerns can be mitigated to some degree by supplementing the medium with a high concentration of all 20

amino acids (Sreenath et al. 2005) since amino acid biosynthesis is regulated by feedback inhibition. The degree of isotope scrambling depends largely on the amino acid used for residue-specific labelling. The use of auxotrophic strains is highly recommended if a high occupancy of a labelled residue is needed, especially if feedback inhibition of the biosynthesis of this specific amino acid is not sufficient.

This paper focuses on methodologies that have been developed for the selective deuteration of amino acids within MBP—an approach that has potential application for all of the neutron scattering techniques mentioned above. The work is based on a high-yield expression system for this model protein, and an optimized deuteration procedure using auxotrophic strains of *E. coli* and the relevant amino acid precursors. While tryptophan labelling presents no particular problems since it can be efficiently incorporated into essentially any strain (auxotrophic or not) of *E. coli*, methionine labelling does require an auxotrophic strain and we have used the B834(DE3) strain commonly used for the preparation of selenomethionine-labelled proteins. For the double labelling our approach has been to make use of the B834(DE3) strain auxotrophic for methionine and rely on back regulation for the incorporation of tryptophan. All of the procedures have been developed in the ILL-EMBL Deuteration Laboratory. Although small-angle X-ray scattering (SAXS) studies of the wild type protein have been described by Shilton et al. (1996a), and of a mutant of the periplasmic MBP (Shilton et al. 1996b), there appear to have been no previous studies of MBP by SANS. The labelling methods described here may provide valuable information that can be exploited in such studies.

## Materials and methods

### Design of an expression system for the model protein MBP

For protein expression in perdeuterated media, the coding sequence for the mature form of *E. coli* MBP was subcloned from pMAL-c2E vector (New England Biolabs). Site-directed mutagenesis was carried out removing pMAL-c2E vector sequence coding for a C-terminal vector-specific extension. From the resulting pMAL-c2E clone an *Nde*I-*Hin*dIII fragment was inserted into pET-28a (Novagen) vector that confers kanamycin resistance to the expression construct and an N-terminal histidine-tag to MBP to facilitate large-scale purification. The resulting construct was called pMBP/kana. The primary sequence of the histidine-tagged MBP is shown in Fig. 1.

### High-cell density cultures for the expression of hydrogenated and perdeuterated MBP

Histidine-tagged perdeuterated MBP (D-MPB) was obtained by expression in *E. coli* BL21(DE3) at the ILL-EMBL Deuteration Laboratory in Grenoble, France using high-cell density cultures. Cells were grown in minimal medium: 6.86 g $L^{-1}$ $(NH_4)_2SO_4$, 1.56 g $L^{-1}$ $KH_2PO_4$, 6.48 g $L^{-1}$ $Na_2HPO_4 . 2H_2O$, 0.49 g $L^{-1}$ diammonium hydrogen citrate, 0.25 g $L^{-1}$ $MgSO_4 . 7H_2O$, 1.0 mL $L^{-1}$ (0.5 g $L^{-1}$ $CaCl_2 . 2H_2O$, 16.7 g $L^{-1}$ $FeCl_3 . 6H_2O$, 0.18 g $L^{-1}$ $ZnSO_4 . 7H_2O$, 0.16 g $L^{-1}$ $CuSO_4 . 5H_2O$, 0.15 g $L^{-1}$ $MnSO_4 . 4H_2O$, 0.18 g $L^{-1}$ $CoCl_2 . 6H_2O$, 20.1 g $L^{-1}$ EDTA), 5 g $L^{-1}$ glycerol, 40 mg $L^{-1}$ kanamycin [17,18]. For preparation of fully deuterated medium, mineral salts were dried out in a rotary evaporator (Heidolph) at 333 K and labile protons exchanged through dissolution in and redrying from $D_2O$. Perdeuterated $d_8$-glycerol was used as a carbon source. Adaptation of BL21(DE3) cells to deuterated minimal medium was achieved by a multi-stage adaptation process (Artero et al. 2005). Typically, 1.5 L of deuterated medium was inoculated with 100 mL preculture of adapted cells in a 3 L fermenter (Labfors, Infors). During the batch and fed-batch phases the pH was maintained at 6.9 (by addition of NaOD) and the temperature was adjusted to 30°C. The gas-flow rate of sterile filtered air was 0.5 L $min^{-1}$. Stirring was adjusted to ensure a dissolved oxygen tension (DOT) of 30%. The fed-batch phase

**Fig. 1** Primary sequence of His-tagged *E. coli* maltose-binding protein. Methionine residues (M) are in bold, tryptophan residues (W) are underlined

**M**GSSHHHHHHSSGLVPRGSH**M**KTEEGKLVI<u>W</u>INGDKGYNGLAEVGKKFEKDTGIKVTVE
HPDKLEEKFPQVAATGDGPDIIF<u>W</u>AHDRFGGYAQSGLLAEITPDKAFQDKLYPFT<u>W</u>DAV
RYNGKLIAYPIAVEALSLIYNKDLLPNPPKT<u>W</u>EEIPALDKELKAKGKSAL**M**FNLQEPYF
T<u>W</u>PLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKH**M**NADTDYSIAEA
AFNKGETA**M**TINGP<u>W</u>A<u>W</u>SNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKE
LAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAAT**M**ENAQKGEI**M**PNI
PQ**M**SAF<u>W</u>YAVRTAVINAASGRQTVDEALKDAQT

was initiated when the optical density at 600 nm reached 3.2. $D_8$-glycerol was added to the culture to keep the growth rate stable during fermentation. When $OD_{600}$ reached 13.6, MBP over-expression was induced by the addition of 0.5 mM IPTG over a period of 24 h. Cells were then harvested, washed with 10 mM HEPES (pH 6.4), and stored at −80°C.

## Amino-acid specific single and double labelling of MBP using auxotrophic strains

Cells were grown at 30°C in the presence of 40 mg $L^{-1}$ kanamycin to an $OD_{600}$ of 0.6 in a modified M9 medium according to (Ramakrishnan et al. 1993), 3 g $L^{-1}$ $KH_2PO_4$, 6.8 g $L^{-1}$ $Na_2HPO_4$, 1 g $NH_4Cl$. Doubly concentrated M9 medium was supplemented with 2 mM MgSO4, 25 mg $L^{-1}$ $FeSO_4 \cdot 7H_2O$, 0.4% glucose 40 mg $L^{-1}$ amino acid mix 1 (all amino acids except methionine, tyrosin, tryptophan and phenylalanine) and 40 mg $L^{-1}$ amino acid mix 2 (methionine, tyrosin, tryptophan and phenylalanine). Riboflavin, niacinamide, pyridoxine monohydrochloride and thiamine were added at 1 mg $L^{-1}$ each and the pH kept at 7.4. Before induction the bacterial culture was cooled down to 22°C and MBP expression induced by the addition of IPTG to a final concentration of 0.5 mM. For each of the selectively labelled analogues the cultures were supplemented as follows: (a) D-*trp* MBP: KS463(DE3) pMal/kana cells were grown in Enfors medium supplemented with 60 mg $L^{-1}$ $d_5$-indole tryptophan, (b) D-*met* MBP: B834(DE3) pMal/kana cells were grown in M9 medium supplemented with 100 mg $L^{-1}$ $d_3$-methyl-methionine instead of methionine, (c) D-*trp*/*met* MBP: B834(DE3) pMal/kana cells were grown in M9 medium supplemented with 60 mg $L^{-1}$ $d_5$-indole tryptophan and 100 mg $L^{-1}$ $d_3$-methyl-methionine. Figure 2 shows the compounds used for the amino acid specific labelling.

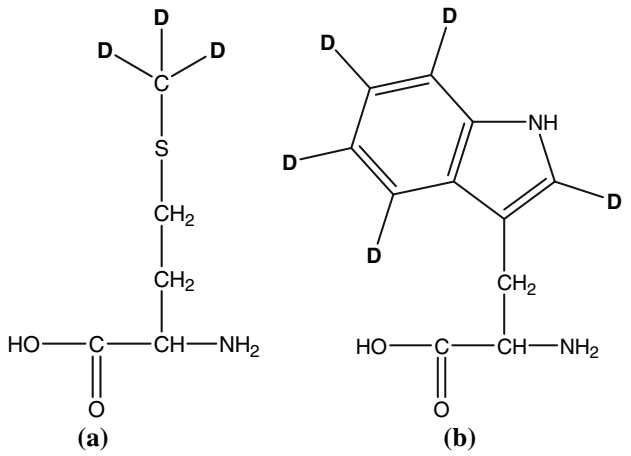


**Fig. 2** Compounds used for amino acid specific deuterium labelling: **a** L-Methionine-$d_3$ (*methyl*-$d_3$), **b** L-tryptophan-$d_5$ (*indole*-$d_5$)

## Purification of MBP variants

Cells from a 1 L culture were harvested by centrifugation at 9,000 rpm for 20 min and resuspended in 60 mL of 10 mM Tris–HCl pH 7.5, 100 mM NaCl, 10% glycerol and 1 tablet of protease inhibitor mix (EDTA-free) from Roche. Cells were lysed by ultrasound on ice and the lysate centrifuged at 19,000 rpm for 45 min. MBP was purified in two steps using immobilized metal ion affinity chromatography (IMAC) on TALON (Clontech) and gel filtration on Superdex 200 (GE Biosciences). The supernatant was loaded on a column filled with 10 mL of TALON beads, and the column was washed with 20 column volumes of lysis buffer containing 5 mM imidazole. The MBP was then eluted with 100 mM imidazole. Fractions were analysed by polyacrylamide gel electrophoresis (PAGE), pooled and dialysed against 10 mM Tris–HCl pH 7.5, 100 mM NaCl. MBP was concentrated to 5 mg $mL^{-1}$ using ultra-free device from Millipore (MWCO 10 KD). Further purification was carried out in 1 mL portions using Superdex 200 gel filtration column.

## Analysis of MBP variants

Protein purity was assessed by SDS–PAGE (Fig. 3). The molecular weight of hydrogenated, perdeuterated, and amino acid specifically single or double labelled MBP was determined by MALDI mass spectroscopy using the IBS mass spectroscopy service within the Grenoble Partnership for Structural Biology (PSB) (Fig. 4). Gel filtration and analysis on a dynamic light scattering (DLS) device indicated that the MBP samples are monodisperse.

## Preliminary SANS characterisation

Preliminary SANS characterisation was carried out on instrument D22 at the ILL during a short period of test time and aimed to assess the significance of the labelling and the measurability of the differences between labelled MBP analogues. H-MBP and D-MBP samples were dialysed against 10 mM Tris–HCl pH7.5, 10 mM NaCl. Samples of the selectively labelled D-*trp* MBP, D-*met* MBP, and D-*trp*/*met* MBP were dialysed against a buffer containing 10 mM Tris–HCl pH7.5, 10 mM NaCl in 40% $D_2O$. Just before the neutron experiment the samples were centrifuged at ultra high speed in an airfuge system (Beckman Coulter) as a precaution to remove potentially aggregated protein. The sample-detector distance was 2 m with 2 m collimation at a wavelength of 6 Å, covering a Q range of 0.026–0.3 Å. Data for H-MBP and D-MBP (each at a concentration of 1.5 mg $mL^{-1}$) in $H_2O$ solvent buffer were collected for 15 minutes with a neutron count rate on the detector of $4.4 \times 10^5$ neutrons $s^{-1}$. D-*met* MBP, D-*trp* MBP, and D-*trp*/*met* MBP (each at a concentration of 1.5 mg $mL^{-1}$)

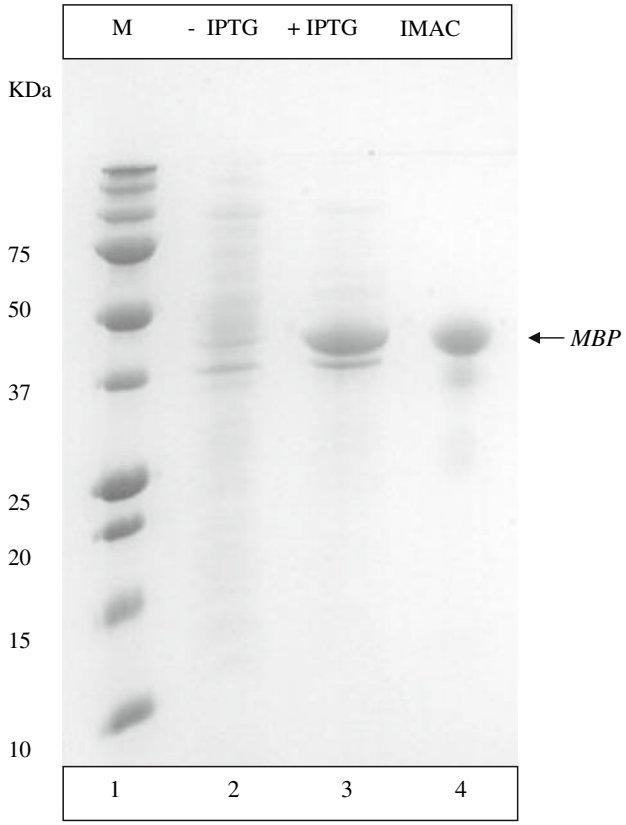


**Fig. 3** SDS-PAGE analysis of labelled MBP expressed in *E. coli* B834(DE3). Proteins were separated on a 12% SDS-PAGE gel and visualised by Coomassie brilliant blue R250 staining. *Lane 1* protein markers (Precision plus, Biorad). *Lane 2* total protein from uninduced bacteria. *Lane 3* induced bacteria. *Lane 4* MBP fraction after IMAC purification

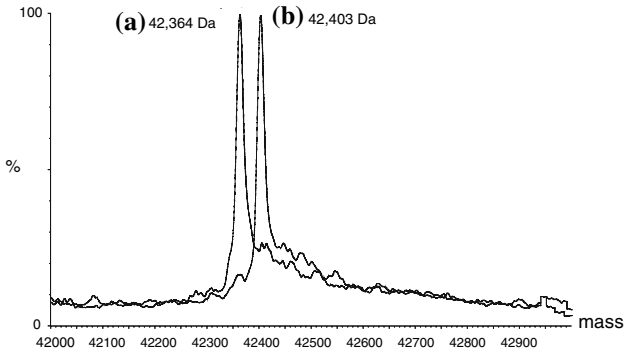


**Fig. 4** Mass spectrometic (electrospray Q-TOF) analysis of **a** unlabelled (42,364 Da) and **b** indole-$d_5$ tryptophan labelled (42,403 Da) MBP. The observed mass difference of 39 Da is very close to the expected mass difference of 40 Da between 8 $d_5$-indole tryptophan residues and 8 tryptophan residues

in 40% $D_2O$ solvent buffer were measured for 220 min with a detector count rate of $3.2 \times 10^5$ neutrons $s^{-1}$. Data treatment was performed using the $GRAS_{ans}P$ software package (Dewhurst, 2006; http://www.ill.fr/lss/grasp/grasp_main.html). Scattering curves of the available ligand-free MBP high-resolution structure (Sharff et al. 1992) were calculated using CRYSON (Svergun et al. 1998).

## Results

Histidine tagged MBP (whose primary sequence is given in Fig. 1, with the methionine and tryptophan residues indicated) was used for all amino acid specific labelling experiments. Our electrophoresis results show that all of the MBP analogues studied were of high purity (as shown in Fig. 3 for the D-MBP). In the case of methionine labelling, tests showed no significant incorporation of $d_3$-methyl-methionine into MBP using the wild-type strain as observed by mass spectroscopy (data not shown), and effective labelling of methionine was only achieved using the auxotrophic strain. However the successful incorporation of indole-$d_5$ tryptophan using the *trp* auxotrophic strain KS463(DE3), the *trp* wild type strain BL21(DE3) and the *met* auxotrophic strain B834(DE3), demonstrates the feedback inhibition of tryptophan biosynthesis. The results from electrospray ionization mass spectroscopy shown in Fig. 4 indicate increases in molecular mass that correspond well to the presence of 8 indole-$d_5$ tryptophan residues in the D-*trp* MBP samples and of $d_3$-methyl-methionine in the D-*met* samples. Table 1 summarizes the results of mass spectroscopic analysis for all different labelled and unlabelled MBP variants. In each case, the methionine aminopeptidase (Ben-Bassat et al. 1987) processed form, which was from the mass spec data by far the dominant form, was analysed. The molecular mass for unlabelled MBP was calculated using ProtParam (Gasteiger et al. 2005). A mass of 40 Da corresponding to 8 times (8 tryptophan residues in MBP) the mass difference between indol and $d_5$-indol was added to the value obtained for unlabelled MBP for the indole-$d_5$ labelled MBP(D-*trp* MBP). In a very similar way the mass for the $d_3$-methyl-methionine labelled MBP (D-*met* MBP) was calculated (7 methionines in the methionine aminopeptidase processed form and a mass difference of 3 Da between methyl- and $d_3$-methyl leading to an extra mass of 21 Da). For the double labelled MBP (D-*trp*/*met* MBP) both mass differences (40 and 21 Da) were added to the mass for the unlabelled enzyme. For the calculation of mass for perdeuterated MBP (D-MBP) the value of 2,952, corresponding to the number of hydrogens in the unlabelled MBP was added to the molecular mass of the unlabelled MBP. Since the mass spectroscopic analysis was carried out in $H_2O$ buffers, the obtained value for the perdeuterated MBP (D-MBP) in $D_2O$ was corrected by subtracting the exchangeable hydrogens to give the value of D-MBP in $H_2O$ of 44,666 Da. Measured and calculated values for each MBP variant are within the experimental error range, demonstrating the quality of the isotope labelling procedures.

**Table 1** Calculated and measured molecular masses of hydrogenated (H-MBP), perdeuterated (D-MBP) and amino acid specific deuterium labelled MBP dMet, H-MBP dTrp and H-MBP dTrp + dMet) maltose-binding protein variants

| MBP | Calculated mass (Da) | Measured mass (Da) |
|---|---|---|
| H-MBP | 42,360 | 42,364 |
| D-MBP | 45,312($D_2O$) | ND |
| | 44,666($H_2O$) | 44,664 |
| H-MBP dMet | 42,381 | 42,383 |
| H-MBP dTrp | 42,400 | 42,403 |
| H-MBP dTrp + dMet | 42,421 | 42,424 |

For each variant the methionine amino-peptidase processed form without the N-terminal methionine has been

The preliminary SANS data (Fig. 5) collected from the MBP analogues show a marked difference between data recorded from the H-MBP and the D-MBP, and in each case there is good agreement with the SANS curve calculated from the model structure. For the selectively labelled analogues, the counting statistics were, as expected, poor, given the limited measuring time and the relatively low concentration of the MBP samples. However, despite this there are clear differences between the data recorded for the labelled analogues.

## Discussion

The protocols described illustrate how high levels of selective deuteration can be achieved in a protein through the use of auxotrophic bacterial strains, both for single labelling and double labelling of MBP with tryptophan and methionine. These methods add to a steadily expanding base of labelling approaches that are being developed for general exploitation not only by biological neutron scattering community, but also for solution and solid state NMR (Varga et al. 2007). The labelling approach described here can be generalized for labelling with any other deuterated amino acid. Most amino acids with deuterium labels in non-exchangeable positions (e.g. $d_7$-L-alanine, $d_7$-L-arginine, $d_5$-glycine, $d_{10}$-L-leucine, $d_8$-L-valine etc.) are commercially available. By using *E. coli* strains that have been modified to contain the appropriate genetic lesions to control amino acid biosynthesis, dilution of the isotope and scrambling of the label to other types of residues can be avoided (Waugh 1996).

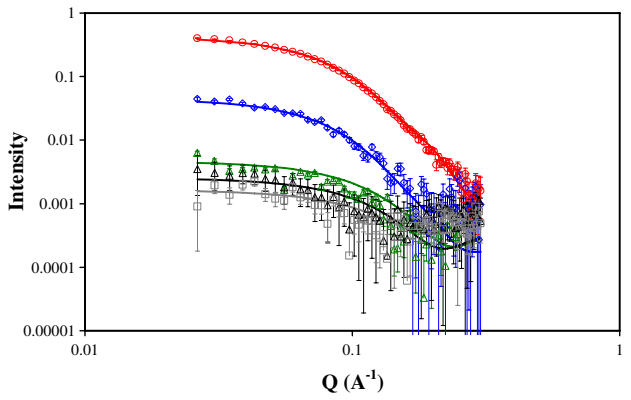


**Fig. 5** Preliminary SANS data recorded from the MBP analogues. *Diamond* hydrogenated MBP in $H_2O$ buffer; *circle* perdeuterated MBP in $H_2O$ buffer; *thin triangle* D-*trp*/*met*-MBP in 40% $D_2O$ buffer; *thick triangle* d-Tryp-MBP in 40% $D_2O$ buffer; *square* dMet-MBP in 40% $D_2O$ buffer. Following the same colour scheme, *solid lines* show the respective calculated scattering curves using CRYSON

We are also evaluating the possibility of MBP labelling by chemical modification with deuterium enriched reagents. Reductive methylation of lysine residues with isotope ($^{14}C$)-enriched formaldehyde and $NaCNBH_3$ has been described first by Dottavio-Martin and Ravel (1978). $^{13}C$-enriched formaldehyde has been used by Huque and Vogel (1993) to label lysine residues of calmodulin. It has been shown that reductive methylation of lysine residues does not change the structure of calmodulin, the protein studied, or abolishes calmodulin's activity. Recently lysine methylation has been proposed as routine rescue strategy for protein crystallization (Walter et al. 2006). We propose to adapt these methylation methods to introduce, with $^2H$-enriched formaldehyde, residue specific deuterium labels into proteins for neutron scattering applications.

The labelling methods described here may be important for the exploitation of SANS data in the modelling of protein tertiary structure. Methods have been developed (Svergun et al. 2001; Petoukhov et al. 2002) in which a protein structure is represented by an assembly of dummy residues so that the low resolution structure can be reconstructed ab initio. These methods were further developed to build models of missing fragments in high-resolution structures, also using residue-specific information (Petoukhov et al. 2002; Petoukhov and Svergun 2006). Most of these approaches are based on the fitting of a single (usually, X-ray scattering) dataset, and, of course, the information content in the scattering experiment was only sufficient to reconstruct the overall conformation at low resolution without any residue-specific detail. Although SAXS data were useful in assisting fold prediction for small proteins (Zhang 2002), general algorithms have not yet been proposed. The use of multiple scattering patterns increases the information content and allows more detailed models to be built. In the case of SAXS studies, this is possible by simultaneous fitting of the scattering patterns from deletion mutants (Petoukhov et al. 2006; Bernado et al. 2007). The deuteration of selective residues in SANS may allow the extraction of information about interresidue distances in the protein,

providing significant restraints on the possible protein fold topology and hence improving the potential of SANS for the tertiary structure analysis. Importantly, many of the theoretical approaches (Petoukhov et al. 2002; Petoukhov and Svergun 2006) already have the capacity to utilise the information from selective labelling. The preliminary SANS data we have recorded show that, even at relatively low concentrations, detectable differences can be observed between SANS data recorded from D-*trp* MBP, D-*met* MBP, and D-*trp*/*met* MBP samples. We are currently planning SANS work on the same labelled samples but at much higher concentrations (up to 20 mg $mL^{-1}$) and using much longer exposure times on instrument D22 at ILL. Our initial modelling work will focus on data collected for the double-labelled D-*trp*/*met* MBP samples. At the first stage, using the decomposition into the basic scattering functions (Stuhrman 1974) we shall extract the scattering contribution solely due to the labelled residues and validate the results by comparison with the scattering computed from the atomic models. The approach will then be extended to the data from the single labelled MBP constructs to yield the information about the spatial arrangement of the residues of interest. This information will be further incorporated into the general methods for tertiary structure modelling and we expect that the simultaneous use of the SANS data from differently labelled mutants will assist the ab initio and homology-based algorithms for tertiary structure modelling.

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