Detection of a Conformational Change in Maltose Binding Protein by $^{129}$Xe NMR Spectroscopy

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Since the observation of a xenon binding site in the interior of myoglobin,¹ the interactions of proteins with xenon have been studied with the goal of using the inert gas as a biomolecular probe. Xenon is relatively small and hydrophobic, and it binds weakly ($K < ~10^3$ M$^{-1}$) to the nonpolar interiors of many proteins with little perturbation to the protein structure.² The sensitivity of the xenon chemical shift to its local environment³ has motivated magnetic resonance studies of xenon in biological systems.⁴ More recently, the intense $^{129}$Xe NMR signals attainable with optical pumping techniques⁵ have been used to probe cavities in lyophilized lysozyme and lipooxygenase,⁶ detect blood oxygenation levels,⁷ and identify xenon binding sites in a lipid transfer protein⁸ by the spin-polarization induced nuclear Overhauser effect.⁹ Here we report the dependence of the $^{129}$Xe chemical shift on the chemical shift to its local environment ³ has motivated magnetic resonance studies of xenon in biological systems.⁴ More recently, the intense $^{129}$Xe NMR signals attainable with optical pumping techniques⁵ have been used to probe cavities in lyophilized lysozyme and lipooxygenase,⁶ detect blood oxygenation levels,⁷ and identify xenon binding sites in a lipid transfer protein⁸ by the spin-polarization induced nuclear Overhauser effect.⁹ Here we report the dependence of the $^{129}$Xe chemical shift on the potential use of $^{129}$Xe NMR for direct assessment of protein functional states and ligand binding events.

MBP is a periplasmic protein in Gram-negative bacteria that plays a role in active transport and serves as an initial receptor for chemotaxis.¹⁰ MBP (MW $\approx 47000$) binds maltose, other linear maltodextrins, and cyclodextrins with high affinity, but it binds glucose with low affinity.¹¹ The three-dimensional structures of MBP, both unligated and complexed with maltose, have been determined by X-ray crystallography to 1.8 and 2.3 Å respectively.¹² The sugar binding site is located in a cleft between the two domains of the protein; maltose binding ($K \approx 9 \times 10^3$ M$^{-1}$) induces a large structural change from an “open” unliganded conformer (Figure 1a and 1b) to a “closed” structure of the complex (Figure 1c). The conformational change of MBP upon addition of maltose has been detected in solution by a number of physical measurements including fluorescence, electron paramagnetic resonance, small-angle X-ray scattering, and $^1$H NMR.¹¹,¹³ Figure 1 shows $^{129}$Xe spectra of laser-polarized xenon in a 350 μM solution of MBP in the absence of ligand (a) and in the presence of 1 mM β-cyclodextrin (b) and 1 mM maltose (c).¹⁵ The $^{129}$Xe resonance is shifted by 0.35 ppm in the spectrum of xenon in solution with the closed MBP conformation. This change in the spectrum upon addition of maltose indicates that the $^{129}$Xe chemical shift is sensitive to the conformational state of MBP.¹⁵ To characterize further the effect of MBP conformation on the $^{129}$Xe chemical shift, shift measurements were made on a series of MBP solutions at varying protein concentration in the absence and presence of maltose; the results for the titrations are plotted in Figure 2. The single resonance observed in each spectrum indicates that xenon is in fast exchange between the buffer and protein interaction sites. Analogous behavior has been observed for a number of proteins.⁵,¹⁶ The observed shift is the average of the $^{129}$Xe chemical shift in each environment weighted by the occupancy of xenon in that environment. As described previously, these environments can all be treated as weak binding sites that correspond either to diffusion-mediated nonspecific interactions between xenon and the protein surface or to specific xenon binding sites in the protein.¹⁶ As observed in Figure 2, the overall chemical shift changes linearly with protein concentration in the limit where a small fraction of xenon is bound to any site. The concentration-normalized shift, $\alpha$ (units of ppm/M), has a characteristic value for a protein that depends on the number, strength, and effect on the $^{129}$Xe shift of all the nonspecific and

¹⁵MBP was expressed from a PET vector in E. coli BL21(DE3) cells and purified using DEAE ion-exchange and Superdex 75 size-exclusion chromatography (Pharmacia Biotech). Lyophilized protein was dissolved in a buffer containing 50 mM Tris-HCl pH 7.6, 100 mM KCl, 20% D.O, and 1 mM sugar when indicated. In a manner similar to that previously reported,¹⁶ protein samples were rapidly mixed in a 2:1 ratio with buffer containing $\approx 3$ mM laser-polarized xenon (natural abundance $^{129}$Xe, isotopic). After the mixture was allowed to equilibrate for 1 ms, it was transferred into the magnet for immediate data acquisition.¹⁵ $^{129}$Xe NMR spectra were acquired on a 300 MHz Varian Inova spectrometer using a single 90° pulse and a xenon polarization pulse and a xenon 90° pulse. Spectra were referenced to the 1H NMR resonance of DMSO-d$_6$ as an external reference. Ten to twenty scans were collected and baseline corrected. Variable temperature experiments were acquired at 200 (20°C), 250 (25°C), 300 (30°C), and 350 (35°C) K. Figure 1 shows $^{129}$Xe spectra of laser-polarized xenon in a 350 μM solution of MBP in the absence of ligand (a) and in the presence of 1 mM β-cyclodextrin (b) and 1 mM maltose (c).¹⁵ The $^{129}$Xe resonance is shifted by 0.35 ppm in the spectrum of xenon in solution with the closed MBP conformation. This change in the spectrum upon addition of maltose indicates that the $^{129}$Xe chemical shift is sensitive to the conformational state of MBP.¹⁵ To characterize further the effect of MBP conformation on the $^{129}$Xe chemical shift, shift measurements were made on a series of MBP solutions at varying protein concentration in the absence and presence of maltose; the results for the titrations are plotted in Figure 2. The single resonance observed in each spectrum indicates that xenon is in fast exchange between the buffer and protein interaction sites. Analogous behavior has been observed for a number of proteins.⁵,¹⁶ The observed shift is the average of the $^{129}$Xe chemical shift in each environment weighted by the occupancy of xenon in that environment. As described previously, these environments can all be treated as weak binding sites that correspond either to diffusion-mediated nonspecific interactions between xenon and the protein surface or to specific xenon binding sites in the protein.¹⁶ As observed in Figure 2, the overall chemical shift changes linearly with protein concentration in the limit where a small fraction of xenon is bound to any site. The concentration-normalized shift, $\alpha$ (units of ppm/M), has a characteristic value for a protein that depends on the number, strength, and effect on the $^{129}$Xe shift of all the nonspecific and
specific interactions of xenon with that protein.\textsuperscript{16ac} A linear fit of the data in Figure 2 yields an \( \alpha \) value for the unligated, open conformer of MBP (\( \alpha = 2.1 \pm 0.2 \) ppm/mM) that is measurably greater than that of the closed MBP–maltose complex (\( \alpha = 1.2 \pm 0.1 \) ppm/mM).

A likely explanation for the difference in \( \alpha \) values of the two conformers of MBP is distinct specific xenon–protein interactions.\textsuperscript{17} For example, xenon binding sites may be unique to a particular conformer, or the affinity of an identical site may significantly alter the xenon–protein interactions that give rise to the \( ^{129} \text{Xe} \) shift.

We further demonstrate that changes in the \( ^{129} \text{Xe} \) shift that mark a conformational change due to ligand binding can be detected in the presence of a large number of other species in solution; this observation is possible because all contributions to the shift (in the limit of weak xenon binding) are additive. Figure 3 shows \( ^{129} \text{Xe} \) NMR spectra of laser-polarized xenon dissolved in crude cell lysates from two samples of \( \text{E. coli} \) with (a) and without (b) the expression plasmid for MBP.\textsuperscript{19} As expected, the spectra indicate that the \( ^{129} \text{Xe} \) chemical shift is indeed sensitive to the addition of maltose (spectra with broken lines) but only in the lysate that contains overexpressed MBP.

The sensitivity of the \( ^{129} \text{Xe} \) chemical shift to the conformation of MBP suggests that xenon is useful as a probe of protein functional states and ligand–protein binding interactions in solution. \( ^{129} \text{Xe} \) NMR has many advantages for use in a biochemical assay, and the employment of laser-polarized xenon ensures rapid acquisition of data. The fact that biomolecular states are reported through an independent, chemically inert species in solution alleviates the need for labeling molecules of interest with radioisotope or fluorescent probes and allows for full recovery of biomolecular samples in their native state. Furthermore, the detection of \( ^{129} \text{Xe} \) NMR signals from xenon in solution is facilitated by a simple spectrum with no background. We anticipate that the ability to detect protein conformational changes through the \( ^{129} \text{Xe} \) chemical shift will be quite general and are currently investigating other protein systems.

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(17) The difference in \( \alpha \) for two protein conformers may result solely from a change in the chemical character or area of the protein surface accessible to xenon. However, the change in the \( \alpha \) value of MBP upon changing its conformation (\( \Delta \alpha = 0.9 \) ppm/mM) is likely too large to be accounted for by changes in nonspecific interactions alone.\textsuperscript{16ac}


(19) Lysate samples were made by suspending the washed cell paste of 1 L cultures in 5 mL of lysis buffer containing 50 mM Tris-HCl pH 7.6, 100 mM KCl, 1 mM DTT, 5 mM EDTA, and 20% D2O. Cells were lysed by sonication and spun at ~5000g for 30 min. The resulting supernatant was used to make 0.5 mL samples for data acquisition; where indicated, concentrated maltose was added to a final concentration of 1 mM. MBP concentrations in the lysates from cells containing the plasmid for overexpression were estimated to be ~200–300 \( \mu \)M by comparison with purified MBP on an SDS-polyacrylamide gel.