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Applications of laser-polarized ^{129}Xe to biomolecular assays

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Abstract

The chemical shift sensitivity and significant signal enhancement afforded by laser-polarized ^{129}Xe have motivated the application of ^{129}Xe NMR to biological imaging and spectroscopy. Recent research done by our group has used laser-polarized ^{129}Xe in biomolecular assays that detect ligand-binding events and distinguish protein conformations. The successful application of unfunctionalized and functionalized ^{129}Xe NMR to in vitro biomolecular assays suggests the potential future use of a functionalized xenon biosensor for in vivo imaging.

Keywords: Xenon binding; Ligand-protein interactions; Biosensor; Protein conformation assay

1. Introduction

Since the advent of laser-polarized xenon for use in imaging void space of human lungs [1,2], several researchers have been working to realize the potential of laser-polarized xenon in tissue imaging. Many xenon tissue images have been generated using xenon in a nonselective manner, allowing the monatomic species to freely diffuse and partition in tissue according to its inherent properties. To improve interpretation of xenon tissue images, several studies have identified characteristics of xenon-tissue and xenon-biomolecule interactions [1,3-6]. These results, as well as the current state of xenon NMR, are thoroughly described in B.M. Goodson's recent comprehensive review [7]. This article briefly summarizes recent work done in Berkeley using xenon either as an unfunctionalized or functionalized sensor for protein conformational changes and ligand-binding events. The potential applications of functionalized xenon as a molecular tag to selectively probe specific tissue types or biomolecules for in vivo imaging are also discussed here.

2. Experimental method

Xenon is an attractive biomolecular probe for several reasons. Most striking is the extraordinary sensitivity of xenon's chemical shift to its local environment, which arises from the high polarizability of xenon's electron cloud. The chemical shift range of xenon in tissue and solution is ~200 ppm [7,8]. Another advantage of xenon is the sensitivity enhancement made possible by optical pumping. Optical pumping uses circularly polarized light to induce a nonequilibrium electronic spin distribution in an alkali metal that is subsequently transferred to xenon nuclei via spin-exchange processes [9]. Optical pumping affords signal-to-noise gains of four to five orders of magnitude relative to thermally polarized xenon [7]. Accordingly, optical pumping significantly decreases both the detection limit and spectrometer time required for xenon NMR. Preparation and use of polarized xenon are relatively convenient due to its rather long

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longitudinal relaxation time, which ranges from hours in the solid phase to minutes in water to tens of seconds in tissue [3,7].

The technical aspects of xenon polarization, xenon delivery to samples, magnetic resonance detection, and sample preparation for experiments discussed here will not be addressed in this article. The reader is referred to the original reports cited below or to recent reviews for this information [3,4,7,8].

3. Detecting conformational changes with unfunctionalized xenon

Because xenon is small and hydrophobic, it can be used directly as an unfunctionalized probe for tissues and biomolecules. Xenon is known to bind to amphiphilic membranes and small hydrophobic cavities in proteins [10,11]. Protein-xenon interactions were first studied with NMR by Tilton and Kuntz, who characterized xenon-globin interactions [11]. Later work conducted by our group and others showed that xenon-protein interactions can be categorized as either specific or nonspecific interactions [12,13]. Specific interactions consist of xenon binding to particular hydrophobic cavities present in many proteins, which are observable in crystal structures [14,15]. Although specific, these interactions are weak, with xenon association constants that are typically $\sim$200 M^{-1} or lower. The size, shape, and lining residues of a cavity determine how it affects the xenon chemical shift [16]. On the other hand, nonspecific interactions primarily consist of xenon interacting with surface residues on a protein. Such interactions are even weaker than specific interactions and also occur with denatured proteins and free amino acids in solution [17]. Relative to the chemical shift of xenon in aqueous solution, nonspecific interactions typically induce downfield xenon chemical shifts, whereas specific binding interactions may induce either upfield or downfield xenon chemical shifts [12,16].

For both specific and nonspecific interactions, xenon exchange between protein and solvent environments is in the fast limit on the chemical shift time scale, resulting in the observation of only one xenon NMR peak in the spectrum. Increasing protein concentration gives rise to an increase in protein-associated xenon and a shift in the xenon solution peak. The chemical shift of the xenon peak is linearly related to protein concentration, Eq. (1), in the limit where a small fraction of xenon is bound to any site.

$$\delta_{obs} = \left(\sum_i \delta_i K_i \right) [Protein]_{total} \quad (1)$$

Here, δ_{obs} is the observed chemical shift of xenon in protein solution, δ_i is the chemical shift of the *i*th xenon binding site (specific or nonspecific), K_i is the association constant of the *i*th site, and $[Protein]_{total}$ is the total protein concentration [16]. For denatured proteins, the average, concentration-normalized chemical shift ($\Sigma \delta_i K_i$) is equal to $\sim$0.005 ppm/mM per amino acid [17].

Specific binding cavities can shift the xenon solution peak either up-field or down-field relative to the xenon solution peak; hence, their presence can either increase or decrease the concentration-normalized xenon chemical shift [12,16]. A significant difference between the concentration-normalized xenon chemical shifts of a protein in folded vs. unfolded states usually correlates with the presence of a specific xenon-binding cavity [11,16]. This trend allows one to deduce the presence of a specific binding site by comparing the xenon chemical shift for natively folded protein to that predicted or measured for denatured protein. Numerous proteins have been reported to contain a specific xenon-binding cavity [14]; those studied by xenon NMR include myoglobin, hemoglobin, maltose binding protein, and T4 lysozyme. The xenon binding cavities in these proteins are usually lined by hydrophobic residues and are larger than that of the xenon atom, $\sim$45 $Å^3$ (r = 2.2 Å), ranging in size from 47 $Å^3$ to 165 $Å^3$ [11,16].

Changes in the size, shape, or affinity of a xenon binding cavity change the dependence of xenon chemical shift on protein concentration. Accordingly, xenon-binding cavities that change with protein conformation allow xenon NMR to detect biomolecular events that involve protein conformational changes. The first conformation-sensitive xenon-binding site was reported for *E. coli* maltose binding protein (MBP) and enabled the detection of maltose binding by MBP [18]. MBP undergoes a significant conformational change upon maltose binding that changes the structure of the xenon-binding cavity. The change in cavity structure is associated with a decrease of the xenon binding constant. Both the change in affinity and cavity structure upon maltose binding are thought to contribute to the change in the xenon observed chemical shift [16].

Current work in our laboratory is showing that xenon NMR can detect more subtle protein conformational changes. The *E. coli* protein CheY, an essential protein involved in bacterial chemotaxis, undergoes a localized conformational change upon activation. The main structural change involves changes in position of threonine 87 and tyrosine 106 with minor alterations in nearby residues [19]. Although the conformational change is small, a significant change in xenon chemical shift is observed upon CheY activation as shown in Fig. 1. These preliminary results are currently under further investigation to determine how xenon senses this subtle protein conformational change and will be fully reported elsewhere.

Because xenon-protein interactions are additive in the limit of weak xenon binding, xenon NMR can detect protein conformational changes in heterogeneous media. This made it possible to use xenon NMR to detect maltose binding by MBP in *E. coli* cell lysate [18]. This observation suggests that xenon-mediated detection of biomolecular events may be possible in vivo.

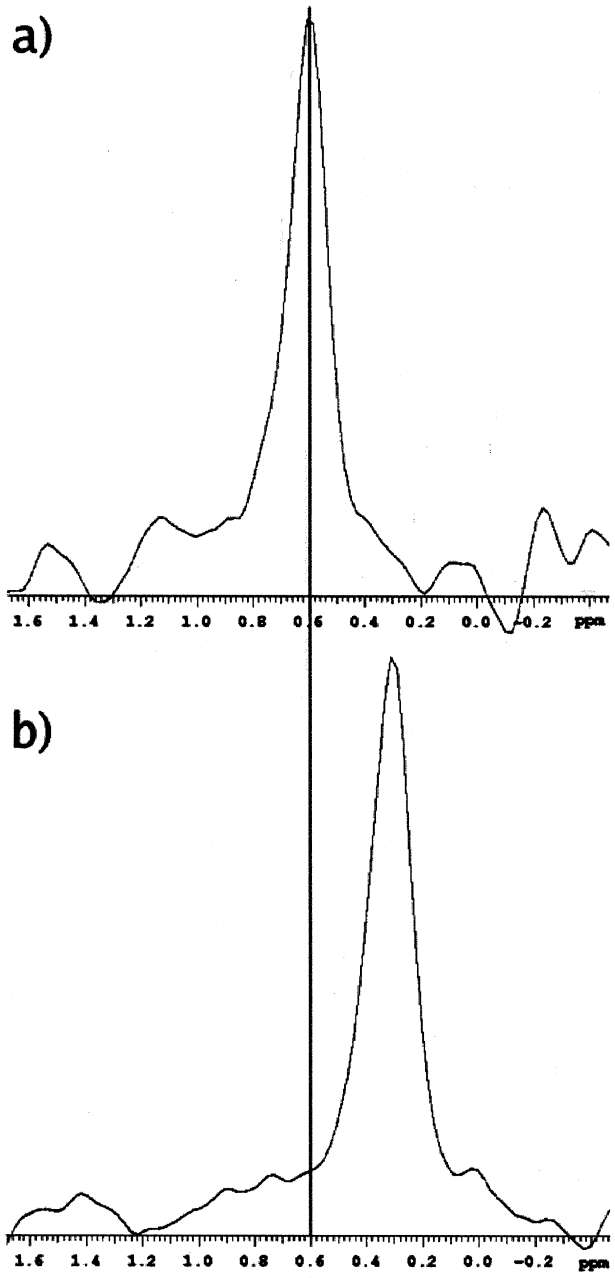

Fig. 1. ^{129}Xe spectra of laser-polarized xenon dissolved in a solution containing 360 μM inactive CheY (a), and 370 μM activated CheY (b). Inactive CheY buffer was 50 mM Tris at pH 6.7, and 20% $^{2}H_2O$. Activated CheY buffer was 50 mM Tris at pH 6.7, 20% $^{2}H_2O$, 16 mM $BeCl_2$, 100 mM NaF, and 20 mM $MgCl_2$. CheY undergoes a subtle conformational change upon activation by BeF_3^- [19], which results in the 0.31 ppm change in xenon chemical shift. Each spectrum is referenced to its corresponding ^{129}Xe chemical shift in buffer solution without protein.

Detection of protein conformational changes and ligand binding events by xenon NMR is different from approaches based on fluorescence or radiolabelling. Xenon NMR detects ligand binding via an independent species in solution, requiring no derivatization and leaving the protein unmodified and recoverable. However, when considered for multiplexed assays or tissue imaging, there are limitations for the "unfunctionalized" xenon as a sensor. First, not all proteins have a conformation-sensitive xenon-specific binding cavity, which leaves them undetectable with unfunctionalized xenon. Second, only protein-associated xenon acts as a reporter, requiring a substantial protein concentration for detection due to small induced shifts and the generally low xenon binding affinity of proteins. Third, unfunctionalized xenon detection relies on measuring chemical shift changes in the xenon solution peak, so that it responds to multiple events simultaneously but cannot discriminate their individual contributions.

4. Detecting ligand-binding events with functionalized xenon

More experimental flexibility and higher sensitivity are possible if the xenon analyte peak is separate from the xenon solution peak. A "functionalized" xenon biosensor has been developed to circumvent the limitations of unfunctionalized xenon sensing making it applicable to a wider variety of biomolecules [20].

The functionalized xenon biosensor consists of a supramolecular cage that binds xenon with relatively high affinity ($>$1000 M^{-1}), which is tethered to a ligand that targets a protein of interest (Fig. 2). The rate of xenon exchange between the cage and solute is slow on the chemical shift timescale. Functionalized xenon NMR spectra exhibit two separate xenon peaks, one for xenon in solution and one for xenon bound by the biosensor (Fig. 2). The first functionalized xenon biosensor used to detect a ligand binding event was cryptophane-A tethered to biotin, which targeted the protein avidin ($K_a = 10^{15}$ M^{-1}). Upon avidin binding, the free xenon biosensor peak decreases in amplitude and a new, broader peak appears slightly down field, as shown in the inset of Fig. 2. The new peak only appears when unliganded avidin is added, confirming the identity of this new peak to be xenon biosensor bound to avidin. The mechanism of xenon chemical shift change may involve either cage contact with the protein surface, or changes in the vibrational and rotational motions of the cage as a result of ligand binding [20].

As described in the initial biosensor report, the functionalized xenon biosensor is readily amenable to detecting multiple ligand binding events simultaneously. By tethering different cages, each giving a distinct bound xenon chemical shift, to different ligands the presence of their binding partners could be detected in parallel [20]. Such an approach is made possible by the sensitivity of xenon chemical shift to even slight modifications of cage structure [21].

5. Potential as a biomolecular affinity tag

Several properties of the functionalized xenon biosensor are promising for its development as a selective biomolec-

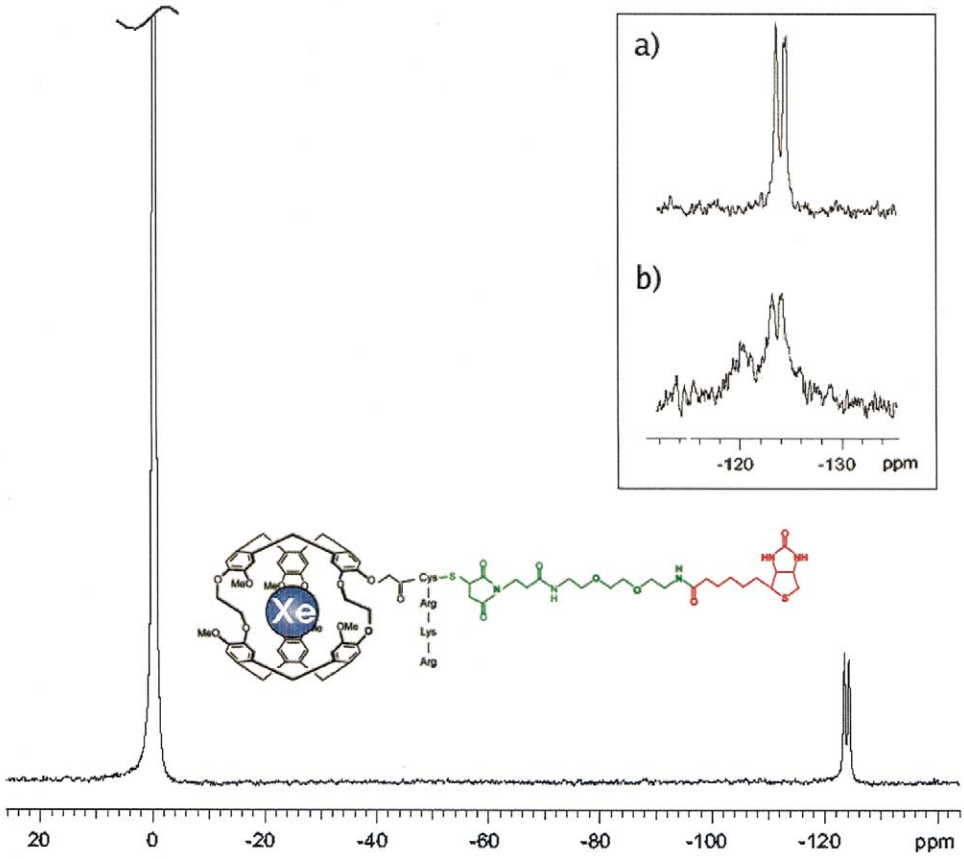

Fig. 2. ^{129}Xe spectra monitoring the binding of biotin-functionalized xenon biosensor to avidin. The main figure shows the spectrum of ~100 μM functionalized xenon biosensor in the absence of avidin. The peak at −124 ppm corresponds to biosensor-bound xenon and is referenced to the xenon in aqueous solution peak. Also shown in the main figure is a schematic of the biotin-functionalized biosensor, in which the xenon-containing cryptophane-A supramolecular cage is black, the tether green, and the biotin ligand red. The inset shows the biotin-functionalized xenon biosensor peak before the addition of avidin (a), and with one half equivalent of avidin (b). Addition of avidin decreases the intensity of the free biosensor peak and introduces a new broad peak ~1.5 ppm downfield that corresponds to biosensor bound to avidin.

ular affinity tag for cell or tissue spectroscopy and imaging. There are already many small molecule ligands and antibodies that bind selectively to specific receptors, giving specificity for specific tissues. Tethering these to the supramolecular cage that encapsulates xenon would make xenon a selective agent for those tissues. One advantage of the functionalized xenon biosensor is that the signal from the target of interest would have no background. The xenon biosensor chemical shift is separated by 124 ppm from the xenon in aqueous solution peak (Fig. 2). Similarly, the chemical shifts of xenon in blood, tissue, and protein are distinct from the chemical shift of xenon biosensor [4,5,20]. Multiple biomolecules or tissue types could be selectively targeted simultaneously using different cages, each with no background.

To actually exploit these advantages, there remain several issues of compatibility and delivery that must still be resolved. From an NMR perspective, the one in need of most improvement is sensitivity. As is apparent in Fig. 2b, the detection limit of target-bound functionalized xenon biosensor is currently on the order of 100 μM (5 mm NMR tube). Typical effective concentrations that would be encountered in cell or tissue imaging are much lower and the detection threshold must be reduced to match these [22].

Several methods of signal enhancement are currently being explored. One aspect under investigation entails optimizing the supramolecular cage. Alternative supramolecular cages that bind multiple xenon atoms may offer higher sensitivity and narrower lines. Another development being pursued is selective signal averaging. Conventional signal averaging cannot be applied to optically polarized xenon because the optical polarization is lost after a single 90° pulse. However, selective pulse sequences can circumvent this problem by exciting bound xenon for detection, but leaving the reservoir of polarized xenon free in solution unaffected to replenish the bound signal by chemical exchange. These methods of sensitivity enhancement will be fully discussed elsewhere.

6. Conclusions

Xenon NMR has proved to be a novel and selective sensor to probe protein conformational changes and ligand

binding events in homogeneous and heterogeneous in vitro solutions. Further improvement in sensitivity may allow for the application of the functionalized xenon biosensor for in vivo spectroscopy and imaging.

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References

[1] Mugler JP, Dreihuys B, Brookeman JR, et al. MR imaging and spectroscopy using hyperpolarized 129 Xe gas: preliminary human results. Magn Reson Med 1997;37:809–15.

[2] Albert MS, Cates GD, Driehuys B, Happer W, Saam B, Springer CS, Wishnia A. Biological magnetic resonance imaging using laser-polarized 129 Xe. Nature 1994;370:199–201.

[3] Goodson BM, Song YQ, Taylor RE, Schepkin VD, Brennan KM, Chingas GC, Budinger TF, Navon G, Pines A. In Vivo NMR and MRI using injection delivery of laser-polarized xenon. Proc Natl Acad Sci USA 1997;94:14725–9.

[4] Bifone A, Song YQ, Seydoux R, Taylor RE, Goodson BM, Pietrass T, Budinger TF, Navon G, Pines A. NMR of laser-polarized xenon in human blood. Proc Natl Acad Sci USA 1996;93:12932–6.

[5] Wolber J, McIntyre DJO, Rodrigues LM, Carnochan P, Griffiths JR, Leach MO, Bifone A. In vivo hyperpolarized 129 Xe NMR spectroscopy in tumors. Magn Reson Med 2001;46:586–91.

[6] Swanson SD, Rosen MS, Agranoff BW, Coulter KP, Welsh RC, Chupp TE. Brain MRI with laser-polarized 129 Xe. Magn Reson Med 1997;38:685–8.

[7] Goodson BM. Nuclear magnetic resonance of laser polarized noble gases in molecules, materials, and organisms. J Magn Reson 2001; 155:157–216.

[8] Ratcliffe CI. Xenon NMR. Annu Rep NMR Spectrosc 1998;36:124–208.

[9] Walker TG, Happer W. Spin-Exchange optical pumping of noble gas nuclei. Rev Mod Phys 1997;69:629–42.

[10] Xu Y, Tang P. Amphiphilic sites for general anesthetic action? Evidence from 129 Xe-{1H} intermolecular nuclear Overhauser effects. Biochim Biophys Acta 1997;1323:154–62.

[11] Tilton RF, Kuntz ID. Nuclear magnetic resonance studies of xenon-129 with myoglobin and hemoglobin. Biochemistry 1982;21:6850–7.

[12] Rubin SM, Spence MM, Goodson BM, Wemmer DE, Pines A. Evidence of nonspecific surface interactions between laser-polarized xenon and myoglobin in solution. Proc Natl Acad Sci USA 2000;97: 9472–5.

[13] Locci E, Dehouck Y, Casu M, Saba G, Lai A, Luhmer M, Reisse J, Bertik K. Probing proteins in solution by (129)Xe NMR spectroscopy. J Magn Reson 2001;150:167–4.

[14] Prange T, Schiltz M, Pernot L, Colloc'h N, Longhi S, Bourget W, Fourme R. Exploring hydrophobic sites in proteins with xenon and krypton. Struct Funct Genet 1998;30:61–73.

[15] Soltis SM, Stowell MHB, Wiener MC, Phillips GN, Rees DC. Successful flash-cooling of xenon-derivatized myoglobin crystals. J Appl Crystallog 1997;30:190–4.

[16] Rubin SM, Lee SY, Ruiz EJ, Pines A, Wemmer DE. Detection and characterization of xenon-binding sites in proteins by 129 Xe NMR spectroscopy. J Mol Biol 2002;322:425–40.

[17] Rubin SM, Spence MM, Pines A, Wemmer DE. Characterization of the effects of nonspecific xenon-protein interactions on 129 Xe chemical shifts in aqueous solution: further development of xenon as a biomolecular probe. J Magn Reson 2001;152:79–86.

[18] Rubin SM, Spence MM, Dimitrov IE, Ruiz EJ, Pines A, Wemmer DE. Detection of a conformational change in maltose binding protein by ^{129}Xe NMR spectroscopy. J Am Chem Soc 2000;123:8616–7.

[19] Cho HS, Lee SY, Yan D, Pan X, Parkinson JS, Kustu S, Wemmer DE, Pelton JG. NMR Structure of activated CheY. J Mol Biol 2000; 297:543–51.

[20] Spence MM, Rubin SM, Dimitrov IE, Ruiz EJ, Wemmer DE, Pines A, Yao SQ, Tian F, Schultz PG. Functionalized xenon as a biosensor. Proc Natl Acad Sci USA 2001;98:10654–7.

[21] Brotin T, Lesage A, Emsley L, Collet A. 129-Xe NMR spectroscopy of deuterium-labeled cryptophane-A xenon complexes: investigations of host-guest complexation dynamics. J Am Chem Soc 2000;122: 1171–4.

[22] Lehinger AL, Nelson DL, Cox MM. Lehninger principles of biochemistry, 3rd ed. New York: Worth Publishing, 2000.