Xenon NMR: Chemical shifts of a general anesthetic in common solvents, proteins, and membranes

(129Xe/131Xe/myoglobin/lipid bilayers/biomembranes)

KEITH W. MILLER*, NICHOLAS V. REO†, ANTONIUS J. M. SCHOOT UITERKAMP‡, DIANE P. STENGLE§, THOMAS R. STENGLE‡, AND KENNETH L. WILLIAMSON§

*Departments of Pharmacology and Anesthesia, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts 02114; †Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003; ‡Interdisciplinary Programs in Health, Harvard School of Public Health, Boston, Massachusetts 02115; and §Department of Chemistry, Mount Holyoke College, South Hadley, Massachusetts 01075

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ABSTRACT The rare gas xenon contains two NMR-sensitive isotopes in high natural abundance. The nuclide 129Xe has a spin of 1/2; 131Xe is quadrupolar with a spin of 3/2. The complementary NMR characteristics of these nuclei provide a unique opportunity for probing their environment. The method is widely applicable because xenon interacts with a useful range of condensed phases including pure liquids, protein solutions, and suspensions of lipid and biological membranes. Although xenon is chemically inert, it does interact with living systems; it is an effective general anesthetic. We have found that the range of chemical shifts of 129Xe dissolved in common solvents is ca. 200 ppm, which is 30 times larger than that found for 13C in methane dissolved in various solvents. Resonances were also observed for 131Xe in some systems; they were broader and exhibited much greater relaxation rates than did 129Xe. The use of 129Xe NMR as a probe of biological systems was investigated. Spectra were obtained from solutions of myoglobin, from suspensions of various lipid bilayers, and from suspensions of the membranes of erythrocytes and of the acetylcholine-receptor-rich membranes of Torpedo californica. These systems exhibited a smaller range of chemical shifts. In most cases there was evidence of a fast exchange of xenon between the aqueous and organic environments, but the exchange was slow in suspensions of dimyristoyl lecithin vesicles.

Xenon NMR spectroscopy is a potential probe of the structural and dynamic aspects of the molecular environment of the xenon atom in physical and biological media. Natural xenon contains 126% 129Xe which has spin I = 1/2 and 21% 131Xe which is a quadrupolar nucleus and has spin I = 3/2. The NMR sensitivity of 129Xe is relatively large and the solubility of xenon in most liquids is high for an apolar gas—e.g., from 4.3 mM in water to 166 mM in isooctane at 1 atm and 273 K—so the NMR spectra of both isotopes can be observed with commercial multinuclear spectrometers. The chemical shift of the xenon atom is especially reflective of its environment due to its large, polarizable, electron cloud. In xenon compounds, shifts up to 4000 ppm have been observed (1). In the free xenon atom, the effects of the medium can produce sizable shifts. Such shifts have been observed in pure liquid and gaseous xenon as well as in gas mixtures of xenon with a second component (1). However this effect had not been studied in condensed phases. We have observed solvent-dependent shifts over a range of ca. 200 ppm, a range that is much larger than the solvent shifts of 13C and 19F (2, 3).

Xenon interacts with many biological systems including myoglobin (4) and hemoglobin (5). It is also soluble in lipid bilayers: membrane/gas partition coefficients vary from 0.4 (at 20°C) in erythrocytes (6) to 1.3 (at 25°C) in egg lecithin (unpublished data). Its most striking pharmacological property is its ability to induce general anesthesia; its efficacy is comparable to that of nitrous oxide (7). The physicochemical mechanism of anesthetic action is controversial, but it is likely that the locus of action is either in the lipid or the protein region of excitable membranes (8). If the NMR characteristics of xenon in these environments differ widely, then this would have obvious implications for distinguishing among theories of anesthetic action.

MATERIALS AND METHODS

Xenon gas (Linde, 99.995%) was dissolved in the various liquid solvents at 1 atm (101 kilopascal) by shaking the gas with the liquid in a syringe. Solutions containing xenon at higher pressures were prepared on a vacuum line. Known amounts of xenon were distilled into heavy-walled NMR tubes containing the solvents and sealed off under vacuum. Reagent-grade solvents were used without further purification. Samples of biological materials that could be denatured by freezing were prepared by condensing the xenon at the top of the tube. Most aqueous samples were run at elevated pressures (5–15 atm). Most xenon spectra were obtained at ambient probe temperature (23.5°C) on a JEOL FX-90Q FT spectrometer at a centerband frequency of 24,789,225.0 Hz for 129Xe and 7,349,500.0 Hz for 131Xe. Usually a 30° pulse and a 1- to 2-sec repetition time were used. With a 2000-Hz sweep width, 4000 data points were collected, giving a resolution of ±0.5 Hz. A concentric capillary filled with 3H2O was used as a spectrometer lock. The observed resonance frequencies were compared to a sample of xenon gas at 10 atm, and the shift with respect to the gas at zero pressure (24,784,771.0 Hz) was calculated from the known pressure dependence of the 129Xe shift in the pure gas (9). The shifts quoted here are given in ppm downfield from the frequency of the pure gas at zero pressure.

Phospholipid vesicles were prepared in aqueous solution as described (10). Whale myoglobin (Sigma) was purified by chromatography and converted to the ferricyanide form. Acetylcholine receptor-rich membranes were prepared from fresh Torpedo californica electroplaques by adaptation of the method of Cohen et al. (11).

RESULTS

129Xe gave resonances with a linewidth in the range 1–2 Hz whereas the quadrupolar nucleus 131Xe gave much broader resonances in the range 20–30 Hz. Spectra of xenon solutions in benzene and water, taken with a Bruker WM-250 at 69.17 MHz (129Xe) and 20.51 MHz (131Xe), showed single resonances with linewidths similar to those found with the JEOL FX90Q. These solvent shifts cover a range (ca. 200 ppm) which is about 30 times

Abbreviations: (Myr)2Lec, dimyristoyl lecithin.
larger than the range found for the $^{13}$C resonance in solutions of methane (2) or for $^{19}$F in solutions of the anesthetic halothane ($\text{CF}_3\text{CHClBr}$) (3). The concentration dependence of the shifts is small, so that these values are essentially at infinite dilution. However the shifts are temperature sensitive: e.g., the $^{129}$Xe shift shows a linear temperature dependence of $-0.4 \text{ ppm/°C}$ in CC1$_4$ between 30°C and 37°C and of $-0.2 \text{ ppm/°C}$ in olive oil between 13°C and 50°C. Relaxation studies of xenon in benzene show relaxation times ($T_1$) at 20°C of approximately 5 msec for $^{129}$Xe at 7.35 MHz and of approximately 175 sec for $^{129}$Xe at 24.79 MHz and 69.17 MHz.

In aqueous solutions of myoglobin, only a single $^{129}$Xe signal is observed; this indicates that xenon exchange between protein and solvent is fast. This conclusion is supported by the effect of myoglobin concentration on the Xe shift. The shift ranges from 197 ppm at infinite dilution to 202 ppm at high myoglobin concentration (750 mg/ml).

The spectrum of $^{129}$Xe in a 10% suspension of dimyristoyl lecithin [(Myr)$_2$Lec] vesicles at 35°C showed two peaks, 24 Hz apart, with shifts of 197.2 ppm (narrow peak) and 196.2 ppm (broad peak) (Fig. 1). At 50°C, the two peaks coalesce into one with a shift of 193.7 ppm. The spectrum at 41°C showed an intermediate situation in which the two peaks are only partially resolved. Spectra of $^{129}$Xe have been observed in several other systems of biological interest. Addition of cholesterol to a lecithin/cholesterol ratio of 2:1 gave a line with width at half-height of 3.7 Hz at 198 ppm. A suspension of egg lecithin run at low resolution (1 atm partial pressure) showed a single line of 199 ppm. A suspension of T. californica membranes in 1.2 M sucrose exhibited a single peak at 209 ppm compared with a shift of 211 ppm for the 1.2 M sucrose solution alone.

**DISCUSSION**

Our work clearly demonstrated that the chemical shift of xenon is exquisitely sensitive to its environment in simple solvents. Moreover, the presence of the two isotopes in high natural abundance provides a powerful approach to dynamic properties because the two isotopes have different spins and their relaxation rates will be sensitive to different characteristics of the environment. Preliminary studies indicate that $^{129}$Xe has a relaxation rate 4 orders of magnitude higher than that of $^{129}$Xe in solution. The possibility of exploiting these advantages to explore the nature of the solvent shell in complex solvents such as water is particularly intriguing.

**Pure Solvents.** The NMR chemical shifts of solutes produced by pure liquid solvents have been treated theoretically with some success. The difference in shielding between an isolated molecule and the same substance immersed in a solvent is taken to be the sum of several terms (2). These include a contribution from the bulk susceptibility of the sample, the effects of electric fields generated by any permanent dipole moments of the solute or solvent, the effect of any magnetic anisotropy of the solvent, and the "van der Waals" term which arises in the dispersive and repulsive solute–solvent interactions. In xenon spectra, in
which the shifts are large, the van der Waals term dominates and
the other terms can be ignored to a first approximation.
Linder (12) assumed that the dispersive part of the van der
Waals shift arises in the reaction field that is produced in the
solvent by spontaneous electronic fluctuations of the solute.
This model predicts that there is a correlation of the shift with
a simple function of the refractive index of the solvent (13). Fig.
2 shows the data of Table 1 plotted against a refractive index
function proposed by Rummens (14). Although there is a fair
correlation between the two quantities, there are some notable
exceptions. Aqueous media, in which the deshielding is ca. 45
ppm greater than expected, are interesting, especially in view
of the unique nature of aqueous solvation of apolar solutes.
An important deviation from the reaction field model is seen in Fig.
2: the best straight line does not pass through the origin as pre-
dicted. These deviations probably contain information about
specific solute–solute interactions. It is also likely that repul-
sive interactions play a role in some cases. We find that these
deviations can be minimized if the correlations are limited to
groups of related solvents. This has important implications
for the reaction field model, and it will be treated more fully
elsewhere.

An alternative approach is the attempt to correlate solvent
shifts with the solubility parameter (3). This quantity, \( \delta \), is re-
lated to the cohesive energy of the solvent according to \( \delta^2 =
\Delta E_c/V_m \) in which \( \Delta E_c \) is the heat of vaporization at constant
volume and \( V_m \) is the molar volume of the solvent (15). This
approach might be expected to work best with nonpolar sol-
vents, and the \(^{199}F\) halothane shifts do produce a fair correlation
in such circumstances (2). We also observe a fair correlation (\( r = 0.71 \)), passing close to the origin, when xenon shifts are com-
pared with solubility parameters for a wide range of solvents if
hydroxyl solvents are excluded. The hydroxyl solvents can be
brought more closely into line with other solvents if a semiempiri-
cal method is used to subtract the hydrogen bonding con-
tribution to the solubility parameter (16).

**Biological Systems.** Xenon interacting with biological sys-
tems exhibits spectra that are more difficult to interpret because
of the heterogeneous nature of the medium. Often there are
distinct environments that can contain xenon, and the spectrum
will be affected by the chemical exchange of xenon between
these environments. If the exchange is slow, separate signals
from the xenon in the two environments will be observed. If
the exchange is fast, only a single signal will appear. Its chemical
shift will be the weighted average of the shift in the different
environments.

Crystalline myoglobin is known to contain one site (or two
sites at high pH) that can add xenon atoms under a partial pres-
sure of 2 atm (4). Only a single xenon line broadened at high
myoglobin concentrations is observed in solutions under high
oxenon pressure (5–12 atm). However there is a variation of the
shift with protein concentration which indicates that xenon is
in fast exchange between the bulk aqueous environment and a
site on the protein. If the concentration of xenon in the aqueous
phase is known from its solubility, and the chemical shift in the
protein site can be obtained by extrapolation to high
protein concentration, the usual fast exchange expression can
then be used to determine the remaining quantity—i.e., the number
of xenon atoms associated with the protein. Our data suggest
that ca. 10 xenon atoms interact with each myoglobin molecule.
This implies considerable nonspecific binding in addition to the
site observed by X-ray analysis and agrees with earlier obser-
vations (17). This interpretation of the myoglobin–xenon inter-
action is corroborated by the fact that the resonance of \(^{131}Xe\)
is considerably broadened by the presence of small amounts of
myoglobin (1mg/ml).

The effect of exchange on the spectrum of xenon in a heter-ogenous medium is illustrated in Fig. 1 which shows the
spectrum of xenon at 10 atm in an aqueous suspension of
(Myr)\(_2\)Lec vesicles above their native gel–liquid crystalline
phase transition (24°C). At 35°C the xenon is in slow exchange,
as indicated by the two peaks. The sharp peak at 197.3 ppm
can be assigned to xenon dissolved in water; its chemical shift is very
close to that observed in pure water at the same temperature and
pressure (197.4 ppm). The broad peak at 196.3 ppm must be
due to xenon associated with the (Myn)\(_2\)Lec. Presumably, the
high local viscosity causes an enhanced relaxation rate and con-
comitant line broadening. This assumption is consistent with
the finding that the ratio of peak areas in Fig. 1 (1:11) is not propor-
tional to the ratio of moles of xenon in each phase (1:1).
We did not attempt to collect fully relaxed spectra. The spec-
trum at 35°C indicates that the rate of exchange of xenon be-
tween the two phases is smaller than 140 sec\(^{-1}\). Only a single
broad peak at 193.7 ppm is observed at 50°C. This cannot be
caused by an overlap of the two individual signals because the
xenon shift in pure water is 197.5 ppm at 50°C. Thus, xenon is
in fast exchange and the two peaks have merged into one.

Similarly, fast exchange was also observed in the natural lipid
bilayers and in the biomembrane. A similar pattern in which
only fully saturated lipid bilayers show slow exchange has been

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**Table 1. Chemical shift of \(^{13}Xe\) in pure solvents**

<table>
<thead>
<tr>
<th>Key</th>
<th>Solvent</th>
<th>Chemical shift, ppm*</th>
<th>Refractive index, (n^\prime)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol</td>
<td>148</td>
<td>1.3286</td>
</tr>
<tr>
<td>2</td>
<td>Water</td>
<td>196</td>
<td>1.3330</td>
</tr>
<tr>
<td>3</td>
<td>Methyl chloride</td>
<td>153</td>
<td>1.3389</td>
</tr>
<tr>
<td>4</td>
<td>Diethyl ether</td>
<td>160</td>
<td>1.3526</td>
</tr>
<tr>
<td>5</td>
<td>n-Pentane</td>
<td>156</td>
<td>1.3577</td>
</tr>
<tr>
<td>6</td>
<td>Tetramethylsilane</td>
<td>158</td>
<td>1.3586</td>
</tr>
<tr>
<td>7</td>
<td>Acetone</td>
<td>175</td>
<td>1.3590</td>
</tr>
<tr>
<td>8</td>
<td>Ethanol</td>
<td>165</td>
<td>1.3614</td>
</tr>
<tr>
<td>9</td>
<td>Ethyl acetate</td>
<td>168</td>
<td>1.3724</td>
</tr>
<tr>
<td>10</td>
<td>n-Heptane</td>
<td>168</td>
<td>1.3878</td>
</tr>
<tr>
<td>11</td>
<td>n-Octane</td>
<td>171</td>
<td>1.3976</td>
</tr>
<tr>
<td>12</td>
<td>1-Butanol</td>
<td>176</td>
<td>1.3990</td>
</tr>
<tr>
<td>13</td>
<td>2,2,4-Trimethylpentane</td>
<td>192</td>
<td>1.3916</td>
</tr>
<tr>
<td>14</td>
<td>Cyclopentane</td>
<td>158</td>
<td>1.4063</td>
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<tr>
<td>15</td>
<td>n-Decane</td>
<td>177</td>
<td>1.4121</td>
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<tr>
<td>16</td>
<td>Methylene chloride</td>
<td>192</td>
<td>1.4243</td>
</tr>
<tr>
<td>17</td>
<td>Cyclohexane</td>
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<td>1.4263</td>
</tr>
<tr>
<td>18</td>
<td>Ethylene glycol</td>
<td>199</td>
<td>1.4319</td>
</tr>
<tr>
<td>19</td>
<td>1-Octanol</td>
<td>187</td>
<td>1.4295</td>
</tr>
<tr>
<td>20</td>
<td>n-Hexadecane</td>
<td>186</td>
<td>1.4344</td>
</tr>
<tr>
<td>21</td>
<td>Chloroform</td>
<td>217</td>
<td>1.4457</td>
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<tr>
<td>22</td>
<td>Oleic acid</td>
<td>193</td>
<td>1.4582</td>
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<tr>
<td>23</td>
<td>Carbon tetrachloride</td>
<td>222</td>
<td>1.4603</td>
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<tr>
<td>24</td>
<td>Olive oil</td>
<td>198</td>
<td>1.4663</td>
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<tr>
<td>25</td>
<td>Fluorobenzene</td>
<td>176</td>
<td>1.4659</td>
</tr>
<tr>
<td>26</td>
<td>Toluenes</td>
<td>190</td>
<td>1.4969</td>
</tr>
<tr>
<td>27</td>
<td>Benzenes</td>
<td>195</td>
<td>1.5011</td>
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<tr>
<td>28</td>
<td>Pyridines</td>
<td>197</td>
<td>1.5102</td>
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<tr>
<td>29</td>
<td>Chlorobenzene</td>
<td>202</td>
<td>1.5346</td>
</tr>
<tr>
<td>30</td>
<td>Methyl iodide</td>
<td>209</td>
<td>1.5314</td>
</tr>
<tr>
<td>31</td>
<td>m-Dichlorobenzene</td>
<td>207</td>
<td>1.5459</td>
</tr>
<tr>
<td>32</td>
<td>Nitrobenzene</td>
<td>189</td>
<td>1.5524</td>
</tr>
<tr>
<td>33</td>
<td>Bromobenzene</td>
<td>219</td>
<td>1.5602</td>
</tr>
<tr>
<td>34</td>
<td>Bromoform</td>
<td>285</td>
<td>1.5977</td>
</tr>
<tr>
<td>35</td>
<td>Iodobenzene</td>
<td>248</td>
<td>1.6195</td>
</tr>
<tr>
<td>36</td>
<td>Carbon disulfide</td>
<td>225</td>
<td>1.6279</td>
</tr>
<tr>
<td>37</td>
<td>Methylene iodide</td>
<td>335</td>
<td>1.7464</td>
</tr>
</tbody>
</table>

*Chemical shifts are reported as parts per million downfield from xenon gas at zero pressure.*
observed with $^{19}$F NMR of halothane (18, 19).

For those systems in fast exchange, the intrinsic shift in the lipid phase can be calculated if the lipid bilayer/water partition coefficient is known. This is so in the case of egg lecithin and egg lecithin/cholesterol (unpublished data), and the value in (Myr)$_2$Lec can be assumed to be equal to that in egg lecithin (20). We calculate that in our suspensions the proportion of dissolved xenon in the lipid phase is close to 50%. The shifts in the lipid phase so calculated are shown in Table 2 compared to olive oil. Quite remarkably the shifts in the oil parallel those in the bilayers over the whole temperature range.

With biomembranes one cannot achieve such high concentrations of material as with lipid bilayers and furthermore the partition coefficients are smaller. In a suspension of T. californica membranes (membrane concentration, 2%) in sucrose, we observed a chemical shift of 209 ppm which is 2 ppm lower than that of the sucrose solution used as the suspending medium. We estimate that, at most, 10% of the xenon was in the membrane fraction. More detailed studies will be necessary to establish the intrinsic shifts under fast exchange conditions in biomembranes. In particular, varying the solvent shift by changing solvent composition offers a promising approach. Use of enriched $^{129}$Xe would enhance sensitivity and would also allow more physiological concentrations of xenon to be used.

The potencies of general anesthetics such as xenon are known to correlate with their solubilities in olive oil, but whether this correlation implies an interaction with a lipid bilayer region of a neuronal membrane or a hydrophobic region in a protein contained in the membrane has remained controversial. Table 2 shows unequivocally that olive oil is a good model of lipid bilayers over a wide temperature range in so far as the chemical shift is concerned. The range of chemical shifts we have observed in bilayers, biomembranes, and myoglobin, although large compared to previous probes (3, 19), is small relative to the range in pure solvents. Thus, even though the chemical shift for myoglobin is 3.5 ppm higher than in olive oil at the same temperature, we cannot rule out the possibility that some hydrophobic regions of proteins will also resemble olive oil. Systematic studies of a wider range of bilayers, biomembranes, and proteins with greater attention to the determination of intrinsic shifts and relaxation times therefore will be required to ascertain if these three environments can be differentiated on the basis of their $^{129}$Xe and $^{131}$Xe NMR characteristics. If this can be accomplished, further progress may be made with the problem of characterizing the site of anesthetic action, but our results at the present time certainly do not support recent attempts, based on correlations between potency and bulk solvent solubility, to rule out lipid as the site of anesthetic action (21). Given xenon’s unusually high sensitivity to its environment, it is unlikely that NMR studies of any other anesthetic would be capable of resolving this problem.

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Table 2. $^{129}$Xe shifts in bilayer systems and olive oil

<table>
<thead>
<tr>
<th>Bilayer</th>
<th>Temp., °C</th>
<th>$^{129}$Xe shift in bilayer, ppm</th>
<th>Equivalent $^{129}$Xe shift in olive oil, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Myr)$_2$Lec</td>
<td>50</td>
<td>193.7</td>
<td>193</td>
</tr>
<tr>
<td>(Myr)$_2$Lec</td>
<td>35</td>
<td>196.2</td>
<td>196</td>
</tr>
<tr>
<td>Egg lecithin</td>
<td>25</td>
<td>200.6</td>
<td>198</td>
</tr>
<tr>
<td>Egg lecithin/cholesterol</td>
<td>25</td>
<td>198.9</td>
<td>198</td>
</tr>
</tbody>
</table>