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

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

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ABSTRACT **The rare gas xenon contains two NMR-sensitive isotopes in high natural abundance. The nuclide ^{129}Xe has a spin of ½; ^{131}Xe 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 ^{129}Xe dissolved in common solvents is *ca.* 200 ppm, which is 30 times larger than that found for ^{13}C in methane dissolved in various solvents. Resonances were also observed for ^{131}Xe in some systems; they were broader and exhibited much greater relaxation rates than did ^{129}Xe. The use of ^{129}Xe 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 26% ^{129}Xe which has spin I = ½ and 21% ^{131}Xe which is a quadrupolar nucleus and has spin I = 3/2. The NMR sensitivity of ^{129}Xe 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 ^{13}C and ^{19}F (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 ^{129}Xe and 7,348,500.0 Hz for ^{131}Xe. Usually a 30° pulse and a 1- to 2-sec pulse 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 ^2H_2O 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 ^{129}Xe 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

^{129}Xe gave resonances with a linewidth in the range 1–2 Hz whereas the quadrupolar nucleus ^{131}Xe 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 (^{129}Xe) and 20.51 MHz (^{131}Xe), 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

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Abbreviations: $(Myr)_2Lec$, dimyristoyl lecithin.

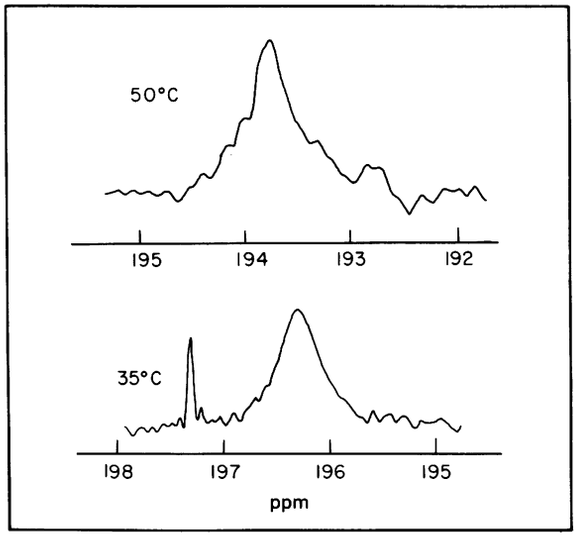

FIG. 1. Temperature dependence of ^{129}Xe spectrum in a 10% suspension of $(Myr)_2Lec$ vesicles. Amplitude settings for the two spectra are slightly different. At 35°C the areas of the two peaks have a ratio of 1:11. The xenon pressure is 10 atm.

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 ($CF_3CHClBr$) (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 ppm/°C in CCl_4 between 30°C and 37°C and of −0.2 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 ^{131}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 [$(Myr)_2Lec$] 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 ^{131}Xe has a relaxation rate 4 orders of magnitude higher than than 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

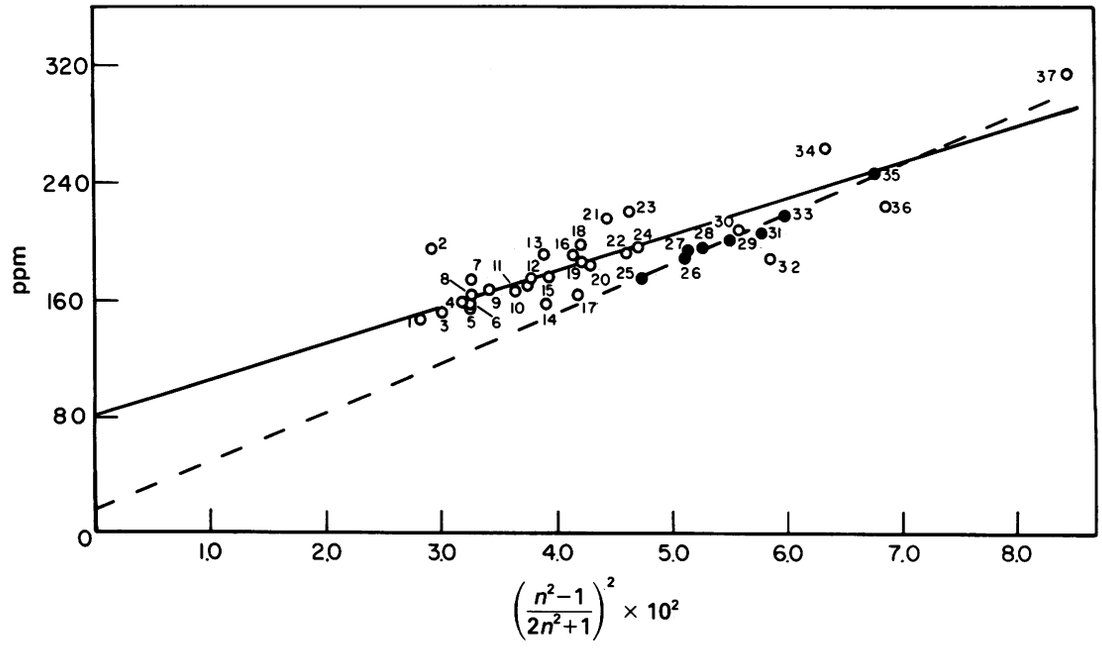

FIG. 2. Gas-to-solution shift of ^{129}Xe in common solvents. Solid line, best fit to all the points; dashed line, best fit to the aromatic solvents (indicated by filled-in circles) with nitrobenzene excluded. Numerals refer to items in Table 1.

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, especiallly 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 predicted. These deviations probably contain information about specific solute–solvent interactions. It is also likely that repulsive 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, δ, is related to the cohesive energy of the solvent according to $\delta^2 = \Delta E_v/V_m$ in which ΔE_v 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 solvents, and the [^{19}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 compared 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 semiempirical method is used to subtract the hydrogen bonding contribution to the solubility parameter (16).

Table 1. Chemical shift of ^{129}Xe in pure solvents

Key	Solvent	Chemical shift, ppm*	Refractive index, n_D^{20}
1	Methanol	148	1.3286
2	Water	196	1.3330
3	Methyl chloride	153	1.3389
4	Diethyl ether	160	1.3526
5	*n*-Pentane	156	1.3577
6	Tetramethylsilane	158	1.3586
7	Acetone	175	1.3590
8	Ethanol	165	1.3614
9	Ethyl acetate	168	1.3724
10	*n*-Heptane	168	1.3878
11	*n*-Octane	171	1.3976
12	1-Butanol	176	1.3990
13	2,2,4-Trimethylpentane	192	1.3916
14	Cyclopentane	158	1.4063
15	*n*-Decane	177	1.4121
16	Methylene chloride	192	1.4243
17	Cyclohexane	165	1.4263
18	Ethylene glycol	199	1.4319
19	1-Octanol	187	1.4295
20	*n*-Hexadecane	186	1.4344
21	Chloroform	217	1.4457
22	Oleic acid	193	1.4582
23	Carbon tetrachloride	222	1.4603
24	Olive oil	198	1.4663
25	Fluorobenzene	176	1.4659
26	Toluene	190	1.4969
27	Benzene	195	1.5011
28	Pyridine	197	1.5102
29	Chlorobenzene	202	1.5246
30	Methyl iodide	209	1.5314
31	*m*-Dichlorobenzene	207	1.5459
32	Nitrobenzene	189	1.5524
33	Bromobenzene	219	1.5602
34	Bromoform	285	1.5977
35	Iodobenzene	248	1.6195
36	Carbon disulfide	225	1.6279
37	Methylene iodide	335	1.7464

*Chemical shifts are reported as parts per million downfield from xenon gas at zero pressure.

Biological Systems. Xenon interacting with biological systems 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 pressure of 2 atm (4). Only a single xenon line broadened at high myoglobin concentrations is observed in solutions under high xenon pressure (8–12 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 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 observations (17). This interpretation of the myoglobin–xenon interaction 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 heterogeneous medium is illustrated in Fig. 1 which shows the spectrum of xenon at 10 atm in an aqueous suspension of $(Myr)_2Lec$ 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 $(Myr)_2Lec$. Presumably, the high local viscosity causes an enhanced relaxation rate and concomitant line broadening. This assumption is consistent with the finding that the ratio of peak areas in Fig. 1 (1:11) is not proportional to the ratio of moles of xenon in each phase (1:1). We did not attempt to collect fully relaxed spectra. The spectrum at 35°C indicates that the rate of exchange of xenon between 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

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

Bilayer	Temp., °C	^{129}Xe shift in bilayer, ppm	Equivalent ^{129}Xe shift in olive oil, ppm
$(Myr)_2Lec$	50	193.7	193
$(Myr)_2Lec$	35	196.2	196
Egg lecithin	25	200.6	198
Egg lecithin/ cholesterol	25	198.9	198

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 $(Myr)_2Lec$ 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 region 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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