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Evidence of xenon transport through the gramicidin channel: a ^{129}Xe-NMR study

Steve McKim, James F. Hinton *

Department of Chemistry / Biochemistry, University of Arkansas, Fayetteville, AK 72701, USA

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Abstract

Evidence is presented for Xe transport through the gramicidin A channel. This evidence for Xe transport through gramicidin A channels has been obtained using ^{129}Xe-NMR spectroscopy. Three experiments were utilized. The first experiment involved monitoring the change in the chemical shift of ^{129}Xe in the presence of increasing gramicidin A concentration, the second observed the effect on the ^{129}Xe chemical shift with gramicidin A channels photochemically altered by UV light and the third determined the effect of gramicidin A channels blocked by Ba^{2+} on the ^{129}Xe chemical shift. The results of these three experiments indicate that Xe transports through the gramicidin A channel.

Key words: Gramicidin; NMR, ^{129}Xe-; Transport; Ultraviolet photolysis; Blocking

1. Introduction

Gramicidin, a polypeptide of 15 alternating D-L amino acids, is known to form monovalent cation conducting channels in bilayers or membranes [1–4]. The form of the channel which spans the membrane consists of two gramicidin monomers linked by hydrogen bonds at their NH_2 terminal ends [5–9]. The length of the gramicidin dimer spanning the membrane is 26 Å and its diameter is 4 Å [10,11]. The ability of gramicidin to function as a channel for the transport of monovalent cations across a lipid bilayer or membrane has been studied extensively. Although transport of a neutral species through the gramicidin channel, such as water, has been shown to occur [12–15], it was of interest to determine if another neutral species, Xe, transports through the gramicidin channel as well.

Five steps are postulated for the movement of a cation (e.g., Na^+) through a gramicidin channel [16]: (1) diffusion through the aqueous phase to the channel opening; (2) loss of hydration shell (dehydration) which results in association with the channel; (3) transport through the channel; (4) dissociation from the channel which results in regaining of hydration shell (hydration) and (5) diffusion through the aqueous phase away from the channel opening. Since it is uncharged, Xe, unlike a monovalent cation, does not possess a very strongly bound hydration shell. Therefore, the energy involved in the partial dehydration process and binding at the channel entrance should be small compared to the same step involving a cation. Also, the interaction of the Xe atom with the carbonyl groups lining the channel interior should be smaller than that of a monovalent cation. The main obstacle to Xe transport would appear to be the size of the Xe atom (diameter 4.46 Å) [17] when trying to fit into the gramicidin channel (diameter 4 Å). However, the gramicidin channel is not a rigid structure. It has been shown to be permeable to ions with a diameter larger than the channel diameter, such as the formamidinium ion (diameter 4.5 Å) and the guanidinium ion (diameter 5.4 Å) [18,19]. This being the case, Xe transport should be possible in spite of its size.

The experiments described in this paper were done to determine if Xe atoms transport through gramicidin A channels incorporated into the lipid bilayers of vesicles. The evidence obtained from these studies leads to the conclusion that Xe does transport through the gramicidin A channel.

* Corresponding author. Fax: +1 (501) 5754909.

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2. Materials and methods

The preparation of the large unilamellar vesicles (LUV) was that of the reverse phase evaporation technique [20,21]. These vesicles were comprised of a 4:1 mole ratio of L-α-phosphatidylcholine (PC) (type V-E: from frozen egg yolk, Sigma Chemical Co., St. Louis, MO) and L-α-phosphatidylglycerol (PG) (egg-sodium salt, Avanti Polar Lipids, Alabaster, AL), respectively. Each ml of LUV prepared contained a total of 66 μmoles of lipid. Gramicidin A (from gramicidin D, a mixture consisting of 80% gramicidin A, 5% gramicidin B and 15% gramicidin C) (Sigma Chemical Co., St. Louis, MO) was incorporated into the vesicles using a thermal incubation procedure [20]. The salts K_2HPO_4, $BaCl_2 \cdot 2\ H_2O$, NaCl (Aldrich Chemical Co., Milwaukee, WI) and NH_4Cl (Fisher Scientific, Fair Lawn, NJ) were used without further purification.

Xenon gas (99.99%, Spectra Gases, Newark, NJ) was introduced into Wilmad 5 mm o.d. screw cap NMR tubes fitted with air tight Teflon-Silicon septa (Wilmad Glass, Buena, NJ) that contained the various solutions of interest using the basic method described by Miller et al. [22]. The air above the solution in the NMR tube with the septum cap was removed and then 15 ml of Xe gas was injected with a syringe fitted with a 26 gauge needle. The solution and Xe contained within the NMR tube were then vigorously shaken to insure the proper solubilization of Xe. The samples were kept at probe temperature, in a constant temperature bath, before being placed into the spectrometer. The samples were then allowed to thermally equilibrate for at least fifteen minutes in the probe before the start of data collection.

^{129}Xe has a spin quantum number of $I = 1/2$, a natural abundance of 26.44%, a magnetogyric ratio, γ, of $-7.4521 \cdot 10^7$ rad s^{-1} T^{-1} and a receptivity relative to ^{13}C of 32.4 [23]. The ^{129}Xe-NMR spectra were obtained at 138.300 MHz using a Varian VXR 500S NMR spectrometer (Varian, Palo Alto, CA). Typically, 250 FIDs were acquired for each sample. ^{23}Na-NMR spectra were also obtained using the Varian VXR 500S NMR spectrometer. The ^{23}Na-NMR spectra were acquired at 132.218 MHz.

In order to use ^{129}Xe-NMR spectroscopy for the study of the transport of Xe atoms through the gramicidin channel, a chemical shift reagent had to be found that would provide a means for differentiating the ^{129}Xe atoms on the inside of the vesicles from those on the outside of the vesicles, allowing a transport experiment, similar to that performed with $^{23}Na^+$ [20], to be duplicated with ^{129}Xe atoms. Hydroxypropyl-β-cyclodextrin (HPBCD) (Amaizo, Hammond, IN) was chosen as the ^{129}Xe chemical shift reagent. HPBCD is very soluble in aqueous solution. Since the exterior of the cyclodextrins is hydrophilic (Reed, G.A., personal communication) [24], cyclodextrins tend to be less soluble in non-aqueous solvents (Reed, G.A., personal communication). Thus, it is assumed that HPBCD will have a decreased solubility in and will not penetrate a lipid bilayer. HPBCD is able to complex numerous kinds of molecular and ionic guest molecules within its hydrophobic cavity (Reed, G.A., personal communication), a property inherent to the cyclodextrins [24]. The nonpolar Xe atoms can be accommodated within the cavity because the size of the Xe atom (4.46 Å in diameter) [17] is considerably smaller than the HPBCD cavity whose diameter is approximately 7.8 Å (Reed, G.A., personal communication). This size differential will result in a fast exchange of Xe atoms between the aqueous solution and the cavity. Although Xe is quite inert chemically, the polarizability of the electrons about the nucleus makes the resonance frequency quite sensitive to changes in the physical environment [22]. Since the hydrophobic environment of the cavity is much different than that of the aqueous environment, the resonance frequency of ^{129}Xe atoms within the cavity should be considerably different from that in the aqueous solution outside of the cavity. Because of the solubility of HPBCD in aqueous solution, a high ratio of HPBCD to Xe atoms can be achieved, a condition necessary to produce a relatively large change in the resonance frequency of ^{129}Xe.

To determine if HPBCD has an effect upon the integrity of the vesicles, an experiment was employed using ^{23}Na-NMR spectroscopy. For this experiment two solutions were prepared, one solution being a vesicle blank (containing no HPBCD) and the other solution containing vesicles and 80 mM HPBCD, a much greater concentration of HPBCD than used in the Xe transport experiments. A 100 mM NaCl/10 mM K_2HPO_4/H_3PO_4 (pH 8.06) buffer was used in the preparation of these vesicle solutions. In order to distinguish between $^{23}Na^+$ ions on the inside of vesicles from those on the outside, a cation chemical shift reagent, $Na_7Dy(P_3O_{10})_2$ (from $Dy(NO_3)_3 \cdot 5H_2O$, Alfa Products, Danvers, MA), was placed on the outside environment of the vesicles [20, 25, 26]. The ^{23}Na-NMR spectra of these samples were obtained at 30°C.

Another experiment involving ^{23}Na-NMR spectroscopy was used to discern if gramicidin disrupts the vesicles so as to make them lose their integrity and become porous. Two samples were prepared for this experiment, a vesicle blank that contained no gramicidin and another sample that contained 100 μM gramicidin A incorporated into the vesicles. The concentration of gramicidin A used in this experiment was higher than that used in the Xe transport experiments. The ^{23}Na-NMR spectra of these samples were recorded at 10°C.

To determine if Xe atom transport by gramicidin occurs, three different types of experiments were per-

formed. The first ^{129}Xe transport experiment required the following six solutions, designated as samples 1–6: (1) an aqueous Xe solution; (2) an aqueous solution that contains Xe and vesicles; (3) an aqueous solution containing Xe and vesicles with varying concentrations of incorporated gramicidin A; (4) an aqueous solution containing Xe and 17 mM HPBCD; (5) an aqueous solution containing Xe, vesicles and 17 mM HPBCD and (6) an aqueous solution containing Xe, 17 mM HPBCD and vesicles with varying concentrations of incorporated gramicidin A. All six solutions were prepared using a 10 mM K_2HPO_4/H_3PO_4 (pH 8.25) buffer. The ^{129}Xe-NMR spectra of these six samples were acquired at 10°C. The chemical shift of ^{129}Xe in samples containing vesicles/gramicidin/HPBCD (i.e., sample 6) was always compared to a control sample containing vesicles/HPBCD (i.e., sample 5) so that any difference in the ^{129}Xe chemical shift would be due to the presence of gramicidin and not to a HPBCD–lipid interaction.

The second ^{129}Xe transport experiment involved using ultraviolet (UV) light to photochemically alter the gramicidin channel [20,27–29]. This photochemically altered channel, which has greatly diminished ion transport capability, might be expected to exhibit a decrease in Xe transport.

To make certain that UV photolysis decreases gramicidin transport ability, a Na^+ vesicle solution was prepared using a 100 mM NaCl/10 mM K_2HPO_4/H_3PO_4 (pH 8.06) buffer. The Na^+ vesicle solution contained the $Na_7Dy(P_3O_{10})_2$ shift reagent and was 30 μM in gramicidin A. After the initial ^{23}Na-NMR spectrum of this sample was acquired, at 55°C, it was withdrawn from the NMR tube and placed in a quartz cuvette. The cuvette containing the sample was photolyzed with a 75 W UV lamp (Photon Technology International Model LPS-220 Arc Lamp, Princeton, NJ). After photolysis, the sample was placed back into the NMR tube and a ^{23}Na-NMR spectrum obtained. This photolysis cycle was repeated numerous times. The effect of increasing duration of UV photolysis on this sample was monitored by observing the line-width at half-height of the peak due to the Na^+ ions present on the inside of the vesicles via ^{23}Na-NMR spectroscopy. Additionally, UV absorption data (Hewlett Packard 8452A Diode Array Spectrophotometer, Hewlett Packard, Palo Alto, CA) were obtained of a diluted Na^+ vesicle aliquot, containing no gramicidin, before and after 2 h of UV photolysis in order to determine whether the lipids comprising the vesicles had been damaged by UV irradiation.

The photochemical experiment, the second ^{129}Xe transport experiment, required the following samples: (PS1) an aqueous solution containing Xe and vesicles; (PS2) an aqueous solution that contains Xe and vesicles with 30 μM incorporated gramicidin A; (PS3) an aqueous solution that contains Xe, vesicles and 17 mM HPBCD and (PS4) an aqueous solution containing Xe, 17 mM HPBCD and vesicles with 30 μM incorporated gramicidin A. All four solutions were prepared with a phosphate buffer. The ^{129}Xe-NMR spectra of these four photolysis samples were recorded at 30°C. After the initial ^{129}Xe-NMR spectrum of each sample was obtained, the samples were subjected to UV photolysis. After photolysis in a quartz cuvette, the sample was presumed to have too low a concentration of Xe for ^{129}Xe-NMR. Thus, after being placed back into the NMR tube, Xe was reintroduced into the sample.

The third ^{129}Xe transport experiment used Ba^{2+} to block the gramicidin channel. The Ba^{2+} ion is impermeable to transport through the gramicidin channel, although it binds tightly to the channel entrance [30,31]. This blocking of the gramicidin channel entrance by Ba^{2+} should curtail the transport of Xe through the channel. The four solutions required for the third ^{129}Xe transport experiment, samples BS1–4, were identical to those of the photochemical experiment except that they were prepared using a 100 mM NaCl/10 mM NH_4Cl/NH_3 (pH 8.32) buffer. In addition, 20 mM $BaCl_2$ was present in the outside vesicle environment of each of the four solutions. The presence of NaCl in the buffer served to provide ionic balance to the vesicle solutions. A phosphate buffer was not used due to the insolubility of $BaHPO_4$ [32]. The ^{129}Xe-NMR spectra of these four Ba^{2+} containing samples were acquired at 10°C. Those Ba^{2+} vesicle samples containing gramicidin A (BS2 and BS4) were compared to their respective controls (BS1 and BS3) so as to eliminate the effect of any interaction of Ba^{2+} with the vesicle phosphate headgroups.

3. Results and discussion

The transport of Na^+ ions through gramicidin channels thermally incorporated into large unilamellar vesicles (LUV) has been studied with the use of ^{23}Na-NMR spectroscopy [20]. At ambient temperature the transport process is sufficiently slow on the NMR time scale such that two $^{23}Na^+$ NMR signals, differentiated by the presence of a shift reagent, are observed. However, at high gramicidin concentration and a temperature of 60°C, the two $^{23}Na^+$ signals coalesce into a single signal. Under this set of conditions the fast exchange limit has been reached and the single signal observed has a chemical shift that is the weighted average of the two $^{23}Na^+$ NMR signals in the absence of transport. This is a characteristic of two-site exchange [33]. Since the energy barrier for the transport of Xe atoms through the gramicidin channel is expected to be much smaller than that for the monovalent cations, if a similar experiment were to be performed with Xe

atoms one might expect to find transport rates within the fast exchange limit, even at low temperature and low gramicidin concentration. In fact, this was observed experimentally using ^{129}Xe-NMR spectroscopy. Therefore, techniques such as magnetization inversion transfer or NMR signal line-width could not be used to detect the presence of Xe atom transport through the gramicidin channel. Another factor complicating the analysis of the Xe system for the detection of gramicidin transport was the diffusion of Xe atoms through the vesicle bilayer [34]. To overcome these problems a set of experiments was devised to separate the transport process from that of diffusion. In addition, the channel blocking and photolytic channel alteration experiments were used to corroborate the ^{129}Xe chemical shift results.

Fig. 1 shows the effect of HPBCD on the chemical shift of ^{129}Xe. The ^{129}Xe chemical shift of Xe dissolved in an aqueous solution with 17 mM HPBCD at 10°C is downfield with respect to a similar solution containing no HPBCD. Even though a fast exchange of Xe atoms between the aqueous solution and the cavity occurs, a significant shift is observed in the resonance frequencies of the ^{129}Xe atoms in the presence of HPBCD. Therefore, ^{129}Xe atoms on the inside of the vesicles will experience a different environment than that of the ^{129}Xe atoms on the outside of the vesicles, a necessary requirement for an effective shift reagent.

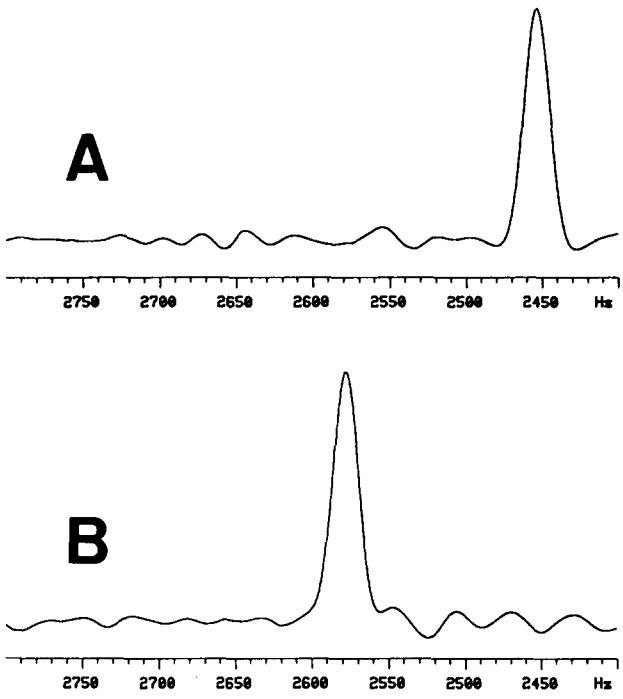


Fig. 1. The effect of HPBCD on the chemical shift of ^{129}Xe in an aqueous phosphate buffer solution at 10°C. Spectrum (A) the ^{129}Xe-NMR spectrum of Xe in buffer and spectrum (B) the ^{129}Xe spectrum of Xe in buffer containing 17 mM HPBCD. The ^{129}Xe chemical shifts were referenced to the right margin of the spectral window, set to 0.0 Hz. The downfield shift induced by HPBCD on the ^{129}Xe resonance frequency is evident.

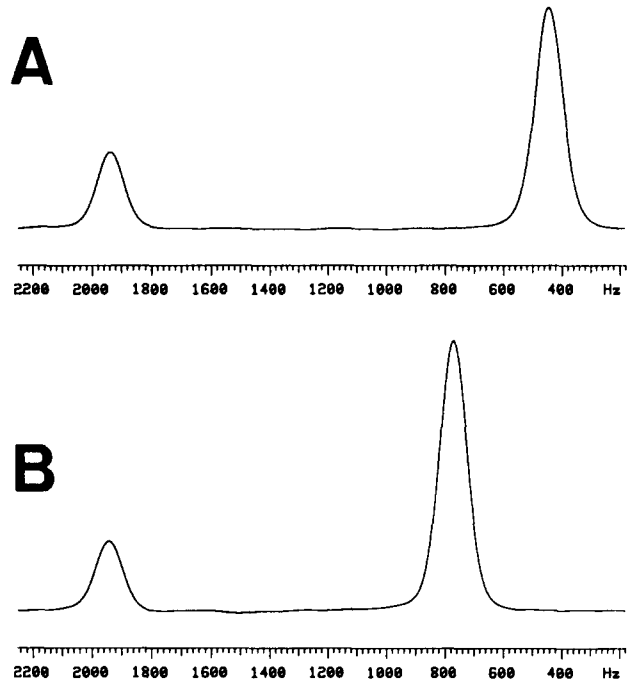


Fig. 2. Effect of HPBCD on the [23]Na-NMR spectra of LUV at 30°C. Spectrum (A) was obtained in the presence of 80 mM HPBCD and spectrum (B) was obtained in the absence of HPBCD. The same vertical scale was used in each spectrum. The ^{23}Na chemical shifts were referenced to the right margin of the spectral window, set to 0.0 Hz. The presence of two peaks in spectrum (A) indicates that HPBCD does not affect the integrity of the vesicle structure.

The result from the ^{23}Na-NMR experiment on vesicles containing HPBCD shows that HPBCD does not disrupt the integrity of the vesicle structure. This can be seen in Fig. 2, where the $^{23}Na^+$ NMR spectra are presented for the Na^+-vesicle system in the presence and absence of HPBCD. The two outside $^{23}Na^+$ signals, the larger of the two signals present in both spectra of Fig. 2, do not have the same chemical shift, unlike the two inside $^{23}Na^+$ signals. The two outside $^{23}Na^+$ signals occur at different chemical shifts, since the outside environment of the sample in spectrum (A) is 80 mM in HPBCD while the outside environment of the sample in spectrum (B) contains no HPBCD. The presence of HPBCD causes the $^{23}Na^+$ ions present in the outside vesicle environment to be shifted upfield. This is in contrast to the downfield effect that HPBCD has on the ^{129}Xe resonance. However, the effect of a shift reagent need not be the same from species to species, particularly in this case since one species is neutral and the other species is charged. If HPBCD were to disrupt the integrity of the vesicle structure one would expect to find only one $^{23}Na^+$ NMR signal or a resonance signal of diminished intensity for the inside $^{23}Na^+$ signal shifted toward high-field. Fig. 2 shows that this is not the case. Therefore, the ^{23}Na-

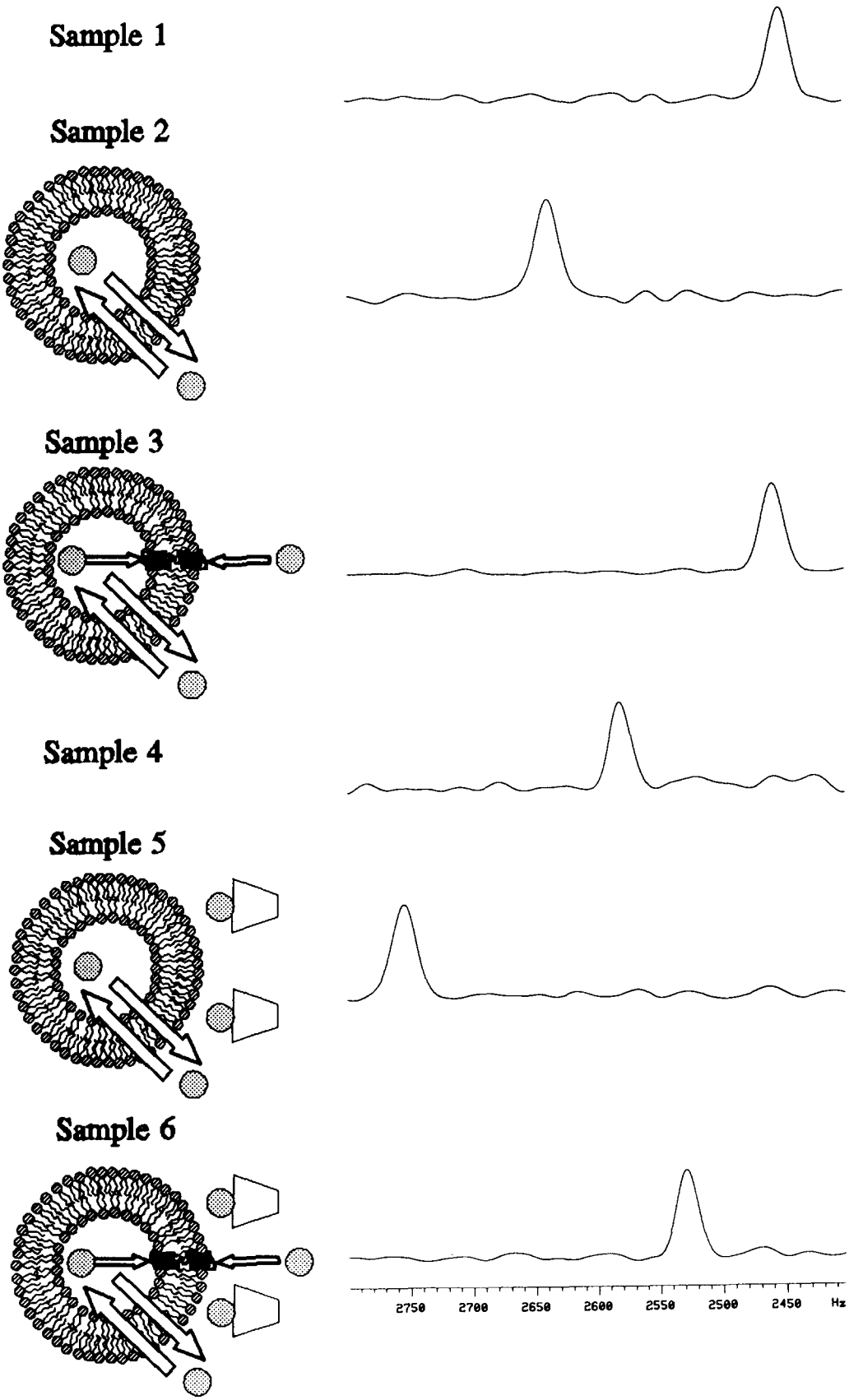


Fig. 3. ^{129}Xe-NMR spectra of (top to bottom) samples 1–6 at 10°C. Samples: (1) an aqueous Xe solution; (2) an aqueous solution containing Xe and vesicles; (3) an aqueous solution containing Xe and vesicles with 65 μM incorporated gramicidin A; (4) an aqueous solution containing Xe and 17 mM HPBCD; (5) an aqueous solution containing Xe, vesicles and 17 mM HPBCD and (6) an aqueous solution containing Xe, 17 mM HPBCD and vesicles with 65 μM incorporated gramicidin A. The ^{129}Xe chemical shifts were referenced to the right margin of the spectral window, set to 0.0 Hz.

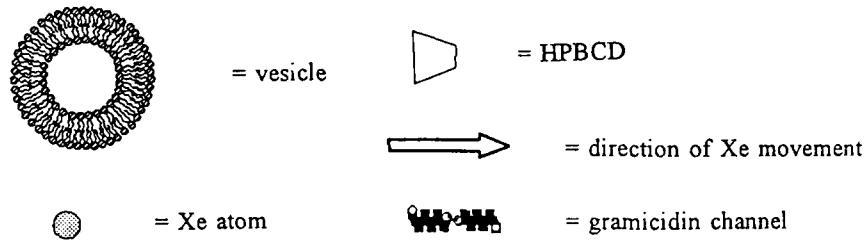


Fig. 3 (continued).

NMR results indicate that HPBCD does not adversely affect the integrity of the vesicles.

^{23}Na-NMR spectra were acquired of two vesicle samples, one containing 100 μM gramicidin A and the other sample containing no gramicidin. The percent encapsulation of $^{23}Na^+$ ions on the inside of the vesicles in the two samples was the same (17%). If a significant fraction of the vesicles had been disrupted by gramicidin then the percent of encapsulation would have decreased. This did not occur. Additionally, if gramicidin makes the vesicles porous then one would expect to observe mixing of the inside and outside aqueous solutions. Such mixing would cause the collapse of the two $^{23}Na^+$ NMR resonance signals into one signal since the cation chemical shift reagent would be on both the inside and outside solutions. However, two peaks were observed even with this high concentration of gramicidin. Obviously the high concentration of gramicidin, higher than that used for the Xe transport experiments, did not appear to disrupt the integrity of the vesicles as monitored by ^{23}Na NMR spectroscopy.

Fig. 3 contains the ^{129}Xe-NMR spectra of samples 1–6, where samples 3 and 6 had a gramicidin A concentration of 65 μM. Diagrams, representing the chemical composition and processes occurring in the vesicle containing samples, are included by the appropriate spectra in Fig. 3. Table 1 contains all the chemical shift data obtained from the ^{129}Xe-NMR experiments on samples 1–6. The other samples are mentioned under the same column since each time a new concentration of gramicidin was chosen, a new series of six samples was prepared. The 47.5 μM gramicidin A experiment differs from the others in that the vesicle samples in this particular case were subjected to a more rigorous sonication procedure.

The presence of gramicidin incorporated into the lipid bilayer of the vesicles may influence Xe transport and/or diffusion across the lipid bilayer in several ways: (1) inhibit the diffusion of Xe atoms through the vesicle bilayer and/or decrease the solubility of Xe atoms in the bilayer; (2) enhance Xe atom diffusion through the vesicle bilayer and/or increase the solubility of Xe atoms in the bilayer or (3) cause Xe transport through the gramicidin channel. Since sonication of the lipid takes place in the absence of Xe (the introduction of Xe gas into the NMR tube is the last thing done before the NMR experiment is performed), the only ways that Xe could move to the inside aqueous vesicle environment from the outside aqueous vesicle environment would be by diffusion through the lipid membrane and/or transport through the gramicidin channel.

In Table 1, a comparison of the ^{129}Xe chemical shifts observed in samples 1 and 2 reveals that there is a lipid induced downfield chemical shift. For this to occur, Xe must be in intimate contact with the lipid bilayer. Xe atoms have been found to diffuse rapidly through a lipid bilayer of incompletely and completely sonicated vesicles [34]. This result has also been confirmed in this study. The high rate of diffusion of Xe through the vesicle bilayer can be said to be in the fast exchange limit, producing only one ^{129}Xe-NMR signal in those samples containing vesicles. In addition, the chemical shift of ^{129}Xe in egg lecithin (which comprises approximately 80% of the lipid material used to make the vesicles in this study), referenced to the ^{129}Xe chemical shift of Xe gas at zero pressure, is downfield (200 ppm) to that observed for Xe in water (196 ppm) [22]. Therefore, the downfield ^{129}Xe chemical shift

Table 1
^{129}Xe chemical shifts (Hz) of samples 1–6 at 10°C

Sample	Gramicidin A monomer concentration Chemical shift (Hz),			
	3 μM	30 μM	47.5 μM [a]	65 μM
1	2452.2	2451.1	2451.3	2452.5
2	2631.5	2632.9	2632.2	2637.3
3	2628.9	2545.6	2512.2	2459.5
4	2576.7	2576.2	2580.9	2580.5
5	2754.9	2742.4	2754.2	2751.6
6	2749.0	2635.7	2615.9	2527.1

Samples: (1) an aqueous Xe solution; (2) an aqueous solution containing Xe and vesicles; (3) an aqueous solution containing Xe and vesicles with incorporated gramicidin A; (4) an aqueous solution containing Xe and 17 mM HPBCD; (5) an aqueous solution containing Xe, vesicles and 17 mM HPBCD and (6) an aqueous solution containing Xe, 17 mM HPBCD and vesicles with incorporated gramicidin A. All ^{129}Xe chemical shifts are reported as being down-field of the right margin of the spectral window, set at 0.0 Hz.
[a] vesicle samples subjected to rigorous sonication.

observed in sample 2 due to the Xe-lipid interaction, as diffusion occurs, is to be expected.

If the addition of gramicidin to the lipid bilayer increases the solubility of Xe atoms in the bilayer or decreases the rate of diffusion across the bilayer, the ^{129}Xe chemical shift in sample 3 should be downfield to that observed for sample 2 and the same effect should be observed with sample 6 compared to sample 5. This does not occur at any gramicidin concentration, as shown in Table 1. Alternatively, if the addition of gramicidin to the lipid bilayer decreases the solubility of Xe atoms in the lipid bilayer or increases the diffusion of Xe atoms across the bilayer or if transport through the channel occurs, the ^{129}Xe chemical shift in sample 3 should approach the ^{129}Xe chemical shift in sample 1 and the ^{129}Xe chemical shift of sample 6 should approach that in sample 4. The data in Table 1 show that as the gramicidin concentration increases, the ^{129}Xe chemical shift of sample 3 does approach that of sample 1 and that of sample 6 approaches the chemical shift of sample 4 (actually the chemical shift of sample 6 moves to high field of sample 4 because the HPBCD effect is diluted by the aqueous environment on the inside of the vesicles). These results appear to indicate that the reversal of the ^{129}Xe chemical shift, with increasing concentrations of gramicidin, is due to access through the channel being greater for ^{129}Xe atoms than diffusion through the lipid. Although these experiments provide evidence for Xe transport through the gramicidin channel, in order to more fully resolve the question, one needs to examine the results obtained with the photochemical and divalent cation blocking experiments.

In a sample containing vesicles and gramicidin, Xe has two potential routes available to go from the outside to the inside vesicle environment, diffusion through the lipid and transport through the gramicidin channel. However, if the gramicidin channel in a vesicle can be modified in such a way that Xe transport no longer occurs or is reduced significantly, then diffusion becomes the dominant route for the movement of Xe atoms from the outside to the inside vesicle environment. This is what occurs in a vesicle sample without gramicidin, where diffusion through the lipid is the only route available for Xe to go from the outside to the inside of the vesicle. Table 1 reveals that the ^{129}Xe chemical shift of a vesicle sample containing gramicidin is always upfield of the ^{129}Xe chemical shift of a similar vesicle sample not containing gramicidin. Therefore, a vesicle sample where the gramicidin channel has been altered to hinder Xe transport should have a ^{129}Xe chemical shift moving downfield towards or equal to the ^{129}Xe chemical shift of a vesicle sample containing no gramicidin. The magnitude of this downfield shift will depend on the degree of the alteration of the channel.

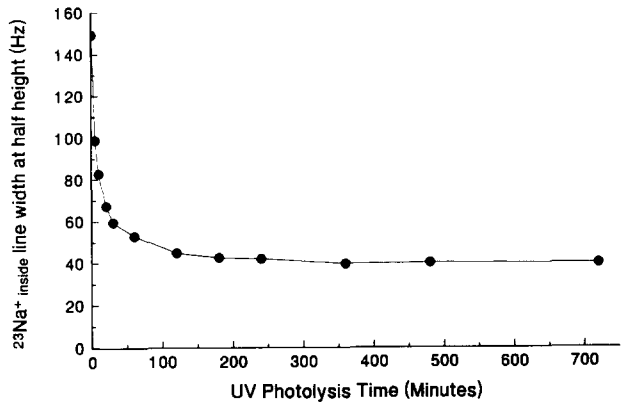
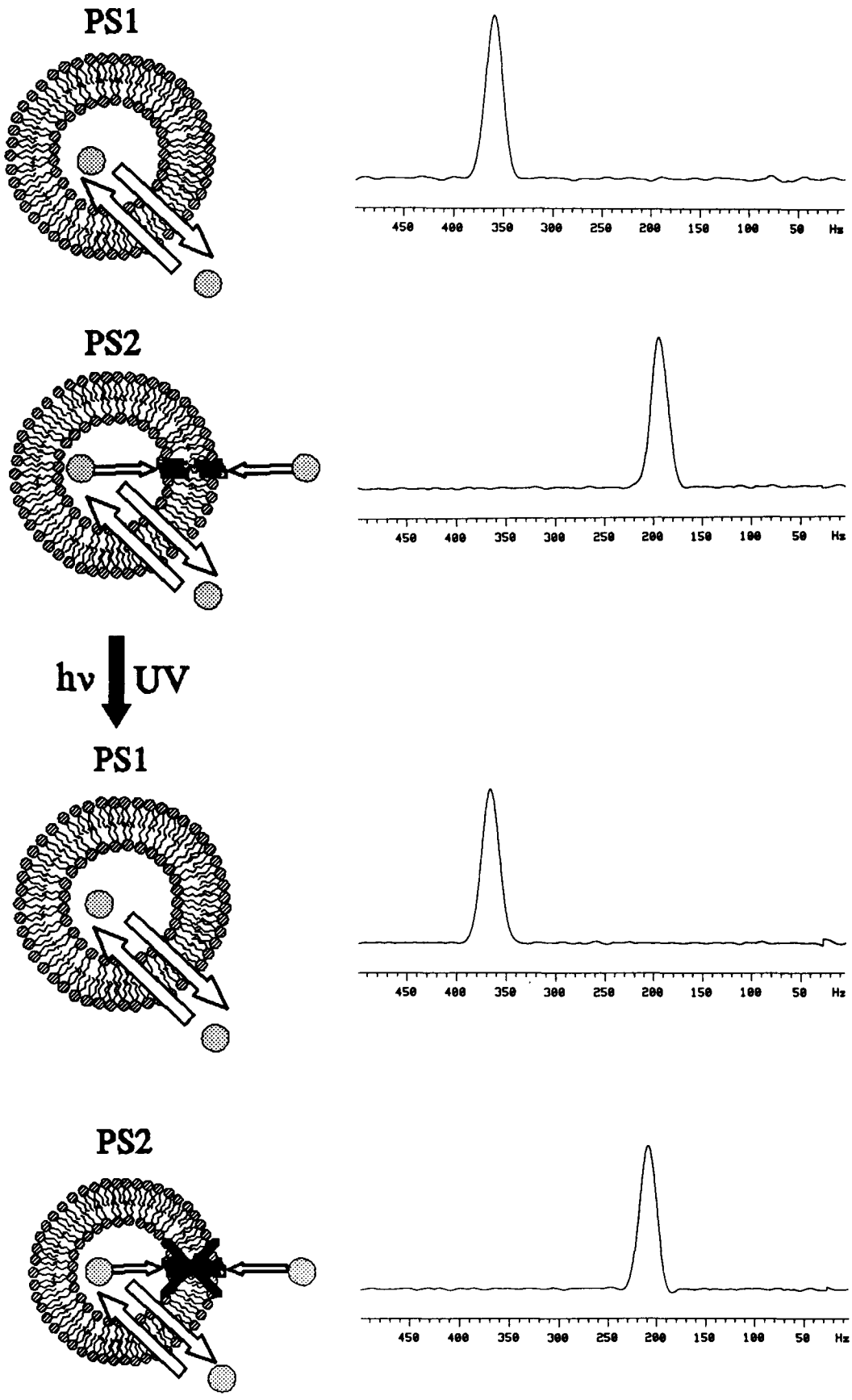


Fig. 4. Plot of the UV photolysis times of the Na^+ vesicle/ 30 μM gramicidin A sample vs. the ^{23}Na-NMR line-width at half-height of the inside $^{23}Na^+$ peak at 55°C. The line-width can be seen to decrease with longer photolysis times due to the slowing of $^{23}Na^+$ exchange through the photochemically altered gramicidin A channel.

Gramicidin A is known to be affected by UV light, due to the absorption by the channel tryptophans. When exposed to UV light, the tryptophans are photochemically altered in such a way as to cause gramicidin A, incorporated into lipid bilayers, membranes or vesicles, to change into an irreversible non-conducting state, so that ions, like Na^+, are no longer transported through the channel [20,27–29]. To confirm this photolytic effect on the system employed in this study, a Na^+ vesicle sample containing 30 μM gramicidin A was exposed to varying durations of UV light. The ^{23}Na-NMR data for this sample can be seen in Fig. 4. The data contained in the figure show the effect of time of UV irradiation on the line-width at half-height of the peak due to $^{23}Na^+$ present on the inside of the vesicles. There is a decrease in the ^{23}Na-NMR line-width at half-height, $\nu_{1/2}$, of the inside $^{23}Na^+$ peak for the first 2 h of UV photolysis. After 2 h there is no further change in $\nu_{1/2}$. From these results, it appears that 2 h of UV photolysis is the length of time needed to deactivate the channel for Na^+ transport. The decrease in the line-width of the inside signal is caused by a decreased rate of exchange of $^{23}Na^+$ through the gramicidin channel, which is characteristic behavior for a two site exchange system. This decrease in the $^{23}Na^+$ exchange rate is a result of the increasing impermeabil-

Fig. 5. ^{129}Xe-NMR spectra of (top to bottom) samples PS1 and PS2 before and after 2 h of UV photolysis at 30°C. Samples: (PS1) an aqueous solution containing Xe and vesicles and (PS2) an aqueous solution containing Xe and vesicles with 30 μM incorporated gramicidin A. The ^{129}Xe chemical shifts were referenced to the right margin of the spectral window, set to 0.0 Hz.


PS1
450 400 350 300 250 200 150 100 50 Hz
PS2
450 400 350 300 250 200 150 100 50 Hz
hν UV
PS1
450 400 350 300 250 200 150 100 50 Hz
PS2
450 400 350 300 250 200 150 100 50 Hz
= photochemically modified gramicidin channel

ity of the gramicidin channel to ion transport caused by the progressive photochemical modification of the channel. From a UV absorption study on a Na^+ vesicle sample containing no gramicidin, before and after 2 h of UV irradiation, it was concluded that the lipids comprising the vesicles are not damaged by UV light,

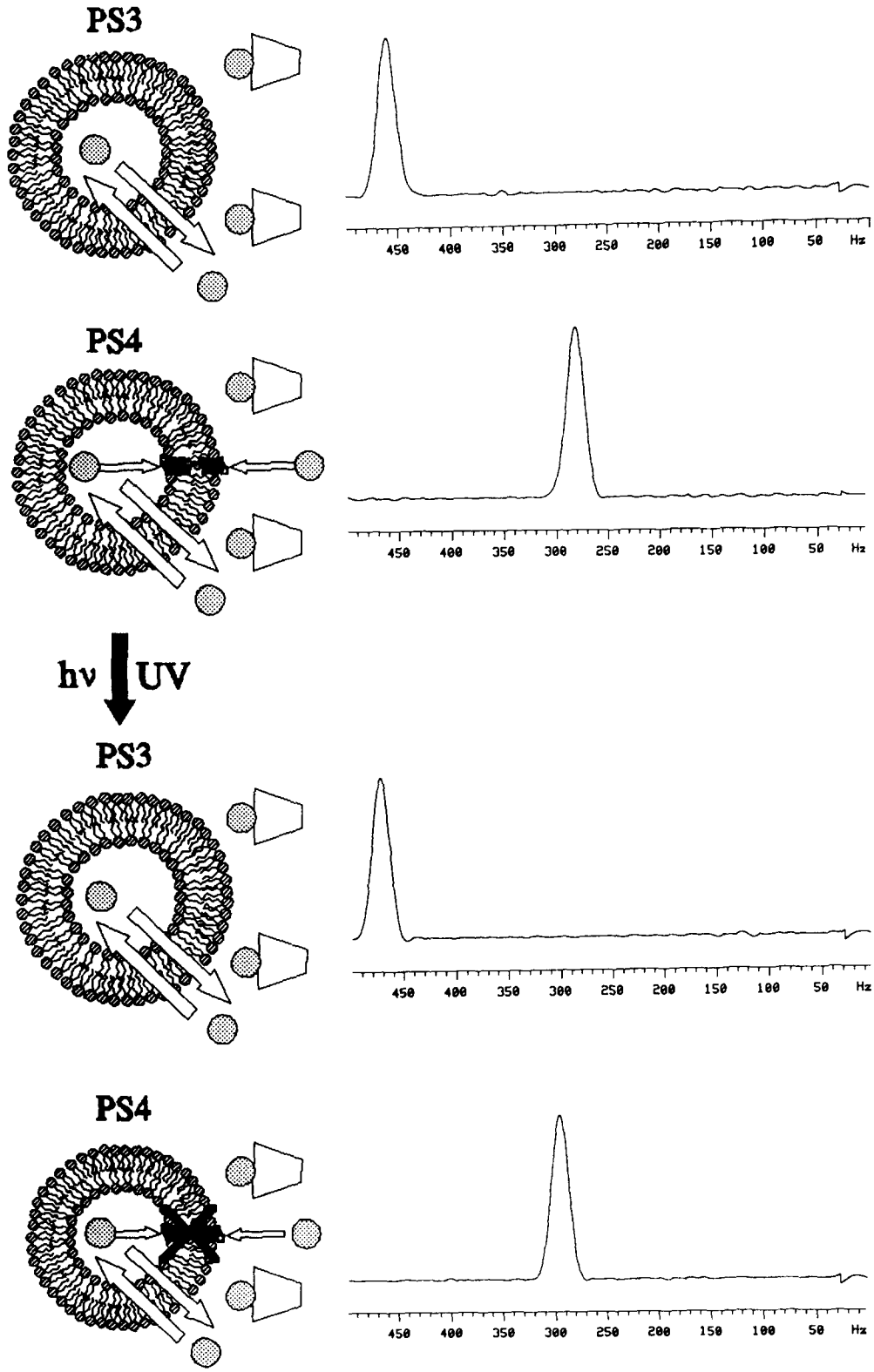


Fig. 6. ^{129}Xe-NMR spectra of (top to bottom) samples PS3 and PS4 before and after 2 h of UV photolysis at 30°C. Samples: (PS3) an aqueous solution containing Xe, vesicles and 17 mM HPBCD and (PS4) an aqueous solution containing Xe, 17 mM HPBCD and vesicles with 30 μM incorporated gramicidin A. The ^{129}Xe chemical shifts were referenced to the right margin of the spectral window, set to 0.0 Hz.

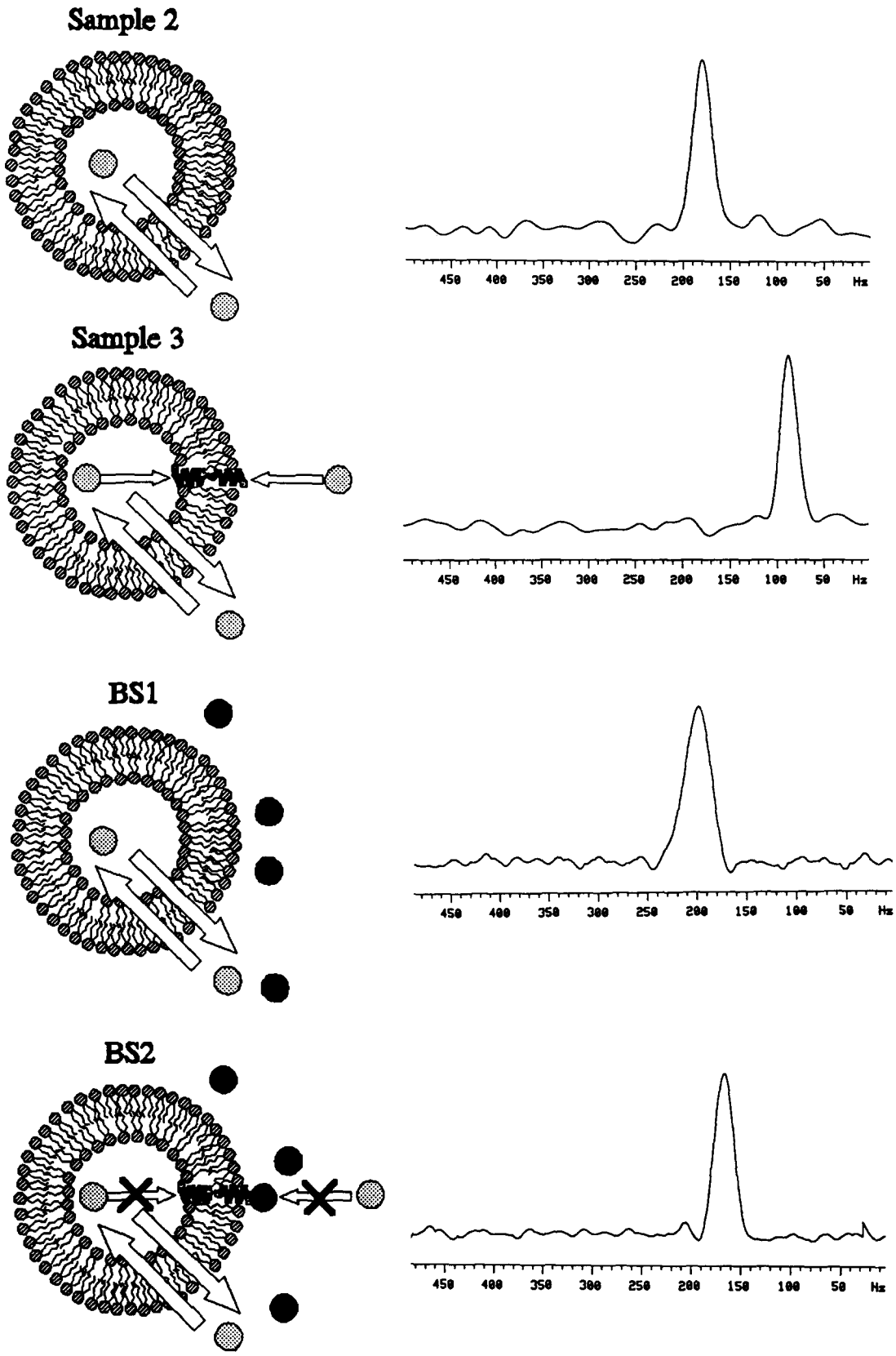

Sample 2
Sample 3
BS1
BS2
450 400 350 300 250 200 150 100 50 Hz
= Ba^{2+} ion
= movement of Xe not permitted in this direction
= gramicidin channel blocked by Ba^{2+}

Table 2
^{129}Xe chemical shift differences (Hz) between samples PS1 and PS2 and samples PS3 and PS4 before and after 2 h of UV photolysis at 30°C

Samples	^{129}Xe chemical shift difference (Hz) before UV photolysis	^{129}Xe chemical shift difference (Hz) after two hours of UV photolysis
PS1–PS2	164.4	157.1
PS3–PS4	177.1	174.6

Samples: (PS1) an aqueous solution containing Xe and vesicles; (PS2) an aqueous solution containing Xe and vesicles with 30 μM incorporated gramicidin A; (PS3) an aqueous solution containing Xe, vesicles and 17 mM HPBCD and (PS4) an aqueous solution containing Xe, 17 mM HPBCD and vesicles with 30 μM incorporated gramicidin A.

the absorbance before and after photolysis being the same.

Using a photolysis time of 2 h, the time required to deactivate the gramicidin channel to Na^+ transport, the ^{129}Xe-NMR spectra of samples PS1 and PS2 before and after photolysis are shown in Fig. 5. Fig. 6 contains the ^{129}Xe NMR spectra of samples PS3 and PS4 before and after UV photolysis. Diagrams are shown in both figures, by the appropriate spectra, representing the composition and dynamics involved in these photolysis samples. Table 2 gives the ^{129}Xe chemical shift differences between the various samples from the photochemical experiment, PS1–4, before and after 2 h of UV photolysis. Samples PS1 and PS2 are the same, except PS2 is 30 μM in gramicidin A. The same relation holds between samples PS3 and PS4, the HPBCD containing samples, with PS4 being 30 μM in gramicidin A. If the ^{129}Xe chemical shift difference between PS1 and PS2 is due to Xe transport through the gramicidin channel, then after 2 h of UV photolysis, the chemical shift of ^{129}Xe in PS2 should move downfield toward that observed in PS1 (i.e., the chemical shift difference between the two should decrease, since the channel has been photochemically altered to prevent Xe transport). For the same reason, the ^{129}Xe chemical shift of PS4 should approach that of PS3. Table 2 reveals that UV photolysis does cause the ^{129}Xe chemical shifts to behave in a manner consistent with Xe transport through gramicidin. Although the UV inactivation effect on the ^{129}Xe chemical shift appears to be small, it must be remembered that a photochemically altered gramicidin channel which is impermeant to cations may still be rather permeant to a neutral species such as Xe.

Table 3
^{129}Xe chemical shift differences (Hz) between samples 2 and 3, samples 5 and 6, samples BS1 and BS2 and samples BS3 and BS4 at 10°C

Samples	^{129}Xe chemical shift difference (Hz)	Samples	^{129}Xe chemical shift difference (Hz)
2–3	87.3	BS1–BS2	27.1
5–6	106.7	BS3–BS4	60.1

Samples: (2) an aqueous solution containing Xe and vesicles; (3) an aqueous solution containing Xe and vesicles with 30 μM incorporated gramicidin A; (5) an aqueous solution containing Xe, vesicles and 17 mM HPBCD and (6) an aqueous solution containing Xe, 17 mM HPBCD and vesicles with 30 μM incorporated gramicidin A. Samples: (BS1) an aqueous solution containing Xe and vesicles and 20 mM Ba^{2+}; (BS2) an aqueous solution containing Xe, vesicles with 30 μM incorporated gramicidin A and 20 mM Ba^{2+}; (BS3) an aqueous solution containing Xe, vesicles, 17 mM HPBCD and 20 mM Ba^{2+} and (BS4) an aqueous solution containing Xe, 17 mM HPBCD, vesicles with 30 μM incorporated gramicidin A and 20 mM Ba^{2+}. All Ba^{2+} was present only in the outside vesicle environment.

Additional evidence for Xe transport through the gramicidin channel can be found in the experimental results obtained from the divalent cation blocking experiment. The gramicidin channel is known to be blocked by divalent cations such as Ca^{2+} and Ba^{2+}, due to their ability to bind to the gramicidin channel entrance [30, 31]. This strong binding of the divalent cations to the channel entrance acts as a barrier, hindering the normal transport of species through the channel [35]. Of the alkali earth metal cations, Ba^{2+} binds the strongest to the gramicidin channel, with a K_i of 122.6 M^{-1} at 34°C [36]. Thus, Ba^{2+} was the ion chosen for the divalent cation blocking experiment.

Fig. 7 displays the ^{129}Xe-NMR spectra of samples 2 and 3 from the first ^{129}Xe transport experiment and samples BS1 and BS2. The ^{129}Xe-NMR spectra of samples 5 and 6, also from the first ^{129}Xe transport experiment and samples BS3 and BS4 are shown in Fig. 8. Both figures display diagrams of the respective composition and processes involved in each sample next to the appropriate spectrum. In Table 3, the ^{129}Xe chemical shift differences between samples 2 and 3 and samples BS1 and BS2 are contrasted, as are the ^{129}Xe chemical shift differences between samples 5 and 6 and samples BS3 and BS4. Samples BS1–4 all have 20 mM $BaCl_2$ present only in the outside environment of the vesicles. Samples 2 and 3 are chemically identical except sample 3 is also 30 μM in gramicidin A. The same relationship applies between samples BS1 and BS2, with BS2 being 30 μM in gramicidin A. Samples 5 and

Fig. 7. ^{129}Xe-NMR spectra of (top to bottom) samples 2 and 3 and samples BS1 and BS2 at 10°C. Samples: (2) an aqueous solution containing Xe and vesicles; (3) an aqueous solution containing Xe and vesicles with 30 μM incorporated gramicidin A; (BS1) an aqueous solution containing Xe, vesicles and 20 mM Ba^{2+} and (BS2) an aqueous solution containing Xe, vesicles with 30 μM incorporated gramicidin A and 20 mM Ba^{2+}. The ^{129}Xe chemical shifts were referenced to the right margin of the spectral window, set to 0.0 Hz. All Ba^{2+} was present only in the outside vesicle environment.

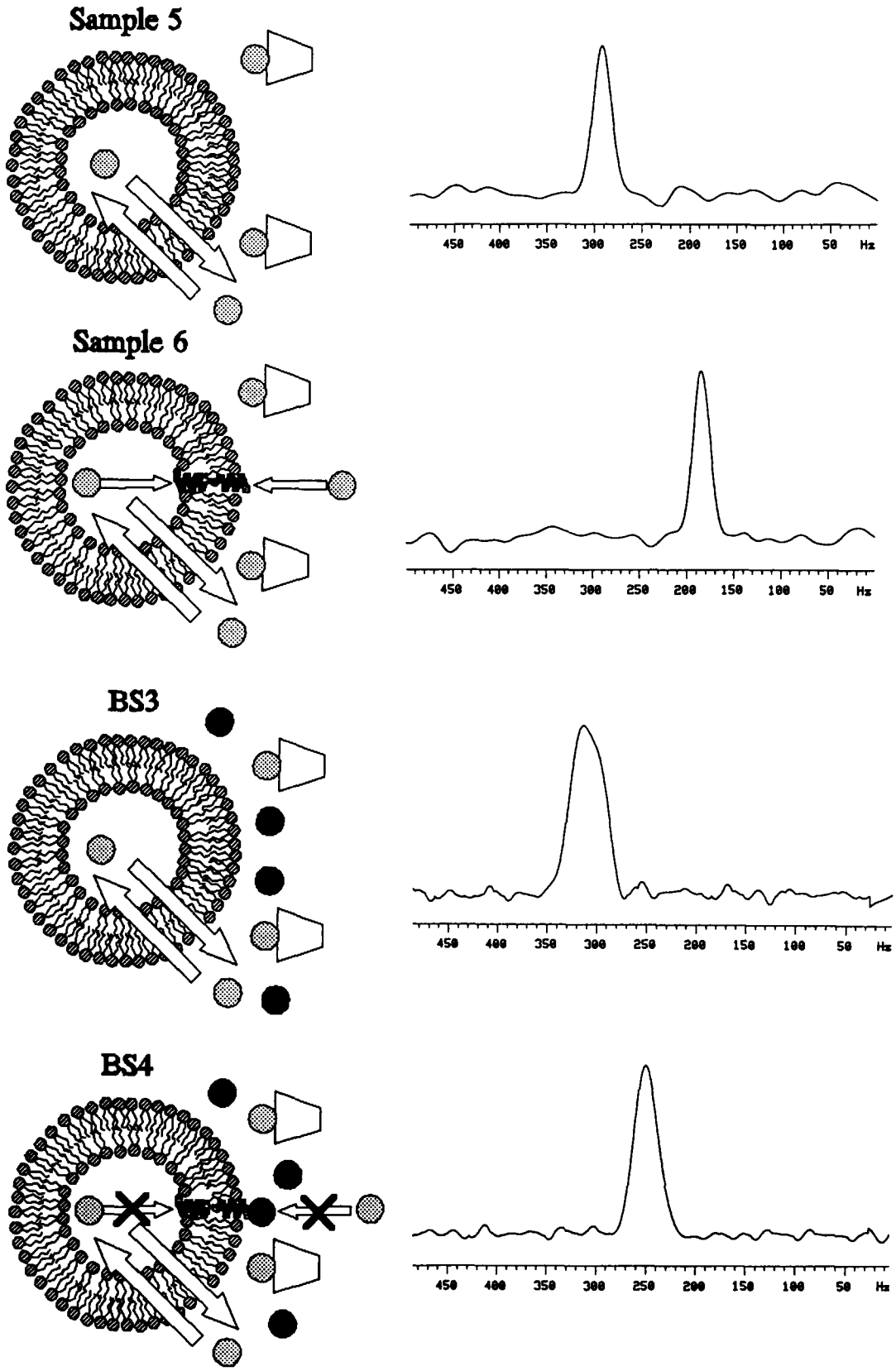


Fig. 8. ^{129}Xe-NMR spectra of (top to bottom) samples 5 and 6 and samples BS3 and BS4 at 10°C. Samples: (5) an aqueous solution containing Xe, vesicles and 17 mM HPBCD; (6) an aqueous solution containing Xe, 17 mM HPBCD and vesicles with 30 μM incorporated gramicidin A; (BS3) an aqueous solution containing Xe, vesicles, 17 mM HPBCD and 20 mM Ba^{2+} and (BS4) an aqueous solution containing Xe, 17 mM HPBCD, vesicles with 30 μM incorporated gramicidin A and 20 mM Ba^{2+}. The ^{129}Xe chemical shifts were referenced to the right margin of the spectral window, set to 0.0 Hz. All Ba^{2+} was present only in the outside vesicle environment.

6, the HPBCD containing vesicle samples, are the same, except sample 6 has 30 μM gramicidin A. This same relationship applies to samples BS3 and BS4, also containing HPBCD, with BS4 having 30 μM gramicidin A. Since Xe transport through the gramicidin channel should be impeded by the blocking action of the Ba^{2+} ion, the ^{129}Xe chemical shift between BS1 and BS2 should decrease, being smaller than the chemical shift difference between samples 2 and 3, which contain no Ba^{2+}. These same relationships hold when comparing the ^{129}Xe chemical shift difference between samples 5 and 6 with that between BS3 and BS4. The ^{129}Xe chemical shift behavior displayed in Table 3 implies that Xe does indeed transport through the gramicidin channel. The ^{129}Xe chemical shift difference between samples BS1 and BS2 is considerably smaller, by 60.2 Hz, than that found between samples 2 and 3. Additionally, the ^{129}Xe chemical shift difference between samples BS3 and BS4 is significantly smaller, by 46.6 Hz, than that observed between samples 5 and 6. Although the presence of Ba^{2+} in these vesicle solutions appears to significantly decrease the amount of Xe transport through the gramicidin channel, transport still appears to occur since the ^{129}Xe chemical shifts of samples BS1 and BS2 are not equal. This is also observed to be the case with samples BS3 and BS4. However, it is known that the presence of the divalent cations does not cause an absolute cessation of transport of species through the gramicidin channel, but rather a diminishment of transport [35].

In summary, evidence has been obtained by three types of experiments that appears to indicate the transport of Xe atoms through the gramicidin A channel. These experiments suggest that the channel is flexible enough to accommodate a species whose diameter is slightly greater than that of the normal channel.

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