Origin and Behavior of Deuteron Spin Echoes in Selectively Labeled Amino Acids, Myoglobin Microcrystals, and Purple Membranes†

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ABSTRACT: We have obtained deuterium NMR spin-echo spectra of crystalline DL-[γ-$^2H_6$]valine, [*S-methyl*-$^2H_3$]-methionine, cyanoferrimyoglobin from sperm whale (*Physeter catodon*), containing deuteriomethyl groups at methionine-55 and methionine-131, and [γ-$^2H_6$]valine-labeled bacteriorhodopsin in the purple membrane of *Halobacterium halobium* R_1. By using 90–τ–$\beta_{90°}$ (XY) and 90–τ–$\beta_{0°}$ (XX) pulse sequences and observing the dependence of the spin-echo amplitude upon the interpulse spacing τ, we have determined that the so-called "quadrupole echoes" obtained in these typical selectively deuterated condensed-phase biological systems are in fact strongly modulated by proton–deuteron and deuteron–deuteron dipolar interactions. The two amino acids and the protein crystals behaved as typical organic solids, with no evidence of "liquid-like" behavior, even in the presence of excess water (in the case of the ferrimyoglobin crystals). However, with the valine-labeled bacteriorhodopsin, the τ-dependence of XY echoes as a function of temperature emphasized the "solid-like" behavior of the membrane "matrix", while the basic nature of the spin-echo response for the narrow central component of the spectrum clearly indicated the very "fluid" or "mobile" nature of a series of residues that are shown elsewhere [Keniry, M., Gutowsky, H. S., & Oldfield, E. (1984) *Nature (London) 307*, 383–386] to arise from the membrane surface. Our results thus suggest that such NMR methods may yield useful information on side-chain dynamics complementary to that of line-shape and spin–lattice relaxation time analyses.

For over 10 years now, there has been considerable interest in the use of deuterium (^2H) nuclear magnetic resonance (NMR)[1] as a tool for probing the static and dynamic structures of cell membranes (Oldfield et al., 1972, 1981; Mantsch et al., 1977, Seelig, 1977). Early studies were hampered by a number of factors, the primary one being the use of low-field NMR instrumentation. As a result, continuous-wave measurements were of very low sensitivity, whilst the Fourier transforms of truncated free-induction decays following single pulses gave gross distortions, due to the long spectrometer recovery times at low field. This latter problem was overcome by Davis et al. (1976), who used a resonant two-pulse sequence, 90–τ–$90_{90°}$, to form a so-called "quadrupole echo", thereby effectively eliminating the problem of receiver overload after a pulse.

Many groups are now using ^2H NMR of selectively ^2H-labeled compounds to study the static and dynamic structures of various biological systems (Oldfield & Rothgeb, 1980; Gall et al., 1982; Kinsey et al., 1981a,b; Rice et al., 1981a,b; Rothgeb & Oldfield, 1981; Smith, 1981; Batchelder et al., 1982; Bienvenue et al., 1982; Keniry et al., 1983, 1984). The basic spin-echo response to a resonant two-pulse sequence has been characterized for only a few perdeuterated organic molecules ([$^2H_3$]acetonitrile at 180 K, [$^2H_6$]benzene at 200 K, and [$^2H_6$]acetone at 150 K; Boden et al., 1978), and it is not described by a purely quadrupolar mechanism, as had been previously considered. Therefore, we felt it important to investigate the basic features of the spin-echo responses in a variety of *partially deuterated* biochemical systems such as those being discussed in the numerous publications cited above. We present in this paper such results on several amino acids, myoglobin, and bacteriorhodopsin of the purple membrane of *Halobacterium halobium* R_1. Overall, our results are consistent with those obtained previously with simple predeuterated organic species at low temperatures (Boden et al., 1978). Also, they confirm the close similarity in dynamics between crystalline amino acids, a protein, and bacteriorhodopsin in the *H. halobium* purple membrane. In addition, our results are consistent with the idea (Keniry et al., 1984) that the surface residues of bacteriorhodopsin in the purple membrane of *H. halobium* are in a highly disordered, "mobile" or "fluid" state, unlike the residues in the membrane matrix.

Theory

NMR spin echoes from a quadrupolar nucleus in a solid were first observed and calculated by Solomon (1958) for the $I = {}^5/_2$ species ^{127}I in KI. The calculations were carried out for a 90–τ–$\beta_{0°}$ (XX) sequence, with β the variable width of the second pulse. The notation $\beta_{0°}$ indicates a zero-degree phase shift between the initial, 90° pulse and the second, $\beta°$ pulse. The analysis assumed explicitly that the dipolar interactions were negligible in comparison with the quadrupolar terms. For spins $I = 1$, eq 13 of Solomon's paper predicts that a sequence of two radio-frequency pulses of the same phase will elicit no spin-echo response for any rotation angle β in the solid state.

Subsequently, however, deuterium NMR spin echoes *were* observed for such a 90–τ–$\beta_0°$ sequence (Boden et al., 1978), when $\beta \neq k \times 90°$ and $k = 0, 1, 2, ...$ the observation of spin echoes with a 90–τ–$\beta_{0°}$ sequence in perdeuterated organic solids proves that dipolar interactions between deuterons, at least in these systems, cannot be neglected since such echoes arise *only* when dipolar interactions are present (Tjon, 1981). For an *infinite* number of identical, dipolar-coupled spins with $I = 1$, this theory predicts that the maximum echo amplitude (E) depends on the width of the second pulse, β, and on τ, the

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[1] Abbreviation: NMR, nuclear magnetic resonance.

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interpulse spacing. For the $90\text{–}\tau\text{–}\beta_{90°}$ (XY) pulse sequence, the dependence is

$$E^{XY}(\beta,\tau) = E_1(\tau)\sin^2\beta + E_2(\tau)\sin^2\beta\cos^2\beta \quad (1)$$

In the absence of dipolar interactions, the second term vanishes (Tjon, 1981). For the $90\text{–}\tau\text{–}\beta_{0°}$ (XX) sequence, in the presence of dipolar interactions between spins with $I = 1$, the dependence is

$$E^{XX}(\beta,\tau) \simeq -E_3(\tau)\sin^2\beta\cos\beta + E_4(\tau)\sin^4\beta\cos\beta \quad (2)$$

Both terms vanish if there are no dipolar interactions (loc. cit.). In these expressions, E_1, E_2, E_3, E_4 are constants for a given pulse spacing τ since they are independent of the rotation angle β. Apart from a change of sign and up to fourth order in time, the theory also predicts that the echo shape following an XX sequence is the same as the shape of the XY echo and that of the nontruncated free-induction decay.

The decay of an XY spin echo due to deuteron–deuteron dipolar interactions is predicted to be (Tjon, 1981)

$$E^{XY}(90,\tau) = \exp\left(-\frac{M_2^{Echo}}{2}\tau^2\right) \quad (3)$$

where $M_2^{Echo} = (8/9)M_2^{VV,DD}$ and $M_2^{VV,DD}$ is the van Vleck second moment of the deuteron spectrum. In the presence of proton–deuteron dipolar interactions as well, the echo-decay coefficient is given by

$$M_2^{Echo} \simeq (8/9)M_2^{VV,DD} + 2M_2^{VV,HD} \quad (4)$$

where $M_2^{VV,HD}$ is the contribution to the van Vleck second moment from $^1H\cdots^2H$ dipolar coupling (Boden et al., 1974; I. C. Baianu, unpublished calculations). The time dependence of an XX echo is predicted to have two components: a rapid initial growth of the echo amplitude is followed by a much slower decay for large τ-values (Tjon, 1981), and similar behavior is expected when heteronuclear, as well as homonuclear, dipolar interactions are present (Boden et al., 1974, 1978).

In order to test these predictions and gain a better understanding of the origins and nature of such spin echoes in typical biochemical systems, we have observed them as a function of the rotation angle (β), phase, and time (τ) in a variety of selectively deuterated amino acids and related compounds. Our results, discussed in detail below, show that dipolar interactions do have a marked effect upon the echo behavior. Our results also demonstrate that such experiments also clearly differentiate between solid-like and fluid-like behavior in the amino acid side chains of a membrane protein.

Experimental Procedures

Nuclear Magnetic Resonance Spectroscopy. Deuterium NMR measurements were carried out on two "home-built" spectrometers, operating at 32.9 and 55.3 MHz, respectively. The design of the 32.9-MHz spectrometer has been published elsewhere (Oldfield et al., 1978). The 55.3-MHz spectrometer consists of an 8.45-T, 3.5-in. bore, Oxford Instruments superconducting solenoid (Oxford Instruments, Osney Mead, Oxford, U.K.), a Nicolet NIC-1180 computer, 293B pulse programmer, Zeta plotter, and Explorer scope (Nicolet Instrument Corp., Madison, WI), and other radio-frequency equipment based on a design described elsewhere (Oldfield & Meadows, 1978). The 90° pulse widths at 32.9 MHz were between 2.6 and 4.0 μs, and at 55.3 MHz they were between 2.0 and 3.6 μs.

Materials. All materials were from batches whose syntheses are described elsewhere: [*S-methyl*-$^2H_3$]methionine, Jones et al. (1976) and Rothgeb & Oldfield (1981) (our compound is in fact an equimolar mixture of *SR* and *SS* forms); DL-[γ-$^2H_6$]valine, Kinsey et al. (1981b); [ϵ-$^2H_3$]methionine-labeled sperm whale (*Physeter catodon*) myoglobin, Rothgeb & Oldfield (1981); [γ-$^2H_6$]valine-labeled bacteriorhodopsin from *H. halobium* R_1, Kinsey et al. (1981b) and M. Keniry et al. (unpublished results).

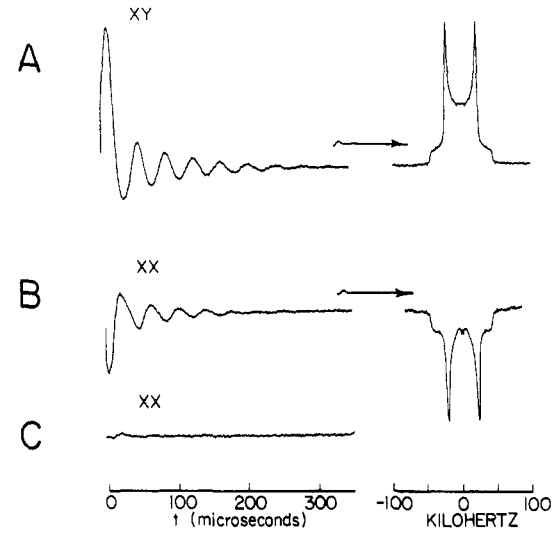

FIGURE 1: Deuterium NMR spin echoes in polycrystalline [*S-methyl*-$^2H_3$]methionine at 293 K for several pulse sequences: (A) spin echo following an XY sequence with $\beta = 90°$; (B) spin echo following an XX sequence with $\beta \sim 55°$; (C) phase test of the XX sequence with $\beta \sim 90°$. The spectra at the right are the Fourier transforms of the spin echoes on the left (with data shifted to the echo maxima for XY echoes, or minima for XX echoes, and no instrumental phase corrections). Data were recorded at 55.3 MHz (corresponding to a magnetic field strength of 8.45 T). The 90° pulse widths were ~2.0–2.5 μs, $\tau = 100$ μs, recycle time 0.5 s, 2-MHz data acquisition rate, 4096 data points per spectrum, no line broadening, and 100 scans per spectrum. Spectra were symmetrized about zero frequency, since single-phase detection was used.

Results and Discussion

Polycrystalline Amino Acids. We show in parts A and B of Figure 1 deuterium NMR spin echoes and their Fourier transforms, respectively, for $90\text{–}\tau\text{–}90_{90°}$ (XY) and $90\text{–}\tau\text{–}55_{0°}$ (XX) pulse sequences applied to polycrystalline [*S-methyl*-$^2H_3$]methionine. The accuracy of the phase setting in the latter case (Figure 1B) was checked with a $90\text{–}\tau\text{–}90_{0°}$ sequence, which, according to eq 2, should give zero spin-echo amplitude. The result of this test is shown in Figure 1C in which the signal level is seen to be about 2% of the maximum echo amplitude of Figure 1A, for a $90\text{–}\tau\text{–}90_{90°}$ sequence. Moreover, an XX ($90\text{–}\tau\text{–}\beta_{0°}$) spin echo was indeed observed for $10° < \beta < 90°$, in close agreement with the predictions of eq 2, which is valid for quadrupolar nuclei of spin $I = 1$ coupled by dipolar interactions (Tjon, 1981). Similar results are predicted for a system of proton pairs dipolar coupled to deuterons (I. C. Baianu, unpublished calculations); the weight of moments of order higher than 2 is increased however, in comparison with the case of deuteron–deuteron dipolar interactions.

The phase of the XX spin-echo response shown in Figure 1B is also as predicted by eq 2 and is opposite to that observed with the XY sequence, Figure 1A. We have made similar observations for [α,*carboxyl*-$^2H_3$]glycine, [α,*carboxyl*,*amino*-$^2H_5$]glycine, DL-[γ-$^2H_6$]valine, and DL-[α,β,γ-$^2H_8$]valine (data not shown). The XX spin echo is, therefore, *dipolar* in origin and should vanish for isolated deuterons in solids. The dependence of ^2H NMR spin echoes upon β for XY pulse sequences is shown in Figure 2 for [*S-methyl*-$^2H_3$]methionine

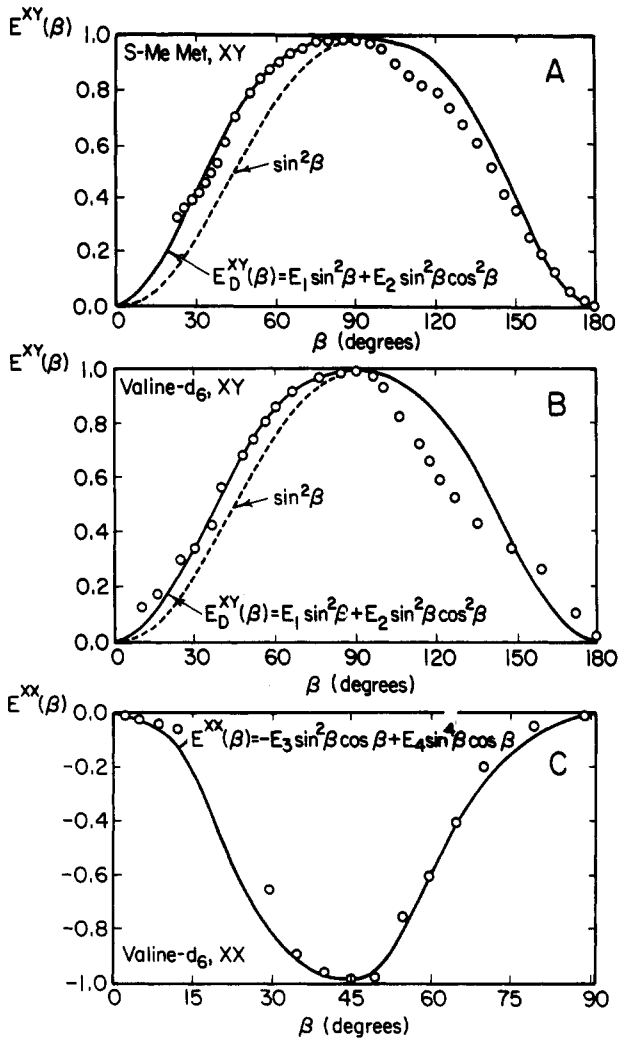

FIGURE 2: Dependence upon the rotation angle β of deuterium NMR spin echoes following XY and XX pulse sequences at 293 K: (A) polycrystalline [*S-methyl*-$^2H_3$]methionine, XY at τ = 50 μs; (B) polycrystalline DL-[γ-$^2H_6$]valine, XY at τ = 50 μs; (C) as in (B) but XX at τ = 150 μs. The solid lines in (A) and (B) are the best fits with eq 1 in the range $0 < \beta < 90°$. Deviations from the solid line for $\beta > 100°$ are due to the fall-off in pulse power at large pulse widths, as discussed by Boden et al. (1978). The solid line in (C) is the best fit with eq 2.

(Figure 2A) and for XY and XX pulse sequences in polycrystalline DL-[γ-$^2H_6$]valine (Figure 2B,C). The XY spin echoes deviate significantly from the $\sin^2\beta$ dependence predicted in the absence of dipolar interactions and can be fitted by adding the second term in eq 1, which describes the effects of dipolar interactions. The additional $\sin^2\beta\cos^2\beta$ term gives much better agreement with the experimental results. Deviations for $\beta > 100°$ are due to pulse-power fall-off (Boden et al., 1978). These findings suggest that the model of an infinite number of spins with I, S = 1, coupled by dipolar interactions is a useful approximation for the behavior of such methyl-labeled solids.

Additional support for a dipolar mechanism of spin-echo generation in our selectively deuterated amino acids come from the dependence upon β of the ^2H NMR spin echoes in polycrystalline DL-[γ-$^2H_6$]valine following XX pulse sequences, Figure 2C. The XX echo response is as predicted by theory (eq 2). Both terms in eq 2 arise only as a result of dipolar interactions, since the isolated spin, I = 1, model gives $E^{XX}(\beta,\tau)$ = 0. It is interesting, therefore, that these calculations predict that the E^{XX} echo shape is the same (apart from a change of sign, and up to fourth order in time) as the E^{XY} echo shape

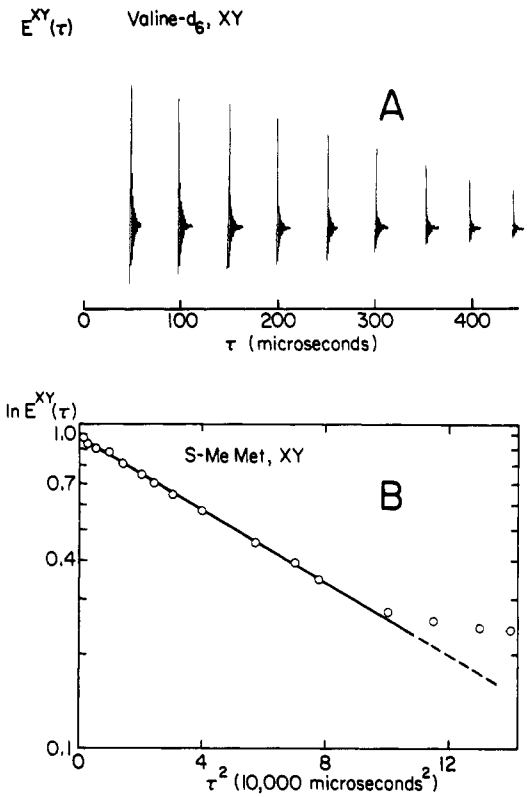

FIGURE 3: Deuterium NMR XY spin-echo decays $E(\tau)$ observed for DL-[γ-$^2H_6$]valine and [*S-methyl*-$^2H_3$]methionine, at 55.3 MHz and 293 K: (A) valine, the experimental spin echoes shown as a function of τ; (B) [*S-methyl*-$^2H_3$]methionine data plotted as ln E^{XY} vs. τ^2. The line is the least-squares fit with eq 3, and the observed slope gives M_2^{Echo} = 0.277 ($\pm$0.004) $\times$ 10^{-8} s^{-2}.

and the free-induction decay signal. The differences observed between the E^{XY} echo shape (Figure 1A) and that of E^{XX} (Figure 1B) are most likely due to the presence of heteronuclear dipolar interactions, which increase the importance of the higher order moments. Moreover, as shown in Figures 1B and 2C, an *inverted* echo is obtained for the XX pulse sequence, with a maximum echo amplitude at $\beta \approx 45°$, again consistent with the predictions of eq 2.

We have also measured the decay of the spin-echo amplitude as a function of the pulse spacing τ in a variety of circumstances (Figure 3). As expected for a dipolar mechanism, the echo-decay rates measured at 32.9 and 55.3 MHz are identical within experimental error. The spin echoes in Figure 3A were obtained following 90–τ–$90_{90°}$ pulse sequences in polycrystalline DL-[γ-$^2H_6$]valine, for τ-values in the range 50–500 μs. The experimental points in Figure 3A are excellently fitted by eq 3, which is based on a dipolar interaction mechanism (data not shown).

Similar observations were made for three other deuterium-labeled amino acids; [*S-methyl*-$^2H_3$]methionine, [α,-*carboxyl*-$^2H_3$]glycine, and [α,*carboxyl*,*amino*-$^2H_5$]glycine. For brevity, we show in Figure 3B only our results for [*S-methyl*-$^2H_3$]methionine. The plot in Figure 3B of the spin-echo decay is Gaussian, as predicted by eq 3, except for a slightly slower decay at $\tau \geq 300$ μs. The non-Gaussian tail of the echo decay is most likely due to higher order moments; these are known to be increased by molecular motion and/or by heteronuclear dipolar interactions [pp 123–124 in Abragam (1961)].

For large τ-values ($\geq$300 μs, when strong heteronuclear dipolar interactions are present), theoretical calculations (Baianu et al., 1981; Tjon, 1982; I. C. Baianu, unpublished results) indicate that the Fourier transforms of the 90–τ–$\beta_{90°}$

Table I: ^{2}H Second Moments Observed and Calculated for DL-[γ-$^2H_6$]Valine (Free Base) Compared with Those Obtained from the Echo Decay of Valine-Labeled Bacteriorhodopsin in the Purple Membrane

system investigated	$2M_2^{Echo}$ ($\times 10^8$ s^{-2})	temp (K)
amino acid	0.217 ± 0.004 (0.201[a])	294
bacteriorhodopsin	0.243 ± 0.004	315
	0.277 ± 0.004	294
	0.322 ± 0.004	287
	0.325 ± 0.004	243

[a] This is M_2^{YY} calculated from the crystal structure of DL-valine (Mallikarjunan & Rao, 1969; Torii & Iitaka, 1970), as discussed by Andrew et al. (1976), and with the proton–deuteron contribution multiplied by 2.0 (eq 4).

echoes will be distorted forms of the powder spectrum. The distortions result from incomplete refocusing described by the increased weight of higher order terms for large τ-values. Such effects have indeed been observed in, for example, DL-[γ-$^2H_6$]valine at 290 K (data not shown). In Figure 3B, the slope of the line is determined by the second moment of the dipolar spectrum. Values of the dipolar second moments obtained from the slopes of such plots are given in Table I.

All of these observations show that the ^{2}H NMR spin-echo behavior of deuteriomethyl groups in polycrystalline amino acids is similar to that observed at low fields in perdeuterated solids (Boden et al., 1978) and that XX echo formation is properly thought of as having a dipolar origin. Further experiments in the presence of proton decoupling may also allow one to distinguish the effects of heteronuclear and homonuclear dipolar interactions upon the XY spin-echo decays.

Myoglobin: A Protein Crystal. We have investigated the responses to XX and XY pulse sequences of a magnetically ordered sample of sperm whale (*P. catodon*) cyanoferrimyoglobin, labeled as C^2H_3 at methionines-55 and -131. With the magnetic-ordering technique (Oldfield & Rothgeb, 1980; Rothgeb & Oldfield, 1981), it is possible to study individually the responses of these two nonequivalent methionines in the microcrystalline protein sample, suspended in 90% saturated $(NH_4)_2SO_4$ solution.

We show in Figure 4 typical ^{2}H NMR spin echoes, $E^{XY}(t)$ and $E^{XX}(t)$, both in the time domain (A and C respectively) and in the frequency domain (B and D, respectively). Figure 4A,B shows the behavior obtained when a conventional 90–τ–$90_{90°}$ (XY), so-called "quadrupole-echo" sequence (Davis et al., 1976) is used. The resulting spectrum contains two doublets (Rothgeb & Oldfield, 1981) arising from Met-55 and Met-131 residues in the magnetically aligned crystals, together with a sharp central component, due to liquid HO^{2}H. Upon application of a 90–τ–$\beta_{0°}$ (XX) pulse sequence, however, the signals originating from Met-55 and Met-131 are inverted, Figure 4D, while that from HO^{2}H remains upright.

This confirms the different origins of the solid XX spin echo and the liquid HO^{2}H echo; in the latter case, dipolar interactions are averaged out by rapid molecular motions, and a Hahn spin echo is obtained. We have also obtained the dependence of XY spin echoes upon rotation angle in this magnetically ordered sample, and our results agree with the predictions of eq 1 (data not shown). Moreover, *both* the Met-55 and the Met-131 peaks have the same dependence on β.

We have also found that the differences between powder spectra observed with an XX sequence and those with an XY sequence are larger in the case of methionine-labeled cyanoferrimyoglobin than with either [*S-methyl*-$^2H_3$]methionine or DL-[γ-$^2H_6$]valine and that the myoglobin echo decay is

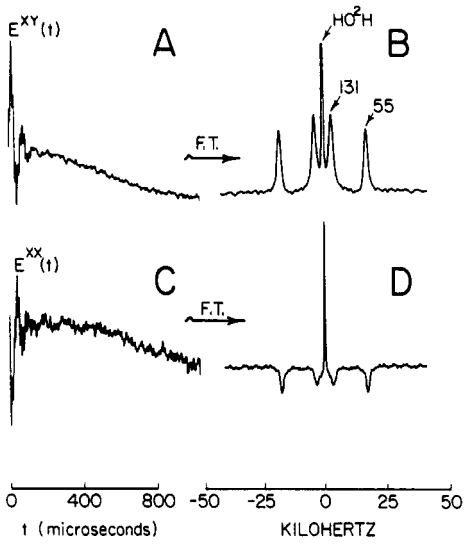

FIGURE 4: Deuterium NMR spin echoes (55.3 MHz), and their corresponding Fourier transforms, in magnetically ordered cyanometmyoglobin microcrystals labeled as C^2H_3 at Met-55 and Met-131, at 293 K: (A) XY spin echo at τ = 200 μs; (B) XX spin echo at τ = 200 μs, with $\beta \simeq 55°$; (C) Fourier transform of the XY spin echo in (A); (D) Fourier transform of the XX spin echo in (B). Spectral conditions were essentially as in Rothgeb & Oldfield (1981), except for a 1.5-s recycle time. Spectra were symmetrized about zero frequency, since single-phase detection was used.

markedly non-Gaussian for $\tau \gtrsim 200$ μs (data not shown). These differences are likely to originate in the proton–deuteron dipolar interactions in myoglobin (J. Jeneer, private communication; I. C. Baianu, unpublished calculations) due, for example, to the extreme dilution in the ^{1}H lattice of our two labeled residues. The occurrence of slow side-chain motion might also contribute to these effects and this aspect will require further investigation.

Overall, the results with ^{2}H-labeled myoglobin microcrystals indicate that dipolar interactions among methyl group deuterons at a given site and with surrounding lattice protons need to be taken into account in order to describe fully the spin-echo behavior and that from the ^{2}H NMR standpoint, the methionine residues in this hydrated protein crystal behave as if they were in a normal solid. A preliminary account of the effects of molecular motion on the spin-echo behavior is given in the following section, on [γ-$^2H_6$]valine-labeled bacteriorhodopsin in the photosynthetic purple membrane of *H. halobium* R_1.

Bacteriorhodopsin: A Membrane Protein. We have recently investigated the dynamics of aliphatic amino acid side chains, including those of valine, threonine, and leucine, in bacteriorhodopsin of the purple membrane of *H. halobium* R_1 (Kinsey et al., 1981b; M. Keniry et al., unpublished results; Keniry et al., 1983). In those studies, we use ^{2}H T_1 measurements to obtain information on the rates of methyl rotation, assuming dominance of the quadrupolar relaxation mechanism. We did not investigate in detail the origins of the ^{2}H spin echoes used to obtain this motional information.

We have now obtained the XX and XY spin-echo responses of [γ-$^2H_6$]valine-labeled bacteriorhodopsin in the purple membrane of *H. halobium*. Typical time and frequency domain spectra are shown in parts A and B of Figure 5, and the τ-dependence of the broad component, $E^{XY}(\tau)$, as a function of temperature, is given in part C. As in the case of deuterated amino acids and cyanometmyoglobin microcrystals, both XY and XX spin echoes are observed, the XX echoes again being inverted with respect to the central component signal, which arises from mobile surface residues (Keniry et al., 1984) (Figure 1,A,B). The Fourier-transform spectra clearly show

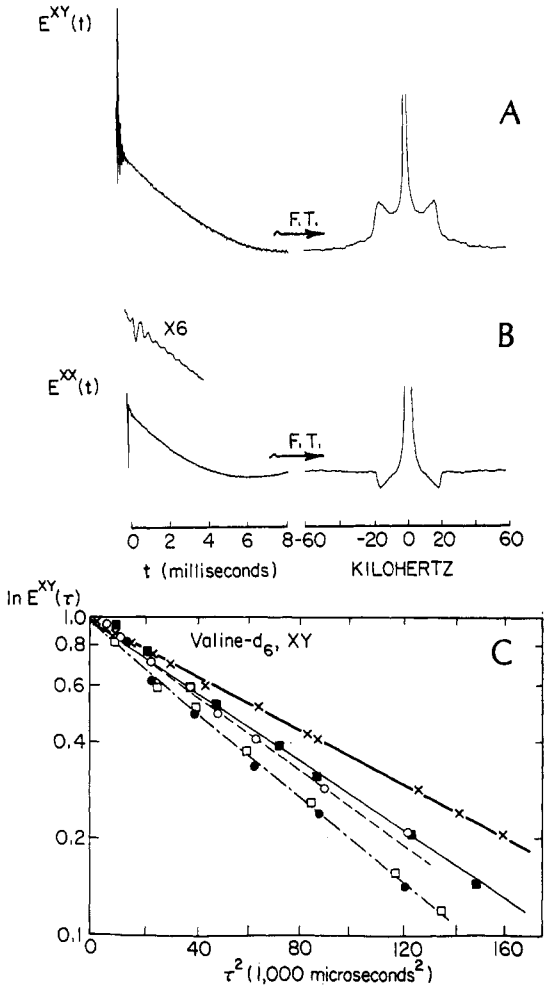

FIGURE 5: Deuterium NMR spin echoes (55.3 MHz) and their τ-dependence as a function of temperature for [γ-$^2H_6$]valine-labeled bacteriorhodopsin in the purple membrane of *H. halobium* R_1: (A) XY spin echo and Fourier transform at τ = 100 μs, recorded with 400-ms recycle time and 800-Hz line broadening; (B) XX spin echo and Fourier transform for $\beta \sim 55°$ and τ = 100 μs; (C) amplitude of XY spin echoes (broad component) observed as a function of the interpulse spacing time interval τ showing linear ln $E^{XY}(\tau)$ vs. τ^2 dependence (eq 3) at (□) 243, (●) 287, (○) 294, and (■) 315 K. Also shown is the XY spin-echo decay for free-base, polycrystalline DL-[γ-$^2H_6$]valine at 294 K (×) (90° pulse widths were 3.2 μs). Dipolar second moments calculated from the echo-decay rates with eq 3 are given in Table I. The sharp central component in the frequency domain spectra arises from mobile surface residues having "liquid-like" behavior (Keniry et al., 1984).

separation of the inverted powder spectrum of the membrane "matrix" [γ-$^2H_6$]valine-labeled residues from the highly mobile surface residues at 290 K.

At temperatures below 253 K, the central component "disappears", due to water freezing, while the ^2H spectrum sharpens and has an increased quadrupole splitting, $\Delta\nu_Q$. At temperatures above 253 K, up to 315 K, XY echo decays show an essentially continuous decrease in the echo-decay rates (Figure 5C), defined as M_2^{Echo} and listed in Table I. This trend is consistent with the presence of additional side-chain motions at higher temperatures, which decrease the second moment of the dipolar spectrum (eq 3), although we cannot dismiss a contribution from quadrupolar relaxation. We include for comparison in Figure 5C the echo decay of DL-[γ-$^2H_6$]valine, which is clearly *slower* than those for the valine-labeled purple membrane. In the latter, the larger values of M_2^{Echo} (given in Table I) are probably due to larger ^1H–^2H dipolar interactions because of molecular packing, analogous to those that we found in myoglobin. One would not expect the CD_3 groups to be more mobile in the valine crystal than in the membrane protein.

These results indicate that the echo responses and their τ-dependencies for [γ-$^2H_6$]valine residues in bacteriorhodopsin in the purple membrane of *H. halobium* are similar to those for the amino acids and protein crystals that we have investigated. The echoes cannot be attributed solely to quadrupolar interactions since they are strongly affected by both homo- and heteronuclear interactions (mostly with protons).

Concluding Remarks. The results described above indicate that, in general, deuterium NMR spin echoes in selectively deuterated amino acids, myoglobin microcrystals, and bacteriorhodopsin of the purple membrane of *H. halobium* show behavior characteristic of restricted motion in solids, with incomplete averaging of both dipolar and quadrupolar interactions. An exception is found for the highly mobile surface residues in bacteriorhodopsin (Keniry et al., 1984). Comparisons with theoretical calculations for $I = 1$ dipolar-coupled spins and proton pairs interacting with deuterons show that hetero- and homonuclear dipolar interactions play a significant role in the formation of deuterium NMR spin echoes in selectively deuterated biological solids. As a result, the direct measurement of the second moments associated with such dipolar interactions is possible in these complex systems, and it provides information complementary to conventional lineshape analyses [e.g., Kinsey et al. (1981a) and Rice et al. (1981a,b)] or spin–lattice relaxation time determinations of molecular motion (Keniry et al., 1983). Because of the utility of ^2H NMR as a structural or motional probe in biological systems, further more quantitative studies of the effects of ^1H–^2H dipolar interactions in specifically deuterated systems are desirable.

Registry No. Deuterium, 7782-39-0; DL-[γ-$^2H_6$]valine, 53497-46-4; L-[*S-methyl*-$^2H_3$]methionine, 13010-53-2.

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