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Dynamics of Amino Acids in Crystals and in Membrane Proteins

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Introduction

Over the past few years much effort has been directed towards characterizing the molecular structure of and intermolecular interactions between individual membrane components, in an attempt to relate the structure of biological membranes to their function.[1,2] Nuclear magnetic resonance (NMR) spectroscopy has proven quite successful in elucidating the effects of membrane proteins on the motions of phospholipid molecules[3-5], but only recently has progress been made in using NMR to determine the dynamic structures of the integral proteins themselves[6-8]. For the past two years we have been using the deuterium(^{2}H) nucleus as a probe in studying the dynamics of membrane proteins, concentrating on the rates and types of motions of the amino-acid sidechains. In this Article we present a summary of our results on the dynamics of deuterated aliphatic, hydroxy- and aromatic amino-acids in the crystalline solid state and when incorporated into bacteriorhodopsin from the purple membrane of the extreme halophile, *Halobacterium halobium* R_1, and into the inner and outer membranes of several *Escherichia coli* auxotrophs.

Amino-acids are the fundamental units of protein structure and their motional properties in the crystalline solid state can be used as a basis for understanding sidechain motions in proteins. In this paper we discuss results for several amino-acids containing deuterated methyl and phenyl sidechains. We have obtained ^{2}H NMR lineshapes as a function of temperature for all systems, and for the methyl containing amino-acids we have measured spin-lattice relaxation times (T_1) as a function of temperature, and from them calculated rotational correlation times using the methods of Torchia and Szabo[9]. Where possible, we have incorporated the deuterium-labelled amino-acids into bacteriorhodopsin and followed the rates and types of sidechain motion by lineshape and T_1 analyses. Overall, the results demonstrate a striking similarity between sidechain mobility in amino-acid crystals and in bacteriorhodopsin.

The simple isolation procedure[10] and the unique function of bacteriorhodopsin make it a most attractive system for study. It is the only protein found in purple membrane[11], its sequence is known[12-14], its three dimensional structure is becoming

available[15,16], and it can also be enriched biosynthetically with a number of deuterated amino-acids without undue label scrambling[6,7,16]. The purple membrane is, however, atypical in its protein content, containing only a single protein. Moreover, it has a low overall lipid content and the lipids are somewhat unusual in structure. Therefore, we have also incorporated our deuterated amino-acids into the cell membranes of *E. coli*. The preparations contain ~80% inner membrane, and have been selectively enriched with either oleic or elaidic acid.

Theoretical Background

We shall give a brief account of the background theory required for an understanding of the spectral lineshapes that may arise from the wide range of motions undergone by different amino-acid sidechains. More detailed analyses of deuterium magnetic resonance spectra are given elsewhere[1,17-19]. In deuterium NMR there are two allowed transitions which, because of the interaction of the nuclear electric quadrupole moment with the electric field gradient at the nucleus, are non-degenerate. This non-degeneracy gives rise to a "quadrupole splitting" of the absorption line with a separation, $\Delta\nu_Q$, between peak maxima of

$$\Delta\nu_Q = \frac{3}{4}\frac{e^2qQ}{h}[3\cos^2\theta\text{-}1\text{-}\eta\sin^2\theta\cos 2\psi] \qquad (1)$$

where θ, ψ define the orientation of the principal axes of the electric field gradient tensor (usually along the C-^{2}H bond vector) with respect to the laboratory frame. For powder samples, all values of θ are possible and one obtains a "powder pattern" (see for example Figure 1A). The resulting quadrupolar splitting for an immobile aliphatic C-^{2}H bond is 126 kHz (Figure 1A). If there is "fast" molecular motion, i.e., motion with a correlation time $\tau_c \leq 1/\Delta\nu_Q$, it is necessary to take a time average over $(3\cos^2\theta\text{-}1)$. Using the notation of Figure 2, one finds that in this event

$$\Delta\nu_Q = \frac{3}{2}\frac{e^2qQ}{h}\frac{(3\cos^2\theta'\text{-}1)}{2}\frac{(3\cos^2\beta\text{-}1)}{2} \qquad (2)$$

where θ' is the angle between the axis of motional averaging and the magnetic field direction and β is the angle between the principal axis of the electric field gradient tensor and the axis of motional averaging (Figure 2). Substituting $\beta = 70.5°$ gives a quadrupolar splitting of ~ 40 kHz for a rapidly rotating methyl C-^{2}H bond (Figure 1B). Any other fast motion along the sidechain superimposed on the methyl rotation would reduce the quadrupolar splitting even more.

The motion most commonly experienced by aromatic amino-acids is a two-fold, 180° flip of the aromatic ring about the C^β-C^γ bond axis, which produces a spectrum similar to that shown in Figure 1C where the singularity separation is 30 kHz, and the simulated quadrupolar splitting and asymmetry parameters are 80 kHz and 0.66, respectively. A flipping aromatic ring is distinguishable from both an immobile ring, which has a quadrupolar splitting of 184 kHz and an asymmetry parameter η =

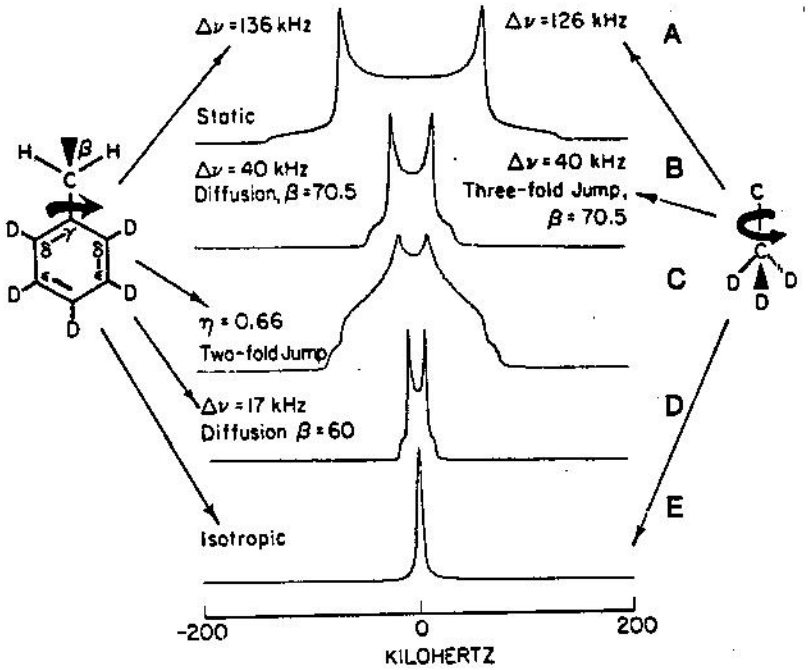


Figure 1. ^{2}H NMR lineshapes simulated for A) rigid C-^{2}H bond. B) a C-^{2}H bond, $\beta = 70.5°$, undergoing either rotational diffusion or a 120° threefold jump about an axis of motional narrowing. C) a C-^{2}H bond, $\beta = 60°$, undergoing a 180° twofold jump. D) a C-^{2}H bond, $\beta = 60°$, undergoing rotational diffusion about an axis of motional narrowing. E) a C-^{2}H bond undergoing fast isotropic motion ($\tau_c \propto 1/\Delta\nu_Q$).

0.05 (Figure 1A), and a ring undergoing fast rotational diffusion (Figure 1D), for which the quadrupolar splitting is 17 kHz. An isotropically moving C-^{2}H bond will give rise to the single narrowed line shown in Figure 1E.

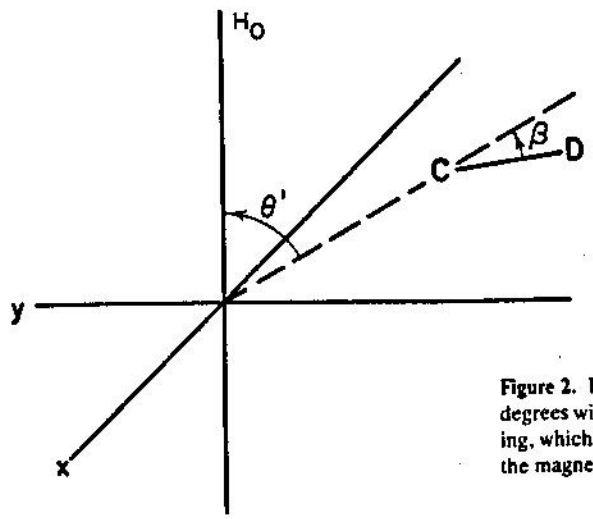


Figure 2. Illustration of a C-^{2}H bond inclined at β degrees with respect to an axis of motional averaging, which is inclined at θ' degrees with respect to the magnetic field direction.

Deuterium NMR of Amino-Acid Sidechains: Static Aspects

Nature has made use of a large variety of chemical functional groups to devise amino acid sidechains that encompass both close relationships and marked contrasts. We are elucidating the similarities and differences in the dynamics of these various amino acid sidechains in crystals and in membrane proteins. The results so far are summarized in Figure 3 and typical spectra are presented in Figures 4 and 5.

[$^2H_2$] glycine in the crystalline state (Figure 4A) and in the purple membrane (Figure 5A) displays a "rigid" splitting of ~ 126 kHz. Although we will refer to glycine and other amino acids as "rigid", we do so in the sense that there are no large angle fluctuations at a rate faster than $10^5 s^{-1}$ and also bearing in mind that there may also be fast small angle librational motions ($\sim \pm 5\text{-}10^\circ$) of the polypeptide chain. There is a large central component in the [$^2H_2$] glycine-labelled purple membrane spectrum contributing to 35% of the intensity. Recent evidence[20,21] has shown that the 21 residue C-terminal fragment of bacteriorhodopsin has considerable freedom of movement, and it is these C-terminal glycine residues that contribute in large part to the intensity of the central component.

Alanine is a metabolically labile amino acid within *H. halobium*[22] and considerable transfer of the deuterium label to other amino acids occurs. For this reason, no spectrum of [β-$^2H_3$] alanine-labelled purple membrane is shown. However, crystalline [β-$^2H_3$] alanine has properties not seen in other amino acids. At 37°C the spectrum (Figure 4B) is that expected for a three-fold methyl jump, however, the jump rate slows so dramatically at temperatures below −133°C (Figure 4C), that the methyl rotor becomes "rigid" on the deuterium timescale. At any given temperature the rate of methyl rotation is slower in alanine than in any other methyl-containing amino acid.[8] This is due presumably to close methyl-methyl contacts and possibly to steric hindrance from the amino acid polar residues.

Valine may be ^2H-labelled at all sites in its sidechain by simple and inexpensive syntheses.[6,23] For this reason we have been able to analyze the motion of each segment in the valine sidechain. DL[α-$^2H_1$] valine is "rigid" in both the crystalline state (Figure 4D) and in the purple membrane (Figure 5B). Virtually identical spectra are found for DL[β-$^2H_1$] valine (Figure 4E, crystalline amino acid and Figure 5C, incorporated into bacteriorhodopsin). The γ-deuterons, however, are undergoing fast three-site jumps due to methyl rotation, as evidenced from the reduction of the quadrupolar splitting from 126 kHz to 40 kHz (Figure 4F, crystalline amino acid and Figure 5D, bacteriorhodopsin). The immobility of the α-deutrons of glycine is a reflection of the immobility of the polypeptide backbone, but the immobility of the β deuteron for valine is a reflection of tight packing within the membrane. Steric hindrance is such that dimethyl substitution of the β-carbon prevents any substantial motion about the C^α-C^β bond. The ^2H spin-lattice relaxation time of the β deuteron is, however, shorter than that of the α deuteron,[24] indicating there may be some small angle motions about the C^α-C^β bond which have, however, little effect on the quadrupolar splitting.

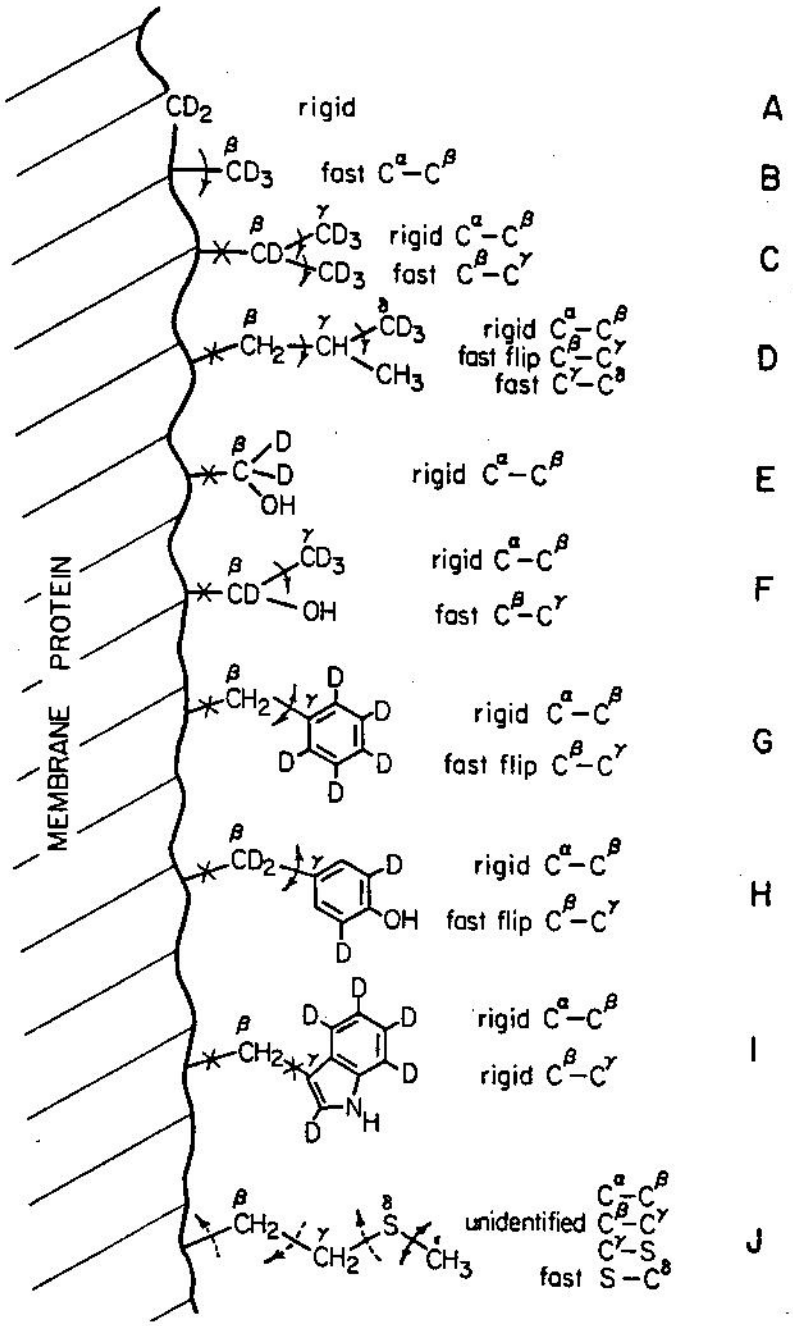


Figure 3. A summary of the motions undergone by Gly, Ala, Val, Leu, Ser, Thr, Phe, Tyr, Trp and Met in polycrystalline amino acids and in bacteriorhodopsin.

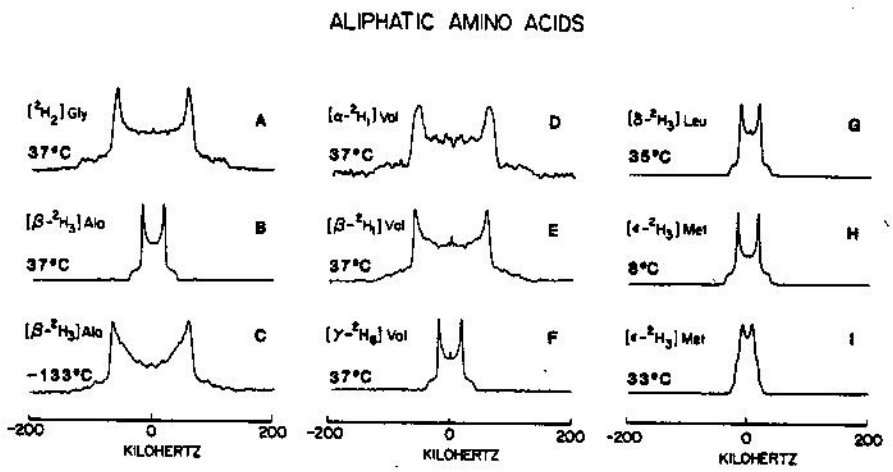


Figure 4. Deuterium NMR spectra of polycrystalline, aliphatic amino acids. A) [$^2H_2$]glycine, 37°C. B) L[β-$^2H_3$]alanine, 37°C. C) L[β-$^2H_3$]alanine, −133°C. D) DL[α-$^2H_1$]valine, 37°C. E) DL[β-$^2H_1$]valine, 37°C. F) DL[γ-$^2H_6$]valine, 37°C. G) L[δ-$^2H_3$]leucine, 35°C. H) L[ϵ-$^2H_3$]methionine, 8°C. I) L[ϵ-$^2H_3$]methionine, 33°C.

Interestingly, the addition of a single methylene segment to the hydrocarbon sidechain to produce leucine modifies the lattice packing enough to permit not only simple three-site methyl jumps but also two-fold jumps about the C^{β}-C^{γ} bond.[8,25] This is evident from the reduced quadrupolar splitting (33 kHz) in the polycrystal-

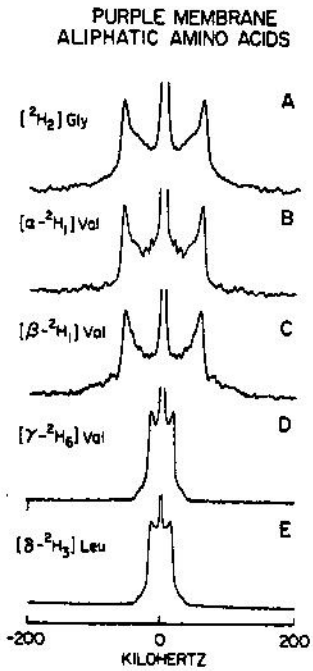


Figure 5. Deuterium NMR spectra at 37°C of aliphatic amino acids incorporated into the purple membrane of *H. halobium*. A) [$^2H_2$]glycine-labelled purple membrane. B) [α-$^2H_1$]valine-labelled purple membrane. C) [β-$^2H_1$]valine-labelled purple membrane. D) [γ-$^2H_6$]valine-labelled purple membrane. E) [δ-$^2H_3$]leucine-labelled purple membrane.

line form of L[δ-$^2H_3$] leucine (Figure 4G), and the even smaller value (31 kHz) found in leucine-labelled purple membrane (Figure 5E). The reduction in the quadrupolar splitting and the increase in the asymmetry parameter (at any given temperature above 0°C) for the leucine-labelled purple membrane is larger than for the crystalline amino acid.[8] This was also found when [$^2H_{10}$] leucine-labelled collagen was compared to the crystalline amino acid.[25] The spectral lineshape variations show that the jump rate about the C^β-C^γ bond at a given temperature is faster in collagen and the purple membrane than in crystalline leucine.[8,25]

The effects of a large heteroatom, such as sulfur, and a long, unbranched chain are evident in the spectra of crystalline L[ϵ-$^2H_3$] methionine (Figures 4H and 4I). Below 8°C the spectrum is that expected for a freely rotating methyl group with no other fast large amplitude motions occuring at preceding bonds. Above 20°C there is substantial narrowing of the spectrum and an increase in the asymmetry parameter, indicating that a large amplitude motion(s) about the C^α-C^β, C^β-C^γ or C^γ-S bonds is beginning to narrow the spectrum. However, we have been unable to simulate the 33°C spectrum to our satisfaction. This could be due to different crystalline forms of the L amino acid which give a composite spectrum, or the motion above 20°C may be more complicated than we have assumed. For example, it could involve jumping between two or more unequally populated sites.

We have synthesized an L[α-$^2H_1$] methionine[26] and have found that its spectrum is not narrowed by whole body motions of the molecules. Moreover when L[ϵ-$^2H_3$] methionine is incorporated into a D,L lattice by co-crystallizing with unlabelled D-methionine, there is no sign at temperatures up to 50°C of the high temperature motions found in the L lattice (Figure 4I). We have, at this time, been unable to incorporate methionine into *H. halobium* without considerable transfer of the label to other amino acids: consequently no spectrum of methionine-labelled purple membrane is presented.

Hydroxy amino acids may have special significance in a proton transport protein such as bacteriorhodopsin.[27] A chain of hydrogen-bonded residues spiralling along the transmembrane pore of bacteriorhodopsin, with retinal forming the light-dependent gate, could conceivably be the route along which protons travel in response to a light impulse.[28,29] With this in mind, we have sought and found evidence for a considerable degree of immobility (hydrogen bonding) in purple membrane labelled with |β-$^2H_2$| serine with |β,γ-$^2H_4$| threonine.

The spectrum of polycrystalline DL[β-$^2H_2$] serine is that expected for a rigid lattice (Figure 6A). Unlike other methyl-like entities, the CD_2OH group is unable to rotate fast enough for motional averaging, even up to 70°C. The most feasible explanation for this is intermolecular hydrogen bonding. Threonine is similar to valine. The γ-methyl deuterons are rotationally narrowed but the β deuteron displays the rigid lattice quadrupolar splitting of 122 kHz (Figure 6B). The immobility of the C^α-C^β bond in threonine could be the result of steric hindrance at the multiply substituted β-carbon, as seen in valine, or through intermolecular hydrogen bonding of the β-hydroxyl.

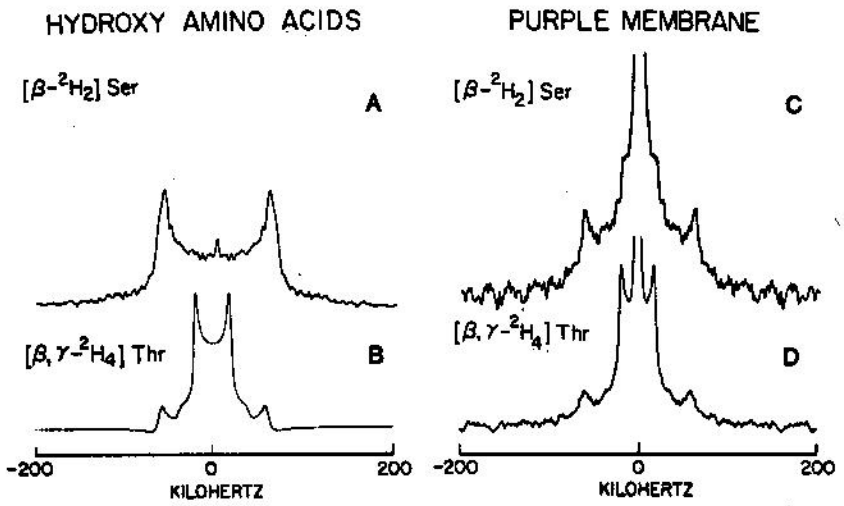


Figure 6. Deuterium NMR spectra of polycrystalline hydroxy amino acids and of hydroxy amino acids incorporated into the purple membrane of *H. halobium*. A) DL[β-$^2H_2$]serine, 37°C. B) DL[β,γ-$^2H_4$]threonine, 37°C. C) [β-$^2H_2$]serine-labelled purple membrane, 23°C. D) [β,γ-$^2H_4$]threonine-labelled purple membrane, 37°C.

The spectrum of serine incorporated into purple membrane (Figure 6C) is complicated by some modification to other amino acids, as revealed by radiotracer experiments.[22] Approximately 20 ± 5% of the β-label is transferred to alanine, which probably accounts for a large part of the intensity of the 38 kHz component seen in Figure 6C. However, the 38 kHz component is small compared to the intensity of the 126 kHz component, so we conclude that for most serine residues (>70%) in the α-helical sections of the polypeptide chain there is no rotation about the C^{α}-C^{β} bond. Those serine and alanine residues in the mobile part of the polypeptide chain may contribute to the central component. Intermolecular hydrogen bonding appears to be the most feasible explanation of the immobility of most of the β-deuterons of serine. Purple membrane labelled with [β,γ-$^2H_4$] threonine (Figure 6D) shows the same trend of immobility seen for the crystalline amino acid and serine, i. e., the only fast motion is methyl rotation.

Aromatic sidechains, because of their bulk, will either be immobile or undergo motions that are allowed by their planar structure. Examination of the spectra in Figure 7, for 20° and 37°C, reveals the presence of some familiar lineshapes, e. g., Figures 7C and 7F for tryptophan in the polycrystalline form and in the purple membrane, and some unusual spectra, e. g., Figure 7D, that of phenylalanine in the purple membrane. The spectra of [$\delta_1,\epsilon_3,\zeta_2,\zeta_3,\eta_2$-$^2H_5$] tryptophan in the polycrystalline form (Figure 7C) and incorporated into the purple membrane (Figure 7F), at 20 and 37°C are essentially the same. Both spectra are simulated well with $\Delta\nu_Q$ ~ 134 kHz and $\eta = 0.05$, the parameters expected for tryptophan in a rigid lattice (except for small-angle torsional or librational motions). These results for tryptophan are not unexpected and are consistent with NMR results for water-soluble globular proteins.[30-32]

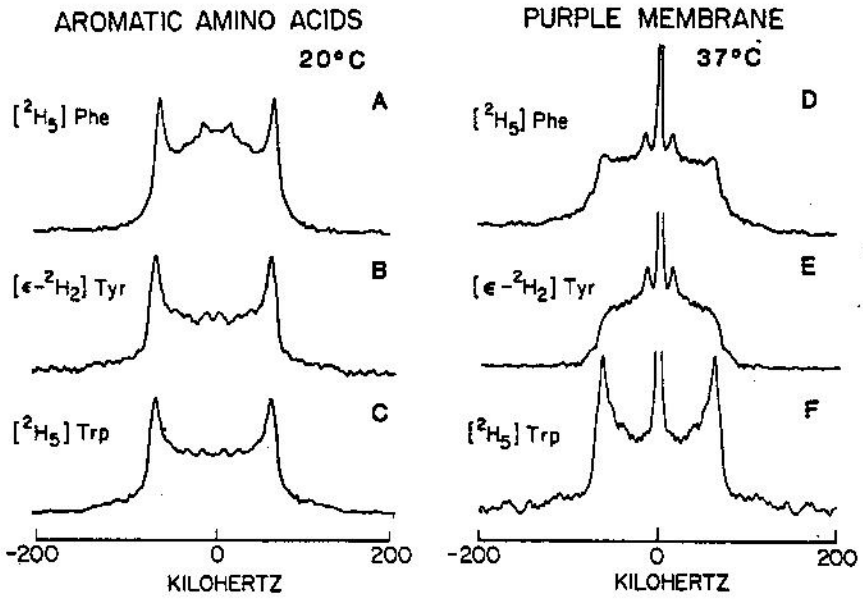


Figure 7. Deuterium NMR spectra of polycrystalline aromatic amino acids at 20°C and of aromatic amino acids incorporated into the purple membrane of *H. halobium* at 37°C. A) $[\delta_1,\delta_2,\epsilon_1,\epsilon_2,\zeta\text{-}^2H_5]$phenylalanine. B) $[\epsilon_1,\epsilon_2\text{-}^2H_2]$tyrosine. C) $[\delta_1,\epsilon_3,\zeta_2,\zeta_3,\eta_2\text{-}^2H_5]$tryptophan. D) $[\delta_1,\delta_2,\epsilon_1,\epsilon_2,\zeta\text{-}^2H_5]$ phenylalanine-labelled purple membrane. E) $[\epsilon_1,\epsilon_2\text{-}^2H_2]$tyrosine-labelled purple membrane. F) $[\delta_1,\epsilon_3,\zeta_2,\zeta_3,\eta_2\text{-}^2H_5]$ tryptophan-labelled purple membrane.

The spectra of $[\delta_1,\delta_2,\epsilon_1,\epsilon_2,\zeta\text{-}^2H_5]$ phenylalanine (Figure 7D) and $[\epsilon_1,\epsilon_2\text{-}^2H_2]$ tyrosine (Figure 7E) in the purple membrane have the appearance expected for "flipping" phenyl groups. Even polycrystalline zwitterionic $[^2H_5]$ phenylalanine (Figure 7A) appears to have some flipping component, however, polycrystalline $[^2H_2]$ tyrosine is rigid (Figure 7B). Indeed, the spectra of $[^2H_5]$ phenylalanine in purple membrane may be simulated assuming 80% of the spectrum has an asymmetry parameter η_{eff} = 0.6 with a splitting between the singularities of 30 kHz. The other 20% of the spectrum is a "rigid spectrum" with $\Delta\nu_Q$ = 130 kHz arising from the $C^\zeta\text{-}^2H$ bond, which lies along the axis of motional narrowing and is thus not affected by the two-site jump motion. Separate experiments with a $\zeta\text{-}^2H_1$ labelled phenylalanine have confirmed that the axis of motional averaging does, indeed, lie along the $C^\zeta\text{-}^2H$ bond. Although 13 ± 2 of the 13 phenylalanine residues are flipping rapidly at 37°C, this is not the case at lower temperatures. Below −25°C all the residues are undergoing slow motions ($< 10^4\ s^{-1}$).[7] Similarly for $[^2H_2]$ tyrosine-labelled purple membrane (Figure 7E) the spectrum may be simulated with an asymmetry parameter $\eta_{eff} \sim 0.6$ and calculations show 9 ± 2 of the 11 tyrosines are flipping rapidly at 37°C. At temperatures below −25°C all rapid flipping motion has stopped.

Polycrystalline, zwitterionic $[^2H_5]$ phenylalanine (Figure 7A) exhibits a composite spectrum which is best simulated assuming 34% rigid spectrum (i.e. η = 0.05 and $\Delta\nu_Q$ = 130 kHz) and 66% flipping spectrum (i.e. $\eta_{eff} \sim 0.6$ and $\Delta\nu_Q$ = 80 kHz). When one takes into account the contribution of the ζ deuteron, it is found that

80% of the phenylalanine rings are flipping and 20% are immobile. Interestingly, the crystalline hydrochloride form of this amino acid exhibits a rigid ^2H spectrum, emphasizing the importance of lattice effects on amino-acid sidechain motion.

A picture of amino-acid dynamics in the integral membrane protein is now evolving. The "rigidity" of the α-deuterons of glycine and valine reflect the relative immobility of the polypeptide chain, except perhaps for some surface residues, especially in the C-terminus region. Our impression of the environment of the amino acid sidechains is one of compactness, without being totally immobile. The valine C^{α}-C^{β} bond is immobile, yet the analogous C^{β}-C^{γ} bond of leucine undergoes two-fold flips. A similar situation is found for aromatic rings: the very bulky tryptophan ring is immobile up to 80°C, however tyrosine and phenylalanine rings become increasingly mobile above 0°C, also undergoing a two-fold flip process. The hydroxy amino acids, although immobile under the conditions used in these experiments, may undergo very rapid, small angle movements in response to light-induced proton transfer. One can easily imagine the making and breaking of hydrogen bonds, causing conformational changes sufficient to expose previously buried amino acids to the pore of bacteriorhodopsin, and in this way propagating the transfer of the proton.[29] Perhaps even transient charge-transfer interactions with rapidly rotating benzene rings may be involved in the H^+-transport!

We complete this section with some general observations and comments on the spectra arising from labelled purple membrane. These spectra are invariably broader and have slightly smaller quadrupolar splittings than their amino acid counterparts. A slight difference in the quadrupolar splitting or a small change in the asymmetry parameter does not necessarily indicate a change in the motional properties of the C-^2H bond vector since peptide bond formation, especially for C-^2H bonds near the backbone, or hydrogen bond interactions may cause a change in the quadrupolar coupling constant. It would be unrealistic to expect a particular amino acid distributed at different sites along a polypeptide backbone, with varied secondary and tertiary structure, to experience the relatively uniform environments found in crystals. A range of closely spaced quadrupolar splittings resulting from local variations of the electric field gradient, and localized small angle librational motions of the polypeptide chain may satisfactorily explain the lineshapes of membrane ^2H spectra.

Amino Acid Dynamics

^2H magnetic resonance may also be used to obtain detailed dynamical information, through the use of spin-lattice relaxation times, provided a mathematical model is available. We have used a model that relates the angular dependent spin-lattice (T_1) relaxation time to a three-fold jump rate for a methyl group hopping between three equivalent sites.[9] If one uses the intensity at the major singularity where the angles of $\theta = 90°$ and 35.3° contribute, then for a methyl group on the "extreme narrowing" side of the T_1 versus correlation time (τ_c) curve

$$1/T_{1(obs)} = 0.55\,\omega_Q{}^2\tau_c \qquad (3)$$

Table 1

Experimental Deuterium NMR Quadrupolar Splittings (QS), Spin-Lattice Relaxation Times (T_1), and Computed Correlation Times (τ_c) for Methyl Rotation in Polycrystalline L[β-$^2H_3$] Alanine, DL[γ-$^2H_6$] Valine, DL[β,γ-$^2H_4$] Threonine and L[δ-$^2H_3$] Leucine, as a Function of Temperature.[a]

L[β-$^2H_3$]Alanine[b]				DL[γ-$^2H_6$]Valine[b]				DL[β,γ-$^2H_4$]Threonine[c]				L[δ-$^2H_3$]Leucine			
Temp (°C)	QS[d] (kHz)	T_1 (msec)	τ_c (psec)	Temp (°C)	QS[d] (kHz)	T_1 (msec)	τ_c (psec)	Temp (°C)	QS[d] (kHz)	T_1 (msec)	τ_c (psec)	Temp (°C)	QS[d] (kHz)	T_1 (msec)	τ_c (psec)
70	40	8.4	335	37	38	29.3	99	37	39	70.6	40	52	31	84.4[b]	34
52	40	5.6	520	22	39	19.9	140	26	39	52.7	53	35	33	76.3[c]	38
38	40	3.9	780	−20	39	7.7	370	− 1	39	28.0	100	16	34	43.7[b]	65
26	40	3.1	1030	−42	40	4.7	620	−29	39	10.4	281	0	35	35.6[c]	83
18	40	2.3	1510					−44	39	6.9	432	−30	36.5	13.0[b]	230
− 7	40	2.3	4000												
−28	40	4.6	14000												
−46	40	8.8	28000												

[a] T_1 determinations were made using an inversion-recovery quadrupole-echo sequence. T_1 and τ_c accuracy is about ±10-20%.
[b] Data obtained at 5.0 Tesla, corresponding to a ^2H resonance frequency of 33.9 MHz.
[c] Data obtained at 8.45 Tesla, corresponding to a ^2H resonance frequency of 55.3 MHz.
[d] Obtained from a spectral simulation; error is about ±2.5%.

and on the "slow" side of the curve

$$1/T_{1(obs)} = 0.13\ \omega_Q^2 \left\{ \frac{1.06\tau_c}{(1+\omega^2\tau_c^2)} + \frac{1.46\tau_c}{(1+4\omega^2\tau_c^2)} \right\} \quad (4)$$

where $T_{1(obs)}$ is the observed spin-lattice relaxation time, τ_c = (3 x jump rate)$^{-1}$, and $\omega_Q = 3e^2qQ/4\hbar$.

As a basis for understanding the dynamics of amino acid sidechains within membrane proteins, we first present the relaxation data for several polycrystalline amino acids in Table I, where correlation times are calculated from Equation 3. It is seen that the jump rate for the methyl group varies widely among these amino acids. For example, the correlation times for Ala, Val, Thr, Leu, S-methyl Met and Met at 37°C decrease by a factor of 200 as the sidechain length increases (Figure 8). It is apparent that there is a considerable intramolecular barrier to methyl rotation.

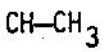
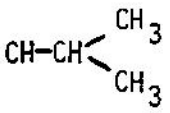
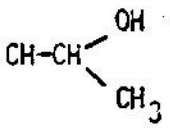
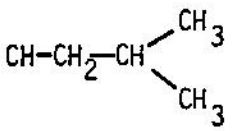
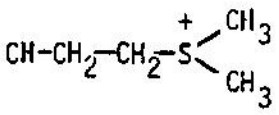
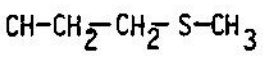
	T_1(msec)	T_c(psec)
	4.8	~800
	29.3	~100
	70.6	~40
	76	~40
	51	52
	800	~5

Figure 8. The dependence of the rotational correlation time for a methyl group upon amino acid sidechain structure, at 37°C.

The very long correlation time of alanine is a result of a large barrier to methyl rotation caused, we believe, both by the close proximity to the $-CO_2^-$ and NH_3^+ substituents, and the presence of Me-Me contacts in the crystal.[33] The low barrier to methyl rotation for methionine is the result of direct bonding to sulfur, and the consequent lack of any substantial intramolecular barriers. *S-methyl* methionine, which has an extra methyl substituent on the sulfur atom, has a much shorter T_1 (51 msec at 37°) and a longer correlation time (52 ps), both values being similar to those observed for the other "isopropyl" containing amino acids valine and leucine. For both alanine and methionine there is a direct correlation between this quantitative dynamical data, and the temperature dependent changes in the lineshapes discussed above.

Intermolecular interactions may also have a considerable effect on the relaxation times. The most dramatic effect is seen upon changing the crystal lattice of methionine, at temperatures above −40°C. In the Arrhenius plot (Figure 9) of [ϵ-$^2H_3$] methionine at temperatures below −40°C, both the L form and the L amino acid in a D,L lattice have a common behavior. Above −40°C, the Arrhenius curve for L[ϵ-$^2H_3$] methionine deviates from linear behavior at the onset of additional sidechain motion. Without further experimentation, using e. g., ^2H, ^{13}C and ^{33}S labelling (experiments in progress), it is not possible to determine the exact nature of this additional motion. The results, however, suggest that methionine may be one of the

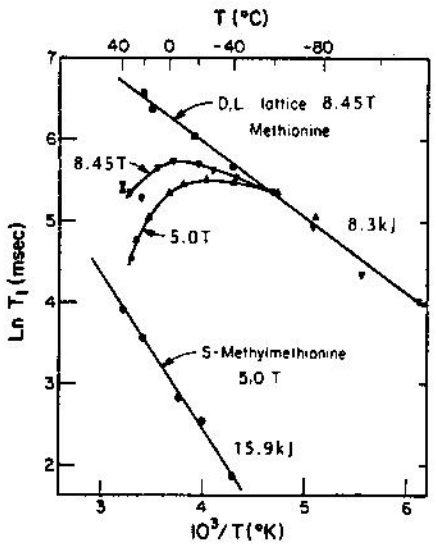


Figure 9. The temperature dependence of the ^2H spin-lattice relaxation time (in milliseconds) for L[ϵ-$^2H_3$]methionine at 8.45 Tesla (▼) and 5.0 Tesla (▲), for L[ϵ-$^2H_3$]methionine in a D,L lattice at 8.45 Tesla (■) and [*S-methyl*-$^2H_3$]methionine at 5.0 Tesla (●).

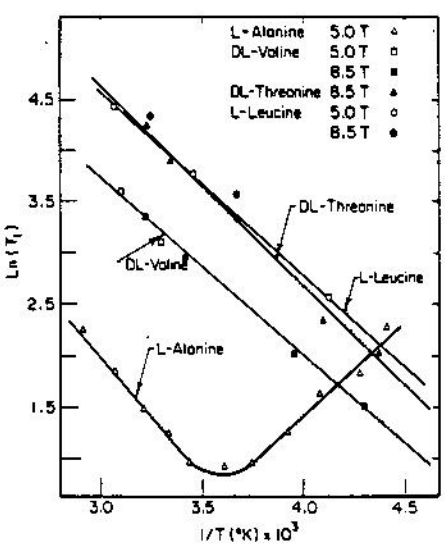


Figure 10. The temperature dependence of the ^2H spin-lattice relaxation time for alanine at 5.0 Tesla (△), valine at 5.0 Tesla (□), valine at 8.45 Tesla (■), threonine at 8.45 Tesla (▲), leucine at 5.0 Tesla (○), leucine at 8.45 Tesla (●).

more interesting residues in membrane proteins, and that differences in crystal packing may result in dramatic changes in ^2H NMR spectra.

The temperature dependence of T_1 of the other methyl-containing amino acids is less complicated (Figure 10). All of the curves are linear, except for alanine which has a T_1 minimum at ~0°C. The results indicate that rapid methyl three fold jumps overwhelmingly dominate deuteron spin-lattice relaxation in these systems. The two-fold jump about the C^{β}-C^{γ} bond that narrows the lineshape of polycrystalline leucine is too slow to contribute significantly to the T_1 relaxation.

Protein Dynamics

Of the methyl-containing amino acids, we have been successful in incorporating only deuterated valine, leucine and threonine into the purple membrane of *H. halobium* without considerable transfer of the ^2H-label to other amino acids. The spin-lattice relaxation times of the methyl group of each of these amino acids have been analyzed using the motional model described above. In the temperature range studied all the Arrhenius curves are linear, with the exception of [β,γ-$^2H_4$] threonine labelled purple membrane, which shows a slight curvature. On lyophilization of this membrane, the curvature disappears but the activation energy also changes. On rehydration of the membrane the activation energy approaches its previous value but the curvature in the Arrhenius curve is not restored. The origin of this behavior is not clear. It may be caused by a distribution of correlation times or the presence

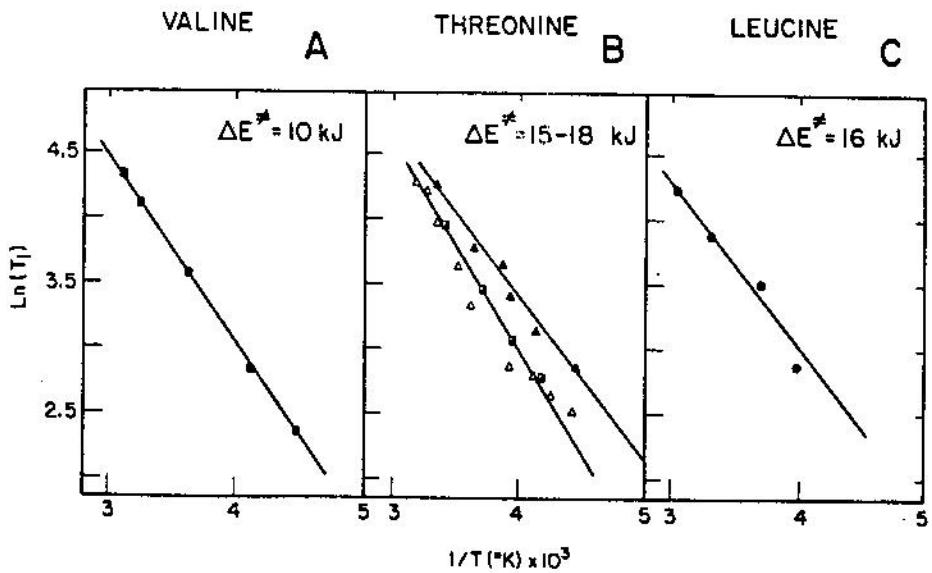


Figure 11. The temperature dependence of the ^2H spin-lattice relaxation time for A) [γ-$^2H_6$] valine-labelled purple membrane. B) [β,γ-$^2H_4$] threonine-labelled purple membrane: (△) native, (▲) lyophilized. (◪) rehydrated. C) [δ-$^2H_3$] leucine-labelled purple membrane.

of a T_1 minimum. Alternatively, freezing of the water in the membrane could cause structural changes and a redistribution of hydrogen bonds.

We have also biosynthetically incorporated [γ-$^2H_6$] valine and [δ-$^2H_3$] leucine into an *E. coli* fatty acid auxotroph enriched with either elaidic acid or oleic acid. The elaidate enriched auxotroph has a broad thermal transition temperature centered around 35°C, and the oleate-enriched auxotroph has a sharper transition centered around 22°C. Despite an observable change in the transition temperature of the *E. coli* membranes, we observe no significant change in the correlation times or the activation energies for [γ-$^2H_6$] valine-labelled membranes. Valine, however, is a short chain amino acid which may not be affected by changes in the fluidity of the membrane. Longer chain amino acids, such as lysine, may be more sensitive to changes in membrane fluidity, as may small proteins, with large surface area/volume ratios, in general.

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