SOLID STATE NMR: MULTINUCLEAR APPROACH TO PROTEINS AND NUCLEIC ACIDS

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INTRODUCTION

DURING the last twenty five years, high resolution NMR has become one of the most powerful tools available for the elucidation of molecular structure and dynamics. The inherent advantage of the technique is that both solids and liquids can be probed, unlike the X-ray and neutron scattering methods which are useful for solid state. The resolution achieved by the recently developed high resolution solid state NMR techniques makes it possible to compare structures in two different states, based on the results obtained with the same technique. The solid state NMR thus bridges the information gap between the solution (NMR, ESR) and solid state structures (x-ray, neutron diffraction).

The primary obstacle to study NMR of solids arises from the broad, featureless lines of typically 10–50 KHz width which masks the informative chemical shift and scalar coupling data. This difference in behaviour compared to NMR in solution arises due to (i) the large dipolar interactions which lead to broad resonances (ii) the chemical shift anisotropy which for powders leads to a spread of resonance requencies even when dipolar broadenings are eliminated and (iii) the long spin-lattice relaxation times ($\sim$ 100s) in solids which necessitate a long inter-pulse time in the FT averaging mode, thus lowering the sensitivity. Rapid molecular tumbling in non-viscous solutions, averages the dipolar interactions to zero, enabling access to the isotropic chemical shift and coupling constant data. Methods are now available to overcome each of these problems in solids by artificially imposing motions on the nuclei either in the laboratory frame by magic angle spinning (MAS) or in the nuclear spin space with multipulse techniques.

(*i*) *Dipolar broadening*[1]: The dipolar interaction Hamiltonian for the nuclear pair i, j can be expressed as

$$\mathcal{H}_D = \left[\gamma_i \, \gamma_j \, \hbar^2 \frac{1}{r_{ij}^3} \left(I_i \, I_j - 3 I_{iz} \, I_{jz} \right) \times \left(3 \cos^2 \theta_{ij} - 1 \right) \right]$$

γ_i and γ_j are the nuclear gyromagnetic ratios, r_{ij} is the internuclear distance and θ_{ij} is the angle between r_{ij} and the static Zeeman field H_0, directed along the z-axis in the laboratory frame. The heteronuclear dipolar broadening, important for the less abundant nuclei (^{13}C, ^{15}N) can be eliminated by the high power gated irradiation of the abundant nuclei (^1H). The homonuclear dipolar broadening in the case of the abundant nuclei (^1H–^1H) can be supressed by reducing the spin operator function ($I_iJ_z - 3I_{iz}I_{zj}$) in equation (1) to zero with multipulse techniques[2]. Here the nuclei are subjected to a repeated sequence of strong resonant radio frequency pulses of a predetermined phase and spacing, thus imposing a time dependence of the nuclear perturbation. These averaging methods can handle large magnitudes of homonuclear dipolar interactions ($\sim$100 KHz) and being selective, allow simultaneous measurements of chemical shift tensors of abundant spins in solids.

(*ii*) *Chemical shift anisotropy (CSA)*: The chemical shielding tensor (σ) gives fundamental information regarding the local symmetry of the electronic charge distribution in a chemical bond and is characterised completely by the three principal elements σ_{11}, σ_{22} and σ_{33} and the direction of the three principal axes. In polycrystalline samples, the random orientation of the chemical bonds with respect to the applied field results in a superposition of all the resonance frequencies leading to the broad asymmetric powder pattern (figure 1a) having $\sigma_{11} \neq \sigma_{22} \neq \sigma_{33}$. In the presence of rapid axial motion *i.e.*, motions fast compared to $| \sigma_{33} - \sigma_{11} |$ an axially symmetric powder pattern (figure 1b) results and the sign of
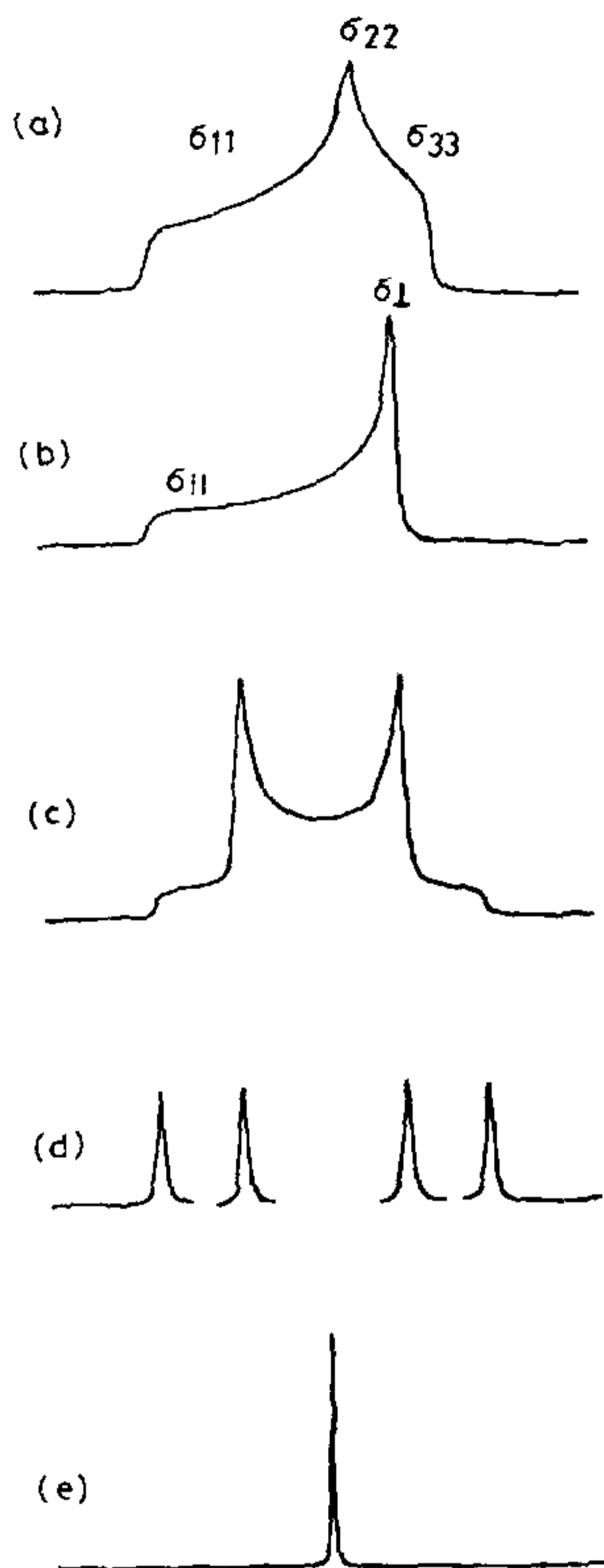
Figure 1. Lineshapes (reference 19b), (a) axially asymmetric CSA (b) axially symmetric CSA (c) axially symmetric $(I = 1)$ ^2H NMR (d) oriented sample ^2H NMR and (e) solution NMR.

the anisotropy depends on the chemical nature of the group and the motions involved. From the polycrystalline spectra, only three principal elements can be determined but not the direction of the principal axes which is obtained only from NMR studies on single crystals. Since the shielding interaction is a second-rank tensor, rapid spinning about an axis inclined by angle θ to the static magnetic field H_0, gives an average spread of chemical shifts reduced by a factor $1/2$ $(3\cos^2\theta - 1)$ and at the magic angle $\theta = 54.7°$, the spread is totally eliminated[3]. In practice, rotation rates upto 10 KHz have been achieved

and when the spinning rate is less than the chemical shift spread due to shielding anisotropy, rotational side bands[4] result in the spectrum. These can be eliminated by synchronizing the spinning speed with data acquisition and this technique is of great value at higher magnetic fields in superconducting solenoids where the practical spining speeds are unable to supress the sidebands. High resolution in solids without the loss of CSA is possible when spinning is synchronized with a variety of coordinating radio-frequency pulses. A further bonus of the magic angle spinning is the averaging of dipolar interactions to zero and the resulting linewidths may be as small as those for solutions and depend on the inhomogeneities in H_0.

(iii) Cross polarization (CP): The long-lattice relaxation times in solid state cause a bottleneck in the time-averaging improvement of signal to noise ratio in the FT mode. In the cross polarization technique[5], the macroscopic magnetization of the rare spins (^{13}C) is generated by a mechanism which is not dependent on its spin-lattice relaxation. The method involves cooling of the rare spins which are at a high temperature by effecting thermal contact in the rotating frame with the abundant spins (^1H) at low spin temperature, resulting in a transer of spin polarization from the abundant to rare spins *via* heteronuclear dipolar interactions (figure 2). Since CP involves no spin-lattice process directly, signal enhancements depending on nuclear Overhauser enhancements (nOe) are not observed and the
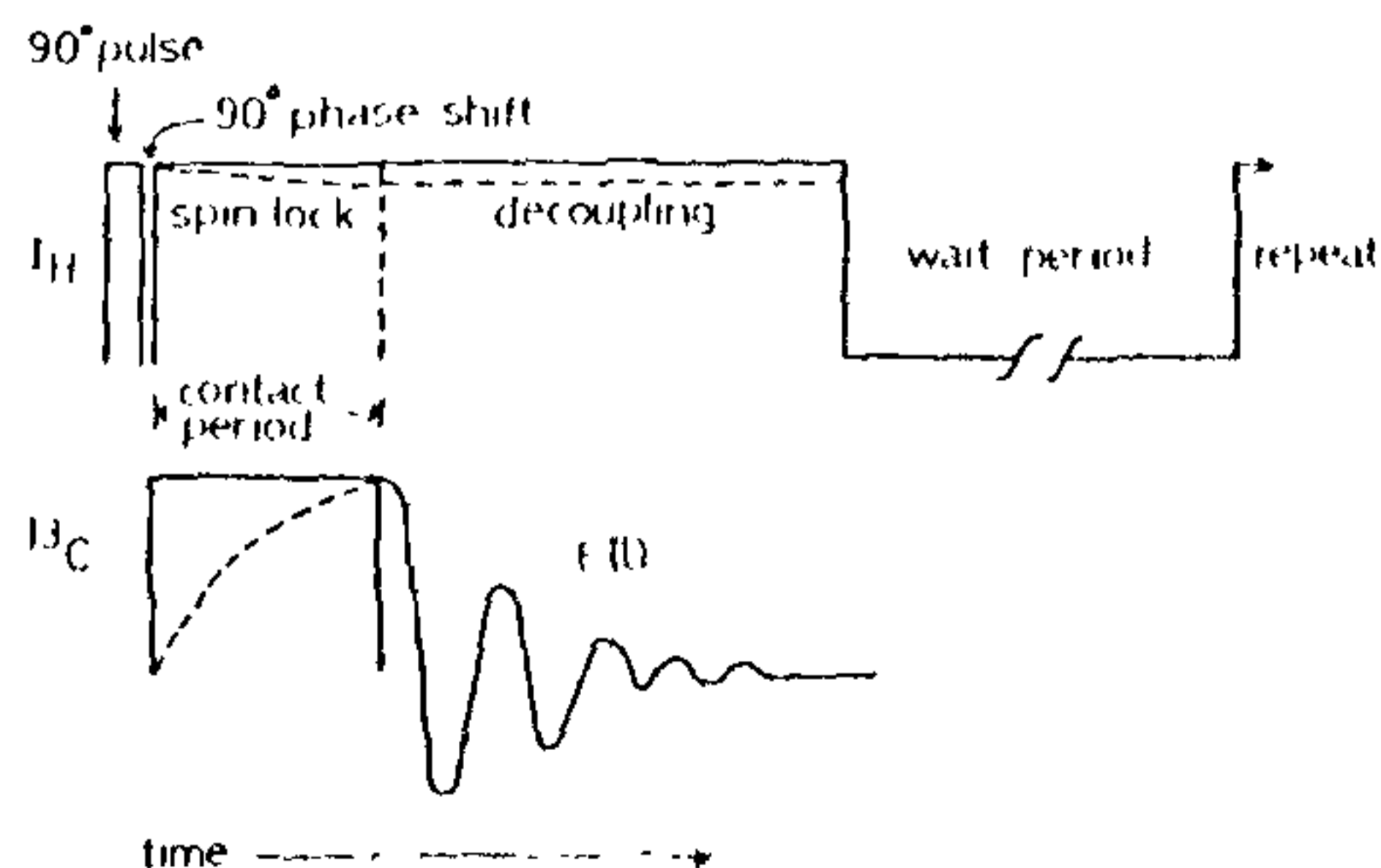

Figure 2. Cross polarization experiment.

optimum magnetization, following a single spin lock CP tranfser is γ_I/γ_S which is about 4 for ^{13}C; this is greater than the magnetization that would be observed in a fully relaxed FT experiment with gated dipolar decoupling. Variations of the original Pines sequence include multiple contact pulses, ADRF spin locking[6] and separated local field spectroscopy[7].

A serial extension to the CP experiment is the double-cross polarization[8] (DCP) to study three hetero spin systems (^{1}H, ^{13}C, ^{15}N), where the initial transfer of polarization from ^{1}H to either ^{13}C or ^{15}N is followed by turning off the proton channel and matching the spin lock contact of carbons and nitrogens: either ^{13}C or ^{15}N is then observed in DCP spectra with higher sensitivities. In the case of ^{15}N, cross polarization not only enhances the sensitivity of ^{15}N but also avoids the negative nOe's which otherwise can completely eliminate the ^{15}N signals.

(*iv*) *Quadrupolar interactions*[1]: For nuclei, with spin greater than $1/2$ (^{2}H, ^{14}N) quadrupolar interactions, arising from the non-spherical nuclear charge distribution leads to the broadening of the order of 100 KHz. The quadrupole splitting of the absorption line is given by

$$\Delta \nu = \frac{3e^2qQ}{2h} \cdot \frac{3\cos^2\theta - 1}{4}$$

where θ defines the angle subtended by the magnetic field H_0 with the principal axis of the electric field gradient tensor. For rigid polycrystalline solids, all values of θ are possible leading to the powder pattern with peak separations for value of $\theta = 90°$, ($\Delta \nu = 3e^2qQ/4h$) and shoulder separations for $\theta = 0°$ ($\Delta \nu = 3e^2qQ/2h$) (figure 1c and 1d).

APPLICATIONS

(*i*) *Carbon* (^{13}C): Some features peculiar to solid state NMR have been noticed in the ^{13}C NMR spectra of solid amino acids[9]. The carbons directly bonded to nitrogen are split into broad

asymmetric doublets for example, glycine, alanine, and valine show splittings of about 100 Hz. The source of splitting is the quadrupole moment of adjacent ^{14}N since it disappears in the ^{15}N labeled amino acids[8]. In the case of peptides, both C^c and $C=$ carbons are split, though to different extents, the magnitude of splitting being a potential source of geometric information about the peptide bond[9,10]. Large differences in chemical shifts of side chain aliphatic groups are observed in ^{13}C NMR of solid valine, attributed to the frozen internal rotations of these groups in solid state, compared to the partial averaging in solution. The splittings of the aromatic carbons in tyrosine have been ascribed to the effect of different substitutent groups and an asymmetric conformation of the ring in solid state. The presence of different crystals forms in a powder sample may also lead to the multiplicity of lines as in the case of leucine where recrystallization simplifies the spectra. The complete analyses of ^{13}C chemical shielding tensors in single crystals of glycine and alanine are now available[11] and from the [13]C—[13]C, [13]C—[14]N dipolar splittings, the C—C, C—N bond lengths and C—C—N bond angles may be determined. In the case of solid cyclic polypeptides structure

Cyclic (D-Phe-Pro-Gly-D-Ala-Pro)   (I)
Cyclic (Gly-L-Pro-Gly)[2]   (II)

two conformationally distinct prolines involved in β (4→1) and γ (3→1) turns have been reconised, based on the C^β chemical shifts of the proline residues[12]. A simple modification of the cross polarization experiment[13], allows observation of non-protonated carbons. Here the proton decoupler is turned off very briefly before FID acquisition and the protonated carbons instantaneously broaden, leaving sharper resonances of quarternary carbons. The utility of this method has been demonstrated in the assignment of ^{13}C resonances of non-protonated ring carbons of aromatic amino acids in solid fd coat protein, both in free and in virus forms[14]. In [13]C—[1]H magnetic double resonance experiment, judicious choice of the contact times during cross polarization, and comparison with the corresponding intensities in the scalar decoupled spectrum allows the mobile ($\tau < 10^{-6}$ s) and rigid

carbons to be distinguished. This idea has been used to study the molecular dynamics in a number of [13]C—enriched structural proteins like elastin, collagen, cartilege and human fetal enamel matrix[15]. In elastin, specifically labeled with [1—^{13}C]-valine, [1—^{13}C]-alanine and [1—^{13}C]-lysine, which usually occur at the crosslinks of the protein chain, such studies suggested that the crosslinks rather than the secondary structures in the extensible region to be the main source of motional restrictions in the protein[15b]. In human fetal enamel protein matrix [15c], 70% of the protein chains exhibit rapid isotropic molecular motion $(\tau < 10^{-6} s)$ while the remaining 30% undergo only anisotropic molecular motion.

The line broadening due to static CSA and dipolar interactions can be effectively removed by the line narrowing techniques described earlier but not those due to relaxation effects. In ^{1}H and ^{13}C NMR studies of high molecular weight DNA, broad lines result due to polymer motions being too slow to effectively average the static dipolar and CSA interactions. [13]C resonances of base residues are further broadened by the adjacent ^{14}N and hence getting useful structural information becomes difficult. ^{13}C NMR of calf thymus DNA has been reported[16] and bacteriophage fd virus has been studied in solution with combined high power decoupling and magic angle spinning techniques.

(*ii*) *Deuterium (^{2}H)*: [2]H has a nuclear spin of I and the associated quadrupolar splittings observed when deuterium undergoes restricted motion can be used to probe the degree of order of environment. This fact together with the ease of incorporation into biological molecules, simplicity of ensuing spectra and straightforward interpretation of relaxation parameters make ^{2}H an ideal nucleus to probe molecular dynamics of membranes and its interaction with sterols and proteins. Based on ^{1}H NMR relaxation times of H_2O in monoclinic lysozyme crystals, Bryant *et al*[17] suggest at least two types of water populations on the protein surface: one type rapidly exchanges with the interior and the other does not. The anisotropy of water molecule motion is

better demonstrated by T_2-resolved ^{2}H NMR of water in lysozyme[17] where the quadrupole splittings observed in the crystal were absent in the powders and frozen protein solutions: this meant that the anisotropy of water motion is induced by the morphology of spaces available in the protein crystal rather than specific water-protein interactions which get rapidly mixed at the surface.

Oldfield *et al*[18] have illustrated the use of high field solid state ^{2}H NMR study dynamics of amino acids side chains, in a membrane protein bacteriorhodops in which was labelled with fully deuterated valine, phenylalanine, tyrosine and tryptophan, each at a time and studied by ^{2}H quadrupolar-echo and ^{13}C FT NMR. The results indicated that the aromatic side chains of the amino acids were rigid at $-30°C$, phenylalanine and tyrosine became highly mobile $(\tau < 10^5 - 10^6 s^{-1})$ with 2-fold jumps about $C^\beta — C^\gamma$ bond, at the growth temperature (30°C). Tryphtophan did not undergo this type of motion even at 85°C, prior to the protein denaturation. In contrast, the aliphatic amino acid valine did not show any evidence for fast motion about $C^\beta — C^\gamma$ at any temperature but the motion about $C^\beta — C^\alpha$ bond is fast $(\tau \leqslant 10^{-6} s)$ at all temperatures.

"Pseudo-single" crystal NMR spectra of paramagnetic proteins may be obtained by ordering the microcrystals of proteins in 90% saturated $(NH_4)_2SO_4$ solution by a static external magnetic field (0.3 to 8.5 T)[19]. The ordering effect due to the large anisotropy in magnetic susceptibility of the protein caused by the paramagnetic centre, aligns the protein crystals in the crystallographic axis (C*) and therefore, the structural parameters obtained are directly comparable to X-ray crystal data. Such ordered crystals of myoglobin from sperm whale was isotopically enriched at C^ϵ methyl groups of methionines 55 and 131 with either ^{2}H or ^{13}C and studied by ^{2}H quadrupolar-echo (figure 3) and ^{13}C FTNMR[19b]. The splitting information from the "single crystal" NMR spectrum coupled with that obtained from the powdered crystals allows a direct determination of (i) spatial orientation of the particular labelled residue within the protein crystal and (ii)
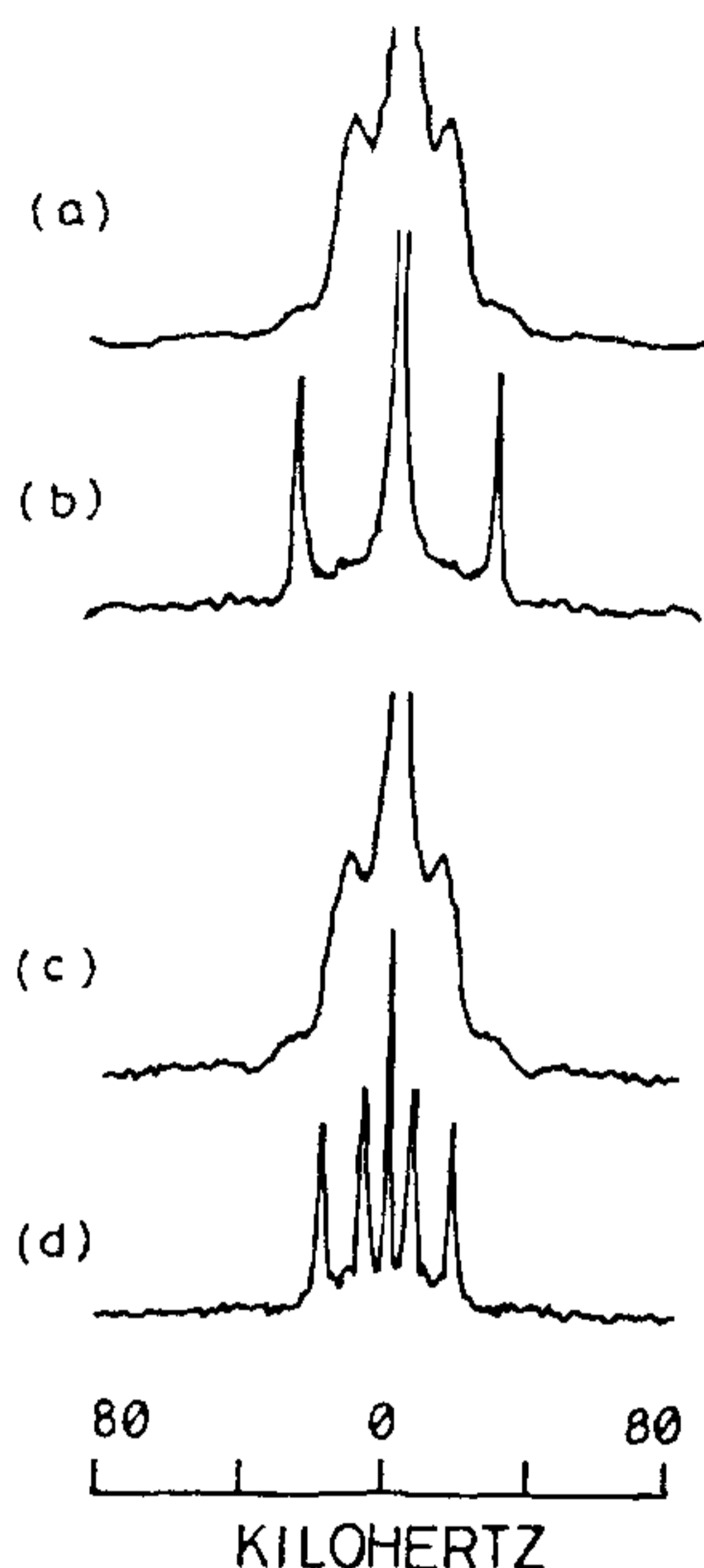
Figure 3. ^{2}H quadruple-echo FT NMR spectra (reference 19b) of ^{2}H-methyl methionione labelled myoglobin, solid hydrated crystal powders, aquo myoglobin (a) and cyanoferri myoglobin (c) and the magnetically ordered samples of (b) and (d) respectively.

the rates and types of side chain motion. The high (S=5/2) and low spin (S=1/2) ferric forms, the cobalt derivative "coboglobin" all show magnetic ordering, indicating the general applicability of this method. The ordered samples may be frozen, spin state transitions altered by pH variation and even the direction of observation may be changed by the sample rotation. The 'ordering' technique opens up new possibilities to investigate substrate binding geometries, multisubunit proteins, relaxation studies to probe side chain dynamics of proteins in membranes and protein-lipid interactions.

(*iii*) *Proton (^{1}H):* The large homonuclear dipolar broadenings of ^{1}H restricts its use in solid state studies. Jardetzky *et al*[20] have compared the high temperature solid state ^{1}H NMR of lysozyme and ribonuclease A with the artificially

broadened liquid spectra. The general features of the two compared well except for certain groups of resonances which were shifted with respect to each other, suggesting different degrees of unfolding of the proteins in the two states. The reorientation of NH^+_3 groups in the zwitterionic form $NH^+_3CHRCOO^-$ is found to be an effective source of proton magnetic relaxation in solid amino acids[21]. Reorientation of CH_3 groups in the side chains provides a second source of relaxation resolvable at lower temperatures. Being hydrogen-bonded, NH_3 groups exhibit higher activation energies (28-52 KJ/mole) for reorientation, compared to non-hydrogen bonded methyl rotors (12 KJ/mole). In the case of proteins lysozyme, ribonuclease A,α-chymotypsin and insulin in powdered form, reorientation of methyl rotors is still the major source of proton relaxation to about 70% with the remaining contribution arising from the side chain reorientations, segmental motions, NH_3 group reorientations, reptation and whole body motions[22]. The absence of methyl groups in adenine (A), guanine (G), cytosine (C) and uracil (U) leads to very long relaxation times ($\sim$100 s) compared to solid thymine (T) (80 ms)[22]. Rotating frame proton magnetic relaxation measurement ($T_{1\rho}$) on powdered samples of A, G, and C have indicated the C_2-axis reorientation for the hindered NH_2 groups in these bases and this motion unlike NH^+_3 group reorientation does not contribute effectively to the relaxation process[23]. In nucleic acids (calf thymus DNA) the effective proton relaxation thus depends on the thymine methyl groups and the relaxation times therefore reflect the amount of AT base pairs present.

(*iv*) *Nitrogen (^{14}N, ^{15}N):* The most important and ubiquitous nucleus after ^{1}H and ^{13}C in biologically interesting molecules is nitrogen: both ^{14}N and ^{15}N are magnetically active. ^{15}N with spin 1/2 and low natural abundance (0.37%) has a long spin-lattice relaxation time and small negative gyromagnetic ratio leading to negative nuclear Overhauser enhancement. ^{15}N can be incorporated into proteins and nucleic acids by growing *E. coli* on a medium with $^{15}NH_4Cl$ as the sole nitrogen source. Opella *et al*[24] have studied ^{15}N-labeled bacteriophage fd virus and the

duplex DNA by solid state ^{15}NMR using separated local field spectroscopy[25], combined with magic angle spinning technique. This is a two-dimensional NMR experiment in which the high resolution dipolar spectra in the absence of CSA and the isotropic chemical shift spectra are resolved in the two domains. In addition, almost single site atomic resolution is possible with 13 of the 14 nitrogen sites of DNA being identified[24a]. Excellent selectivity between the hydrogen bond donor and acceptor nitrogens of the adenine-thymine (AT) and guanine-cytosine (GC) base pairs is possible. ^{15}N NMR offers a possibility to study both DNA and protein components in fd virus, since there is no overlap among the chemical shifts of these constituents (figure 4). The protein spectrum is dominated by the narrow high intensity amide resonances at 98.5 ppm compared to well resolved but broad and low intensity resonances from DNA bases[24c]. The side chain amino groups of glutamine, lysine, the imido nitrogens of proline and amino nitrogen of N-terminal alanine are all well resolved. The single stranded, circular DNA inside the viral coat has ^{15}N line shapes differing from those of duplex DNA, similar to the effects noticed in ^{13}C and ^{31}P NMR due to the influence of the coat protein.

The measurement of the ^{15}N—^{1}H dipolar coupling allows a direct determination of N—H bond lengths in a DNA[24b] in contrast to the X-ray and neutron diffraction studies where the results are from crystals of methylated bases that participate in Hoogsteen rather than Watson-Crick base pairs. The hydration of DNA has no effect on the dynamics of N—H bond lengths of bases, but ^{15}N linewidth changes indicate a reduction of either the CSA in the hydrated DNA or ^{15}N—^{1}H coupling due to motions induced in the backbone.

Solid state ^{15}N NMR is potentially useful to study nitrogen metabolism in plants[26]. A normal ^{15}N experiment on partially double-labeled glycine (25% ^{15}NH$_2$ ^{13}CH$_2$COOH) shows a doublet and a singlet due to ^{15}N—^{13}C and ^{15}N—^{12}C bonds respectively. In a DCP experiment, polarization is drained from ^{15}N coupled directly to ^{13}C thereby decreasing the intensity of the dou-
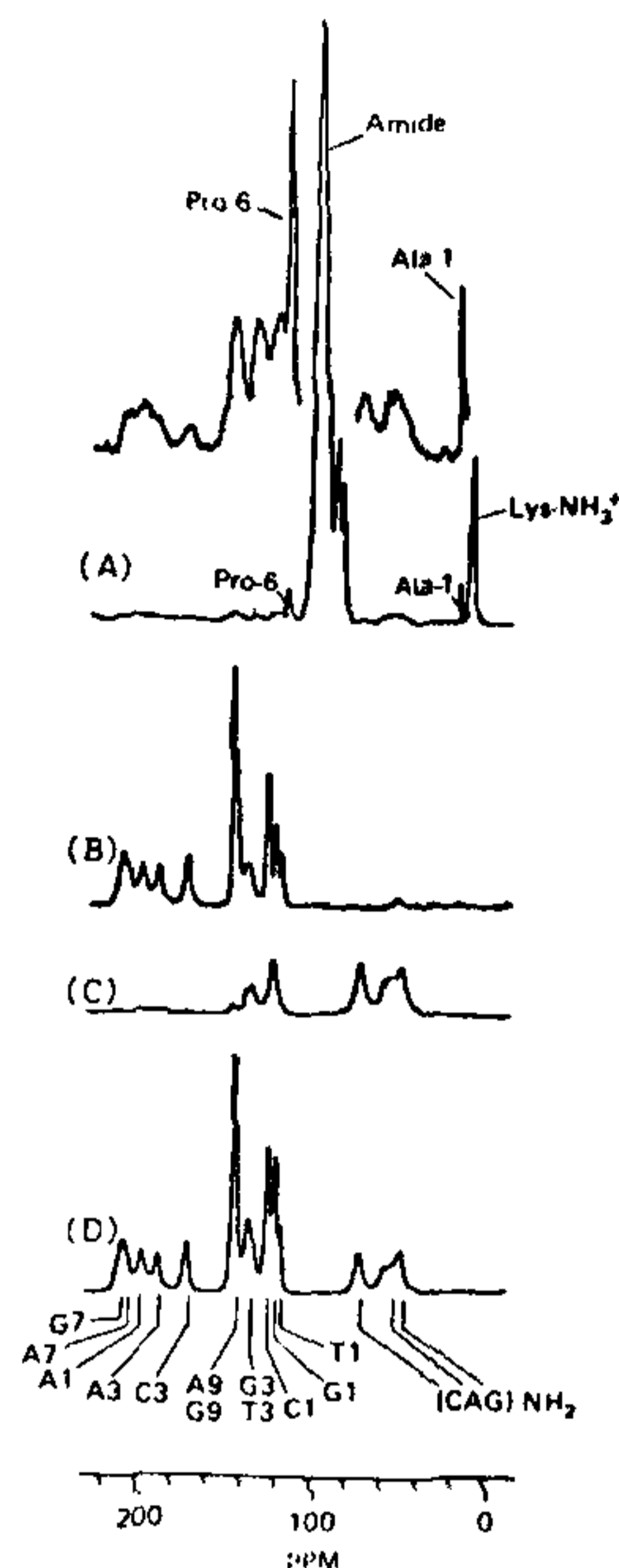

Figure 4. ^{15}N NMR spectra (reference 24) of B-DNA fd virus (a) complete spectrum of virus (b) non-protonated nitrogens of B-DNA (c) protonated nitrogens of B-DNA and (d) complete spectrum of B-DNA.

blet. Shaefer et al[26] have used this idea to trace the history of a chemical bond and thus study protein turnover in soyabean plants grown on ^{15}NH$_4$ ^{15}NO$_3$, exposed to ^{13}CO$_2$ and examining the lyophilized leaves by solid state ^{15}N NMR. The metabolic fate of aspargine which is believed to be an important source of nitrogen for protein synthesis in developing soyabean seeds was studied by ^{15}N NMR of lyophilized cotyledons cultured on media containing [4—^{13}C, amide—^{15}N]-aspargine as the only nitrogen source[26b].

Single crystal ^{14}N NMR of glycine[11a] and ^{15}N NMR of histidine[27] have been reported. ^{14}N with spin I = 1 (quadrupolar) and abundance of 99.4% gives acceptable S/N ratios in short periods of time unlike ^{13}C. In addition, the resolution

is about two orders of magnitude grearter than that obtainable with ^{13}C and is thus a promising technique to study proteins and nucleic acids particularly to derive directly information regarding inter and intra-molecular hydrogen bonds with nitrogens. The disadvantage of ^{14}N experiments at present is a technical one, since the spectra span a large frequence range (~1.5 MHz), they must be obtained in various sections.

(v) *Phosphorous (^{31}P)*: [31]P is an ideal nucleus and a powerful probe to study the intact biological entities including living cells and whole organs. [31]P with spin $1/2$ and a large gyromagnetic ratio yielding a large CSA has several advantages over other nuclei to study nucleic acid structure and dynamics. Its uniform location over the phosphodiester backbone obviates the resolution and assignment problems. Only ^{31}P allows separation of nucleotide and amino acid chromophores and is thus useful in studying protein-nucleic acid interactions in bacteriophage fd virus, chromatin, etc. ^{31}P NMR of nucleic acids in solutions has already been successully employed to monitor the conformation of phosphodiester groups[28], helix to coil transitions in oligo and polynucleotides[29] and nucleic acid-drug complexes[28b].

Terao *et al*[30] have studied the solid state ^{31}P NMR of a number of nucleoside monophosphates, both in salt and free acid forms, cyclic mononucleotides, homopolyribonucleotides, t-RNA and DNA using CP combined with dipolar decoupling in an attempt to interpret the chemical shift tensors in terms of electronic distribution around the phosphorus atom (figure 5). The principal values σ_{11} σ_{22} and σ_{33} of the chemical shift tensor vary widely among the different types of nucleic acids and are sensitive to esterification, protonation and ionization of the phosphate group. Mononucleotide sodium salts exhibit axial symmetric patterns in contrast to free acids and polynucleotides including t-RNA and DNA which are non-axial, the latter having considerably larger anisotropies. Further, the chemical shift tensors are not sensitive to variation in base, sugar or the nature of the strand (single or double). [31]P chemical shift tensors
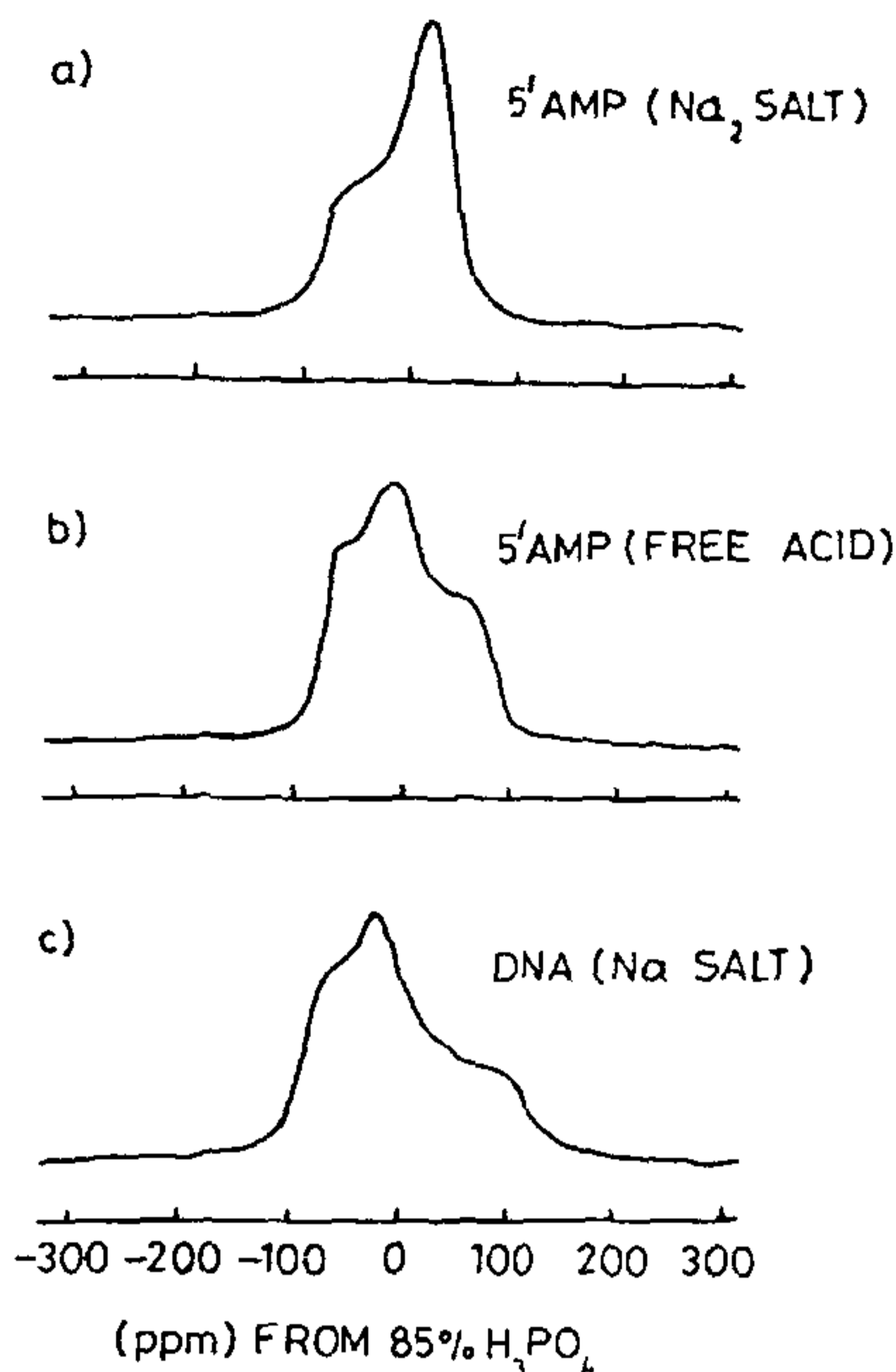

Figure 5. ^{31}P NMR spectra (reference 30) in powdered nucleic acids with cross polarization and proton dipolar decoupling.

reflect conformational variation of the phosphate moiety O-P-O bond angle and rotational angles (ω ω') about P—O bond and hence gives information about the backbone motions in nucleic acids.

A combination of high resolution solid state and solution ^{31}P NMR technique is needed to obtain reliable and interpretable data on DNA dynamics as has been demonstrated by Opella *et al*[31]. No evidence of phosphate backbone motions was obtained for the solid dehydrated calf thymus DNA in B-form even at 25°C in contrast to the hydrated form which shows large amplitude motions at temperatures greater than 5°C. The contributions to line width from [31]P—[1]H dipolar couplings and ^{31}P CSA can be separated by high power proton decoupling and magnetic field dependence experiments[31,32]. The ^{31}P chemical shielding tensor of lyophilized solid fd was identical to that of single or double

stranded DNA without proteins, suggesting no major structural distortion in the phosphate backbone in DNA upon incorporation into virus[31]. However, the line widths in fd were very broad due to motionally unaveraged CSA and relaxation parameters obtained from linewidth measurements indicated DNA packed inside fd to be immobilized by coat proteins.

(*vi*) *Other applications*: Solid state NMR techniques are widely applicable for immobile and slowly reorienting systems and hence very useful in the study of membrane components[33]. ^{15}N, ^{31}P, ^{13}C and ^2H are particularly useful to study head group motions in phospholipids, cerebrosides, etc., whereas ^{19}F and ^2H have played a vital role in probing hydrocarbon chain dynamics to establish the order and the fluidity parameters. Unsonicated intact lipid membranes gave $^{13}C,^{31}P$ spectra by magic angle spinning which were as good as those of sonicated vesicles but without the associated consequences of sonication such as inner-outer symmetry[34].

Smectic lipid-water (dimyristoyl lecithin, potassium oleate, potassium linoleate) systems on orientation between glass plates produce bilayers with rotational symmetry axis normal to the bilayers[35]. Magic angle spinning of such oriented systems produce highly resolved and sharp resonances, permitting measurement of relaxation gradient along the hydrocarbon chain. [26 - ^{13}C, 4 - ^{13}C]-labeled cholesterol incorporated into multilamellar lipid dispersions have been studied by CP combined with high power resonant decoupling[36]. The purple membrane from Halobacterium cutirubrum and aqueous dispersions of its principal phospholipid have shown ^{31}P NMR indicating the absence of phase transitions in the region $5°$ to $60° C$[37]. Other applications of solid state NMR studies include conformational studies of NADH and NAD^+ model systems[38] and gelation of sickle cell-haemoglobin[39].

(*vii*) *Scope of Solid State NMR*: The high resolution solid state NMR techniques now available and the versatility of the multinuclear approach open a new way to study biological structures. The solid state techniques can be extended to study insoluble precipitates, gels, oriented sys-

tems, frozen liquids and hence lack of suitable crystals of many macromolecules is no more a limitation to study them in solid form. The spectral sensitivity and resolution obtained in solid state allows relaxation studies to probe amino acid side chain dynamics in membrane proteins, temporal fluctuations and effect of membrane fluidity. Being non-invasive, solid state NMR will be valuable to study intact biological membranes and the results compared with solution, micellar and crystal data to provide meaningful interpretation of protein-lipid interactions. The technique of magnetic ordering permits resolution of signals from numerous sites in protein crystals with the nuclei ^2H, ^{13}C and ^{15}N and has the potential to study higher oxidation states of metal ions in proteins, and unstable intermediates by freeze-trapping methods. The selective enrichment of ^{15}N and combination with ^{31}P NMR will be valuable to study drug effects, backbone dynamics in nucleic acids and protein-nucleic acid interactions in viruses and chromatin. Studies such as nitrogen metabolism in plant and bacterial systems in real time is possible with solid state NMR and involves least biochemical manipulations of sample preparation.

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