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NUCLEAR MAGNETIC RESONANCE OF E. COLI RIBOSOMES AND VIRUSES

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SUMMARY: ^{1}H Nuclear Magnetic Resonance spectra of a number of viruses and E. Coli ribosomes revealed that experimental values of the linewidth $(\pi T_2)^{-1}$ (< 320 Hz) and T_1 (< 1 sec) of the observable nuclei are too small to be accounted for by the system's molecular weight. The nuclei therefore must be internally mobile. From ^{13}C Nuclear Magnetic Resonance spectra of 12% ^{13}C enriched E. Coli ribosomes, it follows that 30% of the 5000 CH_2/CH_3 groups, 10-20% of the 225 Phe residues, 20% of the δ-Arg and β-Lys carbons, ∿ 100 nucleotides and a number of C_α carbons are internally mobile. It is demonstrated that ^{13}C Nuclear Magnetic Resonance can fruitfully be applied to intact ribosomes.

INTRODUCTION: Nuclear Magnetic Resonance has been applied to a wide range of small biomolecules [1,2]. For large biological systems solid state NMR, such as ^{13}C induction spectroscopy appears to be an attractive technique [3-5]. Even in apparently rigid material, the presence of internal mobility on a time scale of $\sim 10^{-6}$ sec has been demonstrated [6,9], approaching the conditions which apply to conventional Fourier Transform NMR in solution.

Successful application of conventional FT NMR to biomolecules with large molecular weight, such as viruses, phages and ribosomes, depends on the presence of internal mobility and has been recently demonstrated for Tobacco Mosaic Virus (TMV) in some detail [7,18]: Prompted by these results on TMV we have carried out an investigation of a number of other viruses and the E. Coli ribosome, the results of which are reported in this communication.

All particles studied had molecular weights in excess of 10^6 and their NMR spectra have been related to those of dissociated particles. The dissociated protein oligomers then have molecular weights, small enough to be

List of abbreviations: CCMV; cowpea chlorotic mottle virus, BMV; bromo mosaic virus, BBMV; broad bean mottle virus, AMV; alfalfa mosaic virus, C_pMV; cowpea mosaic virus.

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completely observable with NMR [7,18]. For structural details of viruses (TMV, CpMV, BMV, BBMV and AMV) and ribosomes we refer to published data (12, 10, 11, 17).

MATERIALS AND METHODS: Purification and protein preparation of TMV has been described elsewhere (7); the following viruses were obtained as a gift: CCMV, BMV and BBMV, from Dr. B.J.M. Verduin, AMV from Dr. J. Mellema, CpMV from Dr. P. Zabel and adenovirus from Dr. J.M. Vlak. The viruses were purified and their concentrations were determined following published methods, using $E^{1\%}_{1cm;260nm}$ = 58,5, 52, 80 and 120 as a calibration for CCMV/BMV/BBMV(10), AMV(11), CpMV(13) and adenovirus(14), respectively. All other viruses and phages were obtained from Miles Laboratories; concentrations were determined using $E^{1\%}_{1cm;260nm}$ values given by Fraenkel-Conrat and Wagner (14). For E. Coli ribosomes, purified according to Traub (15), concentration was determined using $E^{1\%}_{1cm;260nm}$ = 55 (15). Ribosomal protein denaturation was carried out, following Kaltschmidt (16); experimental details for ^{13}C enrichment of ribosomes will be published elsewhere (manuscript in preparation).

For ^{1}H NMR experiments samples were centrifuged; the resulting pellets were resuspended in D_2O and dialyzed three times against a 100 fold excess D_2O. pD adjustments were made using uncorrected pH meter readings. Before and after each NMR experiment the pD of the unbuffered solutions was checked for changes during the period of measurement. NMR experiments were carried out for ^{1}H at 100 MHz (Varian XL-100) and for ^{13}C at 90,5 MHz (Bruker SPX 360), using 5 Watt broadband noise decoupling at 360 MHz. Temperature was kept constant employing evaporation of liquid N_2 (18). Virus spectral intensities have been calculated relative to the spectral intensities of their dissociated particles by using a planimeter. The essence of this procedure has been discussed elsewhere (7,18).

RESULTS: Table 1 contains the fractions of nuclei, observed by NMR, for different spectral regions. For ^{1}H NMR spectra, only the aliphatic region yields numbers with acceptable accuracy. $C_\alpha H$ regions mostly appear as shoulders on a large HOD resonance and their relative intensity cannot be determined.

From ^{1}H NMR spectra of the various species of Table 1 an upper limit of 320 Hz is found for the linewidth of aliphatic protons, following a procedure similar to that used for TMV and yielding a ^{13}C linewidth $\lesssim$ 160 Hz (18); for protonated ^{13}C nuclei T_1 was found to be $\lesssim$ 1 sec.

Fig. 1 represents a ^{13}C spectrum of native E. Coli ribosomes, containing 12% ^{13}C. Spectral intensities, relative to those of denatured E. Coli ribosomes, have been determined for the aliphatic (150-200 ppm), C_α

Table 1

NMR spectral intensities of protein backbone and side chains in various biological systems, presented as a fraction of the intensity of their dissociated or denatured particles.

system	backbone	side chains	molecular weight($\times 10^6$)
TMV[a]	0.07	0.17	42
TMV protein rod (pH 4.8, 7°C)[b]	0.10	0.10	$\sim$ 42
TMV protein rod (pH 6.2, 20°C)[b]	0.65	0.65	> 2
CCMV/BMV/BBMV[c]	+[e]	0.3	$\sim$ 4.6
AMC[c]	+[e]	0.3	3.7 - 7.3
E.Coli ribosomes[a]	0.27	0.3	2.7
other systems[c]	+[d]	0.05-0.03	4-120

[a] Measured as integrated spectral intensity both for ^{13}C and ^{1}H
[b] Measured as integrated spectral intensity for ^{13}C
[c] Measured as integrated spectral intensity for ^{1}H and corrected for the residual HOD
[d] Backbone hidden under HOD
[e] Backbone is present, but hard to quantify.

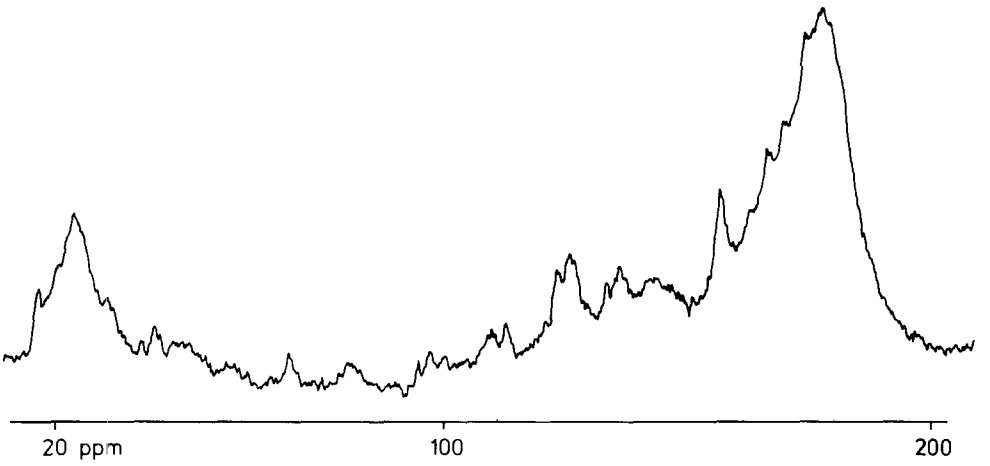

Fig. 1. Broadband noise decoupled ^{13}C NMR spectrum of E. Coli ribosomes at 90.5 MHz. The ribosomes are $\sim$ 12% ^{13}C enriched. Conditions: concn. 38 mg/ml, temp. 5°C, pH = 7, 10 mM $MgCl_2$, 60 mM NH_4Cl, 3mM β-Mercaptoethanol. The ppm scale was referenced to CS_2 assuming 125.8 ppm for β-Thr. Acquisition time plus pulse delay: 1 sec; number of accumulations: 18,700; sensitivity enhancement: 33 Hz.

(130-150 ppm), ribose (100-130 ppm), aromatic (40-100 ppm), and carbonyl (10-40 ppm) regions.

In Fig. 1 we can assign several sharp resonances on the broad background: ϕ-Phe (64 ppm), ε-Arg (36 ppm), δ-Lys (162 ppm), γ-Arg (165 ppm), and ϕ-Tyr (77 ppm), using the position of β-Thr as a reference.

DISCUSSION: From the equations for dipolar relaxation (8,18) we may calculate values for T_1 and the linewidth using the rotational correlation time τ_R, similar to that for aliphatic nuclei of e.g. TMV and its protein (7,18). For spherical molecules τ_R can be obtained from the Stokes-Einstein equation (2), whereas this figure can be used as an upper limit for non-spherical molecules (19).

CCMV, BMV, BBMV and AMV are known to be spherical (10); τ_R for CCMV is $\sim 1.3 \times 10^{-6}$ sec, as is calculated from its known volume and molecular weight (4.6×10^6), yielding a theoretical value for the linewidth $(\pi T_2)^{-1}$ for ^1H resonances of $\sim$ 8 KHz and T_1 = 29 sec. Similarly, we obtain for non-spherical E. Coli ribosomes $\tau_R \sim 7.6 \times 10^{-7}$ sec., yieling $(\pi T_2)^{-1}$ of $\sim$ 4.7 KHz and T_1 = 17 sec. For other systems, mentioned in Table 1, τ_R is obtained by comparison of their molecular weight with that of the above mentioned viruses. TMV and its protein polymers have been previously discussed (7,18,20). The ^{13}C linewidth and the value of T_1 can be derived from the corresponding numbers for ^1H resonances by multiplying with 0.5 (18).

The large discrepancy between experimental and theoretical linewidths and T_1 values leads us to conclude that the observed ^{13}C and ^1H nuclei must be mobile, due to motion within the protein, similar to what has been observed for TMV (7). The presence of motion representing a full 2π rotation of a CH vector about one C-C bond with a correlation time $\lesssim 10^{-7}$sec results in a decrease of the ^{13}C linewidth with $^1/9$, and for protons with $\sim {}^1/4$ (18) w.r.t. the calculated values for the line-

width. Therefore the observed nuclei must be part of a protein region with internal motion involving rotation about at least one C-C bond. The observations of ^{1}H resonances for a wide variety of very large biomolecules, collected in Table 1, indicates that ^{13}C NMR is likely to be a fruitful technique when applied to such objects, since sofar we have found no exceptions to the rule, that they possess considerable internal mobility as a common property, albeit in varying degree.

As a further illustration we discuss the ^{13}C spectrum of $\sim$ 12% ^{13}C enriched (1,7) E. Coli ribosomes (fig. 1) and the data on fractional intensities (Table 1). Several features are worth noticing: (i) the carbonyl spectral intensity is much larger than that of the C_{α} region; (ii) intensity of the Phe ring resonances corresponds to 10-20% of the total number of 225 Phe residues; (iii) the nucleotide intensity corresponds to $\sim$ 100 nucleotides, (iv) the intense peak at 162-164 ppm indicates that 20% of the δ-Arg and β-Lys carbons are mobile; (v) the intensity of the CH_2/CH_3 regions corresponds to $\sim$ 5,000 aliphatic groups, constituting about 30% of the total number, and implying that $\sim$ 2500 of the total number of 7500 aminoacids are mobile. A word of caution is appropriate for the observed RNA resonances: we cannot rigorously exclude that these resonances are due to RNA breakdown, even when all preparations using RNase free E. Coli strains yielded these resonances.

Morrison et al. (21) have studied E. Coli ribosomal protein subunits using ^{1}H NMR. Our results indicate that the use of selectively enriched ribosomes permits the application of NMR to ribosomal protein subunits in their natural environment, e.g. by incorporating a single ^{13}C enriched subunit into a non-enriched ribosome.

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