SPECIFICITY OF INTERPROTON NUCLEAR OVERHAUSER EFFECTS IN GRAMICIDIN-S DISSOLVED IN DEUTERATED ETHYLENE GLYCOL

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ABSTRACT The 250-MHz high-resolution proton magnetic resonance spectra of gramicidin-S in solution in deuterated methanol, deuterated ethylene glycol, and binary mixtures of these solvents have been recorded. Starting from previously published partial assignments for deuterated methanol solution, the solvent transition yields partial assignments in deuterated ethylene glycol solution. In the latter the rotational correlation time for the peptide backbone, τ_c, is calculated to be 14 ns at 25°C. The long τ_c leads to proton spin relaxation behavior that mimics that of moderate-sized proteins in water, and yields negative nuclear Overhauser effects, which have been measured for the protons of the phenylalanine ring. The results suggest that there is rapid and efficient spin-diffusion within closely-connected "islands" of protons, and less efficient spin-diffusion between islands. The results are compatible with the accepted solution conformation of gramicidin-S.

INTRODUCTION

In a growing number of studies, the interproton nuclear Overhauser effect (NOE)[1] has been used in attempts to establish spatial proximity of proton groups in biomolecules (Balaram et al., 1972; Bothner-By and Gassend, 1973; James and Cohn, 1974; James, 1976; Karpeiski and Yakovlev, 1976; Howard et al., 1975; Leach et al., 1977). In proteins, where the reorientational correlation time, τ_c, is usually of the order of a few nanoseconds, NOE's of negative sign are easily observable. This can be explained by assuming that the longitudinal relaxation time of the protons is controlled by magnetic dipole interaction of the nuclei. In a system of two contiguous protons affixed to the proteins, separated by the distance r, the rate of the adiabatic relaxation ($\alpha\beta \rightleftharpoons \beta\alpha$), in which the nuclei exchange excitation without loss or gain of energy to molecular motion, increases with τ_c and r^{-6}, while the rates of relaxation, in which nuclear Zeeman excitation is dissipated into thermal motion of the molecules ($\alpha \rightleftharpoons \beta$ and $\alpha\alpha \rightleftharpoons \beta\beta$), pass through maxima at $\omega\tau_c = 1.0$ and 0.5, re-

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[1] *Abbreviations used in this paper:* $C_2D_6O_2$, deuterated ethylene glycol; CD_4O, deuterated methanol; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; TFA, trifluoroacetic acid; TMS, trimethylsilane.

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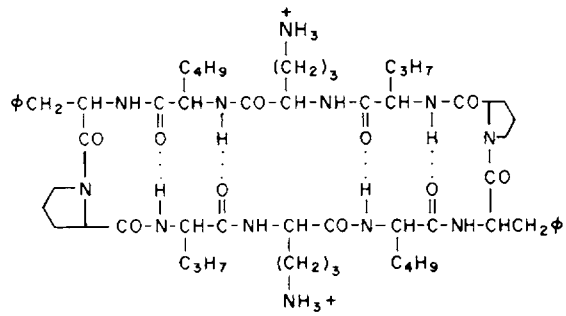

FIGURE 1 Gramicidin S.

spectively (ω = the Larmor frequency of the proton in radians per second). Thus at long τ_c, the adiabatic process dominates, and excitation applied to one nucleus is not dissipated to molecular motion, but is instead transferred to neighboring nuclei, raising their spin temperatures, partially or completely saturating them, and causing their signals to decrease or disappear.

A complication arises at very long τ_c (Kalk and Berendsen, 1976). If all the protons in the system are linked in one group or chain by the efficient adiabatic relaxation process, excitation applied to any nucleus in the group will be distributed to all parts of the system before it is lost. In this case, the intensity of all proton signals will be affected, and the possibility of extracting information identifying protons adjacent to the irradiated proton is lost. Protons and fluorine nuclei have NMR frequencies sufficiently close that systems containing both behave rather like homonuclear systems, and Hull and Sykes (1975) have reported studies on fluorinated alkaline phosphatase (mol wt ~ 84,000) that show a loss of specificity of the $^1H - {}^{19}F$ NOE explicable on the above basis. The rapid conservative transfer of spin excitation within the molecule is termed spin diffusion.

To obtain a picture of the extent and nature of spin diffusion in protein molecules of moderate size, we have made a study of the NOE's observable in gramicidin-S (Fig. 1) dissolved in $C_2D_6O_2$. This solvent is much more viscous than water and has the effect of slowing the rotational motion of gramicidin-S so that it is comparable to that of a protein of mol wt ~ 10,000–20,000 in water solution. Gramicidin-S has been intensively studied by NMR and other means, and the principle features of its conformation are considered well established (Dygert et al., 1975). The relative simplicity of the observed spectrum allows a variety of specific NOE's to be observed. We have measured the effects of irradiation of distinct well-identified resonances on the intensity of the phenylalanine ring protons.

Calculation of the expected effects based on exact geometries and conformational dynamics of gramicidin-S would involve a lot of computation. However, one can make calculations of effects expected in a variety of simplified ideal systems, and we present some here. From the pattern of behavior predicted for these simplified systems, one can understand qualitatively the observed effects in gramicidin-S.

EXPERIMENTAL PROCEDURE

Materials

Gramicidin-S, purchased from Calbiochem (San Diego, Calif.), was used as received. Ethylene glycol-d_4 ($HOCD_2CD_2OH$), 99 atom % D, was obtained from Merck, Sharp and Dohme Canada Ltd., Montreal, Canada. Perdeuteroethylene glycol ($C_2D_6O_2$) was prepared from ethylene glycol-d_4 by exchanging five times with 4 vol of D_2O. After each exchange, water was removed by distillation. Methanol-d_4 (CD_4O) (99 atom % D), D_2O (99.17 atom % D) and neat trimethylsilane (TMS) were purchased from Merck Chemical Div., Merck & Co., Rahway, N.J. Trifluoroacetic acid (TFA) was obtained from Eastman Kodak Co., Rochester, N.Y. Tritration of the ornithine δNH_3^+ groups of gramicidin-S in CD_4O to locate the resonance position of the ornithine δCH_2 protons was accomplished by addition of aliquots of 1 M NaOD/CD_4). This was prepared with NaOD in D_2O (40% wt/wt, 98 atom % D) obtained from Merck Chemical Div.

Methods

All proton magnetic resonance experiments were performed at 250 MHz by the frequency sweep method in the correlation mode (Dadok and Sprecher, 1974; Gupta et al., 1974) using the MPC-high frequency spectrometer at the NMR Facility for Biomedical Studies, Mellon Institute, Carnegie-Mellon University, Pittsburgh, Penn.

Experiments were conducted at the normal ambient temperature of the spectrometer probe (25°C) with 5-mm Wilmad 507-pp NMR tubes (Wilmad Glass Co., Inc., Buena, N.J.) Field-frequency locking as well as chemical shift referencing used an external reference of neat TMS or TFA in a capillary.

A concentration of gramicidin-S in CD_4O of 5% wt/vol was used in the decoupling experiments for the assignment of resonances. Coupled resonances were identified by continuously applying a strong secondary radio frequency field (H_2) at the appropriate resonance frequency while simultaneously frequency sweeping in the correlation mode through the spectral region where the decoupling was expected. Control spectra for monitoring these multiplet perturbations were obtained with the irradiation power turned off.

Negative intramolecular NOE experiments were performed on gramicidin-S in $C_2D_6O_2$ (6% wt/vol) by continuous application of a strong saturating radio frequency field (H_2) at resonance frequencies corresponding to resonances, which correspond to a single proton type. Resonances due to the leucine and valine methyls, and the partially overlapping phenylalanine β CH and ornithine δCH_2 proton resonances were also irradiated. Proton resonance spectra recorded for gramicidin-S in binary solvent mixtures of $C_2D_6O_2$ and CD_4O (25/75, 50/50, 75/25% vol/vol) showed a smooth solvent transition so that resonance assignments in $C_2D_6O_2$ were equivalent to those for solution of the antibiotic in CD_4O. Simultaneously with the saturation of a particular solute resonance, the observing radio frequency field (H_1) was swept in the correlation mode through the phenylalanine ring proton resonance region to observe the NOE. Phenylalanine ring proton control spectra were obtained by positioning the secondary radio frequency field at 5,400 Hz to low frequency of the external TFA lock or 2500 Hz to low frequency of the external TMS lock.

The magnitudes of the NOE's were evaluated by cutting and weighing of Xeroxed spectra to determine integrated resonance intensity changes and by measuring resonance amplitudes. For a given H_2 power level and pulsing, numerous off-resonance control spectra were used to determine the probable error in the NOE's evaluated by the two methods.

RESULTS

The proton NMR spectrum of gramicidin-S in CD_4O is shown in Fig. 2. The assignments given are based on those of Gibbons et al., 1972. We have decoupled all

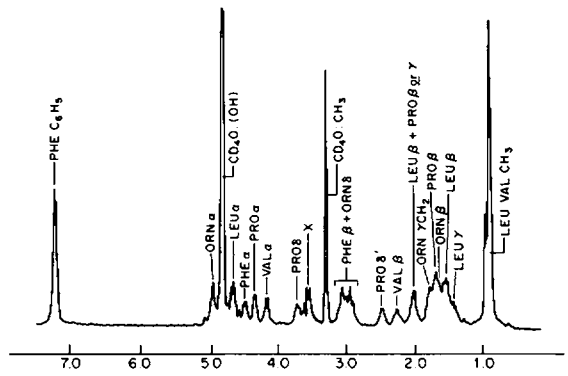

FIGURE 2 250-MHz proton spectrum of gramicidin-S in deuterated methanol solution.

α-CH resonances, the low field δ-Pro resonance, and the δ-Orn resonances, and our results are consistent with those of Gibbons et al. The δ-Orn resonances could be separated from the β-Phe resonances by addition of 1 M NaOH/CD_4O to give a high pH (Fig. 3). The peak at $\delta = 2.04$, corresponding to four protons, is not affected by any of the above irradiations and may tentatively be assigned to overlap of signals from β-Leu (two protons) and either β or γ Pro. Puckering of the proline ring could in either case lead to a situation where the spin-spin coupling with the proton

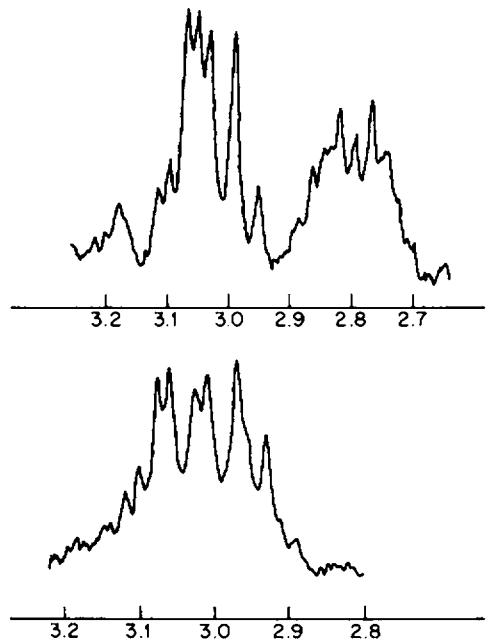

FIGURE 3 Lower spectrum: combined signals from Phe-β and Orn-δ protons. Upper spectrum: spectrum obtained after addition of NaOD, showing Phe-β proton signal on left and Orn-δ proton signal on right.

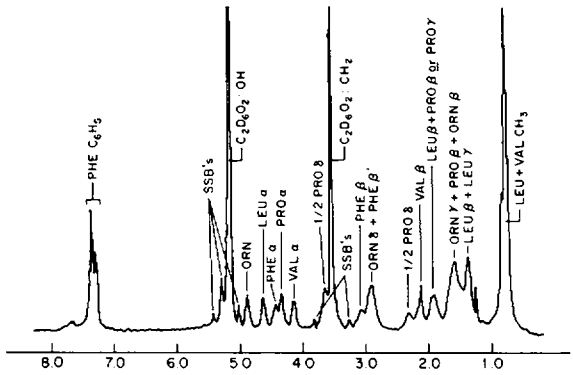

FIGURE 4 250 MHz proton spectrum of gramicidin-S in deuterated ethylene glycol solution.

in the *cis* δ-Pro or α-Pro position would be small, so that decoupling would cause no change in apparent multiplicity.

Spectra were also recorded for gramicidin-S in binary mixtures of CD_4O and $C_2D_6O_2$ with compositions 25–75, 50–50, and 75–25 % vol/vol. There was a smooth and unexceptional change in the appearance of the spectrum; while there were slight shifts in some of the resonances, the major effect was to broaden all resonances somewhat. The spectrum in $C_2D_6O_2$ is shown in Fig. 4 with the assignments obtained.

NOE's on the phenyl-ring proton signals were measured while irradiating each of the other resolved signals in turn. Table I displays the results of these measurements.

The magnitude of the observed effects depends on the conditions of irradiation. Higher power and shorter H_2 pulsing times lead to less selectivity of the effect because additional resonances adjacent to the irradiating center frequency are also excited. Results from the experiments are condensed in Table I; the magnitudes of the effects

TABLE I

INTERPROTON NOE ON THE D-PHE RING PROTON SIGNAL OF GRAMICIDIN-S IN SOLUTION IN $C_2D_6O_2$

Groups (s) irradiated	NOE observed
$-CH_3$ (all)	−0.01
Orn δ	−0.00
Orn α	−0.02
Val α	−0.04
Leu α	−0.08
Val β	−0.09
Pro α	−0.15
Pro δ	−0.15
Pro δ'	−0.18
Phe α	−0.18
Phe β	−0.20

given are less than the maximal observed (−0.43), but the relative magnitudes were reproduced in all measurements. The problem of errors in measurement of NOE's is troublesome. It is difficult to be more than 2–3% precise in peak area measurements, and since differences between peak areas with and without irradiation must be evaluated, the errors in the effects themselves amount to 3–4% of the original peak area. Thus effects of 0–4% are indistinguishable, and can be classified as small or negligible, those of 8–9% as significant, and those of 15–20% as large. A more exact ranking than this does not seem warranted.

DISCUSSION

Calculation of Some Limiting Cases

We first consider some idealized limiting models and calculate the expected degree of spin diffusion in these systems. The computational approach is firmly established and has been applied previously to a number of specific cases (Hull and Sykes, 1975; Gerig, 1977).

The master equation used to predict the behavior is:

$$-\mathrm{d}(I_a)/\mathrm{d}t = \sum_{k \neq a} R_{ak}(I_a - 1) + \sum_{k \neq a} C_{ak}(I_a - I_k). \tag{1}$$

In this equation I_a and I_k represent expectation values of the z component of angular momentum in an ensemble of identical systems, each containing spins a, b, ... k, ... The I's are normalized so that the equilibrium expectation value is 1 (assumed same for all spins). R_{ak} is the rate of relaxation contributing to recovery of spin I_a toward thermal equilibrium as a result of dipolar interaction of spin a with spin k. It is given by

$$R_{ak} = \frac{\gamma^4\hbar^2}{10r_{ak}^6}\left\{\frac{3\tau_c}{1 + \omega^2\tau_c^2} + \frac{12\tau_c}{1 + 4\omega^2\tau_c^2}\right\}. \tag{2}$$

C_{ak} is the cross-relaxation rate affecting both I_a and I_k. It is given by

$$C_{ak} = \frac{\gamma^4\hbar^2}{10r_{ak}^6}\left\{\tau_c - \frac{6\tau_c}{1 + 4\omega^2\tau_c^2}\right\}. \tag{3}$$

An important quantity for the calculation of expected effects is $\rho = C/R$. From Eqs. 2 and 3:

$$\rho = (4\omega^4\tau_c^4 - \omega^2\tau_c^2 - 5)/(24\omega^2\tau_c^2 + 15). \tag{4}$$

ρ varies from $-(1/3)$ in the extreme narrowing case ($\omega\tau_c \approx 0$), through 0 at $\omega\tau_c = \sqrt{5}/2$ (corresponding to no Overhauser effect), to large positive values for long τ_c. For a particular system, the Overhauser effects may be calculated by applying the steady-state condition to Eqs. 1, i.e. by setting all $\mathrm{d}(I)/\mathrm{d}t = 0$, setting one or more $I_i = 0$ (corresponding to saturation of those spins), and solving the resulting

TABLE II

CHANGE IN INTENSITY OF SIGNAL FROM NUCLEUS IN A UNIFORM CHAIN WHEN NUCLEUS "0" IS SATURATED

$\omega\tau_c$	ρ	1	2	3	4
~0	−(1/3)	+0.268	−0.072	+0.019	−0.005
1.118	0	0	0	0	0
2	0.495	−0.170	−0.029	−0.005	−0.000
5	4.016	−0.510	−0.251	−0.125	−0.062
10	16.93	−0.710	−0.505	−0.358	−0.254
20	66.5	−0.841	−0.707	−0.595	−0.500
50	418.5	−0.933	−0.871	−0.812	−0.758

linear equations algebraically. This yields the expectation values of I_a, $I_b \ldots$, which correspond to the expected transition intensities when the normal intensity is unity. The NOE is then calculated by subtracting 1 from these intensities.

LIMITING CASE 1: SEQUENTIAL CHAIN OF SPINS In this case it is supposed that one may delineate a single sequential chain of nuclei through the system so that each proton interacts equally with its nearest neighbors and negligibly with more remote protons. Designating a segment of the chain by I_l, I_m, I_n we have

$$2R(I_m - 1) + 2CI_m - C(I_l + I_n) = 0, \tag{5}$$

whence

$$I_m = [2 + \rho(I_l + I_n)]/(2 + 2\rho). \tag{6}$$

Since the intensities at sufficiently remote parts of the chain must be asymptotic to 1, one can write by symmetry, $I_j = 1 - f^j$, where j is a running index with $j = 0$ corresponding to the saturated proton. This allows the recursion formula to be written

$$(2 + 2\rho)(1 - f^j) = 2 + \rho(2 + f^{j-1} + f^{j+1}), \tag{7}$$

whence

$$f = (1 + \rho - \sqrt{1 + 2\rho})/\rho. \tag{8}$$

Table II shows typical values of Overhauser effects along a uniform chain for various values of $\omega\tau_c$.

In the region $\omega\tau_c > 2$ effects are observable, and extend further along the chains the larger ρ becomes.

LIMITING CASE 2: ISOLATED "ISLANDS" In this system, it is assumed that a group of $(n + 1)$ nuclei all interact equally with each other, and negligibly with protons outside the island. This is an opposite extreme from the chain. Most systems would presumably behave in a way intermediate between the chain and island models.

The master equations give

$$nR(I_a - 1) + CI_a = 0, \tag{9}$$

TABLE III

CHANGE IN INTENSITY WITH n NUCLEI EQUALLY COUPLED TO SATURATED NUCLEUS

$\omega\tau_c$	ρ	$n = 2$	$n = 4$	$n = 6$	$n = 8$
~0	−0.333	+0.200	+0.091	+0.059	+0.0434
1.118	0	0	0	0	0
2	0.495	−0.198	−0.110	−0.076	−0.043
5	4.016	−0.667	−0.501	−0.401	−0.334
10	16.93	−0.920	−0.809	−0.738	−0.679
20	66.5	−0.971	−0.943	−0.917	−0.893
50	416.5	−0.995	−0.990	−0.985	−0.981

since, by symmetry, all nuclei of the group other than the saturated one will have identical expectation values of I.

This yields

$$I_a = n/(n + \rho). \tag{10}$$

Typical values for changes in intensity are given in Table III. Note that in this case the negative effects are very strong. Isolation from other spins that would supply a relaxation sink, combined with efficient cross-relaxation within the group, cause the whole system to be very readily saturated.

LIMITING CASE 3: TWO ISLANDS IN WEAK CONTACT In this model there are two islands of $(n + 1)$ protons each, I_a, I_b, I_c ... and I_q, I_r, I_s. Within each island, all protons are relaxed with rates R and C. Between islands protons are relaxed with rates R' and C'. If a single correlation time, τ_c, applies to all motions $R/R' = C/C' = r_{aq}^6/r_{ab}^6 = \gamma$. Assume one proton in the second island is saturated. Treatment as above then yields

$$I_a = \frac{[(n+1)\gamma + n]^2 + [(2n^2 + 3n + 1)\gamma^2 + (2n^2 + 2n + 1)\gamma + n]\rho}{\left\{\begin{array}{l}[(n+1)\gamma + n]^2 \\ \quad + [(n+1)\gamma + n][2(n+1)\gamma + 1]\rho + (n+1)(\gamma+1)\gamma\rho^2\end{array}\right\}}, \tag{11}$$

$$I_q = \frac{[(n+1)\gamma + n]^2 + 2[(n+1)^2\gamma^2 + n(n+1)\gamma]\rho}{\left\{\begin{array}{l}[(n+1)\gamma + n]^2 \\ \quad + [(n+1)\gamma + n][2(n+1)\gamma + 1]\rho + (n+1)(\gamma+1)\gamma\rho^2\end{array}\right\}}. \tag{12}$$

Eqs. 11 and 12 reduce to the appropriate form of Eq. 10 if $\gamma = 0$ or 1. Some representative values for Overhauser effects $(I_a - 1)$, $(I_q - 1)$ are given in Table IV, for a system of two groups of three nuclei each, with $\gamma = 1/2$, $1/10$, and $1/64$. This corresponds to a ratio of intergroup proton distances to intragroup proton distances of 1.12, 1.47, and 2.00, respectively.

It can be seen that for sufficiently long τ_c (or large ρ), spin diffusion is effective in producing a large NOE in both groups. However, in the region $\omega\tau_c = 5$–10, reasonably good selectivity is obtained if the interisland distance is twice the intraisland distance.

TABLE IV
OVERHAUSER EFFECTS IN WEAKLY COUPLED ISLANDS

		$\gamma = 1/2$		$\gamma = 1/10$		$\gamma = 1/64$	
$\omega\tau_c$	ρ	$I_a - 1$	$I_q - 1$	$I_a - 1$	$I_q - 1$	$I_a - 1$	$I_q - 1$
~0	−0.333	+0.043	+0.117	+0.047	+0.177	+0.008	+0.196
1.118	0	0	0	0	0	0	0
2.0	+0.495	−0.072	−0.116	−0.059	−0.170	−0.011	−0.193
5.0	+4.016	−0.414	−0.481	−0.319	−0.574	−0.083	−0.645
10.0	16.93	−0.750	−0.783	−0.655	−0.823	−0.274	−0.867
20.0	66.52	−0.924	−0.935	−0.881	−0.943	−0.596	−0.953
50.0	416.5	−0.987	−0.989	−0.979	−0.990	−0.902	−0.991

LIMITING CASE 4: SINGLE BRIDGING PROTON In this model there are two groups of m protons each. Within each group all relaxation parameters are identical. Between groups the relaxation parameters are negligibly small. In addition, one proton (I_j) is coupled with the same relaxation parameters to all protons of both groups, forming a bridge between them. With one spin in the second group saturated,

$$I_a = \frac{2m^3 + (2m^3 + 6m^2)\rho + (4m^2 + 4m)\rho^2}{2m^3 + (2m^3 + 6m^2)\rho + (4m^2 + 5m)\rho^2 + (5m + 1)\rho^3}, \tag{13}$$

$$I_m = \frac{2m^3 + (2m^3 + 4m^2)\rho + (2m^2 + 2m)\rho^2}{2m^3 + (2m^3 + 6m^2)\rho + (4m^2 + 5m)\rho^2 + (5m + 1)\rho^3}. \tag{14}$$

Representative values calculated for $m = 3$ are shown in Table V.

Comparison of Models with Gramicidin-S in $C_2D_6O_2$

Previous studies of gramicidin-S by NMR and other techniques have established the main features of its conformation in solution. The cumulative evidence has been considered by Dygert et al., (1975) and compared with the stable conformation derived from potential energy calculations. The main feature of the most stable conformation is the arrangement of the amino acid residues in a cyclic anti-parallel β-pleated sheet with β-turns in the sequences Val-Pro-Phe-Leu. The structure has a twofold axis of

TABLE V
OVERHAUSER EFFECTS IN BRIDGED ISLANDS

$\omega\tau_c$	ρ	$I_a - 1$	$I_m - 1$
0	−0.333	+0.011	+0.156
1.118	0	0	0
2	+0.495	−0.022	−0.143
5	+4.016	−0.462	−0.658
10	+16.93	−0.834	−0.910
20	+66.52	−0.956	−0.978
50	+416.5	−0.993	−0.996

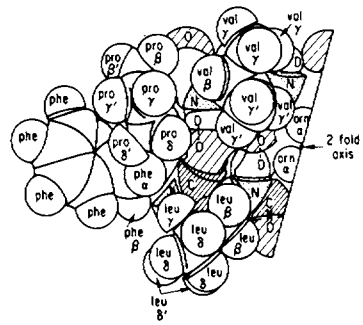

FIGURE 5 View of one-half of the symmetrical gramicidin-S molecule. The accepted conformation is shown, except that the Val side chain has been turned to show the gap separating Val-β from the protons at closest approach.

symmetry (C_2). A portion of a model demonstrating the backbone conformation and disposition of the side chains is shown in Fig. 5. The aromatic ring of Phe overlies the Pro ring, and it is observed that one of the Pro δ protons gives a resonance at higher applied field as a result. This proton is the one *trans* to the proline carboxyl group, and it will be designated Pro δ'. Wyssbrod and Gibbons (1973) have observed that this resonance shifts downfield as the temperature is raised, suggesting that a second orientation of the Phe ring becomes more populated at elevated temperatures. (Dygert et al., 1975).

Allerhand and Komoroski (1973) and more recently Komoroski et al., 1975 have studied the dynamic behavior of gramicidin-S in CD_4O and in dimethylsulfoxide, deducing τ_c for the reorientation of ^{13}CH vectors from measured values of ^{13}C longitudinal relaxation times. They find that the peptide backbone and attached Pro ring rotate almost isotropically, with a τ_c of 0.33 ns in CD_4O at 43°C, and of ~ 0.9 ns in dimethylsulfoxide. The side chains move somewhat more freely as a result of internal rotation.

If overall rotation of the peptide backbone obeys the Debye-Stokes relation, τ_c is expected to increase in proportion to the viscosity of the solvent. The viscosity of methanol at 43°C is 0.435 cP (Isakova and Oshueva, 1966) and of dimethylsulfoxide is 1.454 cP (Yao and Bennion, 1971), so that τ_c should increase by 3.3. The τ_c's are thus in reasonable agreement with expectation. The viscosity of $C_2H_6O_2$ at 25°C is reported by Artemchenko (1972) as 17.0 cP, which yields $\tau_c \sim 14.0$ ns, which at 250 MHz gives $\omega\tau_c \sim 22$ and $\rho \sim 80$. The side chain motions are faster in dimethylsulfoxide than would be calculated from assumption of proportionality to solvent viscosity. This is not unexpected, since (*a*) they will be protected from solvent on one side and (*b*) motions in which solvent is not displaced, such as internal rotations of methyl groups, tend to be little influenced by solvent viscosity, in accord with the "slip" model (Hu and Zwanzig, 1974).

Spin diffusion in the molecule could occur readily within the protons of a single amino acid residue, where nearest neighbors are on the average 1.5–2.5 Å distant, or between protons along the peptide backbone. In $C_2D_6O_2$, the NH protons have all

been exchanged and the distance between the α protons is rather large (~ 4–4.5 Å) so that cross-relaxation by this path is expected to be less effective. Thus if the conformation is such that the side chain does not closely approach another side chain in the peptide, it will behave like an isolated or weakly coupled island (case 2), while if it is in close contact with another side chain, the two will act like tightly coupled islands (case 3).

Examination of the model (Fig. 5), shows that, because of the β-turn, the Phe α proton is in close proximity, possibly van der Waals contact, to the δ' proton. In this way, the Pro island is linked strongly to the Phe island (analogous to case 3 above), and a large effect is produced whenever a Pro proton is irradiated.

In evaluating the NOE's by resonance amplitude measurements, it appeared that the weaker (two-proton) component exhibited larger NOE's than the stronger (three-proton) component. This makes sense if the weaker upfield component is assigned to the *ortho* protons of the Phe ring and the stronger component to the *meta* and *para* protons. Such an assignment has previously been made in polystyrene, where the order of shifts was explained on the basis of ring currents. Another possibility, however, is that the *ortho* protons are less exposed to solvent, and that the shifts are a result of the van der Waals part of the solvent effect. The coupled island model, applied to the five protons of the Phe ring and the five protons of the Pro δ-Phe α-Phe β group, yields a ρ of 16, corresponding to $\omega\tau_c \sim 10$, which is reasonable.

It should be pointed out that the Phe side chain readily adopts a conformation in which an *ortho* proton is in contact both with Phe α and Pro δ'. This could provide a further coupling of the Phe and Pro proton systems. This conformation approximates closely the conformation suggested for the Phe side chain by Dygert et al. (1975). The second orientation of the Phe side chain, deduced by Wyssbrod and Gibbons (1973), could bring the Phe side chain in contact with the Leu side chain and explain the modest NOE observed when Leu α and β protons are irradiated. The Val β protons cannot approach the Phe protons and must operate indirectly by some cross-relaxation with the proline protons. A distinct gap separates the β-Val and Pro protons; the methyl protons of Val can approach more closely, but the rapid methyl rotation should reduce τ_c and ρ so that they are less effective at transferring excitation to the Pro protons. Finally, the Orn protons are located on the opposite face of the gramicidin-S ring, and are relatively remote and isolated from all other protons, and hence ineffective in producing NOE's in the Pro-Phe island.

Implications for the Use of NOE in Proteins

When $\omega\tau_c$ exceeds 25 or so, cross-relaxation becomes very effective, and the probability of observing selective effects is low. Proteins that have a structured hydrophobic core will have these residues in intimate contact, so that spin diffusion within the core will probably be fast and extensive. Side chains will have shorter effective τ_c's than the correlation time for the rotation of the whole molecule, and will have fewer intimate contacts, so they are more likely to exhibit specific effects. It may be difficult, however, to find and assign unique resonances to be irradiated in such large and complex molecules.

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REFERENCES

ALLERHAND, A., and R. A. KOMOROSKI. 1973. Study of internal rotations in Gramicidin S by means of Carbon-13 spin lattice relaxation measurements. *J. Am. Chem. Soc.* **95**:8228–8231.

ARTEMCHENKO, A. I. 1972. Viscosity and structure of potassium iodide solutions in ethylene glycol. *Fiz. Khim. Rastvorov.* 128–34.

BALARAM, P., A. A. BOTHNER-BY, and J. DADOK. 1972. Negative nuclear Overhauser effects as probes of macromolecular structure. *J. Am. Chem. Soc.* **94**:4015–4017.

BOTHNER-BY, A. A., and R. GASSEND. 1973. Binding of small molecules to proteins. *Annu. N.Y. Acad. Sci.* **222**:668–675.

DADOK, J., and R. F. SPRECHER. 1974. Correlation NMR spectroscopy. *J. Magn. Resonance.* **13**:243–248.

DYGERT, M., N. GŌ, and H. A. SCHERAGA. 1975. Use of a symmetry condition to compute the conformation of Gramicidin S. *Macromolecules.* **8**:750–761.

GERIG, J. T. 1977. Fluorine-proton Overhauser effects in fluorine-labeled macromolecular systems. *J. Am. Chem. Soc.* **99**:1721–1725.

GIBBONS, W. A., H. ALMS, R. S. BOCKMAN, and WYSSBROD. 1972. Homonuclear indor spectroscopy as a means of simplifying and analyzing proton magnetic resonance spectra of peptides and as a basis for determining secondary and tertiary conformations of complex peptides. *Biochemistry.* **11**:1721–1725.

GUPTA, R. K., J. A. FERRETTI, and E. D. BECKER. 1974. Rapid scan Fourier transform NMR spectroscopy. *J. Magn. Res.* **13**:275–290.

HOWARD, J. C., A. ALI, H. A. SCHERAGA, and F. A. MOMARNY. 1975. Investigation of the conformations of four tetrapeptides by nuclear magnetic resonance and circular dichroism spectroscopy, and conformational energy calculations. *Macromolecules.* **8**:607–622.

HU, C. M., and R. ZWANZIG. 1974. Rotational friction coefficients for spheroids with the slipping boundary condition. *J. Chem. Phys.* **60**:4354–4357.

HULL, W. E., and B. D. SYKES. 1975. Dipolar nuclear spin relaxation of ^{19}F in multispin systems. Application to ^{19}F labeled proteins. *J. Chem. Phys.* **63**:867–880.

ISAKOVA, N. P., and L. A. OSHUEVA. 1966. Viscosity of liquid methanol at high pressures. *Zh. Fiz. Khim.* **40**:1130–1131.

JAMES, T. L. 1976. Binding of adenosine 5′-diphosphate to creatine kinase. An investigation using intermolecular nuclear Overhauser effect measurements. *Biochemistry.* **15**:4724–4730.

JAMES, T. L., and M. COHN. 1974. The role of the lysyl residue at the active site of creatine kinase. *J. Biol. Chem.* **249**:2599–2604.

KALK, A., and H. J. C. BERENDSEN. 1976. Proton magnetic relaxation and spin diffusion in proteins. *J. Magn. Resonance.* **24**:343–366.

KARPEISKI, M. YA., and G. I. YAKOVLEV. 1976. Application of the nuclear Overhauser effect for the study of the structure of ribonuclease-nucleotide complexes. *Bioorg. Khim.* **2**:1221–1230.

KOMOROSKI, R. A., I. R. PEAT, and G. C. LEVY. 1975. High field carbon-13 NMR spectroscopy. Conformational mobility in Gramicidin S and frequency dependence of ^{13}C spin-lattice relaxation times. *Biochem. Biophys. Res. Commun.* **65**:272–279.

LEACH, S., J. NÉMETHY, and H. A. SCHERAGA. 1977. Use of proton nuclear Overhauser effects for the determination of the conformations of amino acid residues in oligopeptides. *Biochem. Biophys. Res. Commun.* **75**:207–215.

WYSSBROD, H. R., and W. A. GIBBONS. 1973. Conformation-function relationships in peptides and proteins. Part I. Naturally occurring peptides. *Surv. Prog. Chem.* **6**:209–325.

YAO, N.-P., and D. N. BENNION. 1971. Transport behavior in dimethyl sulfoxide II. Viscosity Studies. *J. Phys. Chem.* **75**:1727–1732.