NUCLEAR OVERHAUSER EFFECTS AND THE CONFORMATION OF GRAMICIDIN S

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SUMMARY: Nuclear Overhauser effects have been observed between NH and $C^{\alpha}H$ protons in gramicidin S in dimethyl sulfoxide-d_6. By use of the correlation time (τ_c) and a published relationship between τ_c and maximal nuclear Overhauser effect, the observed changes in intensity have been used to derive internuclear distances, which are compared with those predicted from a calculated low energy structure.

Intramolecular nuclear Overhauser effects have been used to probe the conformations of oligopeptides (1,2), and a recent paper (3) has drawn attention to the possibility that proton-proton distances, together with potential energy maps, might be used to deduce backbone and side-chain dihedral angles (3). When the molecule is tumbling rapidly in solution, it is a relatively straightforward matter to use the nuclear Overhauser effect for estimation of short proton-proton distances (4). When tumbling is slower, the nuclear Overhauser effect is reduced, and even negative "enhancements" may be found (2,5). The expected variation of the maximum Overhauser enhancement with τ_c has been calculated recently (2). We report here some nuclear Overhauser effects for gramicidin S, cyclo(Pro-Val-Orn-Leu-D-Phe)$_2$, in DMSO-d_6 for which slow tumbling and reduced nuclear Overhauser enhancements are observed. The inter-proton distances computed from the nuclear Overhauser effects are compared with those reported recently (6) for a computed structure of this compound.

MATERIALS AND METHODS

The NMR experiments were performed in the correlation mode using the 250 MHz spectrometer at the Mellon Institute, Carnegie-Mellon University. The areas of the $C^{\alpha}H$ peaks were monitored, as specific NH signals were irradiated.

Abbreviations: NMR, nuclear magnetic resonance; τ_c, molecular correlation time; DMSO-d_6, deuterated dimethyl sulfoxide.

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Gramicidin S, as the dihydrochloride salt, was used as a 0.05 M solution in 100% DMSO-d_6 and the solution was degassed by bubbling with nitrogen for ten minutes immediately before the experiments.

The appropriate theory is described by Noggle and Schirmer (7) in their treatment of the linear three-spin case. Since the first and third nuclei (NH_i and NH_{i+1}) are sufficiently far apart so that there is no spin-spin coupling nor dipole-dipole relaxation between them, we can apply equation 3.38b of Noggle and Schirmer (7):

$$f_2(1) \approx (\rho_{21}/2R_2)[1 - f_3(2)f_2(3)]$$

with the terms as defined by these authors. In most of our cases, $f_2(3)$ is observed to be zero, and this equation simplifies to the two-spin case. Even where small values of $f_2(3)$ and $f_3(2)$ are observed, the denominator is close to unity and the approximation as a two-spin system applies.

RESULTS AND DISCUSSION

The signals of the NH protons were irradiated and changes in the areas of the $C^{\alpha}H$ signals were noted. The results are summarized in Table I. One of the peaks is a three-proton conglomerate comprising signals from the $C^{\alpha}H$'s of Pro, Val and D-Phe. In each of the two cases where this conglomerate is involved (see Table I) the interpretation indicated in Table I is reasonable because no other proton is near enough to interfere. However, the calculation of the relative effect in these cases requires a multiplication by three which would magnify any error.

All of the observed intensity changes are negative because of slow tumbling. Using a reported value of $\tau_c = 9 \times 10^{-10}$ sec in DMSO-d_6 (8) and the published curve of Glickson et al. (2) for the dependence of nuclear Overhauser enhancement on τ_c, we have applied a scaling factor to the observed intensity changes to obtain the "rapid-tumbling" (positive) nuclear Overhauser enhancements. The latter have been interpreted by the procedure of Leach et al. (3) in terms of inter-proton distances (see Table I). The curve for the dependence of nuclear Overhauser enhancement on τ_c is so steep in the vicinity of $\tau_c =$ 9×10^{-10} sec that a small change in τ_c produces a large change in the calculated distance, as can be seen from Table I where we have also tabulated estimates based on $\tau_c = 9.5 \times 10^{-10}$ sec. Since the normal precision in the

Table I. Correlation of Nuclear Overhauser Effects with Inter-Proton Distances for Gramicidin S

Signal rradiated	Signal Observed	Intensity Change (%)	Calculated H···H Separation (Å)		Distance in Model (6) (Å)
			τ_c=9.0×10^{-10} sec	τ_c=9.5×10^{-10} sec	
Val NH	All $C^{\alpha}H$'s	None	-	-	2.37, 2.83, 3.39[a]
Orn NH	Val $C^{\alpha}H$	-9.6[b]	2.3_5	2.4_5	2.24
Leu NH	Orn $C^{\alpha}H$	-4.5	2.6_6	2.8_1	2.22
Phe NH	Leu $C^{\alpha}H$	-14	2.2_0	2.3_0	2.19
	Phe $C^{\alpha}H$	-10.5[c]	2.3_1	2.4_2	2.71

) Computed distances (6) from Val NH to Pro $C^{\delta}H$, Val $C^{\alpha}H$ and Pro $C^{\alpha}H$, respectively.

) A 3.2% decrease was observed in the area of the three-proton conglomerate signal from the $C^{\alpha}H$'s of Pro, Val and D-Phe. All of the decrease was attributed to Val $C^{\alpha}H$.

) A 3.5% decrease was observed in the area of the conglomerate signal (see b). All of the decrease was attributed to the Phe $C^{\alpha}H$.

measurement of τ_c is ±10%, this value of τ_c is "equal to" the reported value within the precision of the measurement.

The $C^{\alpha}H$'s of Val, Orn and Leu have, as their nearest neighbor and chief source of relaxation, the NH of the succeeding residue (Orn, Leu and D-Phe, respectively). Irradiation of the Val NH signal had no effect on the areas of other peaks. The nearest neighbors are one of the Pro $C^{\delta}H$'s [2.37 Å away in the model of Dygert et al. (6)], which would be relaxed by its geminal partner, and the Val $C^{\alpha}H$ (2.83 Å away) whose relaxation is dominated by the Orn NH (2.24 Å away) and probably to some extent by Val $C^{\beta}H$ (2.87 Å away) and the C^{γ} methyl hydrogens of the valine side chain which are gauche to the $C^{\alpha}H$. The Overhauser enhancement of the Val $C^{\alpha}H$ signal upon irradiation of the Orn NH is probably reduced by this $C^{\alpha}H/C^{\gamma}H_3$ interaction, and the distance computed from the Overhauser effect is thus an upper limit.

The irradiation of the Orn NH signal produces no change in the Orn $C^{\alpha}H$ (2.86 Å away). The Orn $C^{\alpha}H$ must be relaxed mainly by the Leu NH because we observe a modest Overhauser enhancement for this pair, and our calculated separation for this pair is 2.81 Å, which must represent an upper limit. The value from the model (6), 2.22 Å, is much smaller, and the reduced Overhauser effect must be due to some relaxation of Orn $C^{\alpha}H$ by the adjacent $C^{\beta}H$ hydrogens which are 2.51 and 3.00 Å, respectively, from Orn $C^{\alpha}H$.

Similarly, the Leu $C^{\alpha}H$ is relaxed not by the Leu NH (2.80 Å away) or by $C^{\beta}H$'s (2.39 and 2.94 Å away) but by the D-Phe NH (2.19 Å away), and a strong nuclear Overhauser effect is observed. The D-Phe NH also takes some part in the relaxation of the adjacent D-Phe $C^{\alpha}H$ which has no NH_{i+1} since Pro is the following residue. Again, the interaction seems stronger than would be anticipated from the model (6) especially since the C^{β} hydrogens (2.49 and 3.00 Å away) would also contribute to relaxation of the $C^{\alpha}H$. We believe that the Overhauser results indicate a smaller dihedral angle ϕ, and hence a shorter $NH/C^{\alpha}H$ distance, for D-Phe than that in the model (6). This is also consistent with our observation that $J_{NH-C^{\alpha}H}$ for the D-Phe is 2.9 Hz. Other workers (9) have reported 4.1 Hz for this coupling constant in the derivative, N,N'-diacetyl gramicidin S, and a published spectrum shows no splitting of the D-Phe NH in gramicidin S, but a value of $J_{NH-C^{\alpha}H}$ was cited in the text as <3 Hz (10).

We must use large magnetic fields to obtain sufficient dispersion in the NMR spectra of large molecules, and it is precisely these conditions (2) which lead to negative Overhauser enhancements and the need to adopt the scaling procedure outlined above. Despite the uncertainties of the method, the calculated distances for $NH\cdots C^{\alpha}H$ pairs are sufficiently close to those for the lowest-energy structure of gramicidin S (6) that they lend support to the calculated structure. In particular, they confirm the β-sheet arrangement of the Val-Orn-Leu sections of the molecule.

Some similar nuclear Overhauser results for gramicidin S have been obtained by other workers, but only a preliminary report has appeared (11). The magnitudes of the nuclear Overhauser enhancements were not given, but they were stated to be negative in sign, and no inter-proton distances were reported.

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