atine kinase),[9] or hydrolysis (carboxypeptidase)[10] is involved. These results provide the first direct support for the often quoted hypothesis that a prime function of the metal ion may be to provide useful concentrations of the nucleophile at biologically acceptable pH's. Also, if the above relationship turns out to be a general one[11] it allows the relative efficiencies of different metal ions to be calculated from a knowledge of the pK_a's of the metal-conjugate acid (e.g., water, amines, phosphates, thiols, etc.), and furthermore from a knowledge or estimate of the bimolecular rate constant for the reaction in the absence of the metal (k_N) the rate for the corresponding metal induced reaction (k_{MN}) may be evaluated.

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Delineation of Interactions between Specific Solvent and Solute Nuclei. A Nuclear Magnetic Resonance Solvent Saturation Study of Gramicidin S in Methanol, Dimethyl Sulfoxide, and Trifluoroethanol

Sir:

Detailed information about solute-solvent interactions can aid in the elucidation of molecular conformation in solution and in the development of general concepts of solution structure. Interactions between specific nuclei of the solvent and solute can be detected by NMR solvent saturation experiments, in which intensity changes in the solute spectrum are monitored while resonances of the solvent are saturated. Intensity perturbations of solute resonances result from intermolecular nuclear Overhauser effects[1-4] (NOE's) and from transfer of saturation[5-8] between exchangeable nuclei of the solute and solvent. We have recently demonstrated the application of this technique to studies of the peptide hormones angiotensin II[9] and oxytocin[10] in water. Here we show that with nonsymmetric solvents such as methanol and trifluoroethanol (TFE) preferential interactions between the solute and specific functional groups of the solvent can be detected.

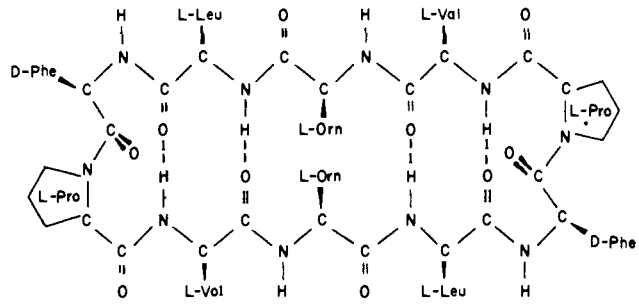

Figure 1. Conformation of gramicidin S.

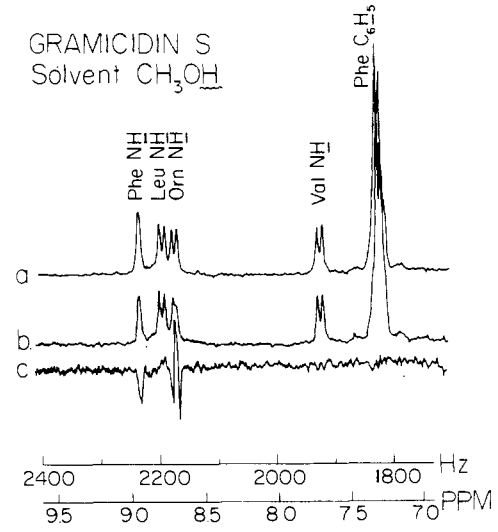

Figure 2. Solvent saturation study of gramicidin S (5% w/v) in methanol at 30 ± 1°C showing the effect of saturation of the solvent O*H* resonance. Spectra were measured at 250 MHz by correlation spectroscopy[15,16] (250 scans/spectrum; 1.6 sec/scan): (a) with off-resonance irradiation 4000 Hz to low field of the CH_3OH peak, (b) with saturation of the CH_3OH peak, and (c) the difference spectrum (spectrum b − spectrum a) amplified three times. Chemical shifts are relative to internal Me_4Si.

To illustrate the type of information which is obtained from solvent saturation experiments in organic solvents, we present a study of gramicidin S in methanol, dimethyl sulfoxide (Me_2SO), and TFE. In each of these solvents the preferred conformation of this cyclic decapeptide antibiotic is the antiparallel pleated sheet structure shown in Figure 1.[11-13] The NH groups of Leu and Val are internally hydrogen bonded to amide carbonyls; the peptide NH's of Phe and Orn are exposed to the solvent.

Figure 2 depicts a typical solvent saturation experiment in methanol. When the CH_3OH resonance is saturated, the intensity of the gramicidin S Phe N*H* peak is decreased by 24 ± 2%. This transfer of saturation results from rapid proton exchange between the methanol OH and Phe NH groups. No significant change in the intensities of the other resonances was measured. The signal with dispersion character in Figure 2c at the Orn N*H* resonance position results from partial decoupling of this proton from its $C^{\alpha}H$, whose chemical shift is the same as that of the methanol O*H*. Because of this effect no attempt was made to measure changes in the intensity of this resonance. Deuterium exchange experiments[12] indicate that the exchange of this proton is significantly slower than that of the Phe NH proton, but several orders of magnitude faster than that of the hydrogen bonded Leu and Val NH protons.

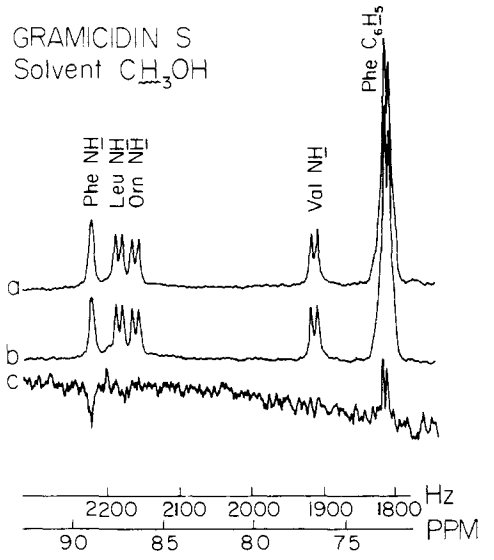

Figure 3. Solvent saturation study of gramicidin S in methanol showing the effect of saturation of the solvent CH_3 peak. Conditions are the same as in Figure 2 except that the CH_3 resonance rather than the OH resonance of methanol is saturated. The amplification factor in (c) is 5.

When the CH_3OH resonance is saturated, the intensity of the Phe C_6H_5 resonance is increased by 5 ± 1%, while the intensity of the Phe NH peak is diminished by 8 ± 2% (Figure 3). The positive NOE experienced by the ring protons results from dipole-dipole interaction between solvent CH_3 protons and phenyl hydrogens. The inverse sixth power dependence of dipolar coupling on internuclear distance[14] indicates intimate contact between the hydrophobic Phe ring and solvent methyl groups. The decreased intensity of the Phe NH resonance results from transfer of magnetization from the CH_3OH proton, which, as indicated in Figure 2, is exchanging rapidly with the Phe NH proton. The methanol OH resonance decreases in intensity by 22% upon saturation of the CH_3 peak, because the hydroxyl proton is relaxed by exchange modulation of its scalar coupling with the methyl hydrogens.[14]

The solvent saturation experiment was repeated in the highly polar solvent, dimethyl sulfoxide. Saturation of the solvent methyl resonance results in a nuclear Overhauser enhancement of 10 ± 1% for the Phe ring protons, suggesting intimate contact between methyl protons and ring CH protons, as was similarly suggested for the methyl group of methanol. Since dimethyl sulfoxide has no exchangeable hydrogens, no perturbation is observed in the intensities of the other resonances. The Orn primary NH_2 resonance was not observed probably because a trace amount of H_2O exchange-broadened this resonance beyond detection.

In TFE an enhancement of 5 ± 1% for the Phe C_6H_5 peak is seen when the solvent ^{19}F resonance is saturated. Irradiation of the methylene or hydroxyl protons of TFE produces no intensity changes in the spectrum of gramicidin S. Even though TFE has an exchangeable OH proton, no transfer of saturation is observed, because of the slow exchange rate of this proton.[13] These observations indicate that in TFE the trifluoromethyl group is closer to the Phe C_6H_5 protons than either the methylene or hydroxyl group.

The intermolecular NOE's are small because of competitive relaxation of the Phe ring protons by mechanisms other than intermolecular dipole-dipole interaction with the solvent. It is likely that these competitive mechanisms are predominantly intramolecular dipolar interactions, which would be enhanced by proximity of solute hydrogens and long intramolecular correlation times. These intramolecular mechanisms could be diminished by selective deuteration of gramicidin S. Other mechanisms, although less likely, cannot be excluded; however, dissolved oxygen does not significantly relax the ring protons, since degassing of samples had no effect on the NOE's. It is evident that the solvent saturation method has considerable potential for elucidating the details of solute-solvent interactions.

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The Mononuclear Nature of the Aquomolybdenum(IV) Ion in Solution

Sir:

Two groups have reported the preparation of the aquo ion of molybdenum(IV) in solution. Souchay and coworkers[1,2] favor a monomeric assignment, while in a later study Ardon and Pernick[3] concluded that the aquo ion is dimeric at $[H^+] > 1\ M$. Using a variety of techniques we have been able to show that the aquo ion exists as a monomeric 2+ species, MoO^{2+} (or possibly $Mo(OH)_2^{2+}$) over the $[H^+]$ range 0.3–2.0 M investigated, in agreement with Souchay et al.[2]

Prior to 1966 molybdenum(IV) was thought to be unstable in aqueous solution.[4,5] It has not for example been possible to obtain chloromolybdenum(IV) complexes in aqueous media, and chloro complexes as well as the thiocyanato complex $Mo(NCS)_6^{2-}$ disproportionate to molybdenum(III) and molybdenum(V).[6] Even the usually very stable $K_4[Mo(CN)_8]$ undergoes disproportionation in concentrated HCl.[7] The only molybdenum(IV) complexes which have been isolated from aqueous solution are the oxalates, $(NH_4)_4[Mo(oxal)_4]$,[8] $M_2[Mo_3O_4(oxal)_3(H_2O)_5]$ (M = K, pyH),[9] and the poorly characterized species $[MoO(oxal)(H_2O)_3]_n$,[10] and various cyano complexes.[4]

In 1973 Ardon and Pernick,[3] reported what they believed to be the first preparation of a stable aquo ion of molyb-