AMMONIUM ION PROTON MAGNETIC RESONANCE PROBE OF CATION-BINDING, CYCLIC ANTIBIOTICS

D. G. Davis
Department of Chemistry
Adelphi University, Garden City, N. Y. (USA) 11530

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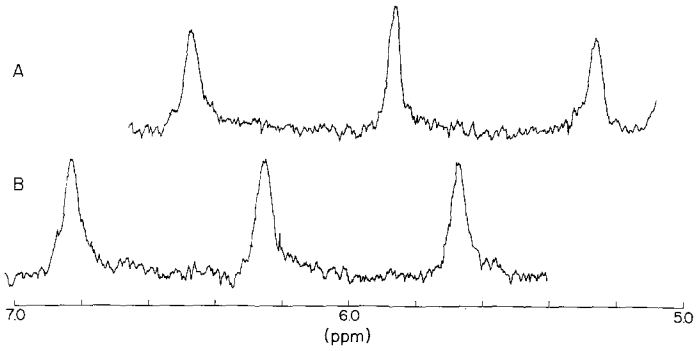
Proton magnetic resonance (PMR) spectra of NH_4^+ ions bound to the cyclic antibiotics valinomycin and nonactin can be analyzed to give, unambiquously, information about the rotational correlation times, τ_R, the Stokes' radii, r_S, and the ^{14}N quadrupolar coupling constants, e^2qQ/h, for the different complexes. For the NH_4^+-valinomycin complex: $\tau_R = 3.2 \times 10^{-11}$ sec; $r_S = 4 \times 10^{-8}$cm and $e^2qQ/h = 1.2$MHz. For nonactin: $\tau_R = 1.3 \times 10^{-10}$sec; $r_S = 6 \times 10^{-8}$ cm and $e^2qQ/h = 0$. It is also found that the proton chemical shift of the ion is 0.6 ppm to higher field when it is complexed to valinomycin as compared to nonactin.

Introduction

Knowledge about the way cyclic antibiotics such as valinomycin and nonactin react selectively with cations and move about in solution is of fundamental importance to understanding the molecular mechanisms of their biological activity[1]. In this communication it is shown how the proton magnetic resonance (PMR) spectrum of NH_4^+ ions complexed to valinomycin and nonactin provides quantitative information about the rotational mobilities and hydrodynamic sizes of these macrocyclic molecules. The PMR data also provides a qualitative comparison of the electrostatic interactions between NH_4^+ and the cation-binding sites of the antibiotics.

Experimental

The NH_4^+ complexes of valinomycin and nonactin were prepared by overnight incubation of 0.01 M solutions of the antibiotics in $CDCl_3$ with crystals of ammonium trinitro-meta-cresolate (NH_4TNC). Both the PMR spectra of the NH_4^+-antibiotic complexes as well as the



90MHz PMR spectra of NH_4^+ ions complexed with (a) valinomycin and (b) nonactin. The chemical shifts are in ppm from TMS. Trintro-meta-cresolate (TNC^-) is the counter-ion and the antibiotic concentrations are 0.01M in $CDCl_3$.

intense yellow-color of the solutions indicated complex formation. (Without added antibiotic, NH_4TNC is insoluble in $CDCl_3$). The solutions were filtered through glass wool into NMR tubes (5mm OD) to which 1% (v/v) tetramethylsilane (TMS) was added as an internal frequency reference. A Bruker Scientific HFX-90 spectrometer with a Fabri-Tex signal averager, was used to record the spectra at 25°C. At least 5 separate spectra were analyzed to give the line width and chemical shift data reported below.

Results

The 90 MHz PMR spectra of NH_4^+, complexed with valinomycin and nonactin, are shown in Figure 1.* The triplet pattern is due to the spin-spin interaction, $J\ \tilde{I}_N \cdot \tilde{I}_H$ ($J = 55Hz$) between the ^{14}N ($I = 1$) and ^1H ($I = 1/2$) nuclei. The outer lines of the triplet are proton transitions with ^{14}N in its $m_I = \pm 1$ spin states and the central line is for $m_I = 0$. Two features of these spectra are to be noted. First, the half-widths of the outer lines, $\Delta\nu_{1/2}(\pm 1)$, for the vali-

*The spectra for the protons of the antibiotics most closely resemble those of their Rb^+ complexes.[2]

nomycin-NH_4^+ triplet are 2.4 (±0.2)Hz and are broader than that of the central line, $\Delta\nu_{1/2}(0)$ = 1.8(±0.2)Hz by 0.6Hz. On the other hand the $\Delta\nu_{1/2}$ of the nonactin-NH_4^+ triplet are all equal to 2.4 (±0.2)Hz. Secondly, the chemical shift of the NH_4^+ protons for the valinomycin complex is 0.6 ppm to higher field than that of the nonactin complex.

Discussion

For protons bonded to a quadrupolar nucleus such as ^{14}N it can be shown[3] that their resonance line widths or spin-spin relaxation rates, T_2^{-1}, are sums of two terms:

$$2\pi\Delta\nu_{1/2}(\pm) = (T_2)^{-1}_{dip} + 3/5\ (T_2)^{-1}_{quad} \qquad (1a)$$

$$2\pi\Delta\nu_{1/2}(0) = (T_2)^{-1}_{dip} + 2/5\ (T_2)^{-1}_{quad}$$

The term $(T_2)^{-1}_{dip}$ is for relaxation via magnetic dipolar interactions among the ^1H and ^{14}N spins, and $(T_2)^{-1}_{quad}$ is for relaxation due to a coupling of the protons to the quadrupolar relaxation of ^{14}N. It can be seen (Eq 1a,b) that dipolar relaxation contributes equally to the width of each transition. By contrast the quadrupolar contribution depends upon the spin state of the ^{14}N nucleus.

Table 1

RESULTS OF LINE WIDTH ANALYSIS FOR NH_4^+ - ANTIBIOTIC COMPLEXES

Compound	$(T_2)^{-1}_{dip}$ (Hz)	$(T_2)^{-1}_{quad}$ (Hz)	τ_R (sec)	r_S (cm)
NH_4^+-valinomycin	0.6 (±0.2)	3.0 (±0.2)	3.2×10^{-11}	4×10^{-8}
NH_4^+-nonactin	2.4 (±0.2)	0	1.3×10^{-10}	6×10^{-8}

It is the latter term that is responsible for the extra broadening of the outer lines of the valinomycin-NH_4^+ triplet.

From the relationships defined by equations 1a,b; the dipolar and quadrupolar contributions to the line widths can be computed separately. Once these relaxation terms have been evaluated (Table 1), the rotational correlation times, τ_R and the equivalent hydrodynamic or Stokes' radii, r_S of the complexes can also be calculated according to Eqs. (2) and (3).†

$$(T_2)^{-1}_{dip} = (9/2\gamma^4\hbar^2 b_{HH}^{-1} + 8/3\gamma_H^2\gamma_N^2\hbar^2 b_{NH}^{-6})\tau_R \qquad (2)$$

$$\tau_R = 4\pi r_S^3/3kT \qquad (3)$$

The computed values of τ_R and r_S for the complexes are listed in Table 1.

As indicated in Table 1 there is a finite quadrupolar contribution to the proton relaxation of the NH_4^+-valinomycin complex. Since τ_R is known one can determine a quadrupole coupling constant, e^2qQ/h, which describes the interaction energy of the ^{14}N nuclear quadrupole moment, eQ and an external electric field quadient according to Eq. 4 and the experimental $(T_2)^{-1}_{quad}$.[3]

$$(T_2)^{-1}_{quad} = 3/8\ (e^2qQ/h)^2\tau_R \qquad (4)$$

For NH_4^+ complexed to valinomycin: $e^2qQ/h = 1.2 \times 10^6$ Hz.

Numerous facts concerning the physical properties of the antibiotics and their interaction with cations can be learned from the analysis of the NH_4^+ PMR spectra. With regard to the size of the

† In equation (2) γ_H, γ_N are the nuclear gyromagnetic ratios, $\hbar$ is (Planck's constant)/(2π) and b_{NH}, b_{NH} are the appropriate internuclear distances ($b_{NH} = 3/8\ b_{HH} = 1.03 \times 10^{-8}$cm).[4] It is also assumed that $(2\pi\nu_H\ \tau_R) << 1$ where ν_H = 90MHz. In equation (3), η is the solvent viscosity (η = 0.54 cP for $CHCl_3$ @ 25°C); kT = 4.11×10^{-14} ergs.

complexes, the estimated Stokes' radius for the valinomycin agrees well with its radius of gyration (r_G = 4.0 x 10^{-8}cm), determined independently by low-angle X-ray scattering methods[5]. Although comparable data is not available for nonactin, its r_S value is compatible with that estimated from its X-ray crystal structure[6]. This agreement on the sizes suggests that the τ_R represent time constants for the rotational diffusion of the entire cation-macrocycle complex. It follows, therefore, that the NH_4^+ ions are held rigidly in the polar cavity of the antibiotics.

With regard to the symmetry of the cation binding site, the value of e^2qQ/h = 1.2MHz for the NH_4^+-valinomycin complex implies that the ligands have a spatial arrangement of less than octahedral symmetry. (For free NH_4^+ ions, e^2qQ/h = 0, owing to their tetrahedral symmetry). By contrast there is no measurable quadrupolar term for the NH_4^+-nonactin complex since the ligand field of the nonactin molecule is nearly cubic[6]. It should be pointed out, too, that an advantage of using the NH_4^+ relaxation to probe the symmetry of the cation binding site is that e^2qQ/h and τ_R are determined independently and unambiguously (cf. ref. 7).

The proton chemical shifts for the complexed ions also reflect the differences in their binding to valinomycin and nonactin and can be understood qualitatively as effects involving both the electric dipoles and the magnetic anisotropy of the ligands[8]. A more quantitative evaluation of these effects involves a theoretical development that is beyond the scope of this communication and requires data about the electric and magnetic properties of the ligands which at present is not available. Nonetheless, the proton chemical shift is a sensitive probe of the NH_4^+ interaction with the cation binding sites and this will be investigated further.

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