NONACTIN AND ITS ALKALI COMPLEXES--A RAMAN SPECTROSCOPIC STUDY*

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Summary: The first laser Raman spectra of the ionophorous antibiotic nonactin and its complexes with Na^+, K^+, Rb^+, Cs^+, and NH_4^+ are reported. Changes in the Raman spectra indicate that the crystalline conformation of nonactin differs appreciably from those which it assumes in solution. In the alkali ion complexes, the frequency of the ester carbonyl stretch varies with the unhydrated radius R, approximately as 1/R. The frequency of this mode in the nonactin-NH_4^+ complex is significantly higher than would be expected for an alkali ion of the same radius. Other spectral changes attendant on complexation are discussed.

Several macrocyclic antibiotics are known to facilitate selective ion transport in biological and model systems[1]. Because of their utility as models of selectivity in biological membranes, these antibiotics have been intensively investigated by a variety of techniques. The actins are of particular interest because their internal cavities are sufficiently flexible to accommodate ions of different radii[2,3]. Complexation is nonetheless selective; for example, Li^+ is not appreciably complexed by nonactin, while the Na^+, K^+, Rb^+, and Cs^+ complexes have formation constants (in methanol) of 240, 3800, 3400, and 900 liters $mole^{-1}$ respectively[4].

Uncomplexed nonactin, shown in Fig. 1a, contains alternating ester linkages and tetrahydrofuran rings[5]. The analogs monactin, dinactin, trinactin, and tetranactin have increasing numbers of the asterisked methyl groups (Fig. 1a) replaced with ethyl groups. The origin of the biological activity of nonactin and its ability to extract salts of monovalent cations and lipid soluble anions (e.g., picrate) into organic solvents is suggested by the X-ray crystallographic structure of the nonactin-K^+ complex[6] (Fig. 1b). The ester and

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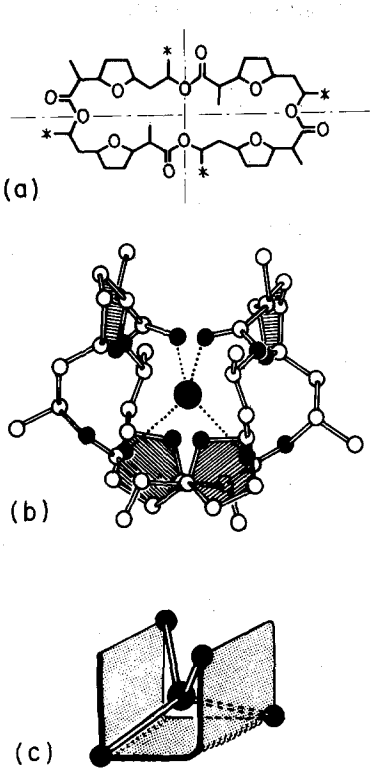

Figure 1.
(a) Chemical structure of nonactin. In monactin, dinactin, trinactin, and tetranactin one, two, three, and four of the asterisked methyl groups, respectively, are replaced with ethyl groups.
(b) X-ray crystallographic structure of the nonactin-K^+ complex (after Ref. 6).
(c) Simplified schematic drawing of the coordination of a cation to the ester carbonyl groups of nonactin, emphasizing the tetrahedral symmetry of the ester carbonyl groups.

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tetrahydrofuran oxygens each occupy tetrahedrally symmetric positions around the K^+ ion (Fig. 1c). The nonactin molecule, shaped somewhat like the seam of a tennis ball, surrounds the cation, providing an exterior of lipophilic alkyl groups. Proton NMR studies[7] indicate that this tetrahedral symmetry is maintained in acetone solutions of the Na^+, K^+, and Cs^+ complexes of nonactin.

Laser Raman spectroscopy has recently been applied to the study of the complexed[8] and uncomplexed[9,10] conformations of valinomycin and other antibiotics. We here report the first Raman spectra of nonactin and its Na^+, K^+, Rb^+, Cs^+, and NH_4^+ complexes in 15:1 M/M (4:1 v/v) methanol:

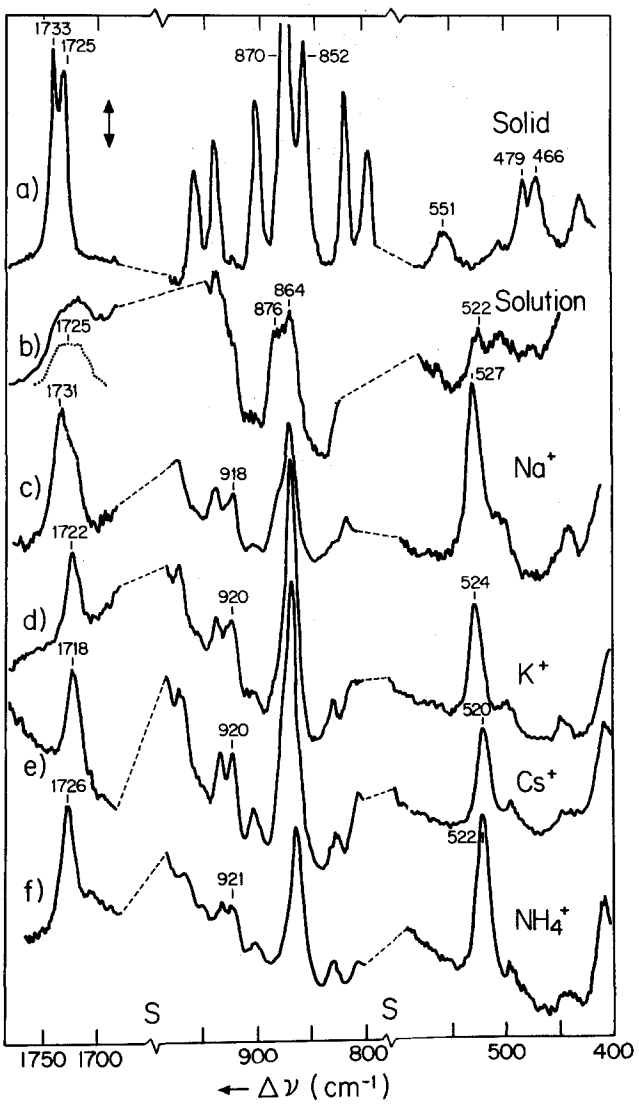

Figure 2. Raman spectra of uncomplexed nonactin (a) in the solid state, (b) in 15:1 M/M $CH_3OH:CHCl_3$ solution (dotted insert includes background correction) and nonactin complexed with (c) Na^+, (d) K^+, (e) Cs^+, and (f) NH_4^+. Arrow indicates (a-c) 100 counts/second, (d-f) 300 counts/second. Laser lines used were (a, b) 5145Å, (c-e) 4880Å, (f) 4579 Å at powers of (a) 100 mW, (b) 120 mW, (c) 100 mW, (d) 120 mW, (e) 140 mW, and (f) 80 mW. Numbers indicate peak frequencies after correction for background. Spectral regions obscured by solvent peaks indicated by S.

chloroform solutions. The solvent was chosen to increase the solubility of the nonactin complex; with 0.5M salt, 0.01 M nonactin-complex solutions were readily obtained. The uncomplexed nonactin was noticeably less soluble.

Raman spectra were measured using a Spectra Physics Model 164 Ar^+ laser and SPEX Ramalog 4 double grating monochromator system described previously[10]. Figure 2 presents spectra of the 400-600 cm^{-1}, 800-1000 cm^{-1}, and 1650-1800 cm^{-1} regions, which are free of solvent peaks.

The Raman spectrum of uncomplexed nonactin in solution (Fig. 2b) differs considerably from that obtained in the solid state (Fig. 2a), which suggests that the conformation of crystalline uncomplexed nonactin differs significantly from the conformation(s) occurring in more biologically relevant environments. We find no Raman spectroscopic evidence for the formation of a nonactin-Li^+ complex in 0.5 M LiCl or $LiNO_3$. This is consistent with experimental findings in other solvents[1]. The addition of water (1:5 v/v) had little effect on the spectra of the Li^+ solutions. Hence the spectral changes observed in solutions of the other alkali salts do not arise from changes in the ionic strength of the solution.

The 1700-1800 cm^{-1} region contains the conformation-sensitive ester carbonyl stretch vibrations[8-11]. A sharp doublet (1725, 1733 cm^{-1}) in the Raman spectrum of crystalline nonactin may reflect the effects of intermolecular forces in the crystal. In contrast, a single broad line centered at 1725 cm^{-1} is found in solution (Fig. 2b). This line broadening may represent increased flexibility of the nonactin molecule in solution or perhaps the simultaneous presence of several related conformers as in valinomycin[12]. (A 1712, 1735 cm^{-1} doublet has been reported in recent infrared measurements[3] of nonactin in CH_3OH; a singlet at 1720 cm^{-1} is observed in $CHCl_3$.)

In 0.5 M solutions of KSCN (Fig. 2d), KCl, and CsCl (Fig. 2e), or in saturated solutions of RbCl, the broad ester carbonyl band (1725 cm^{-1}) of uncomplexed nonactin (Fig. 2b) is replaced by a single narrow, intense peak. A similar spectral change in valinomycin-KSCN solutions demonstrates the presence of a single, rather rigid, conformation, that of the valinomycin-K^+ complex.[8] At Na^+ concentrations of 0.5M or above, a complicated spectral line centered at 1731 cm^{-1} is observed. Since the line shape is in-

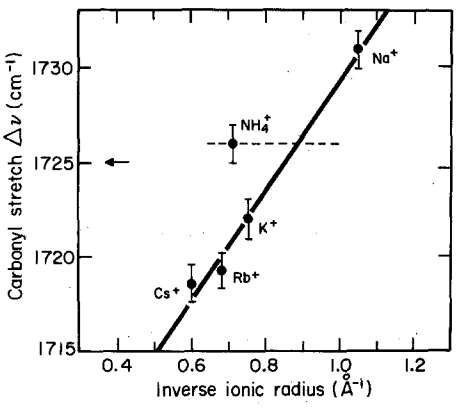

Figure 3. Dependence of the ester carbonyl stretch frequency of nonactin-alkali cation complexes on the unhydrated cationic radius R. The ester carbonyl stretch frequency found in the nonactin-NH_4^+ complex corresponds to that expected for an alkali cation of smaller radius ($1/R \sim 0.9 Å^{-1}$). The arrow indicates the frequency of uncomplexed nonactin in solution.

dependent of Na^+ concentration, it presumably arises entirely from complexed nonactin. The low-frequency shoulder may indicate that the Na^+ ion is inequivalently coordinated to the four ester carbonyl groups in some of the ester vibrations.

The ester carbonyl stretch vibration frequencies (1725-1735 cm^{-1}) of the complexes of nonactin with Na^+, K^+, Rb^+, Cs^+ (unhydrated radii R=0.95, 1.33, 1.48, 1.69 Å) exhibit a remarkable regularity: they are a linear function of 1/R (Fig. 3). This effect could arise from changes in the positions of the carbonyl groups as they coordinate ions of different sizes. It is interesting to note that Krasne and Eisenman[13] calculate the electrostatic interaction energy of an alkali cation with the nonactin carbonyl group to be nearly proportional to 1/R. Such a trend was not reported in recent infrared studies[3].

The 820-890 cm^{-1} region of uncomplexed nonactin powder (Fig. 2a) contains a prominent doublet at 852, 870 cm^{-1} which shifts to 864, 876 cm^{-1} in solution (Fig. 2b). The intensity ratio of the 864 cm^{-1} peak to that at 876 cm^{-1} is a function of solvent; it is ~ 0.35 in pure $CHCl_3$ and ~ 1.2 in 15:1 M/M CH_3OH:$CHCl_3$. In 0.5 M Na^+ solution (Fig. 2c) this ratio is further increased to ~ 1.9; and in 0.5 M K^+, Cs^+, Rb^+ (Figs. 2d-e) the

876 cm^{-1} peak is reduced to a barely observable shoulder on a greatly enhanced 864 cm^{-1} peak. This again suggests that complexation is not complete in 0.5 M Na^+, a fact consistent with its smaller formation constant[4]. The frequency of the 864 cm^{-1} peak is independent of ionic radius.

The 450-600 cm^{-1} region of uncomplexed nonactin powder (Fig. 2a) contains a prominent doublet at 466, 479 cm^{-1} and a prominent singlet at 551 cm^{-1}. Comparisons with model compounds, such as 2,5-dimethyltetrahydrofuran, suggest that these peaks represent vibrations of the tetrahydrafuran ring. In solution (Fig. 2b) this region contains a weak, broad doublet near 500, 522 cm^{-1} that is unaffected by 0.5 M Li^+. Complexation dramatically enhances the relative intensity of this peak (Figs. 2c-e). The frequency of this peak shifts downward with increasing ionic radius, from 527 cm^{-1} for the nonactin-Na^+ complex to 518 cm^{-1} for the nonactin-Cs^+ complex. Increasing the diameter of the enclosed alkali cation may facilitate interaction with the tetrahydrofuran oxygen atoms.

In isosteric complexes, the formation constant is expected to be proportional to the amount of cation extracted into a bulk organic phase[13]. The extraction of NH_4^+ by nonactin from water into dichloromethane is far greater (9000 liters $mole^{-1}$) than that of the alkali cations Li^+, Na^+, K^+, Rb^+, and Cs^+ (0.05, 3.2, 190, 90, and 11.5 liters $mole^{-1}$ respectively)[2]. Complexation is readily observed in solutions of 0.5 M NH_4^+ with characteristic changes occurring in the 1700-1750 cm^{-1}, 450-600 cm^{-1}, and 800-950 cm^{-1} regions (Fig. 2f). Complexation of nonactin with NH_4^+, but not with the alkali cations, is observed in 0.05 M salt solutions. Raman measurements on the nonactin molecule thus yield the same $NH_4^+ > K^+ \sim Rb^+ \sim Cs^+ \sim Na^+ > Li^+$ selectivity implied by macroscopic measurements of salt extraction into bulk phases.

The preferential complexation of NH_4^+ by nonactin may arise from the tetrahedral symmetry of the NH_4^+, which matches that of the coordinating ester carbonyl groups (Fig. 1c). Notice that the ester carbonyl stretch frequency of the nonactin-NH_4^+ complex lies to the left of the solid line in

Fig. 3; its frequency corresponds to that expected for a considerably smaller alkali cation ($1/R \sim 0.9 Å^{-1}$). Further experimental and theoretical work is underway to determine the effects of ion shape and size on nonactin complexation.

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