

## UVRR spectroscopic studies of valinomycin complex formation in different solvents

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### Abstract

We investigated the complexation of valinomycin (VM) in different solvent environments with the aid of the UVRR spectroscopy. By probing the 206.5 and 229 nm excited Raman spectra, we showed that new bands are observed around 1700 and 1290  $\text{cm}^{-1}$ . We assigned the 1700  $\text{cm}^{-1}$  band to the hydrogen bonded ester carbonyl stretching vibration. In a polar solvent, VM– $\text{K}^+$  complexation shows significant intensity changes in amide and ester carbonyl stretching region. Because of the small amount of conformational interconversion, complexation has a negligible effect on other band intensities including, the amide III,  $\text{C}_\alpha\text{H}$ , and amide II. We also showed the effects of the solvent polarity on the solution conformation of VM.

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**Keywords:** Resonance Raman; Valinomycin; Complexation

### 1. Introduction

The cation binding properties of ionophoric peptides (depsipeptides) are well known and they form complexes at very low ion concentrations in solution. The conformational structure of depsipeptides changes depending on the solvent polarity. The solvent polarity also determines the amount of complex formation. The complex structure remains questionable in that the site binding of metal ions may be related to the denaturation of depsipeptides. This phenomenon is not well understood. One way of solving this kind of problem would be to monitor the structural changes of depsipeptides with the addition of salts at varying concentrations.

The remarkable ability of the membrane bound ionophores (ion carriers) such as valinomycin is to differentiate selectively between various cations. Valinomycin, so called because of its high valine content and microbial origin, is known to increase the permeability of  $\text{K}^+$  over  $\text{Na}^+$  in biological membranes [1]. VM is the most important of the antibiotic-ionophores. At rather low concentrations ( $10^{-8}$  M) it selectively induces the potassium permeability

of a wide variety of biological membranes [2]. Understanding of the complexation basis of valinomycin (VM) with different metal ions is not completely resolved. However, introducing new techniques to science gives more opportunity to elucidate the complexation basis of valinomycin. Considerable work has been done on the structure and the complexation of valinomycin [2] to get more insight into the biological activity of valinomycin.

Valinomycin is a 12 membered cyclic depsipeptide. VM, cyclo-(L-val-D-hy-D-val-L-Lac)<sub>3</sub> plays an important role in the selective enhancement of permeability of biological membranes for potassium ions [1]. The VM– $\text{K}^+$  complex is very stable [3] and is weakly solvent dependent.

The conformation of the free VM has been shown to be highly solvent dependent [4]. A variety of different valinomycin conformations exist in solution; their structure and relative concentrations at equilibrium depend on the polarity and hydrogen-bonding ability of the solvent. The ion selectivity of VM depends on the nature of the ligands and the conformational states of the molecule [6,7]. To get more insight into the conformational structure and complexation properties of VM, it is important to study the titration of the VM with  $\text{K}^+$  ion. The titration study gives information about the initial conformation of VM and also complexation property of VM. We show here the applicability of UVRR

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to understand conformational structure of valinomycin and the complex formation of valinomycin. In this paper, we report our results on the conformation studies of VM and its complexed formation in polar and apolar solvents.

## 2. Experimental

VM was purchased from Sigma Chemicals and used without further purification (95% pure). Methanol and cyclohexane were obtained from Fisher Scientific. Deuterated  $\text{CD}_3\text{OD}$ ,  $\text{D}_2\text{O}$ , and  $\text{KCH}_3\text{COO}$  were obtained from Aldrich Chemical Co.

The concentration of VM solution used for Raman and absorption measurements was 4.5 mM. Solutions for the first part of titration experiments were prepared by appropriate mixing of the stock solutions of VM and  $\text{K}^+$  in methanol. Deuteration was accomplished directly in a 1 ml volumetric flask. Apolar deuteration was done by exchange across a  $\text{D}_2\text{O}$ /cyclohexane interface. Also, apolar complexation was done by transfer across a water- $\text{K}^+$ /cyclohexane interface.

UVR spectra were obtained by using a ca.  $135^\circ$  back scattering geometry, and by exciting samples spinning in a quartz NMR tube. The UVR spectrometer is described in detail elsewhere [8]. UV excitation at 206.5 and 229 nm were obtained from intra-cavity frequency doubled CW krypton and argon ion lasers, respectively. We used the  $1029\text{ cm}^{-1}$

methanol band as an internal standard. The Raman scattered light was dispersed by a Spex Triplemate monochromator and detected by an intensified CCD detector (Princeton Instrument Co.).

## 3. Results

### 3.1. Polar solvent

The Raman spectra of VM and VM- $\text{K}^+$  complex excited at 206.5 and 229 nm in methanol is shown in Figs. 1 and 2, respectively. Marked changes were observed in the Raman spectra of the free VM with the stepwise addition of the VM solution containing excess of the potassium acetate salt. The Raman spectra for three different molar concentration ratios of VM to potassium salt are shown in Fig. 2. The change in the band intensity and band position between complexed VM and free VM are monitored between  $1100$  and  $1800\text{ cm}^{-1}$ . The VM bands above  $1200\text{ cm}^{-1}$  are assigned to the amide I ( $1639\text{ cm}^{-1}$ ), II ( $1555\text{ cm}^{-1}$ ), III ( $1245\text{ cm}^{-1}$ ),  $\text{C}_\alpha\text{H}$  ( $1389\text{ cm}^{-1}$ ), and ester carbonyl vibrational modes ( $1744\text{ cm}^{-1}$ ). There are two adjacent amide I bands that occur at  $1680$  and  $1639\text{ cm}^{-1}$ . This is because of the presence of both intramolecular and free hydrogen-bonded amide  $\text{C}=\text{O}$  groups in VM. In Fig. 2, the  $1700\text{ cm}^{-1}$  band is not enhanced by 206 nm excitation light and combined with the

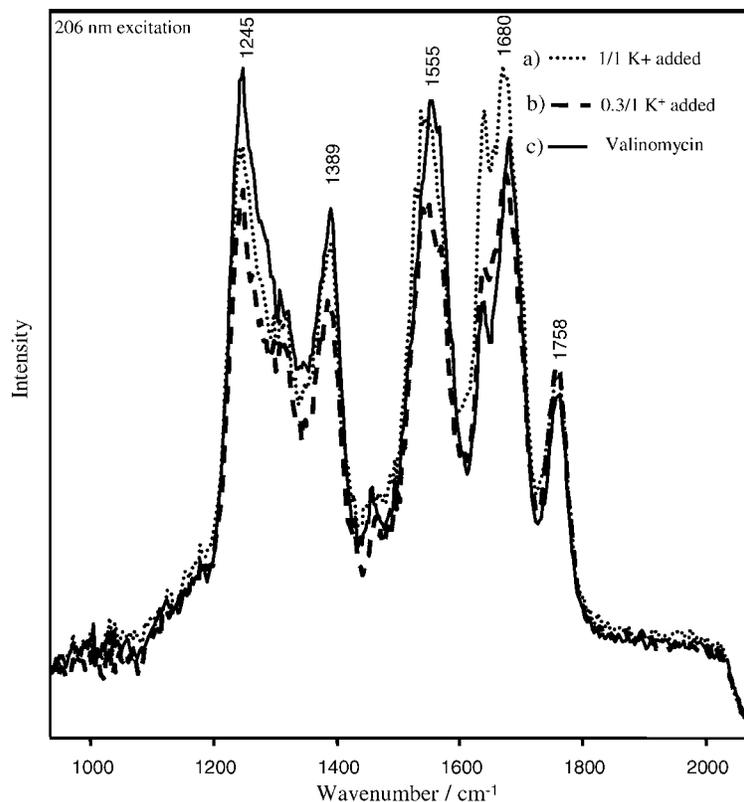


Fig. 1. UVR spectra of valinomycin complexation steps with  $\text{K}^+$  (206 nm excitation wavelength): (a) complexed valinomycin (1/1); (b) complexed valinomycin (0.5/1); (c) valinomycin.

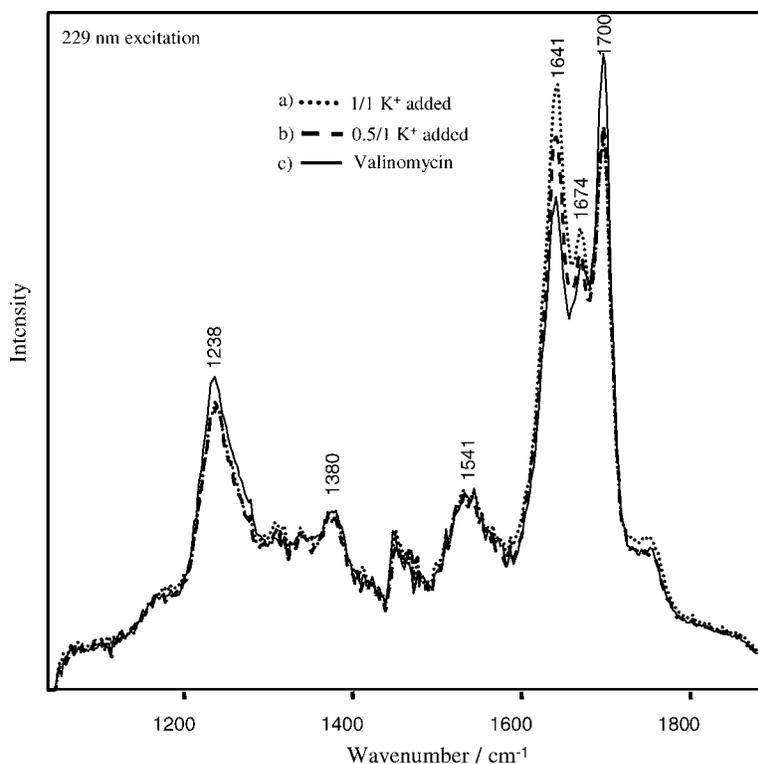


Fig. 2. UVRR spectra of valinomycin complexation steps with  $K^+$  (229 nm excitation wavelength): (a) complexed valinomycin (1/1); (b) complexed valinomycin (0.5/1); (c) valinomycin.

amide I band. The same band is enhanced by 229 nm excitation wavelength in Fig. 2 and band intensity decreases with complex formation of VM. This shows that the ester C=O groups exist in a variety of different local environments with varying degrees of exposure to the solvent. Free ester carbonyl stretch vibration arises at  $1755\text{ cm}^{-1}$ . The band intensity increases with complexation. In Fig. 2 this band is seen as a small shoulder. Another spectral difference between complexed and the free form of VM spectra is the downshift of amide I band (Fig. 1).

The amide II band is primarily the combination of amide C–N stretch and C–N–H in-plane bend. This band also shows marked changes in its relative intensity as a function of  $K^+$  concentration (Fig. 1). Amide II band shows a down shift from  $1555$  to  $1542\text{ cm}^{-1}$  and the band intensity increases with increasing  $K^+$  concentration.

The amide III band is mainly C–N stretching and N–H in-plane bending displacement. This band arises around  $1245\text{ cm}^{-1}$  is very broad and appears to be composed of overlapping sub-bands. We used  $1028\text{ cm}^{-1}$  methanol band to normalize our spectra, because there is no overlap between amide bands and this band; hence, this band can be used for normalization without interfering amide bands. The new band in amide III region arises around  $1290\text{ cm}^{-1}$  and its intensity increases with complexation. The  $C_{\alpha}H$  bending vibrational band arises around  $1389\text{ cm}^{-1}$  and the intensity of the band does not change significantly.

### 3.2. Deuterated spectra

The Raman spectra of VM dissolved in  $CD_3OD$  dramatically differ from those observed in  $CH_3OH$  because the increase in the mass of the deuterium atom exchanged for the amide hydrogen dramatically alters the vibrational normal mode distribution (Figs. 3 and 4). In the resulting spectra, the amide II' band vibration dominates. The amide II' band is pure C–N stretching and appears around  $1461\text{ cm}^{-1}$ . The amide I bands observed in  $CD_3OD$  solution are weaker and shifted to slightly lower frequency (Fig. 3). Table 1 lists the detailed Raman band frequencies. While  $1674\text{ cm}^{-1}$  band in Fig. 2 is not affected by deuteration,  $1700$  and  $1641\text{ cm}^{-1}$  amide I bands show a down shift to  $1690$  and  $1632\text{ cm}^{-1}$ , respectively.

### 3.3. Apolar solvent

We monitored the VM conformational structure change with complexation in the apolar solvent cyclohexane. Ester carbonyl and amide I bands appear at same position as the bands in methanol. Other bands are mainly downshifted. Figs. 5 and 6 show the Raman spectra of VM in polar and apolar solvents. When we use  $206\text{ nm}$  excitation wavelength, we are able to see one very strong amide band in an apolar solvent, but in the case of  $229\text{ nm}$  excitation, two different amide I bands can be seen. These bands occur near  $1674$

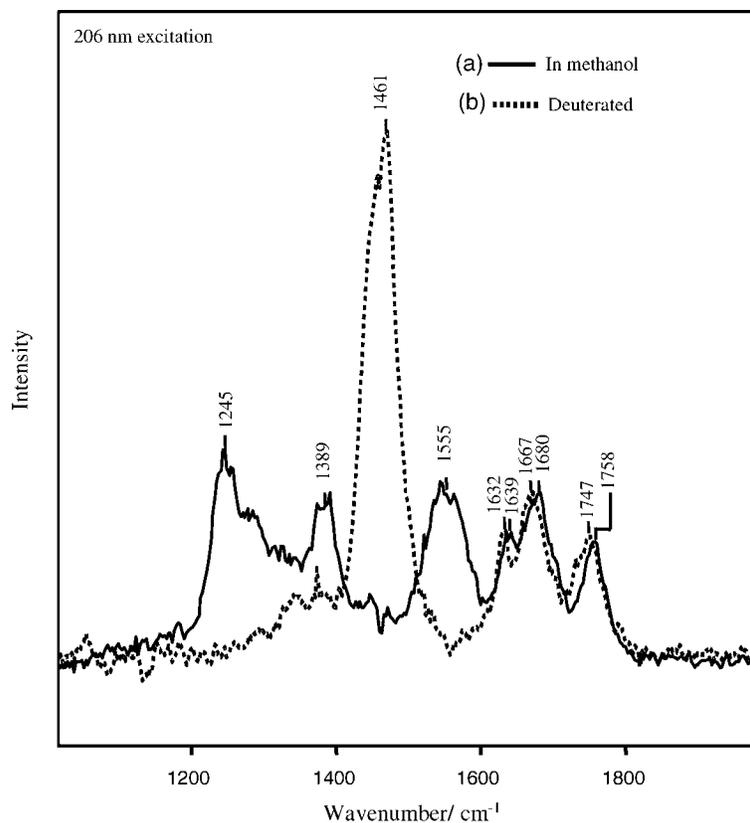


Fig. 3. UVRR spectra of (a) valinomycin and (b) deuterated valinomycin (206 nm excitation wavelength).

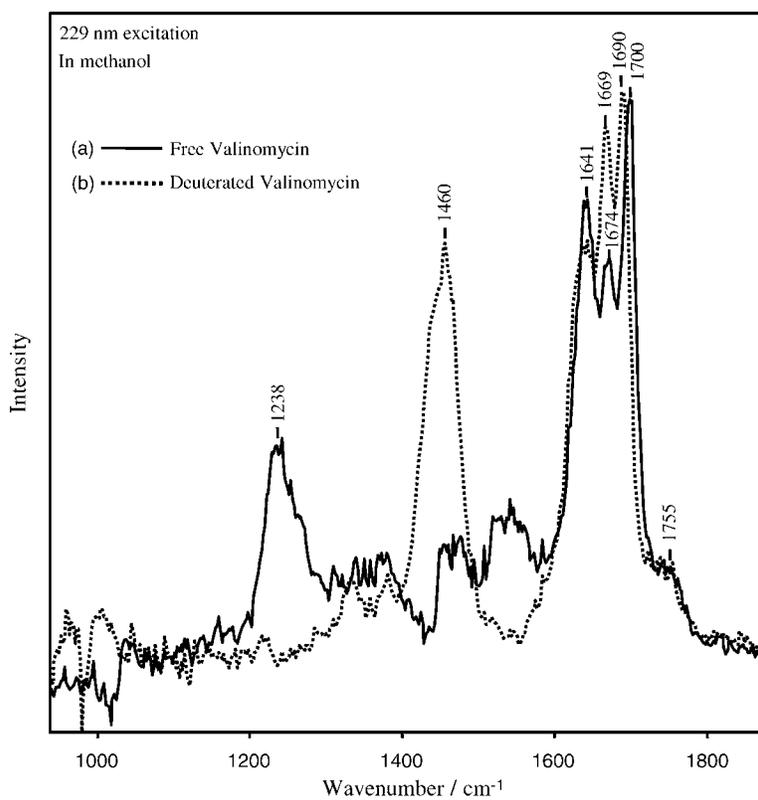


Fig. 4. UVRR spectra of (a) valinomycin and (b) deuterated valinomycin (229 nm excitation wavelength).

Table 1  
Spectral peak assignment for valinomycin and valinomycin complexes

Assignment	206 nm excitation			229 nm excitation				
	VM in methanol	VM–K in methanol	VM deuterated in methanol	VM in cyclohexane	VM in methanol	VM–K in methanol	VM deuterated in methanol	VM in cyclohexane
Amide III	1245	1245		1241	1238	1238		1247
C <sub>α</sub> H bending	1389	1389		1370	1380	1380		1375
Amide II	1555	1542		1532	1541	1541		1525
Amide II'			1461				1460	
Amide I non-hydrogen bonded	1680	1673	1667		1674	1670	1669	1674
Amide I hydrogen bonded	1639	1640	1632	1667	1641	1642	1632	1646
Ester C=O stretch free	1758	1759	1747	1756	1755	1751	1748	1749
Ester C=O stretch H bonded					1700	1697	1690	1696

Excitation wavelengths are 206 and 229 nm.

and 1646 cm<sup>-1</sup>. This shows also that amide C=O groups exist in a variety of different local environments with varying degrees of exposure to the solvent [4]. The ester carbonyl contains two distinct bands in the 1650–1800 cm<sup>-1</sup> region, indicating the presence of both free and intramolecularly hydrogen-bonded ester carbonyl groups (1696 and 1749 cm<sup>-1</sup>).

Raman spectra of cyclohexane solutions of the VM–K<sup>+</sup> complex show only band intensity decrease and no other apparent spectral information in Fig. 2. The VM–cyclohexane solution in Fig. 6 provides information on the extent of hydrogen bonding. For example, the relative intensity of the 1646 cm<sup>-1</sup> peak of VM–cyclohexane is more intense than the 1696 cm<sup>-1</sup> band. The same band in VM–methanol is less intense than the 1700 cm<sup>-1</sup> band because of the different number of intramolecular hydrogen bonds.

The amide II band (~1541 cm<sup>-1</sup>) appears in both VM–methanol and VM–cyclohexane solutions, but the relative band intensity of these is different. In the apolar medium this band has lower intensity than the amide I band.

#### 4. Discussion

Raman spectra obtained with 206.5 and 229 nm excitation are similar in terms of the vibrational modes observed but resonance enhancement and resolution are different. Because the resonance Raman process reflects the molecular geometry change, the intensities of the Raman peaks can provide information about the geometry of the resonant excited state.

The conformational structure of VM is strongly solvent-dependent. The spectra reveal that in solution VM has three major conformers [2]. Depending on solvent polarity from apolar to polar, VM could have a different number of intramolecular hydrogen-bonds from 1 to 6 and the number of hydrogen-bonds determine the conformational structure of VM.

The structures of the VM and VM–K<sup>+</sup> complexes have been studied previously by spectroscopic methods

[3,5,6,9,10]. NMR studies show that the uncomplexed VM molecule is conformationally so flexible [1]. In the complexed form of VM, the K<sup>+</sup> ion is coordinated by six carbonyls from the valine groups. We have monitored the complexation of VM in polar and apolar solvent by UVRR between 1200 and 1800 cm<sup>-1</sup> and results are reported in Table 1. The spectral changes reflect the breaking of weak hydrogen bonds and formation of new interactions in solution.

##### 4.1. The ester and amide carbonyl region

Valinomycin and its metal complexes contain six amide bonds and six ester bonds. The C=O stretching and N–H bending vibration appear as the amide band. In earlier studies it was shown that VM has two amide I bands at 1651 and 1676 cm<sup>-1</sup> in crystal form [4,5]. In solution Asher et al. [4] reported that uncomplexed valinomycin has only one amide I while the complexed form has two amide I bands. In this study we have shown that whether it is complexed or not, VM has two amide I bands in methanol and cyclohexane.

It is known that the ester C=O vibration usually appears at a higher frequency than the amide carbonyl stretch in the vicinity of 1750 cm<sup>-1</sup>. For the uncomplexed VM, two bands at 1700 and 1755 cm<sup>-1</sup> are assigned to the ester carbonyl stretching vibration (Fig. 2). The energy and geometry of the amide ππ\* excited state in the depsipeptides are likely to differ from that of peptides, because the electronic interactions occur between the adjacent ester carbonyl and amide chromophore. These interactions may shift the ππ\* electronic transition or create new electronic transitions. For the first time we propose this 1700 cm<sup>-1</sup> band as a hydrogen-bonded ester carbonyl and 1755 cm<sup>-1</sup> non-hydrogen bonded ester carbonyl. When we compare the ester bands relative intensities and the area under the bands, we can say that most of the ester carbonyls adopt intramolecular hydrogen bonding. While the 1700 cm<sup>-1</sup> band is clearly seen in the spectrum excited at 229 nm, this band is absent in the 206 nm excited spectrum. Most probably, this band overlaps with the 1680 cm<sup>-1</sup> amide I band. Examination of the

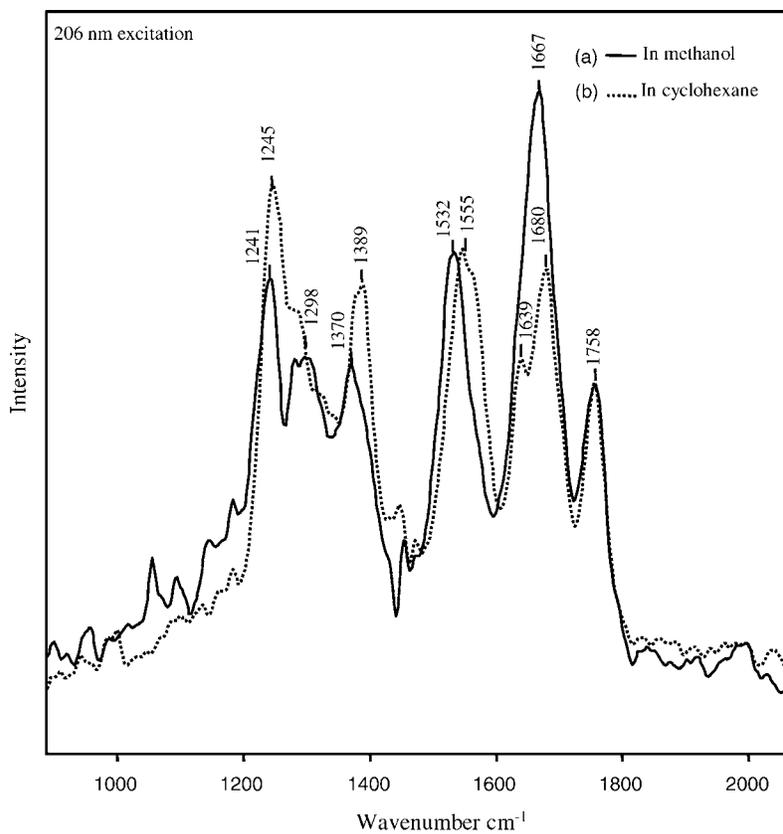


Fig. 5. UVRR spectra of valinomycin in (a) methanol and (b) cyclohexane.

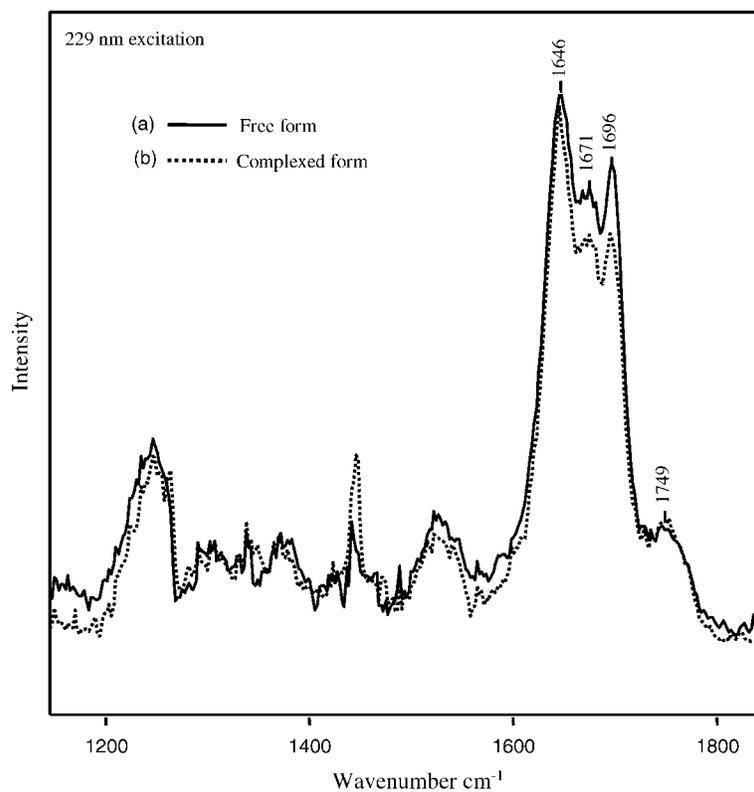


Fig. 6. UVRR spectra of valinomycin in cyclohexane (a) free form and (b) complexed form.

two amide I bands of VM indicate that the  $1680\text{ cm}^{-1}$  band has more than twice the area under the peak as that of the  $1639\text{ cm}^{-1}$  band, so it is possible to assign the  $1680\text{ cm}^{-1}$  band to the four non-hydrogen bonded amide I bonds, and the  $1639\text{ cm}^{-1}$  band to the two hydrogen-bonded amide I bonds. Actually, when we look at the 229 nm excitation Raman spectra, we cannot make this assignment, because the two band intensities are almost equal in these spectra.

The amide bands and ester bands are sensitive to conformational change of VM in solution. The complexation of VM would change the conformational structure. The addition of potassium salt to the solution initiates a conformational structure change. The most apparent change is the intensity increase in the amide I bands and an intensity decrease in  $1700\text{ cm}^{-1}$  ester carbonyl band (Fig. 2). Depending on this result, the  $1755\text{ cm}^{-1}$  ester carbonyl band intensity should increase (Fig. 1). The intensity changes of  $1639\text{ cm}^{-1}$  amide I band and  $1700\text{ cm}^{-1}$  band can explain the complex formation of VM. When the VM- $\text{K}^+$  complexation occurs, there is a change in the orientation of the ester C=O groups. As the ester carbonyl groups are oriented toward the cation, they cannot make intramolecular hydrogen bonding anymore and the number of amide intramolecular hydrogen bonds increases. The reason is that the conformational structure of VM changes completely and VM surrounds the  $\text{K}^+$  ion like a tennis ball seam and the location of carbonyl groups change. With addition of an excess of potassium salt, the  $1700$  and  $1680\text{ cm}^{-1}$  bands do not disappear, because there are still free amide carbonyls, and hydrogen-bonded ester carbonyls. This result tells us that VM does not make 1:1 complex with  $\text{K}^+$  in methanol.

The complexation of VM also affects the band frequencies of the amide vibrational modes. The conformational dependence of the amide band frequencies results from the dependence of the amide bond force constants on the polypeptide geometry and the differences in hydrogen-bonding of the different secondary structure forms. In the case of VM complexation, the ester carbonyl displacements are not significant and the interconversion between the free form and complexed form is not very high [2], so we cannot expect much frequency shift. The small amount of frequency shift (Fig. 2) may come from this small conformational change.

Deuteration of VM (dissolving in  $\text{CD}_3\text{OD}$ ) results in a complete spectral change, because the increase in the mass of the deuterium atom exchanged for the amide hydrogen dramatically alters the vibrational normal mode distribution. The resulting amide II' vibration dominates the UV Raman spectra of peptides [11] and proteins [12]. The frequency downshift of  $1700\text{ cm}^{-1}$  shows that this band comes from intramolecular hydrogen bonded ester carbonyls. The amide I band and ester carbonyl bands also show a down shift with deuteration (Fig. 3). Table 1 lists the frequency values of these bands.

#### 4.2. C–N stretching region

In the C–N stretching region, there are three bands. One is the amide III band and this vibration is known to be extremely sensitive to hydrogen bonding and the conformation of polypeptides [5]. The other two bands are  $\text{C}_\alpha\text{H}$  bending and amide II vibrational modes. We have already explained that the conformational structure of VM shows a small change with complexation in methanol. The complexation mostly affects the number of intramolecular hydrogen bonds. Because of this we do not expect much spectral change in the amide III region. The significant change in this region is the appearance of a new band at  $1290\text{ cm}^{-1}$ . The intensity of this band increases as the complexation ratio increases. Rothschild et al. [5] assigned this band to C–H vibration. The band position does not change during the complexation of VM.

The amide II band is affected by complexation. The band position shows a down shift from  $1555$  to  $1542\text{ cm}^{-1}$  and the band intensity increases slowly with increasing ion concentration. During the complexation process the band intensity ratio between amide I and amide II changes. The amide I band intensity becomes more intense than amide II (Fig. 1).

Wang et al. [13] reported that the hydrogen bonding strongly affects the amide I frequency and that the frequency shift was correlated with the intensity shift from amide I and amide II. Our results show that the amide I band intensity is strongly affected by intramolecular hydrogen bonding. The complexation affects both the amide I and amide II band intensity but we have not completely explained this intensity change. The general result is that both amide C=O and N–C bond orders increase with complexation, so we can see an intensity increase for both amide bands.

#### 4.3. Apolar complexation and conformational change

Solvents of different polarity are known to alter the conformation of VM [5]. VM was studied in cyclohexane and methanol. Cyclohexane is an apolar solvent while methanol is a medium polar solvent. Ovchinnikov and Ivanov [2] reported that VM could have six intramolecular hydrogen bonds between amide chromophores, but we showed here that VM has two different amide bands at  $1646$  and  $1674\text{ cm}^{-1}$  for the hydrogen-bonded and free amide carbonyls, respectively. In the Raman spectra of VM, we expected one ester carbonyl band, but in this region, two ester bands appear in the  $1650$ – $1800\text{ cm}^{-1}$  region, indicating the presence of free and intramolecularly hydrogen-bonded ester carbonyls (Figs. 4 and 6). The  $1696\text{ cm}^{-1}$  band intensity is lower than the hydrogen-bonded amide I band at  $1646\text{ cm}^{-1}$ . This indicates that the number of intramolecularly hydrogen-bonded amide carbonyls is higher than the number of the intramolecularly hydrogen-bonded ester carbonyls. In our apolar complexation studies, we have not obtained complete complexation of VM, so we are not able

to give detailed information about the apolar complexation of VM.

The solvent polarity affects the Raman spectra of VM. The increase in solvent polarity may shift the conformational equilibrium of the system toward conformations with fewer intramolecularly bonded amide carbonyl groups with a consequent rise in the average frequency of the amide carbonyl stretch bond. Fig. 4 shows the Raman spectra of VM in two solvents. All the amide and  $C_{\alpha}H$  bands show down shift in wavenumbers and the ester carbonyl band frequency does not significantly. The band intensity of amide I becomes intense in an apolar solvent. Wang et al. [13] reported the solvation and hydrogen-bonding effects on amide band intensities and frequencies; a shift in the character of the ground electronic state toward resonance form of amide should make the ground state more like the excited state along the C–N coordinate, diminishing the displacement along the C–O bond in the resonance Raman excitation and increasing the displacement along the C–N bond. Thus, hydrogen bonding, which stabilizes the resonance form of the amide, should result in less activity, that is less resonance enhancement, in the carbonyl amide I vibration and higher resonance enhancement for the amide II vibration (Fig. 4).

## 5. Conclusion

We have used Raman spectroscopy to study VM– $K^+$  complexation and conformational structure in apolar and polar solvents. We focused especially on the amide and ester carbonyl stretch frequencies of VM and have shown that new bands are observed around 1700 and 1290  $cm^{-1}$ . We assigned the 1700  $cm^{-1}$  band to the hydrogen-bonded ester carbonyl stretching vibration. In a polar solvent VM– $K^+$  complexation shows significant intensity changes in the amide and ester carbonyl stretching region that the intensity of other bands does not change significantly. Because of small amount of conformational interconversion, the band frequencies do not change too much.

We showed also that the solvent polarity effect on the conformational structure of VM. The solvent polarity change shows the same effect on the depsiptides Raman spectra as in peptides.

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