BRIEF COMMUNICATION

STRUCTURE OF VALINOMYCIN-K^+ COMPLEX IN SOLUTION BY EXTENDED X-RAY ABSORPTION FINE STRUCTURE

H. W. HUANG AND C. R. WILLIAMS, *Physics Department, Rice University, Houston, Texas 77001 U.S.A.*

ABSTRACT We used synchrotron radiation to measure the K-edge absorption spectra of the potassium ion in valinomycin-K^+ complexes dissolved in ethanol and methanol. Our motivation is to study the structure of valinomycin around the potassium ion and the effect of solvents. From the extended x-ray absorption fine structure, we found that the mean distance from potassium to its coordination atoms, oxygen, is the same for both solvents, 2.79 ± 0.02 Å, compared with 2.76 Å in crystal. The K-edge threshold spectra of the two solutions are almost identical but have a small difference in their relative peak intensities. The coincidence of their corresponding peak positions indicates that the strength of ligand field is about the same in these two samples. This agrees with the known binding energies of potassium ion to valinomycin in solutions. The difference in the relative peak intensities suggests a perturbation of ligand symmetry by solvents.

Molecules capable of selective complexation with alkali and alkaline-earth cations are of great interest in biology and chemistry. In particular, naturally occurring ionophores are valuable for studying transmembrane ion transport. One of the most frequently used and studied ionophores is valinomycin, which has a marked specificity for potassium ion (1–10). To understand its ability to enhance respiration in mitochondria and its strong preference for complexing potassium ion (1–10), the structure of valinomycin has been studied by using x-ray crystallography (11, 12) and the minimum energy model (13).

While it is reasonable to assume that the structure of an ionophore-ion complex in solution is similar to that in crystal, it has never been shown directly. More importantly, it is well known that solvents affect the ion specificity of ionophores (9). Therefore, it is desirable to study the structure of ionophore-ion complexes in solutions.

In Stanford Synchrotron Radiation Laboratory, we measured the (K-edge) absorption spectra of the potassium ion in valinomycin-K^+ complexes. The complexes were in two different solvents: ethanol and methanol. The spectra from ~100 to 1,000 eV above the absorption edge, called extended x-ray absorption fine structure (EXAFS), can be used to determine the distance from potassium to its coordination atoms, in this case six oxygens. We found the mean K^+-O distance to be 2.79 ± 0.02 Å in both ethanol and methanol. This is to be compared with the x-ray crystallographical distance 2.69–2.83 Å (average 2.756 Å) (12) and

the model calculation 2.85 Å (13). The K-edge threshold spectra of the two solutions are almost identical with a small difference in their relative peak intensities (Fig. 2). The coincidence of their corresponding peak positions indicates that the strength of ligand field is about the same in these two samples. This agrees with the known binding energies of potassium ion to valinomycin in solutions. The binding energy of K^+ to valinomycin in ethanol is only 3.5×10^{-2} eV higher than in methanol (9) (not including the energy difference due to the different solubilities of K^+ in ethanol and methanol [15]). The slight difference in the relative peak intensities between the two samples suggests a perturbation of ligand symmetry by solvents.

The method of measuring the x-ray absorption spectra of liquid samples has been reported elsewhere (16, 17). The samples were prepared by mixing valinomycin and potassium acetate, in one to one ratio, in solvent. The concentration of the molecular complex in each sample is 0.4 M. The measurement was taken at room temperature. From the published equilibrium constants (9), we know that the majority (over 99.99%) of potassium ions are complexed to valinomycin in the solutions. This is confirmed by the marked difference between the spectrum of K^+ in solvent (water) (16) and the spectra of valinomycin-K^+. In fact, the spectra of valinomycin-K^+ resemble the spectrum of crystalline $KTaO_3$ (16); in both cases the potassium is coordinated by oxygens in the O_h symmetry. The spectrum of K^+ in solvent has no sharp peaks.

We analyze the EXAFS according to the standard theory (18): (*a*) the original data (absorption spectra) are expressed as a function of the energy of the incident x-ray, E. The momentum of the photoelectron k is given by $k = 2\pi[2m(E - E_0)]^{1/2}/h$, where E_0 is the energy of K-absorption edge, m is the mass of the electron, and h is Planck's constant. Since E_0 can only be determined to within a few electronvolts, it will be regarded as an adjustable (within a few electronvolts) parameter to obtain the best result. The data are then expressed as a function of k and the smooth background from the absorption spectra is removed to obtain EXAFS, $\chi(k)$. The EXAFS due to the oxygen shell has the form (18):

$$A(k) \sin [2kR + \phi(k)],$$

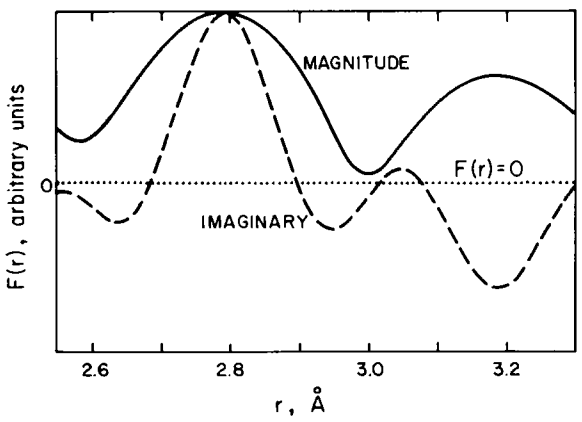

FIGURE 1 Fourier transform of the EXAFS of the potassium ion in valinomycin-K^+ complex dissolved in ethanol. $F(r)$ is the Fourier transform of the original data after the phase shift ϕ_{ex} is removed.

where R is the distance between potassium and the oxygen shell, $\phi(k)$ is the phase shift experienced by the photoelectron when it is ejected from potassium and scattered by oxygen back to the position of the original K-shell electron in the potassium, and $A(k)$ is a nonvanishing, slowly varying function of k. Each atomic shell contributes a term of the same form and $\chi(k)$ is the sum of the contributions from all shells. (*b*) The phase shift $\phi(k)$ can be obtained from the EXAFS of a known sample, such as $KTaO_3$ crystal, for which we know the K-O distance is 2.820 Å (19). Fourier transform the EXAFS of $KTaO_3$ from the k-space to the r-space. The peak corresponding to the oxygen shell is centered at a position of r a few tenths ångström less than R. Fourier transform the oxygen peak back to the k-space and call it $\chi_{K\text{-}O}(k)$. We obtain the phase of $\chi_{K\text{-}O}(k)$, $2kR + \phi(k)$, from the zeroes of $\chi_{K\text{-}O}(k)$. Since R is known for this sample, $\phi(k)$ can be obtained. Our experimental phase shift obtained from the EXAFS of $KTaO_3$ is $\phi_{ex}(k) = 3.415 - 1.234\ k + 0.0324\ k^2 + 16.75\ k^{-3}$, k in $Å^{-1}$. (*c*) Remove $\phi_{ex}(k)$ from the phase of valinomycin-K^+ EXAFS and Fourier transform the result to the r-space. The center of the oxygen peak gives the K^+-O distance R (Fig. 1). Step (*c*) is repeated with a different value of E_0 until the Fourier transform of the valinomycin-K^+ EXAFS has its imaginary part and its magnitude match at their maxima as shown in Fig. 1. Another way to determine the distance R is using step (*b*) to obtain the phase $2kR + \phi(k)$ of

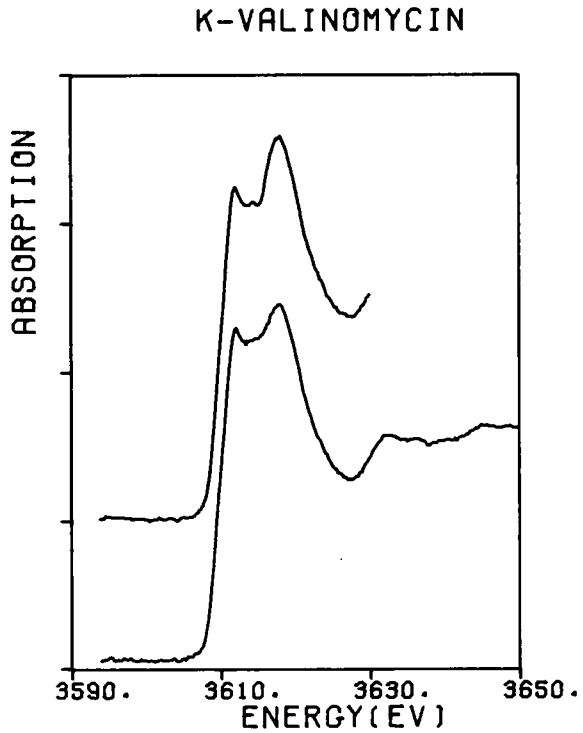

FIGURE 2 Comparison of K-edge threshold spectra of the potassium ion in valinomycin-K^+ complex dissolved in ethanol (upper curve) and in methanol (lower curve). The ordinate is the absorption coefficient in arbitrary units. The abscissa is the energy of the absorbed photon. The background of the pre-edge absorption has been removed from each spectrum.

$\chi_{K\text{-}O}(k)$ for valinomycin-K^+. Since we have $\phi_{ex}(k)$, R can be determined. Both methods yield the same result.

We found the mean K^+-O distance in valinomycin-K^+ to be 2.79 ± 0.02 Å both in ethanol and methanol. Our value is slightly larger than the mean K^+-O distance in crystal, 2.756 Å (12). This may be reasonable, since valinomycin is a rather flexible molecule.

Fig. 2 shows the K-edge threshold spectra of valinomycin-K^+ in ethanol and methanol. The coincidence of peak positions between the two spectra once again indicates that the K^+-O distances are the same in two samples (14). Before we compare these two spectra further, we must assure ourselves that there is no noticeable thickness effect (20, 21). Firstly, since these two samples have the same potassium concentration, 0.4 M, and about the same thickness, the difference between them can not be caused by the thickness effect, if any. The value of $\Delta\mu t$, i.e., the absorption coefficient of potassium above the K-edge minus the value below the edge, $\Delta\mu$, times the thickness t, is ~0.017. In reference 16, we showed that the spectrum of K^+ in water does not vary noticeably from 2 M (with $\Delta\mu t \sim 0.1$) to 0.2 M (with $\Delta\mu t \sim 0.01$).

It was shown in reference 14 that the energy separations between peaks in threshold spectra are sensitive to the ligand field. In that reference, the ligand field due to the coordination oxygens was represented by a step function potential V: $V = 0$ for $r < R_B$, $V = V_B$ for $r \geq R_B$, where r is the radial coordinate from the nucleus of the potassium ion. The energy separation between two peaks is a function of R_B and V_B. The same K^+-0 distance from EXAFS should give the same R_B for the two samples. Consequently, the same peak positions imply the same V_B for both the ethanol and methanol solutions. Since we expect the binding of K^+ to valinomycin to be mainly electrostatic, the ligand field is also responsible for the binding. Indeed the binding energies of K^+ to valinomycin in ethanol and methanol are very close, as mentioned earlier. The question of the relative peak intensities is much more complicated. A theory for the intensity of threshold spectra is still lacking. Experimentally, we know that relative peak intensities of threshold spectra are extremely sensitive to the ligand symmetry. Reference 14 shows many threshold spectra of K^+ with oxygen coordination. They have similar energy separations of peaks but very different relative peak intensities due to different ligand symmetries. It seems reasonable, then, to assume that the different relative peak intensities between the two solutions in Fig. 2 is an indication of a perturbation of the ligand symmetry by solvents.

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