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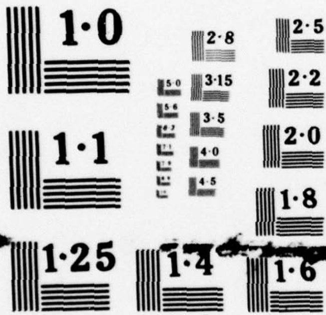

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Studying the State of Cellular Metal Ions by the Extended X-ray Absorption Fine Structure.

by

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Diffusible ions play an important role in resting and action potentials, bioenergetics and other cellular functions. It is therefore of interest to determine their chemical state in the cell. The central issue of past investigations on this subject has been the question of whether or not the majority of intracellular diffusible ions exists in a state of dilute solution (1,2). Intracellular ions are called "free" if their chemical state is similar to that in a water solution at equivalent ionic strengths; otherwise they are regarded as "bound." The chemical potentials of "free" and "bound" ions are expected to be different. Consequently, the thermodynamics of cellular functions depends on the chemical state of diffusible ions (3).

The methods used in the past investigations include electron microscopy, electron probe microanalysis, ion-selective microelectrodes, ion influx and efflux measurements and nuclear magnetic resonance (1,2,4). In a recent review Edzes and Berendsen (1) concluded that the diffusible ions in cells are essentially free. Because the living cell is a heterogeneous system and because the molecular structures within a cell are not known in detail, interpretations of data are not always straightforward. Nonetheless we were surprised by our experimental finding that the X-ray absorption-edge fine structure of potassium in frog blood cells is qualitatively different from that of free potassium in solution. The deviation seems to indicate that the potassium

ions are appreciably influenced by intracellular molecules other than water. At this point, however, due to the lack of quantitative knowledge about this "unfree" nature (other than giving rise to an absorption-edge fine structure substantially different from a free ion) we are not able to estimate the ratio of bound to free ions, if there are indeed simply two such phases.

The importance of our finding stems from the fact that the near-edge structure is a direct measurement of the atomic state of the absorbing element. The spectrum near an absorption edge represents the electronic excited states of the absorbing atom under the influence of its neighbors. We have studied the excited states of the K-shell electron from potassium in various chemical environments including potassium compounds in solid form, potassium-ionophore complexes in solutions, solutions of salts of potassium as well as frog blood cells. Aside from the blood cells, the samples were chosen with the knowledge that the nearest neighbor shells of the potassium are composed of oxygen in each case. The X-ray transmission spectroscopy experiments were performed at the Stanford Synchrotron Radiation Laboratory (SSRL), using the facility of EXAFS Beamline II. The monochromatized incident X-ray beam, off a matched pair of Si(111) crystals, had a resolution of about 1.5 ev. The intensity of the incident beam, I_o, and the transmitted beam, I, were measured with ion chambers. We used a

mixture of He and N_2 in the I_o chamber and pure N_2 in the I chamber. Helium pathways were used to eliminate the X-ray absorption by air. Liquid samples were examined using a specially designed holder (5) in which the liquid sample thickness was adjustable and the X-ray beam passed through a total thickness of about 2 mils (0.002") of polyethylene. The absorption-edge fine structures of potassium in frog blood cells and in the above mentioned samples are expressed in I_o/I in Fig. 1 in the range of photon energy from 3594 ev to 3650 ev (6) (the K-edge of potassium is at 3607 ev).

Since oxygen is electronegative, the oxygen nearest neighbor shell presents a potential barrier to the ejected photoelectron in the absorption process. We expect the pre-edge structure to be dependent not only on the radius of the oxygen shell but also on the charge distribution in the shell. On the other hand, because of the first-shell barrier, the pre-edge structure should be relatively insensitive to the chemistry beyond the first-shell atoms. Thus the pre-edge structure is a sensitive probe of local chemistry. Figures 1A to 1I demonstrate this sensitivity in a sequence of oxygen environments with varying degrees of structural flexibility, ranging from a rigid crystalline arrangement (1A) to much more fluid hydration shells (1G-1I). Between these two extremes are the oxygen cages of ionophores (1B-1F). Since for each sample the spectrum is a statistical average (at room

temperature), the most regular and rigid shell (crystal) gives the sharpest spectrum as one would expect. On the other hand, the hydration shells have the least regular and rigid structure, and consequently give rise to the most smeared spectrum: a largely featureless peak except for a shoulder at ~3610 ev. Among the ionophores, cryptate has the most rigid oxygen cage, so here the spectra of crystal (1B) and solution (1C) are nearly the same. Compared to cryptate, 18-crown-6 has a relatively open or flat structure with the K^+ more exposed to solvent and this situation seems to broaden the main peak (at ~3617 ev). Valinomycin has an approximately octahedral oxygen cage, thereby approximately preserving the $\Delta L = \pm 1$ selection rule and yielding a distinguishable 4p peak (7). In addition valinomycin is a much more deformable or flexible molecule compared with cryptate and therefore the K^+ environment (oxygen cage) is much more susceptible to perturbation through the nature of the surrounding solution. This is seen in the difference between the spectra 1E and 1F. Indeed, the free energies of binding K^+ differ between these two samples by 3.45×10^{-2} ev (not including the difference due to the solubilities of K^+ in ethanol and methanol (8)).

Having studied the general properties of the near edge spectra, we now examine the frog blood cells and aqueous solutions more closely. The 0.2M solution (Fig. 1I) was prepared to roughly

simulate the important diffusible ions in cells. The difference in slope prior to the edge, especially noticeable in 1I and 1J, is due to the enhanced absorption of the non-potassium part of the solution (background) compared with the potassium contribution. In fact the slight difference between 1I and 1G or 1H in the peak region and in the relative height of the shoulder compared to that of the peak is mainly due to this difference in background. Thus Figs. 1G, 1H and 1I provide us with a measure of the uniqueness of the purely aqueous environment. The near edge spectrum of the hydrated potassium is insensitive to both counterion and potassium concentration.

It is this uniqueness which makes it meaningful to compare the spectrum of cells with that of the aqueous solution. Figure 2 shows magnified spectra in the peak region, 3608 ev to 3626 ev. In the blood sample, greater than 95% of the potassium ions are intra-cellular. The intracellular potassium concentration is between 0.1M and 0.2M. The spectrum 2A is the peak region of 1J. The measuring time for each data point on 1J was 10 sec. Figures 2B and 2C are the same spectrum with better statistics, i.e., 30 sec per point and 50 sec per point respectively. One can see that certain features of the potassium spectrum of blood cells are not statistical fluctuations, and they are qualitatively different from the "free" potassium at a similar concentration, 2D. In

particular the height of the low energy peak at ~3610 ev relative to the broad second peak is quite different between the two. The broad peak of the blood cells is shifted to lower energy and is broader indicating perhaps a greater range of heterogeneity of K^+ environment than in H_2O. Because each K^+ environment we have examined appears fairly unique and quite distinguishable, we are led to conclude that the differences represented in Fig. 2 are appreciable and rather characteristic of the complex binding of K^+ in these frog blood cells. For potassium, the cell cannot be regarded as an aqueous solution.

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6. The monochromator was calibrated at the Cu edge (8980 ev). At the potassium edge the scale could be off by a few ev.

7. On the basis of our preliminary theoretical analysis, we have tentatively assigned the first peak or shoulder to a 4p excited state.

8. Stability constants for valinomycin complexes from W. Simon, W. E. Morf, and P. Ch. Meier, Structure and Bonding, Vol. 16, pp. 114-160 (1973). Solubilities of K^+ from "Solubilities of Inorganic and Organic Compounds," ed. H. Stephen and T. Stephen, Vol. 1 (McMillan, N.Y.) 1

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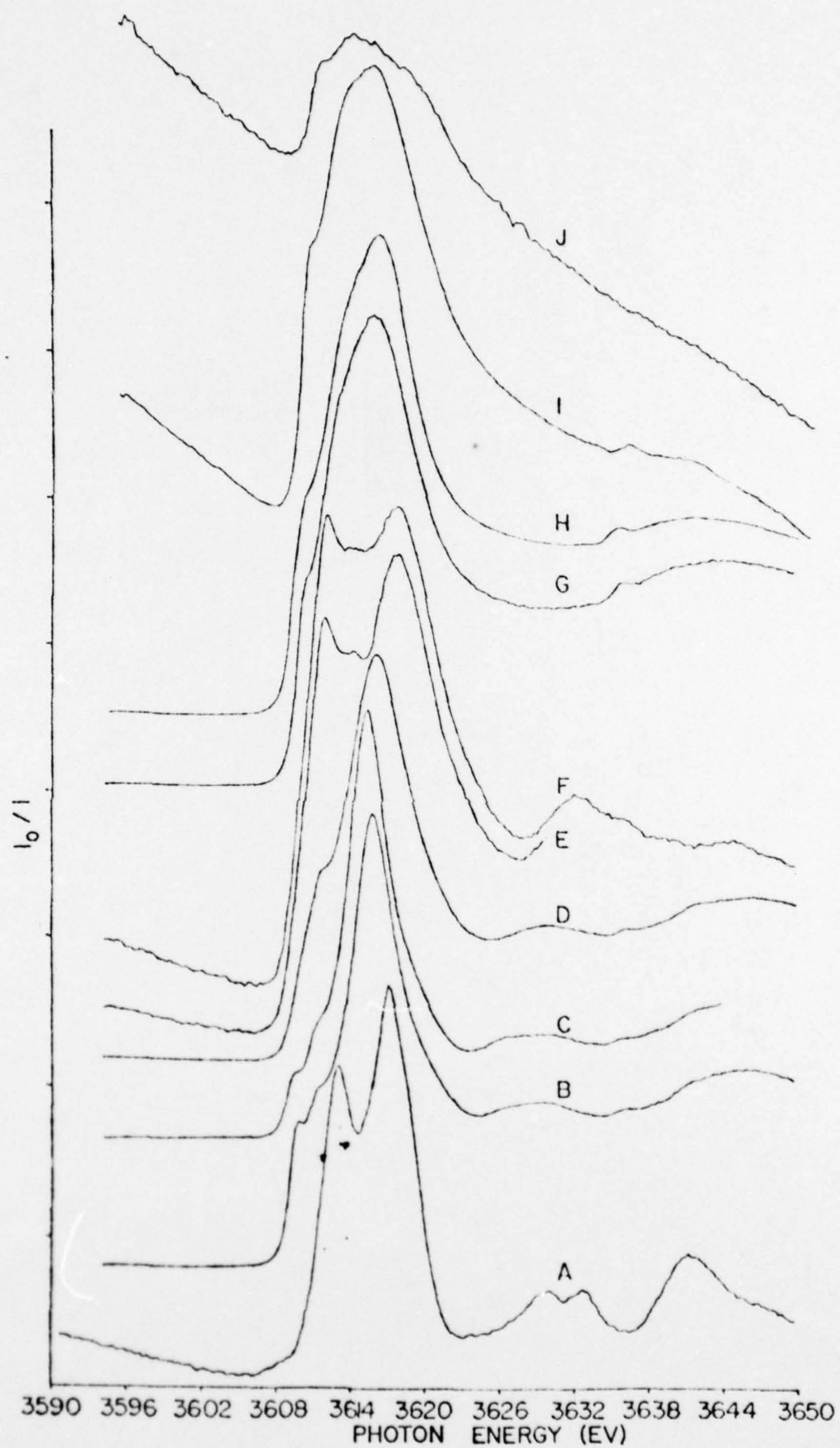
Figure Captions

Fig. 1 Absorption edge fine structure of potassiums I_o/I (in arbitrary units) vs. photon energy (ev). 1A. crystalline $KTaO_3$. For the rest of the samples, the counterion of K^+ is acetate ion, unless specified otherwise. 1B. crystalline K^+-cryptate complex. 1C. 2M (2 Molar) K^+-cryptate complex in acetone. 1D. 2M K^+-18 crown 6 complex in ethanol. 1E. 0.4 M K^+-valinomycin complex in ethanol. 1F. 0.4 M K^+-valinomycin complex in methanol. 1G. 2M potassium acetate (KAc) in water. 1H. 2M KCl in water. 1I. 0.2M K^+ aqueous solution (75% KCl, 15% KH_2PO_4, (15/2)% K_2HPO_4). 1J. blood cells of leopard frog (rana pipiens pipiens, Schreber). In all of these displays the vertical scale has been zero offset for clarity and the absolute value varies considerably from A to J. It is simply the relative edge structure that is of interest.

Fig. 2 The peak regions of the absorption edge fine structures. 2A is the peak region of Fig. 1J, where the measuring time per data point was 10 sec. 2B and 2C are the same spectrum with better statistics, i.e., 30 sec per data point and 50 sec per data point respectively. 2D is the peak region of Fig. 1I.



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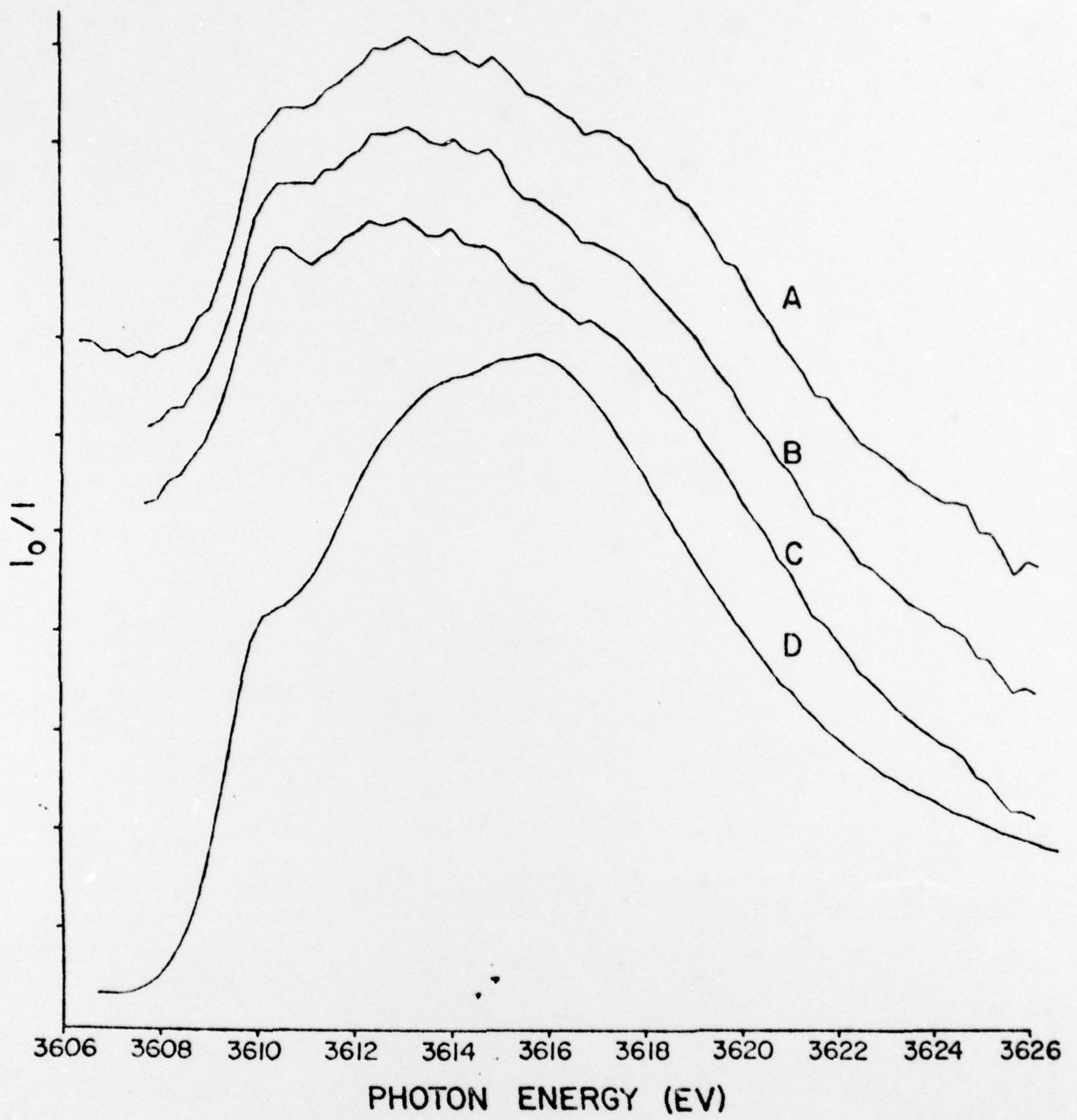

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