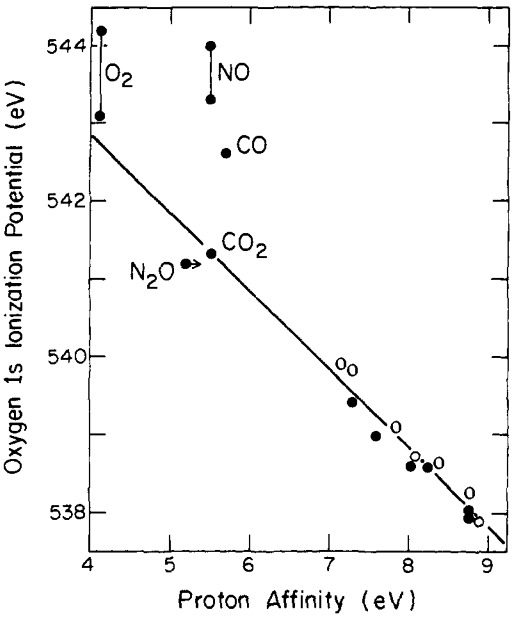

Figure 1. Correlation of oxygen 1s ionization potentials with proton affinities. Open circles represent data previously discussed in ref 1 and 2. Filled circles represent new results. The unlabeled solid circles in the lower right represent, from top to bottom, H_2CO, HCOOH, CH_3CHO, $(CH_3)_2O$, CH_3COOCH_3, and $(CH_3)_2CO$. The open circles represent, from top to bottom, H_2O, CF_3CH_2OH, CH_3OH, CH_3CH_2OH, $(CH_3)_2CHOH$, $(CH_3)_3COH$, and $(C_2H_5)_2O$. For carboxyl oxygens only the lower of the two ionization potentials has been used. The proton affinity for N_2O has been reported only as a lower limit.

and have remeasured this quantity for formic acid (538.92 and 540.60 eV[6]). These ionization potentials also fall quite close to the same correlation line.[7]

We have extended this comparison of proton affinities and inner-shell ionization potentials by considering O_2, NO, CO_2, CO, and N_2O—rather different molecules from those previously considered—together with a recent measurement we have made on methyl ether.[8]

The correlation of proton affinity with core ionization potential is shown in Figure 1. The data previously presented by Martin and Shirley and by Davis and Rabalais are shown as open circles, the additional results[9] mentioned above as filled circles. The line is a least-squares fit, constrained to a slope of -1, to all of the data except those for CO, NO, N_2O, and O_2, and corresponds to the relationship $IP(1s) + PA = 546.84$ eV. We see that, except for the diatomic molecules, all of the points fall within about 0.2 eV of the line, in agreement with the suggestion made by Martin and Shirley that this relationship should hold for a wide class of compounds. Even the diatomic molecules, although falling farther off the line, are in agreement with the general trend.

A closer inspection of the data shows that the points for the double-bonded oxygen fall consistently lower than those for single-bonded oxygen. If these data are fit separately with lines of slope $= -1$, these are displaced from one another by 0.25 eV. The root-mean-square deviation of the points from their respective lines is about 0.1 eV, which is comparable to the experimental error in the measurement of both proton affinity and core ionization potential. This slight difference between the two kinds of oxygen may, as has been pointed out by Davis and Rabalais, arise from the different hybridization involved in single and double bonds.

For formic acid and methyl acetate there are two oxygen 1s ionization potentials, one for the keto oxygen and one for the ether oxygen. The lower of these correlates well with the proton affinity: the higher falls about 1.5 eV above the line. Protonation evidently occurs at the oxygen with the lower 1s ionization potential. Inspection of the other data reveals that keto oxygens have lower core ionization potentials than do ether oxygens in similar compounds. The molecular orbital calculations of Snyder and Basch[10] predict that the keto oxygen in formic acid will have the lower ionization potential. Combining these observations we conclude that protonation occurs at the keto oxygen. This conclusion is the reverse of that reached by Pesheck and Buttrill[11] from ion-cyclotron-resonance results.

We have mentioned above new results and remeasured results for oxygen 1s ionization potentials. These were measured on mixtures of the gas of interest with either carbon dioxide or carbon monoxide as a reference compound so that any drifts in the spectrometer focusing voltage were compensated. The oxygen 1s ionization potential in carbon dioxide is accurately known, having been measured independently in separate laboratories to be 541.32 ± 0.05[12] and 541.28 ± 0.12 eV.[13] We have recently remeasured the oxygen 1s ionization potential in carbon monoxide to be 542.58 eV,[14] somewhat higher than previously reported values.[3,15]

Acknowledgment. This work was supported in part by the U.S. Atomic Energy Commission.

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Received October 29, 1974

Resonance Raman Studies of Nitric Oxide Hemoglobin

Sir:

Resonance Raman scattering from a variety of heme proteins has recently been investigated.[1-3] If the laser wave-

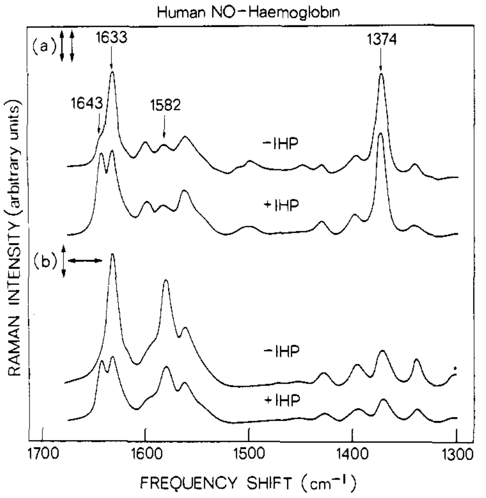

Figure 1. The polarized (a) and depolarized (b) resonance Raman spectra of nitric oxide hemoglobin in the absence and presence of inositol hexaphosphate (IHP). Experimental conditions: 0.6% solution of hemoglobin at pH 6.6 in 0.2 *M* Bis-Tris buffer and 0.04 *M* NaCl in the absence and presence of a sixfold excess of IHP; laser frequency 5145 Å, laser power 500 mW, slit width 800 μ, scan speed 25 cm^{-1}/min.

length lies within an electronic absorption band of the heme, the Raman intensity from the porphyrin can be several orders of magnitude greater than from the protein, allowing the vibrational frequencies of the heme to be observed without interference from the protein. The positions of certain bands in the Raman spectrum of heme proteins have been correlated with the spin and oxidation states of the iron.[1-3] This note reports an attempt to test the reliability of these correlations by studying the resonance Raman spectra of nitric oxide hemoglobin (NO-Hb) solutions.

Perutz[4] found that significant visible and ultraviolet spectral changes occur when inositol hexaphosphate (IHP) is added to NO-Hb, while no changes occur for O_2-Hb and CO-Hb under the same conditions. The interpretation[4] of these results is that IHP, which binds much more strongly to the deoxy than to the oxy quaternary structure of hemoglobin, changes he quaternary structure of NO-Hb from oxy to deoxy. Nitric oxide donates part of its unpaired electron to the d_{z^2} orbital of the iron, lengthening the bond between the iron and the N_ϵ nitrogen of the proximal histidine. Since the allosteric equilibrium of hemoglobin is governed primarily by the distance of N_ϵ from the plane of the porphyrin ring, the longer bond in NO-Hb, as compared with CO-Hb, shifts the allosteric equilibrium less to the oxy quaternary structure, thus allowing IHP to change the quaternary structure of NO-Hb, but not that of CO-Hb, to the deoxy form. This explanation is supported by the electron spin resonance studies of Kon,[5,6] Chien,[7] and Yonetani, *et al.*,[8] who showed that the unpaired electron of the NO has significant d_{z^2} character. The motivation for our study was to use the Raman spectrum to monitor the effect of IHP on the transfer of charge from ligand to metal and the changes of the spin state of the metal.

Yamamoto, *et al.*,[2] found that the peak assigned to C-N vibrations, which occurs at 1365 cm^{-1} in protoporphyrin IX[1], lies between 1370 and 1378 cm^{-1} in a series of *ferric* cytochromes and hemoglobins irrespective of the spin state of the iron, whereas it is found between 1356 and 1361 cm^{-1} in *ferro* cytochromes and deoxy hemoglobin. Since in O_2-Hb this band occurs at 1375 cm^{-1}, they conclude that the iron is essentially ferric (*viz.*, Fe^{3+} O_2^-). These experiments were performed using a laser line at 4416 Å (which excites the Soret band) but the correlation remains using 5145 Å radiation (which excites the visible bands). Spiro and Strekas[3] have found using 5145 Å radiation that an anomalously polarized band lies between 1586 and 1582 cm^{-1} in a series of low spin heme proteins, while it is found between 1555 and 1552 cm^{-1} when the iron is high spin, regardless of the oxidation state.

Figure 1 shows the polarized (a) and depolarized (b) Raman spectra of NO-Hb in the absence and presence of IHP. We could not detect any change in the frequency of the polarized band at 1374 cm^{-1} when IHP was added. Moreover its frequency lies in the region obtained for compounds in which the iron is in the ferric oxidation state. Thus if the correlation mentioned previously is generally applicable, one would conclude that the oxidation state of iron in NO-Hb is ferric and similar to that in O_2-Hb and CO-Hb.[3] Since electron spin resonance shows that about 30% of the unpaired electron occupies the d_{z^2} of the iron,[7] the electron donation from the d_π orbitals of the metal to the p_π orbitals of the ligand would have to be considerably greater for the iron to be formally regarded as ferric. However, it is possible that the position of this band is indicative, not of the total charge on the iron, but of the degree of π back-donation which could be similar for these ligands. In NO-Hb the occupation of the d_{z^2} orbital is expected to have little effect on the C-N vibrations because of the small overlap, expected on symmetry grounds, between the d_{z^2} orbital and the p_π orbitals of the porphyrin nitrogens.

The anomalously polarized band in NO-Hb occurs at 1582 cm^{-1} and remains unchanged when IHP is added. If the correlation found by Spiro and Strekas[3] is applicable, we would conclude that the iron in NO-Hb is low spin and the spin state is unchanged on altering the quaternary structure. This is in agreement with the nuclear magnetic resonance study of Ladner and Perutz,[9] who found no change in the spin state of NO-Hb when IHP is added.

The only significant change observed in the Raman spectrum of NO-Hb on adding IHP is the appearance of a new depolarized band at 1643 cm^{-1} and a weakening of the depolarized band at 1633 cm^{-1}. In fact the new band coincides with a very weak shoulder in the stripped (−IHP) spectrum, so IHP induces a transfer of intensity from the 1633-cm^{-1} band to the 1643-cm^{-1} band with no change of frequency. It is worth noting that in O_2-Hb the corresponding band occurs at 1638 cm^{-1}; this could therefore be a doubly degenerate band that is split in NO-Hb. Whatever the precise mechanism, the bands at 1633 and 1643 cm^{-1} in NO-Hb appear to be very sensitive to changes in the quaternary structure of the protein. Bands in this region do not seem to correlate with either the spin or oxidation state of the iron.[3]

The optical spectra of deoxy Nes-des-Arg hemoglobin[10] and high spin methemoglobins[11] are sensitive to changes of quaternary structure produced by IHP, and it is of interest to study the effect of IHP on the resonance Raman spectra of this series of hemoglobins. In fact we found no difference in the Raman spectra of deoxyhemoglobin NES-des-Arg and deoxyhemoglobin A; also we could detect no effect of IHP on the Raman spectra of fluoromethemoglobin and aquomethemoglobin. However, the quality of our spectra was very poor. The study of the effect of IHP on the Raman spectrum of O_2-Hb at lower salt concentrations than used in this work, where optical difference spectra have been found,[12] would also be of interest.

Complete polarization measurements to separate isotropic, anisotropic, and antisymmetric polarizability contributions to each band,[13,14] together with an understanding of the origin of the new band at 1643 cm^{-1} in NO-Hb, could lead to a detailed knowledge of the changes in the effective symmetry of the heme group produced when the quaternary structure of the protein is altered by IHP.

Acknowledgment. We thank Dr. M. F. Perutz for helpful discussions and for preparing the hemoglobin solutions. A.Sz. thanks the National Research Council of Canada for financial support.

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Received August 1, 1974

Helium(I) Photoelectron Spectra of Organic Radicals

Sir:

We wish to report the results of our investigations of the He(I) photoelectron spectra of methyl and *tert*-butyl radicals. Our spectrum of methyl differs from those previously reported[1] in clearly showing two vibrational spacings for the methyl cation. The spectrum of *tert*-butyl has not previously been reported and our success indicates that the present technique should be useful for intermediates having fairly complicated structures.

The radicals were formed by flash vacuum pyrolysis[2] of azomethane and azoisobutane. The instrument used in this study was a modified Perkin-Elmer Model PS-18 photoelectron spectrometer. The modifications consisted primarily of an enlarged vacuum chamber, to accommodate an increased distance between the He(I) discharge lamp and the analyzer plate-detector assembly, necessitated by the sample heating system. The photoionization chamber was a 14-mm stainless steel tube with a 0.015 mm wide electron exit slit, which could be aimed by rotating the tube. An auxilliary pump and liquid N_2 cold trap served to differentially pump the transient species. Optimum resolution obtained with argon was less than 20 mV, room temperature.

Transient species were produced by resistance heating of a bifilar (noninductive) nichrome coil seated between two concentric quartz tubes, 140 mm in length. A third and innermost concentric tube served as a free photon path from the modified discharge lamp to the photoionization chamber. Temperatures were measured with a junction-type thermocouple in the pyrolysis region. Indicated temperatures in excess of 770° were easily obtained.

Figure 1a shows our spectrum of azomethane[3] at room temperature for calibration. The spectrum at 670° is shown as Figure 1b. The sharp peak at 9.82 ± .02 eV is due to the ionization of methyl radical to give the ground state methyl cation. Much more intense peaks (not shown) for nitrogen at 15.58 ($^2\Sigma_g^+$), 16.69, 16.92, and 17.15 eV ($^2\Pi_u$)[4] were also observed. The intensities of the 9.82 eV and nitrogen bands decreased with decreasing temperature and increased with increasing temperature. The half-width of the 15.76 eV argon peak at 670° was 30 meV.

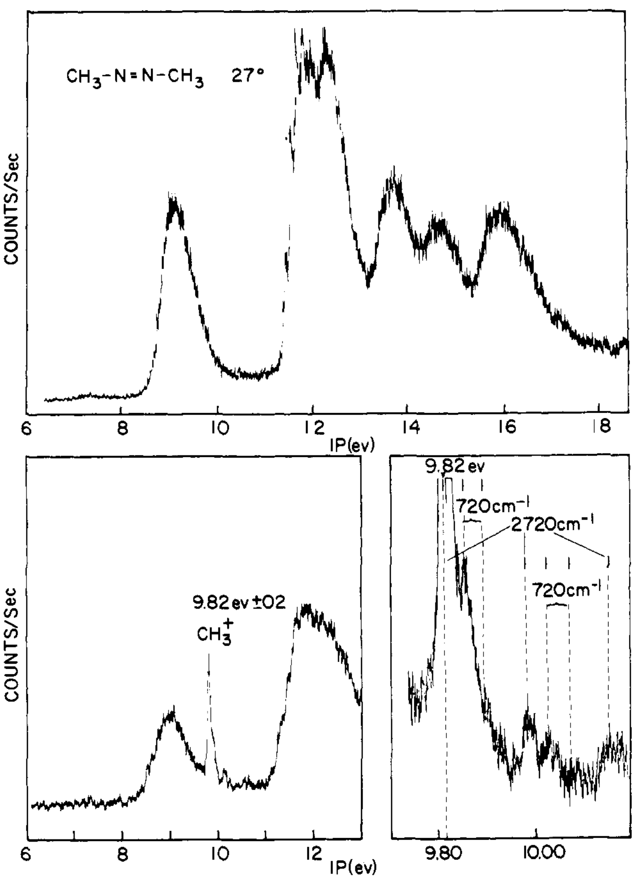

Figure 1. (a) Top, azomethane; (b) bottom left, methyl (9.52 eV) and residual azomethane; (c) bottom right, methyl, higher sensitivity. The main peak is off scale.

The methyl cation band in Figure 1b is qualitatively similar in appearance to that of ref 1a. However, Figure 1c shows that at least two members of a vibrational progression with a frequency of 720 ± 50 cm^{-1} are present in addition to those corresponding to the 2720 ± 30 cm^{-1}. Both of these frequencies are in good agreement with those previously observed[5] for Rydberg transitions.

The 720-cm^{-1} vibrational component can be assigned to the out of plane C–H bending frequency of the carbonium ion. The conclusion from such an assignment is that there must be a change in the degree of planarity upon going from the methyl radical to the methyl cation. All chemical

Table I. Ionization Potentials and Vibrational Spacings

Compound	IP_{vert}, eV	ν_θ, cm^{-1}	ν_R, cm^{-1}
Azomethane	8.95[a]		
Methyl	9.82[b]	720[c]	2720[d]
Azoisobutane	8.33		
tert-Butyl	6.95[e]	410[f]	800[g]
Isobutylene	9.39		1290

[a] ±0.05 eV, lit. 8.95 eV, assignment n^{-3}. [b] ±0.02 eV, lit. 9.83,[1a] 9.843,[5] and 9.86 eV.[1b] [c] ±50 cm^{-1}, out of plane bending mode, intensity ratios in the main ν_R band: $I_0 = 1.0$, $I_1 = 0.3$, and $I_2 = 0.1$. [d] ±20 cm^{-1}, C–H stretching modes, intensity ratios $I_0 = 1.0$, $I_1 = 0.1$, and $I_2 = 0.06$. [e] ±0.05 eV, lit. 6.93 eV.[2a] [f] ±20 cm^{-1}, out of plane bending mode, intensity ratios in the main ν_R band, $I_0 = 0.1$, $I_1 = 0.3$, $I_2 = 0.5$, $I_3 = 0.7$, $I_4 = 1$, and $I_5 = 1.0$. [g] Coupled C–C stretch C–C–H bend; ±100 cm^{-1}.