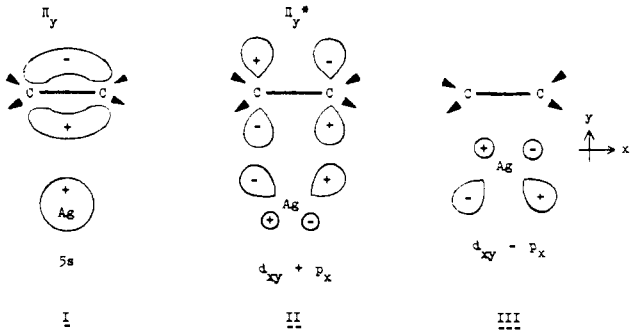
$$|A_1|_{Ag} = 22.5 \pm 0.5 \text{ G}$$
$$|A_2|_{Ag} = 10.0 \pm 0.5 \text{ G}$$
$$|A_3|_{Ag} = 16.5 \pm 0.5 \text{ G}$$
$$|A|_H \leq 1.0 \text{ G}$$

The difference between the coupling constants to the two Ag nuclei could not be resolved. The upper limit of the coupling constant to the protons was estimated from the difference in the apparent line widths of the spectra of the deuterated and nondeuterated species. The computer-simulated spectrum (Figure 1c)[4] based upon the g tensor and the Ag coupling tensor given above and a Lorentzian line shape with the line width of 4 G is in excellent agreement with the observed result (Figure 1b).

The interaction leading to the formation of olefin-Ag^+ cation is thought to involve the π orbitals of the double bond and hybrid orbitals of the cation located equidistant from the two unsaturated carbon atoms.[5] Two dative bonds are thought to be formed, a σ-type bond resulting from migration of electrons from the filled bonding π orbital of the olefin molecule into the vacant 5s orbital of the cation (I), and a π-type dative bond resulting from migration into the vacant antibonding π orbital from a filled d-p hybrid orbital of the cation (II).



In the schematic presentation of the orbitals given above, the d-p hybrid orbital involved in the dative π bond (II) should be given as $\Phi = a(4d_{xy}) + b(5p_x)$. We propose that the structural feature of the Ag atom-ethylene observed here is exactly the same as that of olefin-Ag^+ cation and its unpaired electron is located in the essentially nonbonding hybrid orbital (III) given by $\Phi^* = b(4d_{xy}) - a(5p_x)$. The extremely small hyperfine coupling constants to the Ag and the proton nuclei are thus accounted. The positive g shift ($g_3 = 2.042$) must be caused by the d_{xy} part of the orbital when the magnetic field is perpendicular to the xy plane. The negative g shift ($g_1 = 1.972$) could result from the p_x part of the orbital when the field is parallel to the y axis.

We have also succeeded in generating and observing the ESR spectra of Cu atom-ethylene complex. Gold atoms were found not to complex with ethylene. The detailed analyses of the spectra presented here and those of Cu atom-C_2H_4 will be reported soon.

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Iron to Sulfur Bonding in Cytochrome *c* Studied by X-Ray Photoelectron Spectroscopy[1]

Sir:

A classical problem in the field of metalloproteins is the identification of the groups to which the metal is liganded. Direct chemical evidence is often misleading; positive identification usually requires the elucidation of the three-dimensional structure of the protein using x-ray diffraction methods. The development of alternative methods that are simpler and faster to carry out could therefore have wide-ranging implications.

We wish to report here studies which indicate that x-ray photoelectron spectroscopy (XPS) may be a valuable tool in this context. We have chosen horse heart cytochrome *c*,[2] a heme protein of 12386 molecular weight, to study sulfur coordination to iron.

X-ray diffraction analysis indicates that the axial coordination positions of the heme iron are occupied by an imidazol nitrogen of histidine and a thioether sulfur of methionine.[3] There are four sulfur atoms per molecule of cytochrome *c*. All four are effectively thioethers. Two arise from methionines and two arise from cysteines which are covalently bound to the heme across the double bonds of the vinyl groups.[2]

Therefore three sulfurs in the protein have similar chemical environments while the sulfur coordinated to the heme iron would be expected to have a decreased electron density as a result of electron donation to the metal ion. Since core electron binding energies are a function of the chemical environment of the atom, XPS should be useful in differentiating between these two types of sulfurs and thus provide a means of determining the integrity of the Fe-S bond.

The sulfur 2p electron energy region, 160-170 eV, was examined in cytochrome *c*, the cyano derivative of cytochrome *c*, as well as in the lyophilized protein. No other element in these proteins has a core electron binding energy in the sulfur 2p region.

Spectra were obtained from a Hewlett-Packard 5950A ESCA spectrometer. An electron gun was employed in this work in order to eliminate charging effects. The Al Kα x ray line served as the exciting radiation. The large aliphatic C1s peak was used as the reference signal with a binding energy of 284.0 eV.

Oxidized cytochrome *c* in the solid form was obtained from the Sigma Chemical Co. A solution of the protein was prepared at a concentration of 10 mg/ml in 0.01 *M* phosphate buffer (pH 6.9). To ensure complete oxidation, $K_3Fe(CN)_6$ was added to the solution. For the cyano derivative, a sufficient amount of a neutralized KCN solution was added to give a final CN^- concentration of 0.08 *M*. The absorption spectra of these samples were measured before and after XPS analysis on a Cary 14 recording spectrometer.

A few drops of the sample solution were deposited onto gold platens and then mounted onto a cyrogenic probe. The samples were then inserted into the sample preparation chamber of the spectrometer and frozen under a dry nitrogen atmosphere to 173 K. This temperature was previously determined to be appropriate for this experiment since it allowed for the retention of a layer of ice over the protein thus preventing lyophilization. The ice layer was monitored by scanning the C1s and O1s electron region and observing the rate of growth of these signals. At temperatures higher than 175 K the C1s signal increases rapidly in intensity while at 175 K its rate of growth is markedly reduced indicating that the protein surface is covered by a layer of ice. In addition, the O1s peak increases much more rapidly at 175 K than at higher temperatures. Temperatures lower than 170 K re-

Table I. Sulfur 2p Binding Energies[a]

Oxidized cytochrome *c*	167.8	163.0
Cyanocytochrome *c*	—	162.8
Lyophilized cytochrome *c*	—	162.5
Methionine	—	162.2

[a] Cytochrome *c* concentration was 10 mg/ml in 0.01 *M* phosphate buffer pH 6.9. For the cyano derivative, a sufficient amount of a neutralized KCN solution was added to make a final CN^- concentration of 0.08 *M*. Because of the inherent assymetry of the sulfur 2p peaks all binding energy values are ±0.5 eV.

sulted in the formation of a thick layer of ice on the surface of the protein thus increasing the time required for data acquisition.

The lyophilized sample was prepared by examining the sample at 190 K. Examination of the sample after analysis indicated that lyophilization had occurred. Another sample was studied at room temperature and gave an analogous spectrum. Between 2 and 3 hr of running time was required for each spectrum.

The XPS spectrum of a frozen (173 K), concentrated aqueous solution of oxidized horse heart cytochrome *c* clearly shows two peaks in the sulfur 2p region, differing in energy by 3.8 eV (see Table I). The one at lower binding energy (163.0 eV) corresponds closely with that of free methionine, and agrees with the literature value for a thioether.[4] The peak at higher binding energy must be due to the coordinate methionine sulfur which, because of its electron donation to the Fe(III), would be expected to show a higher binding energy. This seemingly large shift is reasonable in light of the recent work of Solomon et al. with plastocyanin, a "blue" copper protein.[5] In addition, if a shift of 5.2 eV corresponds to a one-electron transfer,[6] then, in the case of cytochrome *c*, 0.7 electron is transferred from the bound methionine sulfur to the iron.

Evidence that the peak at higher binding energy is actually the bound methionine comes from the x-ray photoelectron spectrum of the cyano derivative of cytochrome *c*, for which only one sulfur 2p peak is observed. The binding energy of this peak is 162.8 eV which corresponds to the peak of lower binding energy in the spectrum of native cytochrome *c*. This is consistent with the fact that CN^- replaces the coordinated methionine sulfur.[7] Furthermore, lyophilization of cytochrome *c* also resulted in the disappearance of the peak at high binding energy which is in accord with the belief that lyophilization causes the rupture of this bond.[8] The absorption spectrum of cytochrome *c* after x-ray irradiation showed no evidence of any structural alteration of the native protein.

We conclude that XPS is a valuable tool in determining the integrity of the Fe-S bond in cytochrome *c*. In addition, XPS may be of potential use in determining whether sulfur is liganded to the metals of various other metalloproteins. These include cytochrome *f*,[9] cytochrome p-450,[10] and the copper "blue" proteins.[11]

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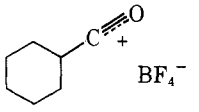
Acylation of Acetylenes. I. Observation of an Intramolecular 1,5-Hydride Shift in a Vinyl Cation Intermediate[1]

Sir:

We recently reported a new and unusual reaction leading to the formation of cyclopent-2-enones via alkyne acylation with acyl tetrafluoroborates in a nonnucleophilic solvent.[2] The purpose of the present communication is to report yet a different reaction pathway that predominates in the acylation of alkynes with the cycloacyl tetrafluoroborate **4**.[3]



4

The formation of the previously reported cyclic substitution products[2] is unusual in that it necessarily involves the saturated chain of the acyl residue. The results can be rationalized in terms of the reactive vinyl cation intermediate A with subsequent conversion, via transition state B, either