duced the expected effect of reducing the kinetics to cleanly first order. Furthermore, the decrease in values of $k_H \approx k_{obsd}/[ArH]$ with increasing added NaBr was steeper than the calculated fall-off of $[Br_2]/[Br_2]_{stoich}$ (see Table I), consistent with an increasing significance of the $k_{-1}[Br^-]$ term of eq 4, i.e., increasing rate-control by the second step.[7] This is verified by the concomitant increase in the observed kinetic isotope effect, k_H/k_D. The fact that k_H/k_D levels off in solutions of highest $[Br^-]$ indicates that essentially maximum rate control by the second step has been achieved in 0.5 *M* NaBr.[8] Finally, in 0.05 *M* NaBr, k_H/k_D was significantly reduced by the replacement of $NaClO_4$ (0.25 *M*) with NaOAc (last row, Table I). The result is consistent with the expected effect of the basic salt, increasing v_2/v_{-1}, thus leading to greater rate-control by the first step. It is to be noted that k_{obsd}, rather than being increased by the substitution of NaOAc for $NaClO_4$, was decreased slightly, indicating the action of significant specific salt effects on individual rate constants[10] and/or K_3.

References and Notes

(1) Further complexity can arise if the fraction of stoichiometric Br_2 tied up as Br_3^- (eq 3) grows to significance during a kinetic run.
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(5) Based on $K_3 = 27.6$,[6] $[Br_2]/[Br_2]_{stoich} = 1/(1 + K_3[Br^-])$ would be 0.97 at 50% reaction, when $[Br_2]_{stoich} = 1 \times 10^{-3}$ *M*.
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(7) A detailed analysis of the data indicates underlying salt effects, either a positive salt effect on the aromatic substitution per se and/or a negative salt effect on K_3.
(8) The maximum isotope effect is comparable to that found by Zollinger and Christen, 2.1, in the actual step of proton removal from the bromoarenium ion of 2-naphthol 6,8-disulfonate in water. In that instance, the arenium ion was not a steady state intermediate but was "instantly" and reversibly formed to greater than 90% from the naphthol plus Br_2 or HOBr.[9]
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Received February 18, 1975

Direct Observation of Sulfur Coordination in Bean Plastocyanin by X-Ray Photoelectron Spectroscopy

Sir:

The ligand environment of "blue" (or type 1) copper proteins has not been established.[1-3] Recent studies in our laboratory on colbalt(II) derivatives of stellacyanin, French bean (*Phaseolus vulgaris*) plastocyanin, and *Pseudomonas aeruginosa* azurin, have suggested that cysteine is a ligand, and that a ligand-to-metal charge transfer (LMCT) transition in the Cu(II)-S(Cys) unit is responsible for the intense absorption band at about 600 nm in each of the native proteins.[2] Resonance Raman spectral experiments on several "blue" proteins have also been interpreted in terms of Cu(II)-S(Cys) coordination.[3]

A direct test of the proposed sulfur-copper coordination in "blue" copper proteins is afforded by X-ray photoelectron spectroscopy (XPS). The sulfur 2p (S2p) binding energy is approximately 164 eV, which is well separated from the core levels associated with the other atoms present in these proteins. The effect of metal incorporation on the sulfur atoms can then be observed by comparing the S2p region of the copper (or cobalt) derivatives with that of the apoprotein. If a sulfur, in fact, coordinates to the metal, its electron density will decrease, thereby increasing the S2p binding energy. We have chosen to perform this experiment on bean plastocyanin, as it contains only one copper and three potential sulfur donor atoms (two methionines and one cysteine).[4] This simplicity allows a number of important conclusions to be drawn about the "blue" copper site.

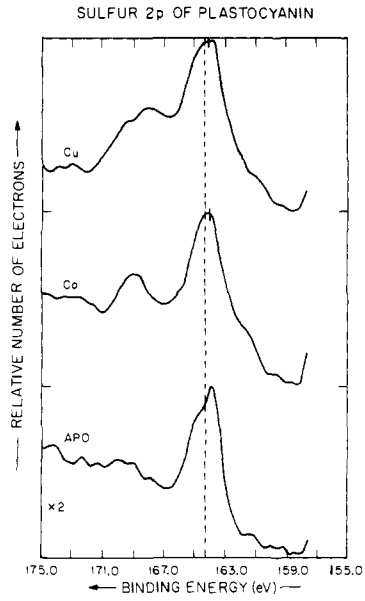

Figure 1. X-Ray photoelectron spectra of copper, cobalt, and apo plastocyanins in the S2p region at 250 K.

The X-ray photoelectron spectra reported here were measured on a Hewlett-Packard 5950 A ESCA spectrometer equipped with a low energy electron source for neutralization of charging effects. This spectrometer utilizes a monochromatic aluminum Kα X-ray source and has an instrumental resolution of 0.55 eV full width at half maximum (FWHM). All metalloprotein spectra reported were taken at 250 K, and 800-W X-ray power. The residual gas pressure in the analyzer chamber was 9×10^{-10} Torr, and the ambient gas consisted primarily of hydrogen, helium, nitrogen, carbon monoxide, and water. All binding energies are referenced to the protein aliphatic carbon 1s signal at 285.4 eV.

The extraction, purification, and metal substitution methods for bean plastocyanin have been described previously.[2] The copper sample had an absorbance ratio A_{278}/A_{597} of 1.1. Protein radiation damage was monitored by following C1s, N1s, and O1s regions. Two 126-sec scans were taken of each region. All sulfur 2p spectra were taken in sets of 30 scans, followed by the carbon, nitrogen, and oxygen sequence. No significant change was found in these regions (including S2p) after continual irradiation for several hours.

The samples were prepared by evaporating a small amount of protein solution (~50 μl) on rigorously clean, gold-plated stainless steel platens in a dry-nitrogen-flushed drybox connected directly to the inlet chamber of the spectrometer. The samples were gradually cooled to 250 K before subjecting them to the X-ray beam. The data were subjected to noise removal procedures using a low pass filter calculated on the basis of 0.8 eV line width.[5]

Figure 1 presents the sulfur 2p binding energy region for copper, cobalt, and apo plastocyanins. One sulfur peak is shifted by approximately 5 eV to higher binding energy (relative to the apo sample) in both the copper and cobalt

Table I. Sulfur 2p Binding Energies in Plastocyanins

Species	S2p (eV)[a]
Cu(II)–S–	169.8 ± 0.3
Cu(I)–S–	167.5 ± 0.3
Co(II)–S–	168.8 ± 0.2
H–S–(apo)	164.5 ± 0.3
CH_3–S–(apo)	164.0 ± 0.2

[a] Referenced to C1s at 285.4 eV.

proteins. The ratio of the intensity of this peak to the one at 164 eV is approximately 1:2, showing that one of the three sulfurs has been affected significantly by metal incorporation.

The S2p energy difference between uncoordinated methionine and cysteine sulfur atoms can be estimated by examining the change in the average energy $\bar{E}$ of the 164 eV peak on metal incorporation. The average energy (first moment) is defined as

$$\bar{E} = \sum_i E_i I_i \Big/ \sum_i I_i$$

where I_i is the electron intensity at a binding energy E_i. The baselines were subtracted, and the high energy tail was estimated to resolve its overlap with the shifted peak. Thus, there is more error in the copper moment due to the greater overlap with the shifted peak. The vertical lines near each peak maximum give the average energy shifts relative to that of the apoprotein (the dotted vertical line). It may be observed that the average energy of the 164 eV peak decreases upon metal incorporation, strongly suggesting that the sulfur at slightly higher binding energy in the apoprotein is the one that coordinates to the metal. It is reasonable to assign the cysteine sulfur at higher binding energy, as a CH_3 group is more electron donating that is hydrogen. Further, the cysteine–methionine energy difference is given by three times this change in average energy, and is found to be 0.56 ± 0.3 eV.

Several comments should be made about the nature of each sample. The broadening of the coordinated sulfur peak in the copper protein is due to partial reduction of the central metal, which shifts S2p to lower binding energy. This effect was monitored, and estimates of the sulfur binding energies of Cu(II) and Cu(I) plastocyanins were made. These binding energies are given in Table I, along with those of the other sulfur species measured. The cobalt plastocyanin samples contained 20–30% apoprotein, which accounts very well for the lower intensity ratio, I_{169}/I_{164}, of the cobalt as compared to the copper spectrum (0.45 vs. 0.32 eV). Finally, a small signal, about one-sixth of that found for the cobalt derivative, is observed in the higher binding energy region of the apoprotein, consistent with the presence of traces of copper and disulfide.

These X-ray photoelectron experiments establish directly that sulfur binds to copper in bean plastocyanin. Further, the sulfur most probably is contributed by the cysteine residue in the protein. The S2p energy shifts that we have found are quite large and indicate that sulfur electron density is substantially delocalized to the copper upon coordination. This evidence for a delocalized Cu(II)–S(Cys) structure accords well with the assignment[2] of the intense, low-energy absorption spectral features in "blue" proteins to one or more S → Cu(II) charge transfer transitions. The XPS studies also show that cobalt(II) occupies the same sulfur binding site as copper(II) in plastocyanin. These experiments are now being extended to other copper-containing proteins.

Acknowledgments. This research was supported by the National Science Foundation and by the Director's Discretionary Fund of the Caltech Jet Propulsion Laboratory.

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*Book Reviews**

Selective Oxidation of Hydrocarbons. By D. J. HUCKNALL (The City University). Academic Press, Inc., London. 1974. vii + 212 pp. $14.00.

The stated objective of this book is to critically review the current knowledge of the catalytic oxidation of C_2–C_5 alkanes and alkenes. The data are purported to be "collated in such a way that the search for a new oxidation catalyst will be less empirical." Unfortunately, the author has allotted only five pages for an introduction to this complex field. There is only a brief mention of the chemical and physical aspects of catalysis and the analytical methods used in this type of research. Only a few more pages of discussion at this point would have made the book of more value to a broader range of readers. The final chapter of the book is devoted to a general discussion of the mechanism of catalysis with the primary emphasis on the application to selective oxidation. Since this chapter is self-consistent, it might well be considered as part of the introduction.

As the author warns, the book may appear to be a "compendium of catalysts and kinetic schemes, etc." Indeed, 121 of the 156 pages of written text are devoted to a systematic discussion of the product distributions, the kinetic parameters, and the reaction schemes for the catalytic oxidation of ethylene (Chapter 2), propylene (Chapter 3), C_4 hydrocarbons (Chapter 4), and C_5 hydrocarbons (Chapter 5).

Although I personally have always been somewhat uncomfortable with the British syntax, I have found their works to contain a

* Unsigned book reviews are by the Book Review Editor.