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Resonance Raman Studies of 'Blue' Copper Proteins

THOMAS M. LOEHR

Department of Chemistry and Biochemical Sciences, Oregon Graduate Center, 19600 N.W. Walker Road, Beaverton, Oreg. 97006, U.S.A.

'Blue' copper proteins possess intense visible absorption bands at ≅600 nm due to $(Cys)S^- \rightarrow Cu(II)$ charge transfer. The type 1 copper in these proteins also exhibits anomalously low hyperfine splitting in the EPR spectra and high redox potentials. Crystallographic and EXAFS investigations on two simple blue copper proteins, plastocyanin and azurin, have provided fairly detailed pictures of their active site structures.

The resonance Raman (RR) spectra of blue copper proteins are remarkably similar to one another; all have three or more strong bands in the 350–450 cm^{-1} range; all exhibit intensity enhancement by resonance with the ≅600 nm LMCT band; all spectra have two nearly constant frequency components at ≅750 and ≅250 cm^{-1} which appear to be vibrational modes of the coordinated cysteinate ligand; and, finally, many weaker spectral features are resolvable in high quality spectra (Fig. 1). The complexity of the RR spectra of the type 1 copper sites suggests that vibrational modes of the chromophore in addition to Cu–S(Cys) stretching must also contribute. Interpretation is complicated by the fact that the principal peaks in the RR spectra are at unprecedently high frequencies for copper complexes involving sulfur and nitrogen coordination.

We recently undertook a normal coordinate analysis of the $CuN_2S(S')$ chromophore structure of *Pseudomonas aeruginosa* azurin. We found that the anomalously high frequencies can be explained by the shortness of the Cu–S (cysteinate) bond and the strongly coupled nature of the vibrations. Good agreement with observed azurin frequencies was obtained for a trigonal pyramidal model with Cu(II) coordinated to two histidines and one cysteinate [1].

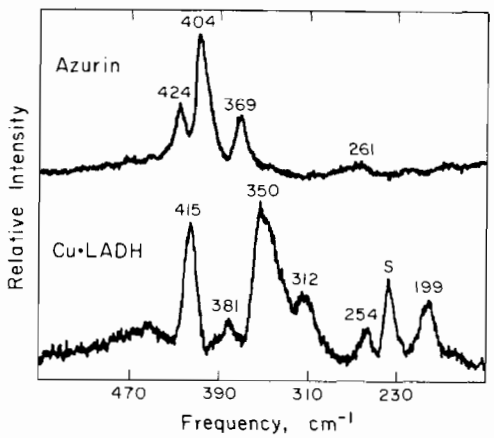

Fig. 1. Resonance Raman spectra of azurin and Cu–LADH excited at 647.1 nm.

TABLE I. Raman Frequencies (cm^{-1}) for Cu–LADH at 77 K.

Protein	ν_a	ν_b	ν_c	ν_d
Cu–LADH	415	350	254	199
+ NADH	420	359	263	202
+ pyrazole	413	368	246	196

The structure of the zinc enzyme, liver alcohol dehydrogenase (LADH), is well documented. Substitution of the catalytic zinc by Cu(II) yields a protein that has type 1 copper properties. We have investigated the RR spectrum of this system and find it to be an excellent model for the spectra of blue copper proteins [2]. A spectrum of Cu–LADH is shown in Fig. 1. The main spectral features are also at high frequencies and correlate well with those observed for azurin. The catalytic Zn(II) ion in LADH is coordinated by two cysteinates, one histidine, and one water molecule in a tetrahedral environment. We attribute the sharp Raman peak at 415 cm^{-1} in Cu–LADH to a predominantly Cu–histidine vibration and the broader band at ≅350 cm^{-1} to contributions from the two Cu–cysteinates. The high frequencies of the chromophore vibrations lead to the prediction that the Cu–S bond distances in Cu–LADH are also close to 2.1 Å, as in azurin and plastocyanin.

Addition of the coenzyme, NADH, or pyrazole, a competitive inhibitor of alcohol oxidation in the native enzyme, to the copper protein causes only minor changes in the RR spectrum of the Cu(II) chromophore (Table I). Thus, despite the large shift of the absorbance maximum from 580 to 495 nm upon pyrazole binding, the copper site appears to maintain its near tetrahedral geometry. Since normal coordinate analyses indicate that vibrational frequencies are sensitive to geometric variations in this type of system [1], any structural changes responsible for the altered λ_{max} and hyperfine splitting in the Cu–LADH/pyrazole complex must be fairly subtle ones.

1 T. J. Thamann, P. Frank, L. J. Willis and T. M. Loehr, *Proc. Natl. Acad. Sci. U.S.A.*, *79*, 6396 (1982).
2 W. Maret, M. Zeppezauer, J. Sanders-Loehr and T. M. Loehr, submitted for publication.