

Resonance Raman Spectra of Cucumber Basic Blue Copper Protein 'Plantacyanin'

TAKESHI SAKURAI*, SATOSHI SAWADA and AKITSUGU NAKAHARA

Institute of Chemistry, College of General Education, Osaka University, Toyonaka, Osaka 560, Japan

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A number of resonance Raman studies on blue copper proteins and multicopper oxidases have been carried out to obtain structural information [1–6]. Satisfactory interpretation of the spectra has not been available, in spite of frequent applications of cryogenic and isotope substitution techniques [7–10]. Thus, the understanding of the resonance Raman spectra remains uncertain for characterizing the blue copper site. Successful measurements of the spectra have hitherto been performed only when laser excitations around 600 nm were employed, since facile reduction of copper leading to the bleaching of the chromophore occurs with Ar⁺-laser excitation.

Plantacyanin isolated from cucumber peel exhibits three absorption peaks at 593 ($\epsilon = 2900$), 740 ($\epsilon = 1600$) and 448 nm ($\epsilon = 1240$) and a shoulder at 410 nm ($\epsilon = 950$). (Although the molar absorptivity of plantacyanin at 593 nm was estimated as 800 in ref. 3, the real value proved to be 2900.) The three bands which comprise the former two peaks have been assigned as the S⁻ (cysteine) → Cu charge transfer bands, the band at 448 nm as the N(histidine) → Cu charge transfer band, and the shoulder at 410 nm as the N(histidine) → Cu or S(methionine) → Cu charge transfer band. The laser excitation at 632.8 nm gave five Raman bands around 400 cm⁻¹ [3].

We are interested in obtaining the resonance Raman spectra of plantacyanin by exciting the N(histidine) → Cu and, possibly, the S(methionine) → Cu charge transfer bands of the protein in order to assign the Raman bands. The excitation of the protein with 488.0 and 457.9 nm lasers gave five bands at 345, 372, 391, 423 and 445 cm⁻¹, similar to those observed using 632.8 nm laser excitation, although Ar⁺-laser excitation gave relatively weak bands compared to the band intensities obtained using He⁺-Ne⁺ laser (Fig. 1). Controversial discussions have been reported about the origin of the bands appearing at around 400 cm⁻¹ [1–10]. In spite of their frequent assignment as Cu–S(cysteine), they seem to be the mixing Cu–S(cysteine) and Cu–N(histidine) vibra-

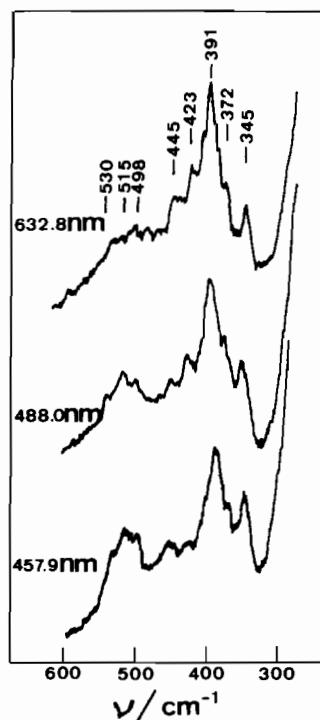


Fig. 1. Resonance Raman spectra of Plantacyanin measured at room temperature (pH 7.0, 0.1 M phosphate buffer).

tional modes, as proposed by Thamann *et al.* [4] for the Raman spectra of azurin. The reason is that the same bands appeared when plantacyanin was excited for its Cu–S(cysteine) and Cu–N(histidine) charge transfer bands. Of the five bands, at least the one at 345 cm⁻¹ may be contributed mainly by $\nu(\text{Cu–N})$ rather than $\nu(\text{Cu–S})$, since the relative intensity of the band was apparently stronger when the laser with shorter wavelength was used. In addition to the five bands, three bands were observed at 530, 515 and 498 cm⁻¹. They may be concerned with Cu–N(histidine) bond, because they were more clearly resolved when the Ar⁺-laser was used. However, the relevant energy region seems to be too high to be attributed to the Cu–N(histidine) bond, and, accordingly, they may be reasonably assigned as deformations of ligand(s) to the copper ion [8]. Every blue copper protein exhibits one or two weak bands at around 260 cm⁻¹; these bands were thought to be Cu–N(histidine) or Cu–S(methionine) stretching [7–10]. The corresponding weak band was observed at *ca.* 265 nm only with the excitation of the 632.8 nm laser (data not shown). This fact suggests that the assignment is not likely, and a deformation like Cu–S–C or S–Cu–N seems to be more probable.

The less significant contribution of the Cu–S(cysteine) bond in plantacyanin may be a reason why

*Author to whom correspondence should be addressed.

this protein exhibits the relatively low reduction potential (0.27 V) and the relatively weak absorptivity around 600 nm ($\epsilon = 2900$ at 593 nm) as a blue copper protein.

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