CHEMISTRY LETTERS, pp. 1297-1300, 1985.

THE TYPE I COPPER OF NITRITE REDUCTASE FROM ALCALIGENES SP. NCIB 11015

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Unique properties of the type I copper from Alcaligenes sp. NCIB 11015 was characterized in detail through curve analyses of absorption and circular dichroic spectra and resonance Raman spectrum.

Nitrite reductase(NIR) from a denitrifying bacterium, Alcaligenes sp. NCIB 11015 contains two type I coppers as sole cofactor in the molecule. Basic properties of this enzyme, absorption, circular dichroic, X-ray absorption and electron spin resonance spectra, redox potential and molecular and enzymatic properties have been reported in some recent papers.[1-3] Here we present curve analyses of absorption and circular dichroic spectra and resonance Raman spectrum of NIR in order to reach a better understanding of the copper binding site.

NIR exhibits three absorption maxima at 470, 594, and 780 nm and the whole feature of absorption spectrum(Fig. 1) is superficially similar to those of plastocyanin and azurin.[4] Curve analysis of the circular dichroic spectrum allows for seven bands at 410(+), 477(-), 599(-), 685(-), 741(-, very weak), 809 (+), and 917 nm(+) in the near ultraviolet to the near infrared region. Three bands appeared at 599, 685, and 809 nm are reverse in the signs from those of plastocyanin or azurin and also stellacyanin or plantacyanin.[5] These bands are assigned to charge transfer bands from S(cysteine residue) to Cu(II) ion. Of these three bands relevant to a cysteine residue, the central one is the "blue band" characteritic of the type I copper. However, as clearly seen from the comparison of curve analyzed absorption and circular dichroic spectra, the central band at 685nm is rather weak while the band at 599nm is most intense. Further, anisotropy factor of the band at 685nm exhibited the greatest value($|\Delta\varepsilon / \varepsilon|$=0.0052) among blue copper proteins(0.0003-0.002). On the other hand, the anisotropy factors of the bands at 599 and 809 nm were 0.0011 and 0.0044, respectively, being in the range of the magnitude of other blue copper proteins, 0.0005-0.002 and 0.002-0.015, respectively. Two bands at 410 and 477 nm due to charge transfer of N(imidazole group of histidine residue)→Cu and/or S(methionine residue)→Cu and the d-d band at 917 nm have normally same signs with those of other blue copper proteins.[5] The origin of the very weak band at 741 nm is not clear at present. This sort of band has never been observed on

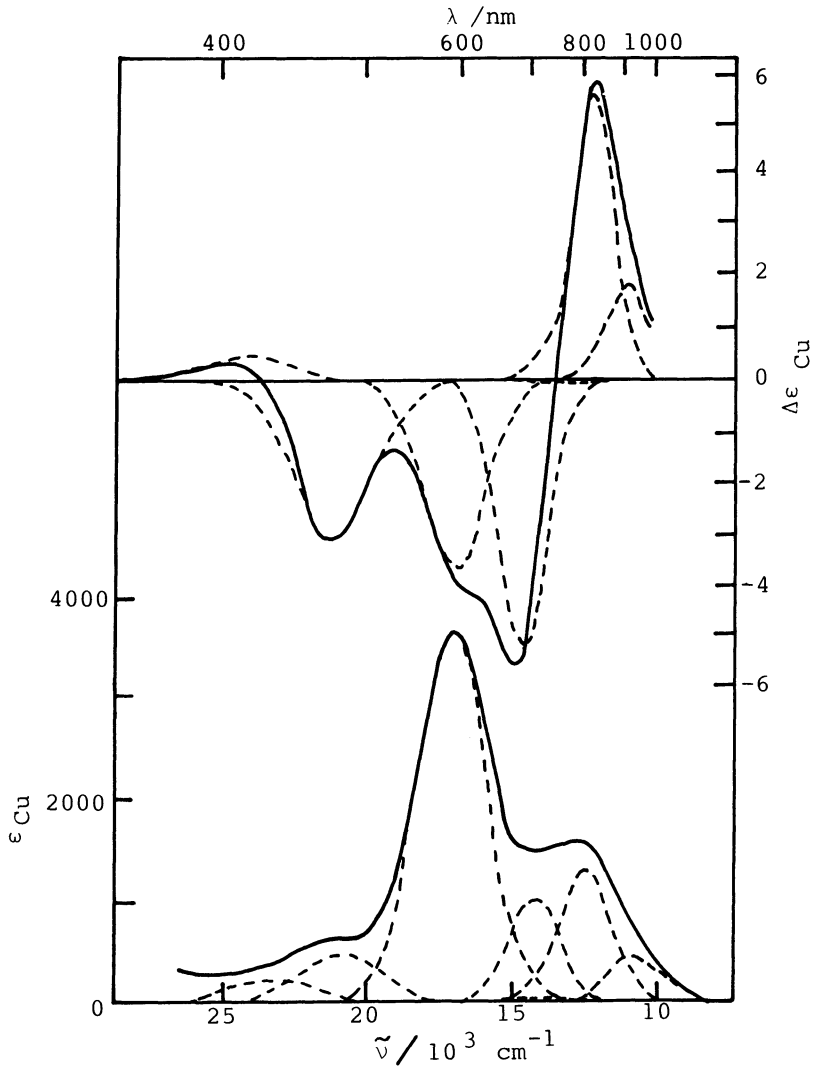

Fig. 1. Curve analyzed absorption and circular dichroic spectra of nitrite reductase.

the absorption and circular dichroic spectra of other blue copper proteins.[6)]

NIR gives the simplest resonance Raman spectrum among blue copper proteins. As indicated in Fig. 2 two strong bands almost overlapped each other are observed at 420 and 414 cm^{-1}, and two weak ones at 378 and 366 cm^{-1}. It is now well accepted that all of these bands around 400 cm^{-1} are mainly attributed to the stretching modes of the Cu-S(cysteine) and Cu-N(histidine) bonds. Since these bands appear at a higher energy region(ca. 20 cm^{-1}) than the relevant bands observed for stellacyanin and plantacyanin while in the same energy region as those for plastocyanin and azurin, Cu-S(cysteine) and Cu-N(histidine) bonds are not considered to be particularly weak. Even in D_2O , these bands appeared at the same energy region, supporting that Cu-ligand stretches contribute mainly to these bands. The shoulder at 260 cm^{-1} is considered to be due to the stretching of the Cu-N(histidine) bond.[7)]

One cysteine, one methionine and two histidines are considered to afford

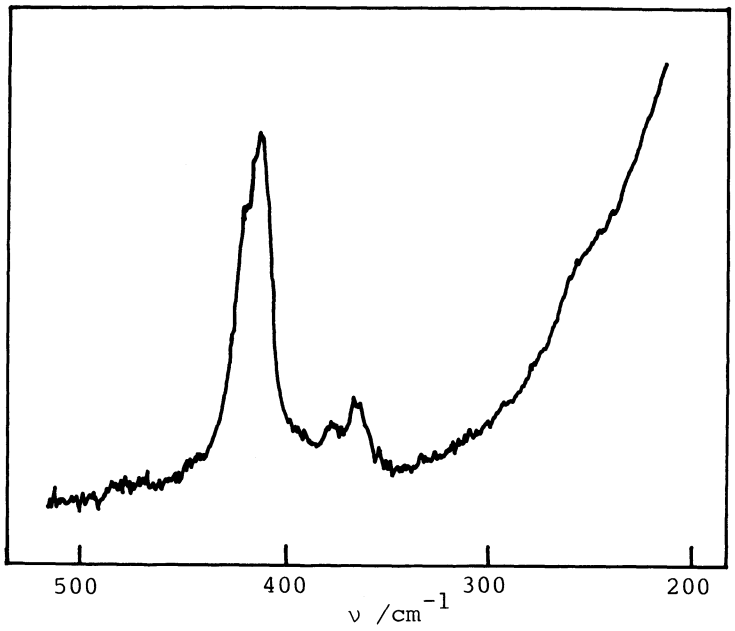

Fig. 2. Resonance Raman spectrum of nitrite reductase(sodium phosphate buffer, pH 7.2, room temperature, 632.8 nm excitation).

the coordinating group around copper ion[2] similarly to the cases of plastocyanin, azurin and plantacyanin. However, the unique spectral feature of NIR suggests that the copper site of NIR has a different steric structure from those of other blue copper proteins or that the asymmetric carbon of the cysteine residue might be located in an entirely differnt position around Cu(II) as compared with the cases in other blue copper proteins. In line with this, Co(II) ion which is introduced in place of the inherent Cu(II) ion has trigonal bipyramidal structure as suggested from the magnetic circular dichroic spectroscopy.[8] In contrast, plastocyanin, azurin and stellacyanin furnish tetrahedral site for Co(II) ion.[4] The Cu-S(methionine) bond of poplar plastocyanin[9] and Alcaligenes denitrificans azurin[10] have been revealed to be unusually long, i. e. near to 0.3 nm and in addition in the latter protein a peptide carbonyl oxygen approaches to Cu(II) ion. Steric structure of the active site of plastocyanin and azurin was described as distorted tetrahedral and possibly distorted trigonal bipyramidal, respectively. Since Cu(II) in NIR has axial symmetry with ESR parameters of $g_{//}$ 2.22, $g_{\perp}$ 2.05, and $A_{//}$ 0.65 mT, the original Cu(II) site in NIR may be near to trigonal monopyramidal taking into consideration that less flexible Co(II) ion is usually able to extremely reflect the original structure of the Cu(II) binding site. The redox potential of Cu ion may be ultimately determined by combined effect of the kind of ligand group set from amino acid residues, their steric arrangement and the extent of hydrophobicity around copper ion. Model studies showed that tetrahedral distortion around Cu ion is one of the important factors to raise the redox potential of blue copper protein.[11,12] The relatively lower redox potential of NIR (+260 mV)[2] as a blue copper protein seems to reflect the trigonal monopyramidal -like(less tetrahedral) character of copper binding site.

This work was supported in part by a Grant-in-aid for Scientific Research B(No. 58470034)(to A. N.) from the Japanese Ministry of Education, Science and Culture. We should like to thank prof. Akira Nakamura, Dr. Norikazu Ueyama and Mr. Mitsuo Ohhama and Drs. Takashi Komorita and Seiko Komorita of Osaka University for measurements of resonance Raman spectra and computer simulations, respectively.

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(Received May 23, 1985)