ON THE STATE OF COPPER IN THE BLUE PROTEIN UMECYANIN

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1. Introduction

A number of copper proteins have a strong blue color due to an absorption band centered around 600 nm [1, 2]. In the proteins showing oxidase activity only part of the copper is responsible for the blue color. This copper has a small hyperfine splitting in its electron paramagnetic resonance (EPR) spectrum and has been designated type 1 Cu^{2+}. Other proteins contain type 1 Cu^{2+} only and show no oxidase activity. Among these are stellacyanin and the azurins.

Recently a new blue copper protein, umecyanin, has been described [3]. It is obtained from horseradish, has a molecular weight of 14,600 and 1 Cu per molecule. The extinction at 610 nm is 3.5 mM^{-1} cm^{-1}, suggesting that the copper has type 1 character. In order to check this classification other spectroscopic properties have now been studied. As the EPR properties of type 1 Cu^{2+} in different proteins show a range of variation, which however follows a certain pattern [2], it would appear to be of interest to see if umecyanin conforms with this as well. It has recently been shown [4] that rather rapid electron transfer can occur between reduced type 1 copper in azurin and fungal laccase and the possibility of such transfer in the case of umecyanin has also been investigated.

2. Materials and methods

Umecyanin was prepared as described earlier [3] except that it was concentrated on carboxymethylcellulose (CMC) rather than with Diaflo after CMC chromatography. For fungal laccase the previously published procedure was followed [5]. Laccase B was used and it was freed from contaminating F^- by dialysis in the reduced state [6]. The reoxidation of reduced umecyanin was followed at 610 nm in a Zeiss PMQ II spectrophotometer.

EPR and optical absorption data were recorded as before [7]. Circular dichroism (CD) spectra were taken with a Cary 60-6002 spectropolarimeter.

3. Results

Fig. 1 shows the EPR spectrum of umecyanin recorded at 9 and 35 GHz. The spectrum is axial ($g_{\|} = 2.317, g_{\perp} = 2.05$) with $g_y - g_x$ less than 0.015. The value for $|A_{\|}|$ is quite small, 30–35 gauss (0.0035 cm^{-1}). The integrated intensity of the 9 GHz spectrum corresponds to about 70% of the chemically detected copper. At pH 9.5, where the optical absorption maximum is shifted somewhat to shorter wavelengths, there is only a very small change in the EPR spectrum.

The optical absorption and CD spectra are given in fig. 2.

The oxidation by oxygen of the reduced protein in the presence of fungal laccase was studied in the following way. About 20 µl of 1 mM ascorbic acid in water was added to 1.1 ml of 50 µM umecyanin in 0.1 M acetate buffer, pH 5.3, containing 0.1 mM EDTA. The absorption at 610 nm was followed

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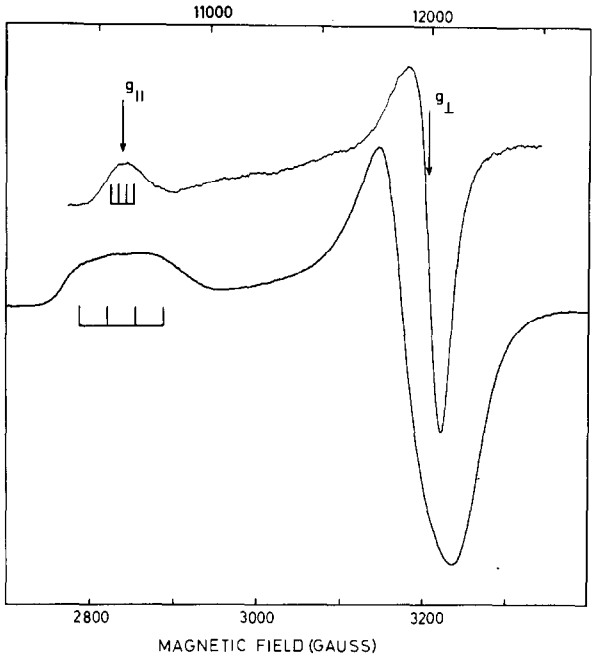

Fig. 1. EPR spectra of umecyanin recorded at 34.40 (upper curve) and 9.201 (lower curve) GHz. The protein was 0.55 mM in 30 mM sodium acetate buffer, pH 5.70. The upper and lower magnetic field scales go with the upper and lower curve, respectively, and have been adjusted so that points with the same g-value coincide. The copper hyperfine splitting around $g_{\parallel}$ is indicated in both spectra. The values of the parameters are $g_{\parallel} = 2.317, g_{\perp} = 2.05, |A_{\parallel}| = 0.0035$ cm^{-1}. The recording temperature was 90 and 77° K for the upper and lower spectrum, respectively.

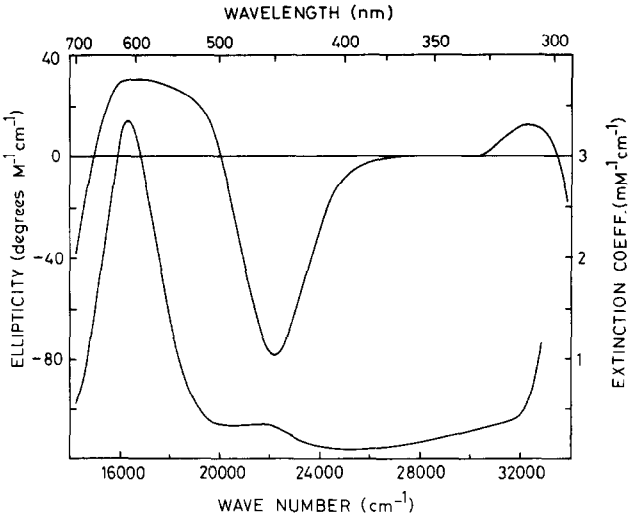

Fig. 2. Optical absorption (lower curve) and CD (upper curve) spectra of umecyanin. The same samples as in fig. 1 was used and the recording temperature was about 25°.

until it reached a stationary level at almost full reduction. This took of the order of 3 min. Then 25 μl of 10 μM fungal laccase B was added and the increase in blue color was recorded as a function of time. At a level of 50% reoxidation another 50 μl of laccase was added. If, in analogy with the results [4] for the laccase-catalyzed oxidation of reduced azurin and cytochrome c, it is assumed that the oxidation rate is limited by the rate for the bimolecular electron-exchange between laccase and reduced umecyanin, a second-order rate constant of about 2×10^4 M^{-1} sec^{-1} could be estimated from the experimental results.

4. Discussion

It is quite clear from the EPR spectra that umecyanin is of type 1 character. The hyperfine coupling is even smaller than for stellacyanin [8]. Unlike this protein, umecyanin shows no departure from axiality in its EPR spectrum, in which respect it more resembles the azurins [9]. As discussed elsewhere [2] there is a linear relation between the values found for $A_{\parallel}$ and $g_{\parallel}$ for the various type 1 Cu^{2+} ions and the values for umecyanin fall close to this line. Also, the general appearance of the CD spectrum, with its relatively strong peak at 450 nm, is similar to that of other proteins [10] containing type 1 Cu^{2+}. The shape of the band at lower energy suggests that there are two peaks present. However, there is no corresponding resolution of the absorption spectrum. This will be further discussed in a separate paper [10] dealing in detail with the CD properties of the blue copper proteins.

Like other reducible metalloproteins containing only one electron-accepting site, such as azurin and cytochrome c, reduced umecyanin is not easily autooxidizable [3]. However, rapid oxidation by molecular oxygen occurs in the presence of laccase, so

that also in this respect umecyanin and azurin are similar. Furthermore, the rate of electron-transfer between the reduced proteins and laccase is of the same order of magnitude in the two cases, second-order rate constants of 2×10^4 and 8×10^4 $M^{-1}sec^{-1}$ being found for umecyanin and azurin [4], respectively.

In view of the stability of the reduced umecyanin towards reoxidation, the low integrated intensity of the EPR signal may be due to partial reduction of the protein. As a consequence one may have to be cautious in assigning all bands in the CD spectrum to be oxidized umecyanin. However, the band at 310 nm is also present in the oxidized form of stellacyanin [10].

In summary, the state of copper in umecyanin appears to comform in its spectral and redox properties with the so-called type 1 Cu^{2+} found in many other copper proteins.

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