X-RAY PHOTOELECTRON SPECTROMETRIC ASPECTS OF THE COPPER CHROMOPHORE IN PLASTOCYANIN

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1. Introduction

The intense multibanded absorption of blue proteins near 600 nm was mainly attributed to a $S(\pi) \rightarrow$ Cu charge-transfer transition [1]. Sulphur 2p (S 2p) X-ray photoelectron spectra of french bean plastocyanin showing two signals at 169 and 164 eV were published [2]. The signal of higher binding energy was interpreted as direct evidence for Cu–S coordination in the protein, the sulphur being present as an S^+ species. The X-ray photoelectron spectra of sulphur in various metal proteins including several plastocyanins were examined [3]. They attributed the higher binding energy component in the S 2p levels observed in some of the samples to extraneous sulphur present in a high oxidation state. Preliminary experiments [4] revealed that reduced plastocyanin lacked the sulphur signal near 168 eV. A theoretical explanation based on a high covalency of the Cu–S bond was forwarded [5]. Such a covalency could be found in several model compounds of blue copper proteins [6]. In this context it should be noted that no such large shifts of the S 2p binding energy was observed in over 20 inorganic compounds by us [7–9] as well as in natural and semisynthetic copper-thiolate-rich proteins [10,11].

The lack of an evidence for either interpretation prompted us to study the X-ray photoelectron spectra of plastocyanin and to detect possible extraneous sulphur and/or breakage of the protein during the measurements.

The X-ray photoelectron spectra of the S 2p levels of native parsley plastocyanin showed in both cases a main peak at 162.2 ± 1 eV and a second one near 166 eV. Employing gel electrophoresis it could be demonstrated that the second signal is caused by a gradual destruction of the native protein during X-ray irradiation in the presence of traces of oxygen and cannot be attributed to an S^+ species. In spite of the low signal-to-noise ratio the binding energy of the copper in both reduced and oxidized plastocyanin was found to be 932.1 eV employing X-ray photoelectron spectrometry. This suggests that the electron transport takes place at the liganded sulphur, the copper remaining in its original electronic configuration.

2. Experimental

Plastocyanin was isolated from parsley following a modified verson of the procedure in [12]. The protein was isolated in the reduced form and was oxidized by $K_3[Fe(CN)_6]$ and desalted on Biogel P-2 immediately before the start of the respective experiment. X-ray photoelectron spectra were run on a Varian V-IEE 15 high resolution electron spectrometer equipped with an on-line 620 L computer (8 K) as in [7–9].

3. Results and discussion

In spite of the low signal-to-noise ratio the main signal of the copper $2p_{3/2}$ levels both in reduced as well as in oxidized native plastocyanin was clearly detectable at 932.1 eV (fig.1). As the copper of plastocyanin can be regarded as an internal standard,

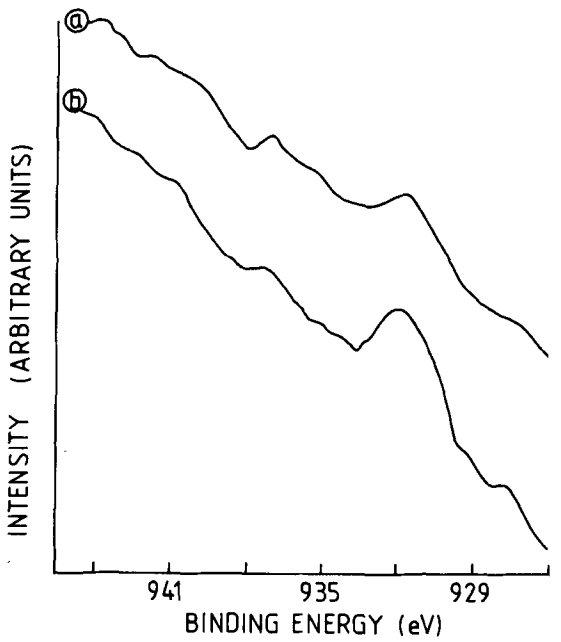

Fig.1. Cu $2p_{3/2}$ X-ray photoelectron spectra of ⓐ oxidized and ⓑ reduced parsley plastocyanin. Recording conditions: work function 6.0 eV; analyzer energy 100 eV; sweep width 30 eV; sweep time 20 s; number of channels 200; pressure range 1–2 μTorr; number of scans 100 in both cases.

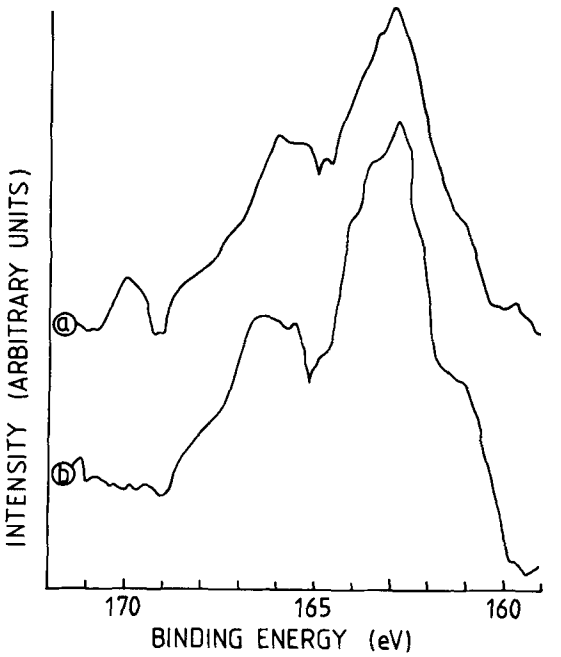

Fig.2. S 2p binding energies of ⓐ oxidized and ⓑ reduced native plastocyanin from parsley as determined by X-ray photoelectron spectrometry. Experimental details are the same as in the legend to fig.1, except that the number of scans was 50.

a shift of the main signal to higher binding energy values upon oxidation should be expected. The absence of such a detectable shift could be considered an indication that electron transport affects the ligands rather than the copper. Unlike with other physicochemical methods, including electron paramagnetic resonance, X-ray photoelectron spectrometry is an extremely fast technique to evaluate the electronic state of the copper. Earlier studies with model compounds revealed that, in fact, Cu remains in blue chromophores in a 3 d^{10} (Cu(I)) configuration [13] which led to the suggestion that the electrons of the metal–sulphur chromophore in blue copper proteins are delocalized and that an equilibrium

$$RS^{-}Cu(II) \longleftrightarrow RS^{\cdot}Cu(I)$$

exists.

X-ray photoelectron spectra of the S 2p levels showed both for oxidized and reduced parsley plastocyanin a main signal at 162.3 and 163.1 eV, respectively, and a second one near 166 eV, even when the protein was isolated in a totally anaerobic manner (fig.2).

Samples of plastocyanin were irradiated for different periods of time in the electron spectrometer, then the samples were dissolved in Tris-buffer (pH 7.5) and examined using disc electrophoresis. In fact, a gradual destruction of both the reduced and the oxidized copper protein was observed as a function of the irradiation time when the gels were scanned after staining with Coomassie blue (fig.3). Besides the main protein band a second, slower moving band was observed, the intensity of which increased with increasing irradiation time.

Cooling the sample during irradiation using liquid nitrogen lowered the rate of destruction of the protein significantly, but did not prevent it.

It is suggested that the higher binding energy signal of S 2p seen with several metal proteins is neither attributable to a S^{+}–Cu coordination nor to extra-

neous inorganic sulphur of higher oxidation state, but to a destruction of the protein. The protein-bound copper in the reduced form reacts with oxygen giving yield to $O_2^{\cdot -}$ [14]. The generated, free or protein-bound, superoxide could be converted into 'OH-radicals [14–16]. Both energy-rich oxygen species are well able to cause the oxidative damage observed during the X-ray photoelectron spectrometric measurements. Parts of the plastocyanin are dissociated during X-ray irradiation and are immediately oxidized giving rise to sulphur of higher binding energy.

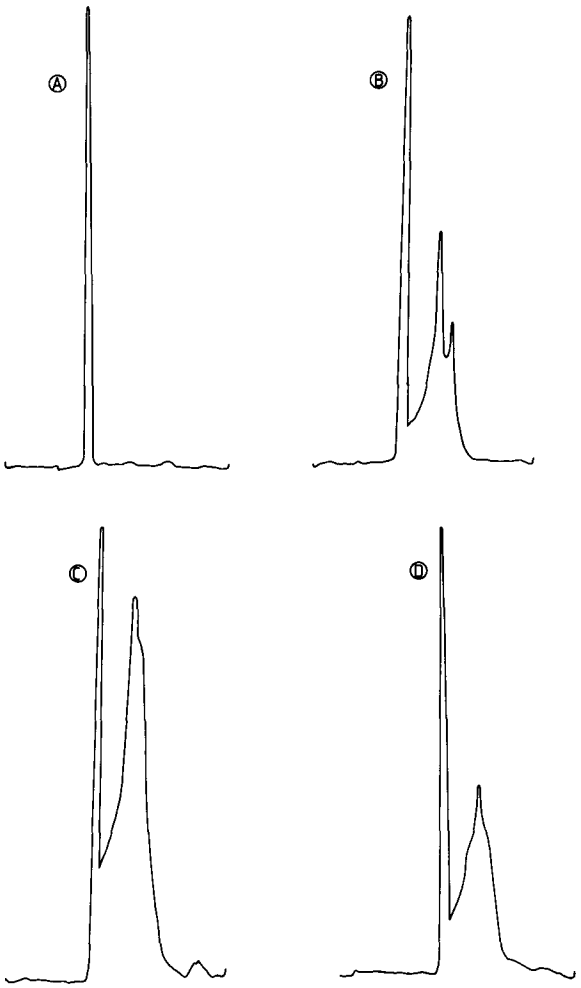

Fig.3. Scanned absorbances of plastocyanin and its degradation products. Ⓐ Untreated protein; Ⓑ after 1 min irradiation; Ⓒ after 10 min irradiation; Ⓓ after 10 min irradiation at liquid nitrogen temperature. The samples were applied on gels containing 7.5% acrylamide and were subjected to electrophoresis at 170 V and 2 mA/gel for 1 h. The gels were stained with Coomassie blue and measured at 560 nm. The scanning speed was 0.2 mm/s, chart speed 10 s/cm; the slit openings were 0.2 mm perpendicular and 2 mm parallel to the gels. A Unicam 1800 spectrophotometer equipped with a scanning unit was employed.

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References

[1] Fee, J. A. (1975) Struct. Bond. 23, 1–60.
[2] Solomon, E. J., Clendering, P. J., Gray, H. B. and Grunthaner, F. J. (1975) J. Am. Chem. Soc. 97, 3878–3879.
[3] Peeling, J., Haslett, B. G., Evans, I. M., Clark, D. T. and Boulter, D. (1977) J. Am. Chem. Soc. 99, 1025–1028.
[4] Wurzbach, J. A., Grunthaner, P. J., Dooley, D. M., Gray, H. B., Grunthaner, F. J., Gay, R. R. and Solomon, E. J. (1977) J. Am. Chem. Soc. 99, 1257–1258.
[5] Larsson, S. (1977) J. Am. Chem. Soc. 99, 7708–7709.
[6] Weser, U., Schubotz, L. M. and Younes, M. (1979) in: Copper in the Environment (Nriagu, J. O. ed) Wiley, London, in press.
[7] Rupp, H. and Weser, U. (1976) Biochim. Biophys. Acta 446, 151–165.
[8] Rupp, H. and Weser, U. (1976) Bioinorg. Chem. 6, 45–59.
[9] Rupp, H. and Weser, U. (1976) Biochem. Biophys. Res. Commun. 72, 223–229.
[10] Rupp, H. and Weser, U. (1974) FEBS Lett. 44, 293–297.
[11] Prinz, R. and Weser, U. (1974) FEBS Lett. 54, 224–229.
[12] Graziani, M. T., Finazzi-Agrò, A., Rotilio, G., Barra, D. and Mondovi, B. (1974) Biochemistry 13, 804–809.
[13] Younes, M., Pilz, W. and Weser, U. (1979) Bioinorg. Chem. in press.
[14] Hartmann, H.-J. and Weser, U. (1977) Biochim. Biophys. Acta 491, 211–222.
[15] Pryor, W. A. (1976) in: Free Radicals in Biology (Pryor, W. A. ed) vol. 1, pp. 1–49, Academic Press, New York.
[16] Paschen, W. and Weser, U. (1975) Z. Physiol. Chem. 356, 727–737.