X-RAY PHOTOELECTRON SPECTROSCOPY (XPS) OF BOVINE ERYTHROCUPREIN

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1. Introduction

Structural aspects and biochemical functions of erythrocuprein have been intensively studied in the last decades [1]. The metalloprotein consists of two 16 300 molecular weight subunits [2, 3] and contains two g-atoms of copper and zinc per 32 600 g of protein. McCord and Fridovich [4] successfully proposed an enzymic function, namely the enzymic catalysed disproportionation of $O_2^-\cdot$ into H_2O_2 and O_2. This biochemical function was critically examined and another reactivity, the quenching of singlet oxygen was presumed the main role of this protein [5]. Nevertheless, the ubiquity of erythrocuprein in many aerobic cells and its reactivity in the biochemistry of oxygen suggests a most important and possible essential function.

In this context we were especially interested in the two metals copper and zinc. At the moment our knowledge of the binding situation of these metallic components is rather poor. Thus we thought it valuable to receive some information regarding their location in the protein portion. Due to the biochemical reactivity it was expected that copper, for the least, should be readily accessible to the substrate.

X-Ray photoelectron spectroscopic measurements gave rise to the conclusion that indeed the cupric ion must probably be located on the surface of the protein portion while Zn^{2+} appears to be bound in the inner sphere of the protein. The ratio of the Me-$2p_{3/2}$ signals was 1:2.5 (Zn:Cu) in erythrocuprein and 1:1 in different amino acid chelates. The Cu-$2p_{3/2}$-value of erythrocuprein (931.9 eV) was lower by 2 eV compared to the corresponding values of different Cu^{2+} chelates (934.2 ± 0.3 eV). Only $Cu[Cu(Asp)_2]$ gave two different Cu-$2p_{3/2}$-values (932 and 934 eV). No marked differences of the Zn-$2p_{3/2}$-signals (1021 ± 0.5 eV) were detected in both erythrocuprein or Zn^{2+}–amino acid chelates.

2. Materials and methods

Erythrocuprein was prepared from citrated bovine blood using the chloroform ethanol procedure described elsewhere [1–3]. During the preparation Cu and Zn were monitored by means of atomic absorption spectroscopy (Zeiss M4 QIII combined with PMQ II and a Linecomp recorder). For XPS-measurements only completely desalted protein was employed. Desalting was performed by Sephadex G-25 gel filtration. The solutions were concentrated using membrane filtration under slight vacuum. $Cu[Cu(Asp)_2]$ was prepared by boiling a solution containing dilute acetic acid, aspartic acid and $Cu(CH_3COO)_2$. After cooling crystals of $Cu[Cu(Asp)_2]$ were separated, washed and recrystallized from dilute acetic acid. The crystals were repeatedly washed with water [6]. The other Cu^{2+}–amino acid chelates were prepared by adding excessive $CuCO_3\cdot Cu(OH)_2$ to a boiling aqueous solution of the respective amino acid. The correspond-

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ing Zn^{2+} chelates were prepared by the method given in [7]. The XPS-spectra were recorded in a Varian IEE-15 photoelectron spectrometer using the Mg-K$\alpha_{1,2}$-line. The electron binding energy of the C-1s signal (284 eV) obtained from Scotch tape served as a standard.

3. Results and discussion

X-Ray photoelectron spectroscopy [8, 9] proved a most convenient new method to study the metal portion in metalloproteins [10, 11]. In comparing the binding energies of different metal compounds even conclusions concerning the metal binding situation are possible. Furthermore, this method is especially suitable for those metals which cannot be studied using Mössbauer or EPR spectroscopy. The binding energies of Cu-2p$_{3/2}$ (fig. 1) and Zn-2p$_{3/2}$ (fig. 2) in erythrocuprein were determined and compared with the corresponding values of Cu^{2+} and Zn^{2+} amino acid chelates.

Marked differences of the intensity of the signals are observed. The intensity ratio of these signals was 1:3.9 (Zn:Cu). This effect was highly reproducible. Even different sample preparations (lyophilized probes or concentrated frozen aqueous solutions) did not affect this ratio. This is contrasted by the observation of the identical intensity using either Cu^{2+} or Zn^{2+} amino acid chelates. Since two g-atoms of Cu and Zn are present in erythrocuprein which yield these different intensities of the Me-p$_{3/2}$ binding energies it can be concluded that Cu^{2+} is probably located in the outer sphere of the protein portion while Zn^{2+} must be bound further inside. This conclusion is reasonable, since the electrons emitted from the zinc ions in the interior of erythrocuprein are more likely to be reabsorbed by the surrounding protein compared to the respective electrons originating from the copper ions. The numerical values of the Zn-2p$_{3/2}$ binding energy was nearly constant at 1021.0 ± 0.5 eV in all the Zn^{2+} chelates using L-ala, L-asp, stearate, the free aquo complex and erythrocuprein. Remarkable differences are observed regarding the Cu-2p$_{3/2}$-value for the Cu^{2+}-amino acid chelates (934 ± 0.3 eV) and erythrocuprein (931.9 eV).

An interesting phenomenon was the recording of two different types of Cu-2p$_{3/2}$ values using the Cu[Cu(Asp)$_2$] complex. The extraneous or salt-like

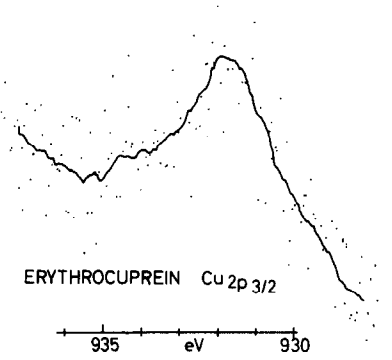

Fig. 1. XPS-Spectrum of bovine erythrocuprein, Cu-2p$_{3/2}$. The temperarure was 25°. Pressure 10^{-5} Torr. Measurements were carried out in a Varian V-IEE 15 photoelectron spectrometer (Mg-K$_{\alpha_{1,2}}$). The C-1s signal (obtained from Scoth tape) at 284 eV served as a standard.

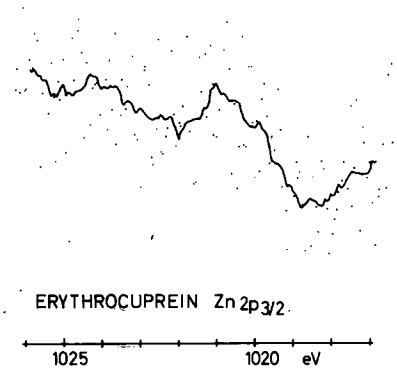

Fig. 2. XPS-Spectrum of bovine erythrocuprein, Zn-2p$_{3/2}$. Experimental conditions as in fig. 1.

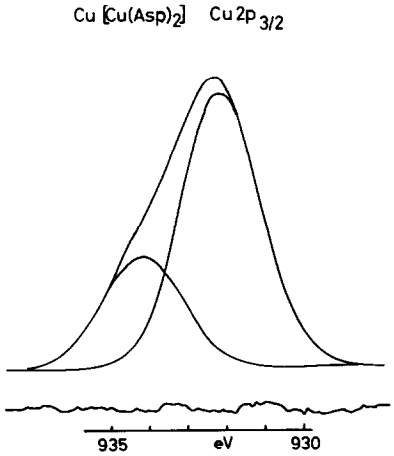

Fig. 3. XPS-Spectrum of Cu[Cu(Asp)$_2$], Cu-2p$_{3/2}$. For experimental details see fig. 1.

Cu^{2+} had an unusual low signal at 932 eV while the binding energy of the chelated inner Cu-$2p_{3/2}$ was measured at 934.1 eV (fig. 3). The binding energy near 934 eV was also obtained using the Cu^{2+}-amino acid chelates of L-lys, L-ala, L-glu, L-his, L-val and gly.

Of further interest was the similarity between the binding enery (932 eV) of the extraneous, salt-like copper in $Cu[Cu(Asp)_2]$ and of erythrocuprein (931.9 eV). However, far reaching conclusions should be made with caution, since no detailed studies regarding crystal field effects upon XPS are available at the moment. The location of the Cu^{2+} ion in the outer sphere of the protein portion supports the finding of Rotilio et al. [12] who determined the enzyme turnover rate of erythrocuprein to be $1.8 \times 10^9\ M^{-1} sec^{-1}$. This second order rate constant is near to the upper limit expected for a diffusion-controlled enzyme–substrate reaction [13]. Neglecting whatever substrate i.e. $O_2^-\cdot$ or singlet oxygen is required [1, 2, 5] a most rapid binding and exchange of the substrate to the cupric ion is an essential condition for the enzymic action of erythrocuprein.

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References

[1] U. Weser, Structure and Bonding, Berlin, in press.
[2] U. Weser, E. Bunnenberg, R. Cammack, C. Djerassi, L. Flohé, G. Thomas and W. Voelter, Biochim. Biophys. Acta 243 (1971) 203.
[3] B.B. Keele, J.M. McCord and I. Fridovich, J. Biol. Chem. 246 (1971) 2875.
[4] J.M. McCord and I. Fridovich, J. Biol. Chem. 244 (1969) 6049.
[5] A. Finazzi-Agro', C. Giovagnoli, P. Del Sole, L. Calabrese, G. Rotilio and B. Mondovi, FEBS Letters 21 (1972) 183.
[6] K. Harada and S.W. Fox, Nature 194 (1962) 768.
[7] G. Weitzel, F. Schneider, A.M. Fretzdorff und H.E. Heyke, Z. Physiol. Chem. 307 (1957) 14.
[8] K. Siegbahn, ESCA, Atomic Molecular and Solid State Structure by Means of Electron Spectroscopy (Almquist and Wiksells, Uppsala, 1967).
[9] C.K. Jørgensen, Modern Aspects of Ligand Field Theory, North-Holland, Amsterdam, 1971.
[10] D. Leibfritz, Angew. Chem. 84 (1972) 156.
[11] G. Jung, U. Weser and W. Voelter, Z. Physiol. Chem. 353 (1972) 720.
[12] G. Rotilio, R.C. Bray and E.M. Fielden, Biochim. Biophys. Acta 268 (1972) 605.
[13] M. Eigen and G.G. Hammes, Adv. Enzymol. 25 (1963) 1.