X-RAY PHOTOELECTRON SPECTROSCOPIC PROPERTIES OF Hg–THIONEIN

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1. Introduction

Metallothionein descriptive for both its unique metal binding capacity and its high sulphur content belongs to a group of most actively studied metalloproteins [1–15]. Some metal ions including Zn^{2+}, Cd^{2+} and to a minor extent Hg^{2+} and Cu^{+} appeared to be preferentially bound by the protein portion [4]. In general, however, the above metal ions are present as arbitrary mixtures in metallo-thioneins isolated from the different biological tissues. Evidence of naturally occurring monodispers cysteine rich proteins exclusively loaded with either zinc or copper ions was recently shown [16–19]. The functional side of all these metalloproteins remains obscure. It was suggested that apart from the metal-free thionein they may act as regulatory components in the biochemistry of the above metal ions. For example, the mercury binding capacity of the thioneins is extraordinarily high [4,5] and it was thought that this kind of mercury chelation would avoid the deleterious reactivity of free Hg^{2+}.

In this context we were especially interested in preparing a monodispers Hg–thionein from rat liver metallo-thionein (Cd–, Zn–thionein). Cd^{2+} and Zn^{2+} were successfully displaced by excessive $HgCl_2$ employing the gel filtration technique [20]. The purified protein contained 8 g atoms of Hg^{2+}. The X-ray photoelectron spectra revealed a marked chemical shift of the $S2p_{1/2,3/2}$ core electrons by 1 eV compared to the binding energy of the corresponding sulphur 2p level of native metallo-thionein. This phenomenon supports the conclusion of a strong polarisation of the mercury-bound sulphur. No such polarisation was seen in the case of the Hg $4f_{5/2,7/2}$ core electrons. Their binding energy remained rather constant at 104.4 ± 0.2 and 100.4 ± 0.2 eV, respectively.

2. Materials and methods

All chemicals employed were of reagent grade quality or better. Cysteine, $HgCl_2$, $CdCl_2$, $ZnCl_2$ were from Merck, Darmstadt; glutathione from Waldhof, Mannheim; Sephadex G-25, G-50 from Pharmacia, Uppsala and DEAE-23 cellulose, Whatman, Maidstone. Metallo-thionein was isolated from rat liver [9,10] and converted into the homogeneous Cd– or Zn–thionein, respectively [21]. Hg–thionein was prepared following the column technique given in ref. [20]. $HgCl_2$ proved a convenient agent for competitive displacement of the more weakly bound Cd^{2+} and Zn^{2+} to metallo-thionein during the chromatographic process employing a Sephadex G-25 column (1.5 × 90 cm). The low mol. wt. metal chelates using cystine, cysteine and glutathione were prepared in accordance with [12].

Zinc and cadmium were determined by atomic absorption spectroscopy [10]. Hg was quantitated using neutron activation analysis equipped with a Ge-Li-semiconducting detecting unit and a multichannel analyser.

2.1. *X-ray photoelectron spectroscopy*

X-ray photoelectron spectroscopy was performed employing a Varian V-IEE-15 spectrometer equipped with an on-line Varian 620 L,8 K computer. The energy of the exciting X-rays was 1253.6 eV (Mg-$K_{\alpha 1/2}$). The C1 S line at 284.0 eV (Scotch tape and/or the protein carbon) served as internal standard.

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3. Results

10 mg of freshly prepared homogeneous Cd–, Zn–thionein [9,10] was applied on a Sephadex G-25 column. The upper 15 cm of the column were previously enriched with excessive $HgCl_2$ (about 100 μmol $HgCl_2$). The Cd–, Zn–thionein was allowed to react overnight with $HgCl_2$. The elution of the protein was successful after 3 hr employing 50 mM phosphate buffer, pH 4.9. The fractions of the protein peak were clearly separated from the Cd^{2+}- and Zn^{2+}-containing fractions. Extraneous protein bound Hg^{2+} was removed from Hg–thionein by repeated gel chromatography. The Hg–thionein proved homogeneous during polyacrylamide disc gel electrophoresis, Sephadex G-25 and G-50 gel filtration and DEAE-23 chromatography. The amino acid analysis was exactly the same as already published [9,10]. The mol. wt. was 11 000. Only 0.1 g atoms of the original Zn^{2+} and Cd^{2+} concentration was detectable. Hg was measured by neutron activation analysis and 8.2 g atoms of Hg per mole of protein were detected.

The optical properties were significantly different to those obtained with native Cd–, Zn–thionein. The shoulder in the UV-absorption spectrum was shifted from 250 to 270 nm. The millimolar absorption coefficient at 270 nm was 12 mM^{-1} cm^{-1}. CD measurements revealed the complete disappearence of the positive extremum at 258 nm while the intensity of the negative Cotton effect was diminished.

The binding energies of the core electrons of Hg $4f_{5/2}$ and Hg $4f_{7/2}$ were determined employing X-ray photoelectron spectroscopy. The corresponding binding energies for Hg using Hg–thionein were compared with the respective data obtained from low mol. wt. Hg-amino acid chelates. In neither complex was any significant change of the binding energies of the Hg 4f levels. Both intensity and line shape were almost identical in all Hg complexes (fig. 1).

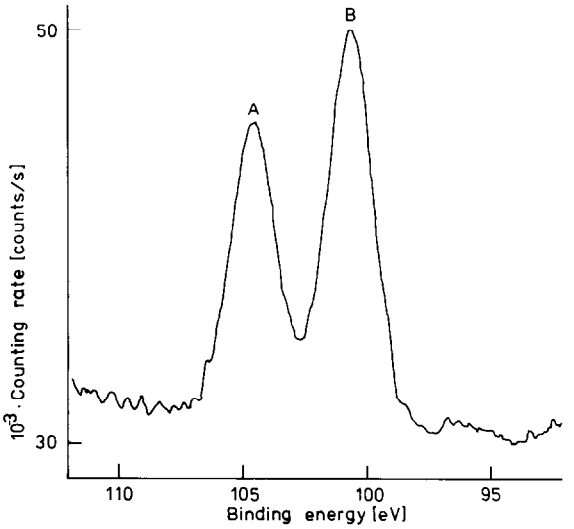

Fig. 1. X-ray photoelectron spectrum of both the Hg $4f_{5/2}$ (A) and Hg $4f_{7/2}$ (B) core electrons using homogeneous Hg–metallo-thionein. X-ray source Mg 11 kV, 130 mA; smoothing over 5 points, work function 4.60 eV; analyzer energy 50 eV; sweep width 20 eV; sweep time 20 sec; no. of scans 50; no. of channels 200; sweep mode, sequential scans. The binding energies are corrected to E (C1s) 284.0 eV using the carbon signal measured during the same recording. The measurements were performed at 1 μtorr and the sample holder was cooled with liquid nitrogen throughout.

The binding energies of the core electrons of Zn,

Table 1
Core electron energy levels expressed in electron volts of $Zn^{2+}(2p_{3/2})$, $Cd^{2+}(3d_{3/2}, 3d_{5/2})$ and $Hg^{2+}(4f_{5/2}, 4f_{7/2})$ determined in the respective monodispers metallo-thioneins. For experimental details see legend to fig. 1.

	Zn	Cd		Hg	
Metallo-thionein	$2p_{3/2}$	$3d_{3/2}$	$3d_{5/2}$	$4f_{5/2}$	$4f_{7/2}$
Zn–Thionein	1020.7	-	-	-	-
Cd–Thionein	-	410.6	403.9	-	-
Zn–, Cd–Thionein	1021.0	411.0	404.0	-	-
Hg–Thionein	-	-	-	104.6	100.6

Cd and Hg were compared using the different monodispers metallothioneins (table 1). As in the case of Hg–thionein no significant chemical shifts were seen employing the respective different low mol. wt. metal chelates. The data listed here are characteristic for the energy levels of Zn $2p_{3/2}$, Cd $3d_{3/2}$ $3d_{5/2}$ and Hg $4f_{5/2}$ $4f_{7/2}$.

Measurements of the binding energies of the core electrons determined in the sulphur $2p_{1/2,3/2}$ levels of different Zn–, Cd– and Hg–thiolate complexes revealed a marked chemical shift (table 2). All X-ray photoelectron spectra of the sulphur levels were of good quality and homogeneity regardless of origin and binding situation of the involved sulphur. In fig. 2 the S $2p_{1/2,3/2}$ binding energies of different metallothioneins known to be representatives for those polymeric species containing high concentrations of RS^- are compared. It was interesting to see that the polarisation of the sulphur was progressively higher with increasing binding strength of the employed metal ion. Out of all metal ions used, mercury proved capable of the strongest electron withdrawing effect. This difference was significant and remained rather constant whatever metal–thiolate complex was examined. The binding energy of the sulphur core electrons using the free metal sulphides was lowest compared to the S $2p_{1/2,3/2}$ levels of the different metallo-thioneins. Due to sulphur bridge formation as it is present in cystine no polarisation of the sulphur was detectable. This is also consistent with the known phenomenon that in these complexes the metal ion is only slightly or even not at all coordinated with the sulphur.

Table 2
Electron binding energies for the unresolved $S2p_{1/2,3/2}$ doublet employing low mol. wt. metal complexes and different metallo-thioneins. The X-ray photoelectron spectra were recorded as described in the caption to fig. 1.

Compound	$S2p_{1/2,3/2}$ (eV)
ZnS	160.9
CdS	160.5
HgS	161.5
Zn–cysteine	161.9
Cd–cysteine	161.6
Hg–cysteine	162.2
Hg–glutathione	162.6
Zn–thionein	161.3
Cd–thionein	161.7
Zn–, Cd–thionein	161.7
Cu–thionein	161.9*
Hg–thionein	162.7
Zn–cystine	163.0
Cd–cystine	162.8
Hg–cystine	163.0

* (taken from ref. [16])

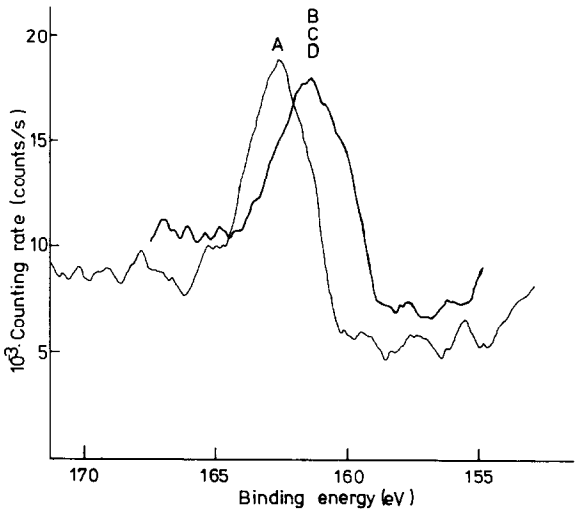

Fig. 2. X-ray photoelectron spectra of the S2p levels of Hg–metallo-thionein (A), Cd–, Zn–metallo-thionein (B), (C), Zn–thionein and (D), Cd–thionein. Smoothing over 15 points; further recording conditions as above.

4. Discussion

The possibility that metallo-thionein is required as a Hg-decontaminating agent is still seriously discussed. However, it was proposed that the metal-free thionein might be an essential biochemically active compound [10,17]. Nevertheless, the complete displacement of Zn^{2+} and/or Cd^{2+} by Hg^{2+} results in dramatic structural changes of the original metallo-thionein. Both the different complex geometry and the increased ionic radius of Hg^{2+} are forcing the polypeptide chain of thionein into a completely different structure. This is also seen in the significant shift of the binding energy of the $2p_{1/2,3/2}$ level of the sulphur. The different electronegativity of Hg^{2+}, at least in part, can be attributed to this marked chemical shift.

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