# X-RAY PHOTOELECTRON SPECTROSCOPY AND THE STRUCTURE OF MELANINS

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## 1. Introduction

For many years studies have been made of the chemical structure, biosynthesis and biological function of melanins [1], a strong motivation for which has been the need to understand and develop methods of control of malignant human melanomas. In the past, structural work has been hampered by the insolubility of these pigments, which has necessitated rather drastic purification procedures and has made most conventional spectroscopic techniques inapplicable. Consequently, the majority of the available structural data has been obtained from chemical identification of functional groups, from the identification of soluble fragments arising from treatment with alkali or permanganate, or from isotopic analysis of melanins from organisms fed radioactive precursors.

The relatively new technique of X-ray photoelectron spectroscopy [2], XPS, has been shown to possess considerable potential for the study of biological materials in the solid state, particularly those which contain metals [3–5]. However, besides studying metals and their binding centres, valuable information about the oxidation state of nitrogen and sulphur can be obtained, such as was recently shown for bovine serum albumin [6]. In XPS the energies and abundance of electrons ejected from the core levels of atoms after X-irradiation of the sample are measured. The binding energies of the core electrons are sensitive to atomic charge and to the oxidation state of the element concerned, and the measurement of these energies thus make it possible to identify elements and, in some cases, to determine their chemical nature. The total number of electrons ejected per element can be used to quantitate the elements present, represented here by the area under the peaks in a plot of the number of electron counts per second versus electron energy. When comparing the intensities of different elements an appropriate correction must be made since the atomic cross section varies with element.

The purpose of this paper is to demonstrate that XPS can yield valuable new information on the structure of isolated melanins and to present Nitrogen 1s and Sulphur 2p binding energies in melanins from different sources and in a number of model compounds.

## 2. Materials and methods

Melanins were isolated from horse melanoma, human hair, watermelon seeds and sunflower seeds by the method of Nicolaus [7]. They were pressed into pellets, using KBr or NaF as a backing when only small quantities of material were available, and were examined in an AEI ES 200A instrument using MgKα radiation. The spectra of some model compounds for comparison were also recorded: these samples were usually mounted on double sided adhesive tape. Binding energies and analytical information derived from XPS peak intensities are given in tables 1 and 2.

Table 1
XPS Data for animal, plant and synthetic melanins

| | Source of melanin | Core electron binding energies/eV[a] | | | Partial elemental analysis[b] | | | | Chemical Analysis[c] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | N 1*s* | S 2*p* (i) | S 2*p* (ii) | N | S (i) | (± 25%) | S (ii) | N | S |
| Animal | Horse melanoma | 400.1 | 168.2 | 164.0 | 6 | 0.13 | | 0.23 | 12.3 | 1.4 |
| | Human hair | 400.1 | 167.9 | 164.2 | 7 | 0.27 | | 0.49 | 11.1 | 0.9 |
| Plant | Watermelon seed | 400 | | | < 0.9 | | < 0.04 | | 0.96 | < 0.5 |
| | Sunflower seed | 400 | | – | < 0.9 | | < 0.04 | | 0.85 | < 0.5 |
| Synthetic | DOPA | 400.1 | | | 7 | | < 0.04 | | | |
| | Dopamine | 400.1 | – | – | 10 | | < 0.04 | | | |

[a] ± 0.4 eV; obtained by assuming the binding energy of the principal C 1s of the melanin to be constant at 285 eV (typical aromatic carbon).
[b] These figures are the number of atoms of the species per 100 C atoms, estimated from the XPS peak areas using previously reported cross-sections (S $2p_{3/2}$ = 0.305, N 1s = 0.376, C 1s = 0.222), and assuming that the electron inelastic mean free path varies as the square root of the energy of the ejected electron [13]. Estimation of oxygen is not given because adventitious oxygen-containing surface contamination is common in XPS.
[c] Expressed as the number of atoms of the species per 100 C atoms.

Table 2
Core electron binding energies (/eV[a]; ± 0.4 eV) for model compounds

| Compound | N 1*s* | | | | $S^{II}$ | S 2*p* | | $S^{VI}$ |
|---|---|---|---|---|---|---|---|---|
| | $NH_2$ | –NHR | $-NR_2$ | Indole –N–H | –SS– | HS– | –S– | $-SO_2-$ |
| L-Cysteine | 402.0 | – | – | – | | 164.5 | – | – |
| L-Methionine | 401.9 | – | – | – | – | | 164.0 | |
| L-Tryptophan | 402.0 | – | | 400.4 | – | – | – | – |
| Tryptamine hydrochloride | 401.9 | – | – | 400.0 | – | – | – | – |
| DL-DOPA | 402.0 | – | – | – | – | – | – | – |
| Dopamine hydrochloride | 401.9 | – | – | – | | – | – | – |
| L-Adrenaline | – | 401.7 | – | – | – | – | – | – |
| DL-Thioctic acid | – | – | – | – | 165.1 | – | – | – |
| Dansyl L-methionine | – | 400.0[b] | 400.0 | – | – | – | 163.7[c] | 168.8[c] |

[a] Obtained by taking C 1s (aromatic) = 285 eV, as in table 1, and C 1s (aliphatic) = 286 eV (internal standards).
[b] $-SO_2-$*NH*–CH(COOH) : not resolved from $-N(CH_3)_2$
[c] S $2p_{3/2}$: baricentre of S 2p ~ 0.3 eV higher (S 2p was not resolved for the other compounds)

## 3. Results and discussion

Typical N1s and S2p spectra obtained from horse melanoma melanin are shown in fig.1a. Both nitrogen and sulphur are detected and on the basis of the peaks at 168.2 and 164 eV we conclude that it contains two chemically distinct sulphur species. Similar spectra were obtained from the other melanin isolated from a mammalian source, human hair melanin. The detection of sulphur is unequivocal despite the low intensity of the signal. The sulphur atoms exhibiting the lower binding energy in these animal melanins are almost certainly $S^{II}$; we obtained binding energies of 163.7–165.1 eV for three different types of $S^{II}$ in model compounds (see table 2). The sulphur atoms with the higher binding energy are probably $S^{VI}$; the sodium salt of thiophene-3-sulphonic acid shows an energy separation between $S^{II}$ and $S^{VI}$ of 4.2 eV [8]

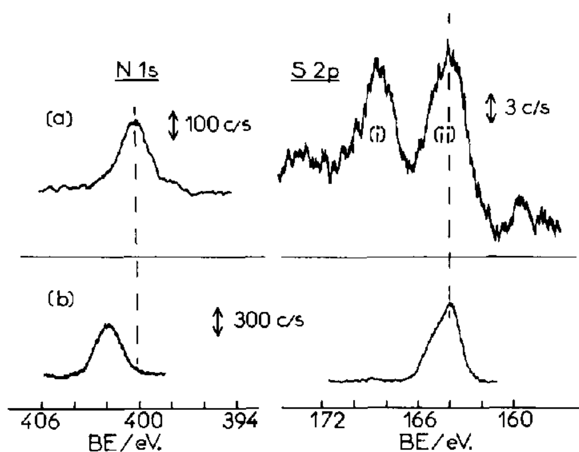


Fig.1. Representative X-ray photoelectron spectra: (a) melanin isolated from horse melanoma; (b) methionine, a typical 'model' compound.

identical to that found in horse melanoma melanin. Dansyl-L-methionine, which also contains both $S^{II}$ and $S^{VI}$, was here found to exhibit a rather larger energy separation, but the extensive compilation of S2p levels by Siegbahn et al. [2] suggests (after allowance for a difference in calibration standards) that $S^{IV}$ species generally possess lower binding energies than the high binding energy sulphur seen in these melanins. We cannot, however, yet eliminate with certainty such possibilities as $>S=O$ or $S\lessgtr^{O}_{OH}$, as binding energies are not solely functions of the immediate chemical environment but depend to some extent on longer range effects.

The more detailed identification of the bonding in these two types of sulphur will await the availability of further model compounds and of further chemical analysis, for which we hope this work will provide a stimulus.

Comparison of the N1s binding energies in tables 1 and 2 shows that the principal N-containing functional groups in horse melanoma and human hair melanins cannot be $-NH_2$; the N1s binding energy ranges of the melanins and of primary amine groups in the model compounds do not overlap. Furthermore we were unable to detect any significant difference in the XPS lineshape between the N1s signals from melanins and those from model compounds containing only one type of nitrogen: in particular no asymmetry was observed. From our lineshape analysis we conclude that the proportion of nitrogen present as $-NH_2$ in these melanins is unlikely to exceed 7% of the total nitrogen content. The majority of the nitrogen is probably present in indole type structures, although we cannot eliminate tertiary or certain secondary amine groupings solely on energetic grounds. These conclusions are in agreement with the chemical determination of free $-NH_2$ in melanoma melanin [9], but would not support the type of structure proposed more recently by Hempel [10] who, on the basis of the incorporation of radioactivity into melanomas in mice from labelled DOPA (3-(3,4 dihydroxyphenyl)-alanine) concluded that melanoma melanin should contain 47% free amine. However, it should be emphasised that the former measurements were made on isolated melanin, the latter on intact material. Since the isolation involves the removal of other cellular materials by hydrolysis in HCl, there may be distinct differences between isolated melanins, such as studied in this paper, and melanins in vivo. In this context we have examined the horse melanoma melanin after a further reflux in 6 N HCl for 168 h (7 × the period used in the initial isolation), and no change in the relative intensities of the two sulphur peaks were observed. Thus it seems likely that two chemically distinct forms of sulphur were present in the original material.

In contrast to horse melanoma and human hair melanins, the XPS spectra of the two plant melanins, from watermelon and sunflower seeds, reveal only traces of sulphur and of nitrogen, in agreement with chemical analysis and, for nitrogen, with the extensive degradation studies which have shown that on alkali fusion animal melanins yield 5,6 dihydroxyindoles whereas plant melanins yield catechols [11,12]. On these grounds animal melanins are considered to be of the DOPA melanin type, that is they show similar degradation products to those obtained from the pigment resulting from oxidation of DOPA. The similarity of the N1s XPS of the horse melanoma and human hair melanins to that of DOPA melanin, synthesised here via oxidation of DOPA with tyrosinase, provides strong confirmation of this concept.

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