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INTERACTION OF Fe^{3+}, Cu^{2+} AND Zn^{2+} WITH MELANIN AND MELANOPROTEINS FROM BOVINE EYES

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Natural melanins are always complexed with proteins, lipids, metal ions and water. Protein incorporated in natural melanin accounts for about 20 - 50% of its weight [1, 2]. It seems probable that such an amount of associated protein can modify metal ion binding to the melanin pigment. The participation of melanosomal protein in the binding of metals to melanin *in vivo* is still an open question.

By the use of radiochemical and e.s.r. (electron spin resonance) methods a high binding capacity of nine metals (Fe, Co, Ni, Cu, Zn, Mn, Cd, Cr and Sn) to melanin was demonstrated [3]. In the majority of the metal ion-DOPA-melanin complexes, the metal can be bound to two classes of independent binding sites [4]. The affinity of metal ions to a large number of various non melanosomal proteins is also well known [5] but the effect of protein on the ion exchange properties of melanin polymers has not been finally established. The participation of melanosomal protein in the zinc(II) -melanin interaction was postulated [6, 7]. On the other hand, most authors suggest that the number and structure of ion exchange centres in melanosomes depend mainly on their melanin content [8 - 10].

It has been earlier demonstrated that the model protein, HSA (human serum albumin), fixed by synthetic DOPA-melanin modifies the binding of metal ion to melanin [11]. The amounts of Fe^{3+}, Cu^{2+} and Mn^{2+} bound to melanin were higher for DOPA-melanin than for DOPA-melanin-HSA

complexes, whereas the amount of bound zinc ions significantly increased in the presence of HSA as compared with protein-free melanin [11]. The obtained results indicate that the protein fixed by melanin may lower the incorporation of metals by blocking sterically certain sites in melanin: however protein can also enhance the overall incorporation by binding itself the metal ions.

Taking into account that the structure of melanosomal protein significantly differs from that of HSA, the aim of the present work was to compare the binding capacity of Fe^{3+}, Cu^{2+} and Zn^{2+} to melanins and melanoproteins isolated from bovine eyes.

Melanoproteins in the form of melanosomes were isolated from the iris and choroid of bovine eyes by two successive sucrose density gradient ultracentrifugations [12]. The protein content in melanosomes determined by the Bradford method [13] was 23.3% (w/w).

Melanin was obtained by solubilization of melanosomal proteins in 0.1% Brij-35/7 M guanidine hydrochloride [14] and 2% sodium dodecylsulphate /5% mercaptoethanol [15].

Melanin-metal ion and melanoprotein-metal ion complexes were prepared as described earlier [11]. The amount of metal ions bound to melanin was determined by radiochemical methods.

For Zn^{2+}-melanin and Zn^{2+}-melanoprotein complexes e.s.r. measurements were performed.

The effect of melanosomal protein on the binding capacity of Fe^{3+}, Cu^{2+} and Zn^{2+} to melanin from bovine eyes is shown in Table 1. For the initial metal ion concentration from 1×10^{-4} M to 1×10^{-2} M the results are expressed as μmoles of bound metal per mg of a sample, either of melanin and melanosomes.

In all analyzed systems the amounts of bound metals were higher for the melanin than for melanoprotein samples. The largest effect of melanosomal protein on the metal to melanin binding capacity was observed for Fe^{3+}. The presence of protein in melanin-Fe complexes caused a decrease of bound Fe^{3+}, depending on its initial concentration, by 18 - 43% as compared with protein-free samples. Similarly, a decrease of metal binding was observed when Cu^{2+} and Zn^{2+} were applied to melanin and melanosomes, but the amounts of bound metals in the melanosome samples were lower by about 10 - 20 % than those in protein-free melanins.

Among the analyzed metals only Zn^{2+} is a diamagnetic ion which can enhance the melanin e.s.r. signal intensity [2]. The e.s.r. data obtained for

Table 1

Amounts of Fe^{3+}, Cu^{2+} and Zn^{2+} bound to melanin and melanosomes from bovine eyes

Initial concentration of metal ion (M)	µmoles of bound metal/mg sample	
	melanin	melanosomes
Fe^{3+}		
1×10^{-4}	0.030	0.017
1×10^{-3}	0.167	0.136
1×10^{-2}	1.207	0.815
Cu^{2+}		
1×10^{-4}	0.080	0.071
1×10^{-3}	0.698	0.559
1×10^{-2}	1.368	1.066
Zn^{2+}		
1×10^{-4}	0.079	0.062
1×10^{-3}	0.718	0.679
1×10^{-2}	1.227	0.958

Table 2

E.s.r. data for Zn^{2+}- melanin and Zn^{2+}- melanosomes complexes

Examined sample	C_o (Zn) • (M)	g_o ± 0.0002	ΔB_{max} (mT) ± 0.01	I_n	I_{rel}
Melanin	0	2.0036	0.43	1.01	1.00
	1×10^{-4}	2.0037	0.47	1.73	1.71
	1×10^{-3}	2.0037	0.48	2.20	2.18
	1×10^{-2}	2.0039	0.48	2.26	2.58
Melanosomes	0	2.0038	0.52	0.88	1.00
	1×10^{-4}	2.0040	0.50	1.67	1.90
	1×10^{-3}	2.0040	0.48	5.11	5.81
	1×10^{-2}	2.0042	0.47	8.01	9.10

Zn^{2+}-melanin and Zn^{2+}-melanosomes complexes are presented in Table 2. The presence of protein in melanin samples caused a decrease of the number of inherent melanin paramagnetic centres by about 13% (cf. I_n if $C_o(Zn) = 0$). Melanin as well as melanosomes exhibited an increase of e.s.r. signal intensity (In) with the increase of concentration of the metal ion added.

However, a much stronger relative effect was found for Zn-melanosome (910%) than for Zn-melanin complexes (260%) as compared with respective Zn-free melanosome or melanin samples.

The described results demonstrate that natural melanin in the form of melanoprotein (e.g. melanosomes) is able to fix somewhat lower amounts of bound metal ions as compared with protein-free melanin (Table 1). The melanosomal proteins linked to melanin probably block certain active binding sites in the melanin matrix, responsible for metal ion binding in free melanin. The blocking effect differs with respect to the analyzed metal ion.

It is known from the literature [5] that, in the metal ion-protein interaction, different metals may occupy different binding sites in the protein molecule. The nature and capacity of binding sites in melanin differ mainly in the content of carboxy-, hydroxy-, amino- and semiquinone groups in the melanin polymer. Therefore metal ion-binding processes in melanins are very complex and thus apt to be strongly influenced by protein binding.

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