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THERMOGRAVIMETRY AND DIFFERENTIAL SCANNING CALORIMETRY OF NATURAL AND SYNTHETIC MELANINS

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The thermal stability of natural melanins from bovine eyes, black human hair and the hard core of banana peel, synthetic melanins obtained enzymatically or by autoxidation of various precursors, and chemically modified synthetic melanins was studied by DSC and TG analysis. It was shown that the resistance of melanins to thermal degradation depends on their origin. Synthetic melanins were found to be more stable to thermal decomposition than natural melanins. Methylation of melanins caused a significant increase in thermal stability. The DSC curves of melanins reveal typical relaxation phenomena in the temperature range 293-413 K.

It is generally believed that melanins are heterogeneous polymers derived from the oxidation of such precursors as tyrosine, 3,4-dihydroxyphenylalanine (dopa), catecholamines or catechol. The chemical and thermal stabilities of melanin polymers were compared with those of soil humic substances [1, 2]. Some similarities, but also distinct differences between fungal melanins and soil humic compounds in their resistance to thermal degradation were demonstrated by Filip *et al.* [3].

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Analysis of the chemical and spatial structures of natural and synthetic eumelanins by differential scanning calorimetry (DSC) and by NMR revealed essential differences between melanin samples when energy was administered to the samples [4].

Evidence exists in the literature that melanins contain water in their structure [5-7]. It was demonstrated that part of the water can be lost from melanin by heating up to 423 K, whereas another part is retained in the inner structure of the melanin polymer, even at temperatures higher than 423 K. Simultaneous DTA, TGA and MS analysis indicate that at least some of the thermally induced effects in melanin samples of various origins result from various concentrations of water present in the analyzed melanin samples [8].

In this paper we try to clarify the differences in DSC and TG curves obtained for various melanin preparations in two temperature ranges, one where no chemical decomposition occurs, and one where the decomposition of melanins is accompanied by the production of various degradation products.

Experimental

Preparation of melanins

Natural melanins were isolated from the iris and choroid with retinal pigment epithelium of bovine eyes according to the method described by Nicolaus [9], from black human hair and from the hard core of banana peel by the procedure described previously [10].

Synthetic melanins from L-tyrosine, L-dopa (β-(3.4-dihydroxyphenyl)-L-α-alanine), tyramine and catechol were prepared by autoxidation of a 5 mM solution of the respective precursor in phosphate buffer (0.067 M, pH 8.0) or enzymatically (pH 6.8) by the use of crude potato tyrosinase or mushroom tyrosinase (Sigma Chemical Co.) according to Binns *et al.* [11]. Adrenaline-melanin was obtained by oxidation of the substrate solution (2 mM) in TRIS-HCl buffer (0.05 M, pH 8.0) in the presence of copper sulfate (0.1 mM) [12]. After 48 h, the oxidation was stopped by acidification with hydrochloric acid up to pH 2 and subsequent centrifugation. The melanin sediments obtained were washed carefully with distilled water until they yielded a negative chloride reaction and then dried over phosphorus pentoxide.

Chemically modified melanins were prepared by treatment of melanin samples with methanol saturated with gaseous HCl or with an excess of diazomethane [13]. Methylation of melanin with methanol-HCl resulted in the esterification of carboxy acid groups, whereas the treatment with diazomethane involved the methylation of carboxy and hydroxy groups as well as the indole and pyrrole NH groups of melanin. The degree of esterified carboxy groups in melanin preparations was determined by potentiometric titration as described previously [14]. The modified melanins used in the experiments were esterified up to 25, 40 and 100%. All melanin preparations were stored in a desiccator over P_2O_5.

Differential scanning calorimetry and thermogravimetric analysis

DSC curves were recorded with a DuPont 990 thermal analyzer (USA). Melanin samples (4-10 mg) sealed in an aluminium sample pan were heated either from 218 K to 423 K or from 218 K to 773 K in nitrogen atmosphere. TG analysis were performed in the temperature range 290-773 K on the 951 thermogravimetric analyzer working on the basis of the Du Pont 990 thermal analyzer. All thermograms were recorded at a heating rate of 10 deg. min^{-1} and at a nitrogen flow of 15-30 $cm^3 \cdot min^{-1}$.

Results and discussion

The obtained DSC curves show for all examined melanin samples a marked endothermal peak in the temperature range 290-420 K and a broad exothermal one in the range 450-700 K, as presented in Fig. 1 for melanins from human dark hair, bovine eyes, dopa obtained enzymatically and dopa obtained by autoxidation. The temperatures of the endothermal peaks for different natural and synthetic melanins are listed in Table 1. It can be seen that the maximum of the endothermal peak was reached at various temperatures (329-353 K), depending on the melanin origin. Chemical modifications introduced in the structure of tyrosine- and dopa-melanin by methylation were manifested by an increase in the endothermal peak temperature.

When melanins after the first DSC run up to 423 K were cooled to 218 K, then heated again and this procedure was repeated several times at various intervals of time, the DSC curves demonstrated typical relaxation phenomena. For example, a set of DSC curves collected for melanin from the hard core of banana peel is shown in Fig. 2. Assuming that the enthalpy of the endothermal peak is 100% in the first run, it was calculated that 37%

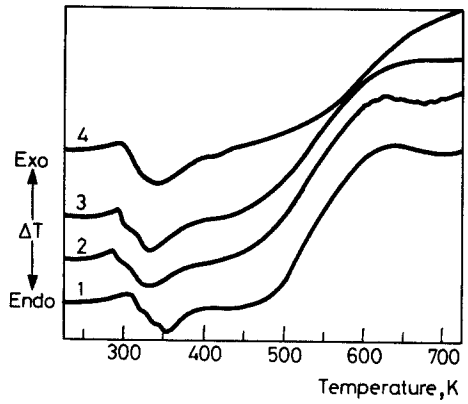

Fig. 1 DSC curves for melanins from the human dark hair (1), from bovine eyes (2), from dopa obtained enzymatically (3) and from dopa obtained by autoxidation (4)

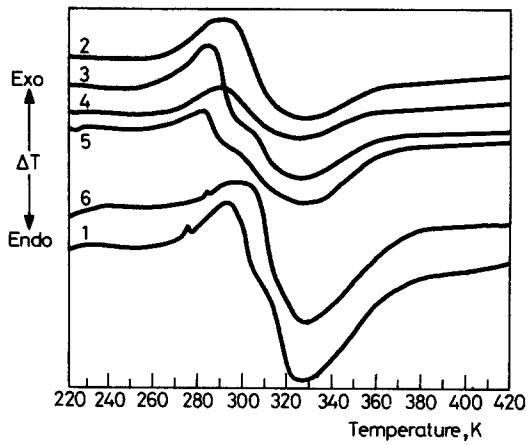

Fig. 2 DSC curves for melanin from the hard core of banana peel registered after successive runs. The time intervals between successive runs were 30 min (2), 50 min (3), 120 min (4), 22 h (5) and 68 h(6). (1) - reference melanin sample

of this value was reached in the second run carried out 30 min later, and that in the time course of the experiment 34%, 39%, 54% and 86% of the initial enthalpy value was recovered in the next runs after 50, 120, 1320 and 4080 min, respectively. Although such behaviour of the DSC curves is characteristic for all examined melanin samples, the recovery of the endothermal peak depends on both the melanin origin and the time elapsed between two

successive runs. For example, the endothermal effect for melanin from black human hair was 42%, 46%, 52% and 30% of the initial value after 30, 60, 120 and 180 min, respectively, and it increased from 30% to 47% in the next run carried out 720 min later. For melanin from bovine eyes, the recovery of the initial enthalpy value was 27%, 22%, 20%, 28% and 24% in the successive runs registered after 30, 40, 60, 180 and 1020 min.

Table 1 TG data on natural and synthetic melanins of various origins

Melanin sample	Temp. of endothermal peak, K	Δm, % at 413 K[a]		
		1st run	2nd run	3rd run
Natural melanins:				
from hard core of banana	329	4.4	0.0	1.0
from black human hair	353	6.2	1.8	0.6
from bovine eyes	332	7.5	3.5	2.7
Synthetic melanins:				
tyrosine-melanin[b]	331	6.2	2.3	1.7
dopa-melanin[b]	329	7.3	2.3	1.9
dopa-melanin[c]	336	13.1	3.8	3.1
catechol-melanin[c]	335	8.4	2,5	6.1
adrenaline-melanin[c]	338	11.4	7.4	7.6
tyramine-melanin[b]	343	6.9	1.4	1.4
Modified synthetic melanins:				
tyrosine-melanin[b] esterified to 25 %	331	7.3	2.3	1.7
tyrosine-melanin[b] esterified to 100 %	337	9.0	1.1	1.3
dopa-melanin[c] esterified to 25 %	336	7.9	1.3	2.0
dopa-melanin[c] esterified to 40 %	338	10.8	1.3	3.3
dopa-melanin[c] esterified to 100 %	340	7.8	1.1	1.1
dopa-melanin[c] methylated with CH_2N_2	350	-	-	-

a) The melanin samples were heated up to 423 K (1st run), cooled to room temperature and immediately heated again to 423 K (2nd run). The third run was carried out 20 h later. During heating and cooling, the samples were kept constantly in a nitrogen atmosphere
b) Melanins obtained enzymatically
c) Melanins obtained by autioxidation

It was shown [8] by the use of simultaneous differential thermal analysis and mass spectrometric analysis that the endothermal effect is related to water desorption from melanins.

It can not be excluded that the water readsorption is, at least in some part, responsible for the observed relaxation phenomenon. it has to be taken into consideration that the analysed melanin sample placed in the sample pan is always (during cooling and heating) in the DSC apparatus under a

stream of nitrogen at a flow rate of from 15 to 30 $cm^3 \cdot min^{-1}$. The water content in the nitrogen is usually in the range of 1-5 ppm. A simple calculation shows that the water present in "dry" nitrogen can be adsorbed on the large melanin surface.

On the other hand, it seems that this phenomenon can not be responsible for such a high enthalpy increase as observed in the present experiment. In our opinion, the total enthalpy should be divided into two parts, where the first is related to the typical hydration phenomenon, whereas the other part can be explained rather as a phase transition phenomenon. This opinion is based on the model of melanin described by Thathachari [15, 16], where it was shown that the melanin granule nucleus is organized in such a way that the short range order in their spatial structure will refer to the parallel stacking of some planar layers. An average interlayer spacing of about 3.4 Å in the lamellar structure of melanin is large enough for water molecules described by a kinetic diameter of 2.8 Å. It can not be excluded that water molecules are present between the layers and therefore direct hydrogen bonds or water bridges between individual water molecules and carboxy, hydroxy and amino groups can be formed. In the course of melanin heating, the breaking of these interplanar couplings and the displacement of stacked layers may occur. The planar fragments of the melanin polymer may partially return to their initial positions after cooling. The proposed explanation of the observed phase transition or thermal relaxation is in good agreement with the results of Albanese *et al.* [17], who demonstrated an increase in the interlayer spacing of 0.2 Å and an increase of about 0.3 Å in the diagonal interplanar distances on the heating of dopa-melanin.

In order to compare the thermal stabilities of natural, synthetic and modified melanins, thermogravimetric analysis was carried out on 14 melanin preparations of various origins. The mass losses measured at 413 K for the analysed melanins are presented in Table 1. After the first run, where the samples were heated up to 423 K, the decrease in mass was from 4.4% (melanin from banana) to 13.1% (dopa-melanin obtained by autoxidation). When the samples were cooled to room temperature and heated to 423 K again, an additional mass loss was registered. As can be seen from Table 1, the mass losses after the second run and after the third run were similar for the given melanin preparation, but they were always lower than on the first heating. The total mass loss at 413 K after three runs was strongly dependent on the melanin origin. The smallest decrease in mass was found for natural melanin from the hard core of banana (5.4% of the initial value). Adrenaline-melanin showed the highest value of Δm (26.4% of the initial mass value). Methyl esters of dopa- and tyrosine-melanin did not show any

significant differences in mass loss at 413 K, depending on the percentage of methylation.

After the third run, the melanin samples were cooled again to room temperature and then heated up to 773 K (4th run). The results of TG analysis at 413 K, 473 K, 573 K, 673 K and 773 K are presented in Table 2. From the data obtained for the fourth run, it can further be seen that there was a small decrease in the mass of the analysed melanin samples at lower temperatures. A significant loss in mass caused by the decomposition of melanin was registered for all investigated melanins at 573 K and at higher temperatures.

The evolution of carbon dioxide, water and ammonia during the heating of melanins at elevated temperatures was demonstrated by the use of simultaneous DTA and MS analysis [8].

Table 2 Results of thermogravimetric analysis of melanins previously heated up to 423 K

Melanin sample	Δm, % - 4th run[a]					$\sum \Delta m$,
	413 K	473 K	573 K	673 K	773 K	%
Natural melanins:						
from hard core of banana	0.6	2.4	17.1	44.9	75.1	80.5
from black human hair	2.3	6.3	25.1	46.8	71.4	80.0
from bovine eyes	2.1	3.4	18.3	39.9	63.6	77.3
Synthetic melanins:						
tyrosine-melanin[b]	1.7	3.4	14.0	35.9	63.9	74.1
dopa-melanin[b]	1.7	3.2	15.1	36.5	63.6	75.1
dopa-melanin[c]	3.0	4.7	15.0	36.5	62.5	82.5
catechol-melanin[c]	4.3	5.7	10.4	28.0	66.9	83.9
adrenaline-melanin[c]	6.8	8.3	21.2	45.1	61.6	88.0
tyramine-melanin[b]	0.9	2.2	12.6	36.3	70.5	80.2
Modified synthetic melanins:						
tyrosine-melanin[b] esterified to 25 %	1.1	3.5	12.0	22.0	32.7	44.0
tyrosine-melanin[b] esterified to 100 %	1.2	3.5	12.2	23.8	36.5	47.9
dopa-melanin[c] esterified to 25 %	1.2	3.9	12.2	22.2	32.8	44.0
dopa-melanin[c] esterified to 40 %	1.6	4.0	12.7	24.1	35.6	51.0
dopa-melanin[c] esterified to 100 %	1.9	4.1	14.3	25.9	37.1	47.1

a) The melanin samples were heated to 423 K, the cooled down to room temperature and heated again three times; described as fourth run, where
Δm% was determined at given temperatures
Δm% = mass loss as compared to the original melanin sample before heating, expressed in per cent
b) Melanins obtained enzymatically
c) Melanins obtained by autioxidation

Striking differences in thermal stability were found between synthetic melanins and their methylated derivatives. Tyrosine- and dopa-melanins lost about 63% of the initial mass at 773 K, whereas their methyl esters lost only about 33-37% of the mass. These differences are easily understandable because the methylation occurs mainly on the carboxylic groups of melanins, and the methylated ones do not undergo decarboxylation under the described conditions.

The data presented in Table 2 indicate that synthetic melanins are in general more resistant to thermal degradation at high temperatures than natural melanins.

The thermal stability of synthetic melanins depends on the precursor used for melanin synthesis. The total mass loss after four runs ranged from 74% for tyrosine-melanin to 88 % for adrenaline-melanin. Natural melanins showed a total mass loss in the range 77.3-80.5% (Table 2).

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Zusammenfassung — Mittels DSC und TG wurde die thermische Stabilität von natürlichen Melaninen aus Rindsaugen, aus schwarzem menschlichen Haar und aus Hartteilen von Bananenschalen, weiterhin von enzymatisch oder durch Autoxidation aus verschiedenen Präkursoren synthetisierten Melaninen sowie von chemisch modifizierten synthetischen Melaninen untersucht. Es zeigte sich, daß die Beständigkeit von Melaninen gegenüber thermischem Abbau von ihrem Ursprung abhängt. Synthetische Melanine erwiesen sich in der thermischen Zersetzung als stabiler als Melanine natürlichen Ursprunges. Durch Methylierung wird eine eindeutige Steigerung der thermischen Stabilität verursacht. Im Temperaturbereich 293-413 K zeigen weisen die DSC-Kurven der Melanine typische Relaxationserscheinungen auf.