INCORPORATION OF L-3,4-DIHYDROXY-[2-^{14}C]PHENYLALANINE INTO HAMSTER MELANOMA MELANOSOMES

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1. Introduction

Melanosomes are specific particles of pigment cells [1]. They have been studied by classical isolation methods [2,3]. The isolation procedures aim at obtaining homogenous samples of melanosomes but neglect quantitative aspects [2]; consequently we lack information both on the amount of melanosomes in pigment tissue and on the recovery by the separation methods.

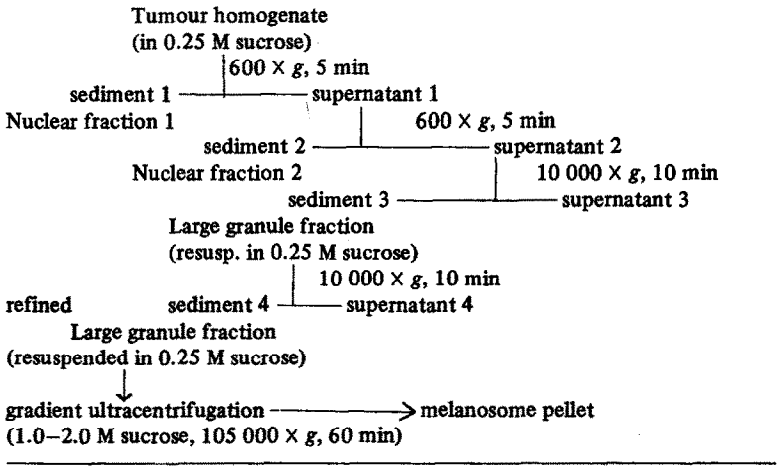
As melanocytes possess a unique metabolic pathway towards melanins with L-3,4-dihydroxyphenylalanine (Dopa) as the specific precursor [1,4,5] and, as on the subcellular level, melanin is produced in melanosomes [1,6], experiments with labelled Dopa could yield both quantitative data, and give more information on the origin of Dopa, repeatedly found in melanosome hydrolysates [7–9].

Here, we report on melanosome concentration in melanoma tissue and show that part of the [2-^{14}C]-Dopa incorporated into melanosomes can be released by hydrolysis.

2. Materials and methods

Six Syrian hamsters (*Mesocricetus auratus*, Waterhouse) bearing a transplantable Bomirski melanoma [10], pigmented line Ma (180th passage), were administered i.p. with L-[2-^{14}C]Dopa (New England

Table 1
Scheme of melanosome isolation



Nuclear, Boston, MA); spec. act. 145 MBq (mM) (296 kBq/animal) and killed 4 days later. The tumours were removed and the melanosomes isoted according to [1,4] as modified in table 1. Separated melanosomes were freeze-dried then kept over $CaCl_2$ until a constant weight was reached.

The amount of melanin in melanosomes was ascertained by means of acid hydrolysis (6 N HCl, 24 h, 112°C) followed by quantitative removal of insoluble melanin [8].

Radioactivity was measured using an ABAC-SL 40 Intertechnique (France) liquid scintillation spectrometer in liquid scintillator SLS-31, Spolana Neratovice, except for dry melanosomes, which were processed by the Intertechnique IN 4101 sample burner then measured in the recommended mixture (400 ml toluene, 330 ml phenetylamine, 220 ml methanol, 7 g butyl-PBD, 50 ml water). Samples obtained during isolation were preincubated with Protosol (6 h, 65°C) before addition of liquid scintillator.

3. Results

The distribution of radioactivity during the isolation procedure is shown in table 2. There was only a moderate loss of radioactivity to nuclear fractions while during the preparation of large granule fractions ~50% of the radioactivity was discarded each time in the supernatant (combined small granule and soluble fractions). The refined large granule fraction (the starting material for gradient ultracentrifugation) contained ~25% of original tumour radioactivity, from which 66% was recovered in the pure melanosome pellet; i.e., ~16% of the original radioactivity.

Table 2
Typical distribution of radioactivity during melanosome isolation

Fraction	% radioact.
Supernatant 1	95.5
Supernatant 2	90.6
Supernatant 3	48.9
Supernatant 4	24.4
Refined large granule fraction	24.9
Melanosome pellet	16.4

100% = radioactivity of original tumour homogenate; fractions were characterized in table 1

Table 3
Attempts to release label from isolated melanosomes

Melanosome treatment	% radioact. released
Acid hydrolysis (6 N HCl 25 h 112°C)	10.7–12.8
Exchange diffusion (against 2.3×10^{-3} M Dopa 16 h)	0.0– 0.5
Extraction with dist. H_2O (16 h)	0.6– 2.5
Extraction with dist. H_2O (15 min)	0.0– 1.5

[2-^{14}C]Dopa-labelled melanosomes (spec. act. 170 430 cpm/g) treated as shown above were removed from the suspensions by centrifugation (20 000 × *g*, 5 min) and the supernatants measured for radioactivity

These results indicate that recovery during melanosome isolation is relatively low; 16.4% in the case of hamster melanoma. From 51.50 g fresh tumour tissue 0.44 g melanosomes were obtained; considering the recovery it means that melanosomes should represents ~5.2% of fresh hamster melanoma weight.

The isolated melanosomes contained 22.71 ± 0.45% ($\bar{x}$ ± SD, $n = 4$) of melanin. The melanin level in the Bomirski hamster melanoma line Ma studied is known to reach 4.45 ± 0.22% of its dry weight [11]. Fresh hamster melanoma contains ~77.7% water; hence it could be calculated that melanosomes should represent ~4.4% of its weight.

[2-^{14}C]Dopa seemed to be tightly bound to the melanosomes. Extraction with water and exchange diffusion against 2.3×10^{-3} M Dopa in water released no significant amount of radioactivity (table 3). However, after hydrolysis 12.04 ± 0.95 % ($\bar{x}$ ± SD, $n = 4$) of radioactivity could be detected in the hydrolysate.

4. Discussion

Isolation of [2-^{14}C]Dopa-labelled melanosomes revealed that the recovery of the classical isolation procedure [1,4] was relatively low; ~16% as for Bomirski hamster melanoma.

Concerning tumour melanosome level, the correspondence between the data derived from [2-^{14}C]-

Dopa experiments and those from melanin contents both in melanosomes and whole melanoma confirms the observed yield, and emphasizes that on a subcellular level Dopa is selectively taken up by melanosomes. This is usually illustrated by autoradiography (e.g., [12–16]) and seldom confirmed by biochemical methods [6,17,18].

These results demonstrate that most of the labelled Dopa was firmly attached to melanosomes and resistant both to exchange diffusion and hydrolysis, little being released on hydrolysis. During melanogenesis of the quinonoid intermediates generated from Dopa by tyrosinase are incorporated into the melanin biopolymer [5,18–20], which is stable to an acid hydrolytic attack [19]. What is the origin of the Dopa reported in melanosome hydrolysates [7–9]? Studies on the binding of Dopa to tRNA indicated that formation of Dopa-containing proteins is most unlikely in animal cells [21]. Dopa residues in proteins may be formed indirectly by post-translational modifications of tyrosyl groups [22]. Under the experimental conditions used, however, such residues would not be radioactive.

At last the affinity of Dopa quinonoid metabolites to thiol groups [23] and to $-NH_2$ groups of lysyl residues [24] should be noted. Such conjugates should be released by hydrolysis from a polypeptide chain as shown on the tissue level in the case of cysteinyldopa [25]. The presented results suggest that such events may occur in melanosomes.

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