INTRACELLULAR DISTRIBUTION OF DOPA AND 5-S-CYSTEINYLDOPA IN HARDING-PASSEY MELANOMA

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Abstract. The concentrations of dopa and 5-S-cysteinyldopa were determined in the various cell fractions of Harding-Passey melanomas. Dopa was present in larger amounts than was 5-S-cysteinyldopa in all cell fractions, but the dopa/5-S-cysteinyldopa ratio was lower in the soluble fraction and in the small-granule fraction than in the large-granule fraction. The soluble fraction contained the greatest amount of catechols. These findings are compatible with high tyrosinase activity not only in the melanosomes but also in the small-granule and soluble fractions.

Key words: 5-S-Cysteinyldopa; Dopa; Melanoma; Aminoacids

Recent investigations have shown that human and animal melanomas contain two major catechols, dopa and 5-S-cysteinyldopa (1, 4, 11). Our ability to measure these catechols in the melanin-producing cells has opened up a new approach to the understanding of pigment synthesis by relating the occurrence of the free catechols to the composition of the pigments formed. It will also be of great interest to compare the content of catechols in cellular fractions with previous findings concerning tyrosinase levels. The present study was performed in order to ascertain the quantities of dopa and 5-S-cysteinyldopa in different cell fractions of Harding-Passey melanomas.

MATERIAL AND METHODS

Melanoma tissue was obtained from DBA mice transplanted with Harding-Passey melanoma 19-21 days earlier. Melanomas from 3 mice were used for each experiment. The procedure for separation of cellular components is given in Fig. 1. After separation, all specimens to be examined were precipitated with 0.4 N perchloric acid (final concentration).

Dopa and 5-S-cysteinyldopa were determined fluorimetrically by methods described previously (3, 5).

RESULTS

The amounts of 5-S-cysteinyldopa and dopa found in the Harding-Passey tumours analysed and in the different cell fractions prepared are given in Table I. The variation between the different experiments was small. It is evident that Harding-Passey melanomas contain more dopa than 5-S-cysteinyldopa: the various fractions showed considerable differences in dopa and 5-S-cysteinyldopa content. The amount of dopa was highest in the soluble and nuclear fractions. The large-granule fraction contained an intermediate amount and the small-granule fraction only a small amount.

The content of 5-S-cysteinyldopa was definitely highest in the soluble fraction. The nuclear fraction contained about half the amount present in the soluble fraction, and the large-granule and small-granule fractions both contained small amounts.

The dopa/5-S-cysteinyldopa ratio was highest in the large-granule fraction, intermediate in the small-granule fraction, and lowest in the soluble fraction.

DISCUSSION

The dopa values found in this study are somewhat higher than those previously reported in Harding-Passey melanomas (4, 10). The concentration of...
TISSUE
(In 5-8 volumes of isotonic sucrose solution)

Homogenization

Centrifugation 700 g for 10 min

Sediment Supernatant

NUCLEAR FRACTION

Centrifugation 11 000 g for 10 min

Sediment Supernatant

Washing in sucrose

Centrifugation 11 000 g for 10 min

Sediment Supernatant

LARGE-GRANULE FRACTION

Centrifugation 100 000 g for 60 min

Sediment Supernatant

SMALL-GRANULE SOLUBLE FRACTION

Fig. 1. Fractionation of melanoma tissue. All procedures were carried out at 0° to 4°C.

5-S-cysteinyldopa was roughly the same as that found by us earlier (6). These results confirm our previous finding that 5-S-cysteinyldopa is a substance characteristic of melanin-forming cells. Its role in the production of phaeomelans is well established. Other biological functions of this amino acid remain unknown, however. We hope that studies on the intracellular distribution of 5-S-cysteinyldopa may prove helpful in elucidating the role played by this compound in the melanocytes.

Four different fractions were analysed with regard to catechol content. The sediment obtained by centrifugation at 700 g contains tissue fragments, whole cells, nuclei, and large melanin particles. The large-granule fraction contains mitochondria and melanosomes and the small-granule fraction has ribosomes and rough-surface and smooth-surface membranes (7, 9). In evaluating the results it is important to realize that the melanomas studied contain not only melanoma cells but also connective tissue cells including phagocytes.

The subcellular localization of tyrosinase in Harding-Passey melanomas has been investigated by Seiji’s group (7), who found most of the tyrosinase in the large-granule fraction in the melanosomes. Some was also found in the small-granule fraction, mostly in the smooth membranes.

Incorporation of tagged dopa into the melanosomes took place very rapidly, whereas rough and smooth membranes showed little uptake of dopa. Melanin deposition was thus dependent on something over and above tyrosinase, and which is present only in the melanosomes.

Our study has shown to our surprise that the largest concentrations of catechols were present in the soluble fraction. The intracellular ratio of dopa to 5-S-cysteinyldopa differed in different fractions, the dopa/5-S-cysteinyldopa ratio being clearly higher in the large-granule fraction than in the small-granule and soluble fractions. The dopa/5-S-cysteinyldopa ratio in the nuclear fraction corresponds to a mixture of whole melanoma cells and particles, in which the dopa/5-S-cysteinyldopa ratio is similar to that in the large-granule fraction.

It is evident from Seiji’s studies that melanin deposition does not occur in all organelles containing tyrosinase. Our results show that the largest concentrations of catechols were present outside the melanin-forming organelles. The importance of the dopa/5-S-cysteinyldopa ratio for melanin formation cannot at present be defined, but the possibility that a higher dopa/5-S-cysteinyldopa ratio may provide chemical conditions favouring melanin deposition must be considered.

Whereas dopa formation in the melanocytes re-

Table 1. 5-S-cysteinyldopa and dopa in cellular fractions of Harding-Passey melanoma (3 experiments)

<table>
<thead>
<tr>
<th>Tumour weight (g)</th>
<th>Fraction</th>
<th>5-S-cysteinyldopa µg</th>
<th></th>
<th>Dopa µg</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6</td>
<td>Nuclear</td>
<td>1.7</td>
<td>26</td>
<td>5.6</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Large-granule</td>
<td>0.54</td>
<td>8</td>
<td>2.3</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Small-granule</td>
<td>0.43</td>
<td>7</td>
<td>0.83</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Soluble</td>
<td>3.8</td>
<td>58</td>
<td>6.0</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>6.5</td>
<td>14.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.6</td>
<td>Nuclear</td>
<td>1.1</td>
<td>25</td>
<td>4.1</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Large-granule</td>
<td>0.33</td>
<td>8</td>
<td>1.4</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Small-granule</td>
<td>0.27</td>
<td>6</td>
<td>0.57</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Soluble</td>
<td>2.7</td>
<td>61</td>
<td>5.0</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>4.4</td>
<td>11.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.9</td>
<td>Nuclear</td>
<td>1.5</td>
<td>28</td>
<td>3.3</td>
<td>34</td>
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<tr>
<td></td>
<td>Large-granule</td>
<td>0.56</td>
<td>11</td>
<td>2.1</td>
<td>22</td>
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<tr>
<td></td>
<td>Small-granule</td>
<td>0.46</td>
<td>9</td>
<td>0.83</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Soluble</td>
<td>2.8</td>
<td>53</td>
<td>3.4</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5.3</td>
<td>9.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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quires only tyrosine and tyrosinase, the formation of 5-S-cysteinyldopa requires dopa, tyrosinase, and cysteine. In the presence of tyrosinase, dopa formed by this enzyme from tyrosine is further oxidized to dopaquinone, which compound on 1,6-addition of cysteine yields 5-S-cysteinyldopa. Furthermore, it is also possible that dopaquinone forms a thioether with glutathione, which peptide by the action of two different enzymes may give rise to 5-S-cysteinyldopa (2).

The availability of SH-groups is a condition for the formation of 5-S-cysteinyldopa. Studies by Seiji have shown that the quantities of protein-like and non-protein-like SH-groups are abundant in the various fractions of Harding-Passey melanomas (8), and that therefore the SH-containing compounds do not seem to be limiting factors in the formation of 5-S-cysteinyldopa. Since excess amounts of SH-groups are present in all fractions, the finding of larger quantities of 5-S-cysteinyldopa in the non-melanosome fractions than in the large-granule fraction is evidence that larger amounts of dopaquinone are available to react with SH-groups of cysteine or glutathione in the small-granule fraction and in the soluble fraction than in the melanosomes. This would imply significant tyrosinase activity, although the presence of this enzyme outside the melanosomes only leads to production of catechols and not to precipitating polymers.

The estimation of dopa and 5-S-cysteinyldopa in the different compartments of melanin-forming cells will undoubtedly prove most helpful in defining the intra- and extramelanosomal conditions for formation of melanin in melanocytes of different origin.

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