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Tyrosinase Activity in a Highly Pigmented Human Melanoma and in Negro Skin[1] (37877)

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Studies dealing with the biochemical aspects of melanin synthesis have been useful in the characterization of melanin pigmentation in both typical and atypical tissues. In mammalian melanomas, studies of melanogenesis have focused on tumors of nonhuman origin. In a highly pigmented human melanoma available to us, the tyrosinase activity, the tyrosinase isozyme pattern, the effect of prolonged freezing upon tyrosinase activation, and solubilization of the particulate tyrosinase were studied.

Methods and Materials. A highly pigmented metastatic melanotic melanoma and skin from a 38-year-old negro male were removed postmortem. The tyrosinase activity in both typical and atypical tissues was determined as described previously (1–3). The studies of tyrosinase isozymes involved homogenization of the tissue in deionized water, preparation of soluble tyrosinase with high-speed centrifugation (144,000*g*, 40 min, 0–4°) of the homogenate or lipase digestion of the particulate tyrosinase, salting out of the soluble or solubilized proteins with ammonium sulfate, acrylamide disc gel electrophoresis of the precipitated proteins, and incubation of the gel with substrate. The details of these procedures are described elsewhere (3). The tyrosinase activities of the melanoma frozen (−27°) for 1 month and for 2 years were compared in order to observe the effect of such storage on tyrosinase activity.

Results and Discussion. The tyrosinase activity in different skin areas varies but was confined to the particulate fraction (Table I). The enzymic range from 24 to 92 tyrosinase units was similar to that previously found in human skin (4). The higher specific activity of the particulate fraction indicated a higher concentration of the enzyme after fractionation. The tyrosine carboxyl-group incorporation into melanin ranged from 12 to 25%, suggesting copolymerization of melanogenic intermediate(s) into melanin in addition to the classic homopolymerization of 5,6-indolequinone units.

The melanoma tyrosinase activity differed from that of the corresponding part of the skin (Table II). The melanoma tyrosinase activity was extremely high with approximately 20% of the total activity in the soluble fraction. The enzymic activity was similar in the various metastases irrespective of the region of occurrence, revealing the similarity of the melanoma despite differences in skin tyrosinase. The high specific tyrosinase activity of the melanoma also indicated a high tyrosinase content. The tyrosinase carboxyl-group incorporation into melanin by melanoma tyrosinase was less than that normally occurring in the skin.

The tyrosinase isozyme patterns of the human melanoma revealed that three isozymes were present in both the soluble and the solubilized enzyme preparations (Figs. 1 and 2). The fastest fraction contained

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TABLE I. Tyrosinase Activity in Various Skin Areas of a 38-Year-Old Negro Male. Tissue Stored for 1 Month at −27°.

Skin area	Tyrosinase units[a] per mg fresh tissue			Specific activity[c]			Tyrosine incorporation with carboxyl group[d] (%)		
	H[b]	P	S	H	P	S	H	P	S
Right arm	44	46	0	7.8	14.0	0	22.2	21.4	0
Left back	48	50	0	10.6	18.2	0	15.4	16.5	0
Anterior abdomen	39	42	0	10.1	18.2	0	18.8	17.9	0
Anterior chest	24	27	0	5.2	10.2	0	25.0	24.3	0
Right palm	92	95	0	7.2	8.8	0	12.0	12.5	0

[a] One tyrosinase unit is that amount of tyrosinase activity required to convert 1 picomole of L-tyrosine to melanin under the conditions of the described assay during a 16-hr incubation period at 30°.

[b] H, Homogenate; P, particulate fraction; S, soluble fraction.

[c] Specific activity: number of tyrosinase units per microgram of protein nitrogen.

[d] L-Tyrosine with carboxyl groups incorporated into melanin as a percentage of the total L-tyrosine converted into melanin. Uniformly labeled L-tyrosine-^{14}C and L-tyrosine-1-^{14}C were utilized as the substrates.

relatively little tyrosinase activity and was clearly visualized in the gel as a melanin band but was less pronounced as a melanin peak when scanned. The middle band was the dominant form and showed distinctly both in the gel and as a peak when scanned. The slowest melanin band was separate in the gel, but the activity overlapped that of the dominant form when scanned. The positions of these isozymes were similar to the clearly separated bands or peaks in B-16 mouse melanoma (5). The dominant form represented in Fig. 3 was isolated from both the soluble and solubilized enzyme preparations by salting out at 50–70% saturated ammonium sulfate (3). The dominant form and the one to its left (Figs. 1 and 2) may correspond to the T^1 and T^2 melanoma isozymes (6, 7).

Prolonged storage (2 years) of melanoma tissue at −27° increased tyrosinase activity (Table III). The tyrosinase activity in the 2-year frozen sample was 196.4% in one case (abdominal melanoma) and 613.5% in another case (chest melanoma) of the enzymic activity in the homogenate of the respective samples frozen for 1 month. The subcellular distribution of tyrosinase activity in the melanoma revealed that the soluble-fraction activity in the 2-year frozen sample was higher than that of the 1-month frozen sample, suggesting "solubilization" of the particulate tyrosinase. Such "solubilization" is present in the abdominal melanoma in which the particulate-fraction activity was decreased

TABLE II. Tyrosinase Activity of Melanoma Metastases in a 38-Year-Old Negro Male. Tissue Stored for 1 Month at −27°.

Location of metastasis	Tyrosinase units[a] per mg fresh tissue			Specfic activity			Tyrosine incorporation with carboxyl group (%)		
	H	P	S	H	P	S	H	P	S
Right arm	19083	15593	3506	1233.7	1349.6	1189.4	10.2	11.2	10.2
Left back	17603	14210	3413	1295.6	1321.5	1179.2	10.0	10.6	10.0
Anterior abdomen	17192	13865	3334	1251.3	1289.2	1177.5	8.4	9.0	8.6
Anterior chest	19660	15635	4013	1221.8	1290.7	1210.4	8.8	8.6	8.8
Average	18385	14826	3567	1250.6	1312.7	1189.2	9.4	9.9	9.4

[a] See footnotes, Table I.

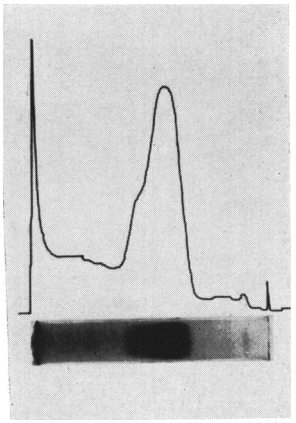

FIG. 1. Acrylamide disc gel electropherogram of the soluble fraction tyrosinase isozymes from a malignant metastatic melanoma. The soluble fraction was obtained after centrifugation of the melanoma homogenate at 144,000*g*, 0–4°, 40 min.

and the soluble-fraction activity increased with prolonged freezing. Both particulate- and soluble-fraction activities were increased during prolonged freezing of chest melanoma, although a comparatively higher percentage of soluble activity resulted. The increased tyrosinase activity after freezing may be produced by membrane and/or cell-organelle rupture with more effective enzyme release as suggested in fish-skin studies (1) or the loss of possible enzymic inhibitors. An inhibitor of integumental tyrosinase in normal primate integument is inactivated by oxidation at low temperatures (0–4°) for 3 weeks (8). Further, this inhibitor occurs in the soluble fraction and prior to oxidation effectively depresses the activity of mushroom tyrosinase. Thus, the very slow loss of enzymic inhibitors under the described conditions, in part, may be responsible for the observed increase in the tyrosinase activity of the human melanoma. Integumental inhibitors of tyrosinase have been found to occur in a variety of vertebrate species from fish to mammal as well as in a number of mammalian melanomas, so that extension of such findings to human material is not unexpected. However, the data (Table III) also indicate that the increase in tyrosinase activity may result from effects other than "solubilization" of the enzyme bound to the particulate fraction and the loss of tyrosinase inhibitor(s), as enzymic activity of the particulate fraction derived from the abdominal melanoma is reduced to approximately one-third of the original activity while the tyrosinase activity derived from the chest melanoma is increased about 4.5-fold. Thus, other as yet undefined factors may also have a role in

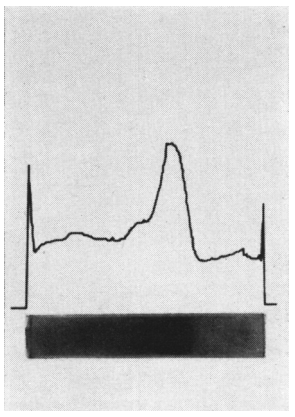

FIG. 2. Acrylamide disc gel electropherogram of tyrosinase isozymes derived from the particulate fraction (144,000*g*, 0–4°, 40 min) after lipase solubilization in a malignant metastatic melanoma. The digest was recentrifuged as above and the released enzyme of the supernatant subjected to electrophoresis.

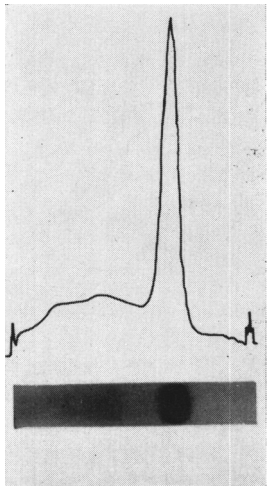

FIG. 3. Dominant form of tyrosinase isozyme as isolated from the soluble and lipase-solubilized fractions of a malignant metastatic melanoma, after centrifugation (144,000*g*, 0–4°, 40 min) and precipitation at 50–70% saturated ammonium sulfate.

TABLE III. Effect of Prolonged (2 Years) Storage at —27° on Tyrosinase Activity and Distribution in Melanomas from a 38-Year-Old Negro Male.

Area of metastasis	Tyrosinase units[a] per mg fresh tissue			Subcellular distribution (%)		Activation[b] (%)
	H	P	S	P	S	H
Anterior abdomen	33,760	9,185	24,600	27.2	72.8	196.4
	(17,192)	(13,865)	(3,334)	(80.7)	(19.4)	
Anterior chest	120,800	69,850	83,200	45.6	54.5	613.5
	(19,660)	(15,635)	(4,013)	(79.6)	(20.4)	

[a] See footnotes, Table I. Values in parentheses are from the appropriate corresponding specimen frozen for 1 month (Table II).

[b] The total tyrosinase activity of 2-year frozen specimens expressed as percent enzymic activity of the corresponding 1-month frozen specimens.

the control of tyrosinase activity in atypical human pigment cells.

Summary. The comparison of the tyrosinase present in the skin of a 38-year-old male Negro with that present in highly pigmented metastatic melanoma in the same individual revealed a number of differences. In the skin, enzymic activity varied with the skin region, showed low specific activity, was confined to the particulate fraction, and showed 12–25% tyrosine carboxyl-group incorporation into melanin. Melanoma tyrosinase activity was similar in the various metastases, showed high specific activity, was present in both the particulate and soluble fractions, and showed only 9.4–9.8% tyrosine carboxyl-group incorporation into melanin. Three tyrosinase isozymes were present in the melanomas. Prolonged storage at −27° of the melanomas increased tyrosinase activity 2–6-fold. Enzymic inhibitors may have a role in the control of tyrosinase activity in melanoma.

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