Melanogenesis in Frog Skin

Recent work has demonstrated that the level of tyrosinase activity is directly correlated with the degree of melanin pigmentation in several goldfish color varieties[1]. The tyrosinase distribution varies within a given color phenotype on anatomic and subcellular levels, and is directly proportional to the degree of integumental melanization. As anuran skin shows pronounced regional color differences and a rather specialized pattern of black spots each surrounded by a light halo of relatively unmelanized integument, it was considered a particularly appropriate system for the study of the control of melanogenesis.

Methods and materials. Six male grass frogs, *Rana pipiens*, (45–53 g), and 1 male and 2 female bullfrogs, *Rana catesbiana*, (260–314 g), were decapitated. The dorsal, lateral and ventral skin areas were removed from 3 *R. pipiens*. The black spots and the associated light areas or halos of the dorsal skin were separated from the remaining 3 animals. The dorsal and ventral skins of *R. catesbiana* were utilized. All skins were immediately frozen at — 27 °C. The procedures and substrates utilized were described previously[2–4]. All assays and assay controls were performed in duplicate. The total number of assays was in excess of 1500. The indicated differences are statistically significant unless otherwise indicated. As the bullfrogs were sexually inactive, sex differences in enzymatic activity were not detectable.

Results. In contrast to vertebrates generally[4] the tyrosinase activity was higher in the ventral skin than in the dorsal skin in both *R. pipiens* and *R. catesbiana* (Table I) although the ventral skin was almost white (relatively melanin poor) while the dorsal skin was comparatively dark (relatively melanin rich). The lateral skin was intermediate in both color and tyrosinase activity. In *R. pipiens*, the tyrosinase levels in descending order of activity were black spots, ventral skin, halo, lateral and dorsal skin. On the other hand, the highest enzymatic activity in the skin fractions was associated with the particulate fraction. The various skin regions, except the halo area, were not appreciably different from one another in this respect; the halo contained a more active soluble fraction. The specific activities of dorsal, lateral and ventral skin homogenates were similar, but were highest in the black spots and lowest in the halo. The distribution of the enzyme was visualized in terms of the particulate fraction/soluble fraction enzyme ratio (Table I). The dorsal skin contained the greatest relative particulate tyrosinase activity with the halo area the least. A more precise measure of enzyme distribution was the particulate fraction/soluble fraction ratio expressed in terms of specific activity (Table I). The dorsal skin was richest in particulate fraction activity with ventral skin poorest.

The tyrosinase activity of *R. catesbiana* generally was lower than in the corresponding regions of *R. pipiens* (Table I). However, *R. catesbiana* skin was higher in specific activity compared to *R. pipiens*. In addition, as the particulate fraction/soluble fraction activities never exceeded unity, the major active enzyme concentrations occurred in the soluble fraction of the skin (Table I).

Table I. Distribution of tyrosinase activity[a] in the skin of the grass frog (*Rana pipiens*) and the bullfrog (*Rana catesbiana*)

Skin region	Tyrosinase units per mg skin			P/S ratio	Specific activity			
	H	P	S		H	P	S	P/S ratio
R. pipiens								
Dorsal	1112[b]	866[b]	250[b]	3.5	47	61	25	2.4
Lateral	1281[b]	869[b]	307[b]	2.8	53	69	33	2.1
Ventral	1510	1099	492	2.2	52	47	66	0.7
Black spots	1999	1459	576	2.5	75	82	54	1.5
Halo	1036[b]	664	407	1.6	33	34	31	1.1
R. catesbiana								
Dorsal	847	396	471	0.8	108	84	155	0.5
Ventral	1114	513	592	0.9	137	94	219	0.4

[a] H, Homogenate; P, particulate fraction; S, soluble fraction. Each value represents the mean of 3 animals. [b] No significant difference.

Table II. Tyrosine carboxyl incorporation (%) into melanin in frog skin[a]

Skin region	*R. pipiens*			*R. catesbiana*		
	H[b]	P	S	H	P	S
Dorsal	37.3	38.4	37.4	21.9	18.5	21.3
Ventral	38.2	39.0	36.9	23.5	24.2	21.8

[a] Expressed as L-tyrosine incorporated without decarboxylation in terms of % of total L-tyrosine converted. [b] See foornote [a], Table I.

The incorporation of tyrosine carboxyl groups into melanin was high in *R. catesbiana* but even higher in *R. pipiens* (Table II). The degree of carboxyl incorporation was similar in all anatomical and subcellular fractions studied.

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Table III. Estimation of D-tyrosine utilization in frog skin homogenates utilizing differently labeled tyrosine-C-14 as substrates[a]

Species and skin area	Tyrosinase activity[b] in cpm/assay				Tyrosinase carboxyl incorporation (%) calculated from	
	ULT-C-14		DLT-2-C-14	LT-1-C-14	ULT-C-14	DLT-2-C-14
	Experiment	Correct[c]				
R. pipiens						
Dorsal	574	617	633	233	37.8	36.8
Ventral	790	845	860	321	38.0	37.3
R. catesbiana						
Dorsal	522	569	567	148	26.0	26.1
Ventral	690	756	746	162	21.4	21.7

[a] ULT-C-14: Uniformly labeled L-tyrosine-C-14, specific activity 0.329 mc/m*M*. DLT-2-C-14: DL-tyrosine-2-C-14, specific activity of L-form 0.329 mc/m*M*; specific activity of D-form, 1.4 mc/m*M*. LT-1-C-14: L-tyrosine-1-C-14, specific activity 0.329 mc/m*M*. [b] Each value was obtained from 1 animal only. [c] Calculated by: $Y = (Z - X/9)9/8$ (from reference [4]); Y = cpm corrected, X = cpm obtained with L-tyrosine-1-C-14, Z = cpm obtained with uniformly labeled L-tyrosine-C-14.

The use of variously labeled tyrosine-C-14 preparations as substrate (Table III) revealed that utilization of D-tyrosine does not occur. The corrected enzymatic activity levels utilizing L-tyrosine approximated that of DL-tyrosine-2-C-14. The lower experimental values obtained with uniformly labeled L-tyrosine-C-14 resulted from decarboxylation.

The enzyme preparations from frog skin were stable (− 4 °C) for at least a month, in contrast to the instability of goldfish enzyme preparations[1,2].

Discussion. Complete correlation of degree of pigmentation with tyrosinase activity as seen in goldfish, is not possible in the anurans studied. The black spots in the dorsal skin of *R. pipiens* have higher tyrosinase activity levels than the adjacent light halo areas, but the relatively heavily pigmented dorsal skin is lower in enzymatic activity than the relatively unpigmented ventral skin in both species. In addition, the distribution of tyrosinase, expressed in terms of particulate/soluble specific activity ratios, can be correlated with the degree of pigmentation in *R. pipiens*, but not in *R. catesbiana*. The possible mechanisms behind these apparent contradictions include: (a) the integumental melanin content may not be correlated with the tyrosinase activity after a given age (size)[5–9]; (b) the apparent degree of melanization may be affected, in part, by the oxidative-reductive state of melanin[8,10]; (c) the dorsal skin is thicker than the ventral skin in older or larger animals so that the tyrosinase activity per unit skin weight or protein nitrogen content is reduced; (d) dilution of in vivo tyrosinase inhibitor(s) in the ventral skin may release tyrosinase activity[11]; (e) a precursor of tyrosinase activity present in the ventral skin may be activated during the course of the in vitro assay period; (f) a portion, at least, of the tyrosinase in the dorsal skin may be inactivated by the deposition of melanin in the granule[12]; and (g) variation in in vivo availability of substrate[13].

In the manometric estimation of tyrosinase distribution in *R. pipiens* skin[11], enzyme levels were not correlated with melanin content. However, it is clear that the methodology involving oxygen consumption of a tissue or organ homogenate may yield data without the requisite degree of precision. Thus, at this time, it is impossible to relate the present findings dealing with *R. pipiens* with those based upon the manometric method.

The incorporation of tyrosine carboxyl groups into melanin is surprisingly high in the anurans studied compared to other vertebrates[4]. In fact, this characteristic feature of melanogenesis in frogs is unique among the vertebrates studied to date. It has been proposed[2,4] that tyrosine-like or dopa-like compounds because of their structural similarities and polarities are independently attracted by the intermediate(s) between dopa and melanin for polymerization and copolymerization. The role of copolymerization in melanogenesis is strongly supported by the high degree of incorporation of substrate carboxyl groups into melanin by the anurans of this study[14].

Zusammenfassung. Es wurden die anatomische und subzellulare Verteilung der Tyrosinaseaktivität im Integument und die Tyrosincarboxylinkorporation in Melanin bei *Rana pipiens* und *Rana catesbiana* festgestellt und gefunden, dass die Tyrosinaseaktivität in der Bauchhaut höher ist als in der Rückenhaut, während die Seitenhaut dazwischenliegende Werte liefert.

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