Activation of Skin Tyrosinase

In the study of melanogenesis in an evolutional series of vertebrates, it was found that the integumental tyrosinase in most vertebrates was stable over a period of time at 0–4 °C[1]. However, during such storage some vertebrate skin preparations showed inactivation of tyrosinase while others showed increased tyrosinase activity (activation). The decreased tyrosinase activity in black goldfish skin enzyme preparations during storage at 0–4 °C has been presented previously[2]. The present report deals with tyrosinase activation in 3 species of primitive vertebrates.

Methods and materials. Three Atlantic hagfish (*Myxine glutinosa*), 5 garpike (*Lepisosteus osseus*), and 3 Australian lungfish (*Neoceratodus fosteri*) were decapitated. The dorsal and ventral skins were removed and frozen at − 27 °C for several weeks. Since garpike and Australian lungfish have thick scales, the skin homogenate preparation was altered from previously described methods[2,3]. Pre-weighed skin was cut while frozen into small strips in the grooves between the scales (garpike) or into small pieces (Australian lungfish). The small strips and pieces of skin (including scales) were homogenized in an ice-chilled micro-Waring blendor for 10 min with 0.1 *M* phosphate buffer, pH 6.8 (1 g skin to 10 ml buffer). The homogenates then were finely reground in a chilled tissue grinder. Since the protein content of the skin in these 2 fishes was unusually low and the tyrosinase activity in the fresh skin homogenate was very small, the amounts of enzyme preparation were increased to 20 mg skin/assay. In the case of the hagfish, weighed frozen skin was sliced on dry ice and homogenized in an ice-chilled micro-Waring blendor for 6 min, with the above buffer (1 g skin to 40 ml buffer), and reground in a chilled tissue grinder. The preparations were immediately assayed (Table I), stored at 0–4 °C, and then assayed periodically (Tables II, III and IV). Determination of tyrosinase activity (by conversion of ^{14}C-labeled L-tyrosine to melanin), specific activity, tyrosine carboxyl incorporation, and protein N determination in the homogenates and fractions has otherwise been described[3,4]. The enzymatic reactions were dopa dependent and inhibited by Na diethyldithiocarbamate (6 m*M*). Approximately 1000 assays were performed. Wherever possible the data are presented as the mean ± standard error of the mean.

The garpike enzyme preparation was used to test a possible mechanism of tyrosinase activation. Portions of fresh enzyme preparations were stored under N_2 at 0–4 °C after being flushed with N_2 for 5 min. Samples were removed and assayed at 3 and 7 days, with the N_2 atmosphere replaced at 3 days. After the 7-day sample was removed, the enzyme preparations were stored under air at 0–4 °C until the tenth day and then re-assayed.

Results and discussion. The tyrosinase activities in the fresh homogenates of all 3 species were low (Table I). The enzymatic activity in the ventral skin was about $^1/_3$ of that in the dorsal skin in garpike and lungfish. No tyrosinase activity was present in the fresh ventral skin homogenates of the hagfish. The tyrosinase activity in garpike and hagfish was present only in the particulate fraction, whereas the enzymatic activity in lungfish was present in both subcellular fractions (soluble and particulate). The lungfish contained only slightly less than 50% of the entire enzymatic activity in the soluble fraction. The tyrosine incorporation into melanin without decarboxylation was approximately 18% in garpike skin and 17% in Atlantic hagfish skin but 30% in lungfish skin. The low specific activity in the homogenates indicates a low active skin tyrosinase content. However, high specific activity of the particulate fraction in Atlantic hagfish indicates a high active tyrosinase content.

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Table I. Skin tyrosinase activity in Atlantic hagfish (*Myxine glutinosa*), long nose garpike (*Lepisosteus osseus*), and Australian lungfish (*Neoceratodus fosteri*)

Species	Tyrosinase activity						Tyrosine carboxyl incorporation[c]		
	T.U.[a]/20 mg skin			Specific activity[b]					
	H[d]	P	S	H	P	S	H	P	S
Atlantic hagfish									
Dorsal	196 ± 100	196 ± 89	0	12 ± 1	27 ± 1	0	17.2 ± 0.4	17.2 ± 0.4	0
Ventral	0	0	0	0	0	0	0	0	0
Garpike									
Dorsal	148 ± 21	147 ± 12	0	2 ± 1	3 ± 0	0	17.9 ± 0.6	17.5 ± 0.3	0
Ventral	48 ± 14	48 ± 10	0	1 ± 0	1 ± 0	0	17.8 ± 0.6	17.5 ± 0.3	0
Australian lungfish									
Dorsal	104 ± 11	54 ± 3	48 ± 1	12 ± 0	9 ± 0	15 ± 1	31.2 ± 0.4	31.3 ± 0.5	28.6 ± 0.6
Ventral	32 ± 2	19 ± 1	17 ± 1	4 ± 0	4 ± 0	6 ± 0	31.6 ± 1.0	27.3 ± 1.0	30.0 ± 1.0

[a] 1 T.U. is defined as the amount of tyrosinase activity required to convert 1 picomole of L-tyrosine to melanin under the conditions of the described assay during a 16 h incubation period at 30 °C. Specific activity of C^{14} labeled L-tyrosine, 0.329 mc/mmole; total of 40 μg of L-tyrosine/ml of incubation mixture. [b] Specific activity is defined as the number of T.U./μg protein nitrogen. [c] Expressed as L-tyrosine incorporated without decarboxylation in % of total L-tyrosine converted. [d] H, skin homogenate; P, particulate fraction; S, soluble fraction (fractionation of homogenate at 0–4 °C, 144,000 *g*, 40 min).

Table II. Alteration in tyrosinase activity[a] (T.U.) at 0–4 °C as a function of time in garpike, *Lepisosteus osseus*, skin homogenates

Enzyme preparation	Time (days)						
	0[b]	3	7	10	14	17	21
Animal 1:							
Dorsal	124	548	8028	23712	10472	9432	–
Ventral	24	880	8128	25192	7952	7448	1232
Dorsal and ventral[c]	92	380	1808	12700	16712	8164	6248
Dorsal (N_2)[d]		130	128	7061			
Ventral (N_2)[d]		26	27	6934			
Dorsal and ventral (N_2)[d]		90	95	1524			
Animal 2:							
Dorsal	176	–	10188	–	13520	3040	2328
Ventral	72	–	1500	–	17492	–	–
Dorsal and ventral[d]	132	–	9912	–	18020	–	–

[a] T.U./20 mg skin. [b] Newly-prepared. [c] The enzyme preparations from the dorsal and ventral skins were combined so that the mixed enzyme preparation contained the same proportion of dorsal and ventral homogenate as in the whole skin. Stored under N_2 for 7 days; stored under air from the seventh to tenth day.

Table III. Alteration in tyrosinase activity[a] (T.U.) at 0–4 °C as a function of time in Australian lungfish, *Neoceratodus fosteri*, skin homogenates

Skin part of one animal	Degree of skin color	Time (days)					
		0[b]	7	11	14	18	21
Dorsal 1	Darkest	83	396	5357	10282	8713	5245
Dorsal 2	Darker	20	73	1782	5923	7160	5154
Dorsal 3	Dark	27	71	593	1006	3398	1596
Ventral 1	White	12	80	323	2334	11574	8529
Ventral 2	White	7	80	294	741	11033	10494
Ventral 3	White	3	17	61	277	1190	255

[a] T.U./20 mg skin. [b] Newly-prepared.

Table IV. Alteration in tyrosinase activity[a] (T.U.) at 0–4 °C as a function of time in Atlantic hagfish *Myxine glutinosa* skin homogenates

Enzyme preparation	Time (days)				
	0[b]	6	10	15	20
Dorsal	102	484	3184	1292	264
Ventral	0	3632	6720	5044	1008

[a] T.U./20 mg skin. [b] Newly prepared.

During the course of the 3-week storage period, tyrosinase activity was increased radically in all 3 species but enzymatic activity eventually decreased (Tables II, III and IV). In garpike skin (Table II) the maximal activity compared with the initially assayed activity was found to increase 191-, 1050- and 182-fold in dorsal, ventral and dorsal-ventral skin homogenates respectively, in one animal. In a second animal, the increases in the above areas were 77-, 243- and 137-fold, respectively. The greatest increase occurred in the ventral skin. In lungfish (Table III) the comparatively darkest, darker and dark parts of the dorsal skin possessed a maximal activity of 105-, 358- and 126-fold the activity of the corresponding fresh enzyme preparations. The 3 white ventral skin portions also increased to maxima of 965-, 1576- and 379-fold the activity in the corresponding fresh skin enzyme preparations. As in the garpike, the increase in activity was the greatest in the ventral part. In hagfish (Table IV), the maximal tyrosinase activity increased to 31-fold the original activity. Although fresh hagfish ventral skin homogenate showed no tyrosinase activity, the enzymatic activity appearing during the course of storage achieved a maximum which was 2-fold greater than that occurring in the dorsal skin at 10 days.

The tyrosinase activity increase during storage may result from the destruction of inhibitor(s) or the production of activator(s) during the course of the gradual oxidation at low temperature. The garpike enzyme preparations stored under N_2 for 7 days showed no change in tyrosinase activity during this interval. However, activity was greatly enhanced 3 days later (stored under air) (Table II). The greater increase in activity in the ventral skin when compared to the dorsal skin suggests a higher concentration of tyrosinase inhibitor(s) in the former than in the latter. It is not surprising, therefore, that the in vivo color of the ventral skin in this species is almost white. As oxidation of some metabolite(s) in the homogenate may take place during storage, their elimination may permit the expression of tyrosinase activity. In addition, the possibility of enzyme liberation or the

activation of pro-tyrosinase during storage at low temperature is not excluded. However, because of the low protein nitrogen content of the skin enzyme preparations derived from the garpike and the Australian lungfish, the loss of inhibitor(s) and/or production of activator(s) appears to be a more reasonable explanation. The ultimate decrease in tyrosinase activity seen after a lengthy period of storage, may be the result of a gradual disappearance of activator(s) but, more probably, the inactivation of tyrosinase itself. In addition, the described findings indicate that the 3 species utilized in the present study, although difficult to procure, may prove to be of considerable utility in the study of natural mechanisms controlling melanogenesis[5].

Résumé. L'activité de tyrosinase intégumentale est augmenté par un séjour de 10–18 jours à 0–4 °C sous oxygène ou en plein air mais pas nitrogène. Les 3 espèces vertébrés qui montrent cette réaction sont *Myxine glutinosa, Lepisosteus osseus* et *Neoceratodus fosteri.*

Y. M. CHEN and W. CHAVIN

Department of Biology[6], Wayne State University, Detroit (Michigan 48202, USA),
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[6] Contribution No. 194, Department of Biology.

Liver Glycogen Concentration in Patients with Chronic Uremia

An impaired galactose tolerance test, decreased hyperglycemic response to adrenaline and glucagon in uremic patients, as well as decreased liver glycogen concentration in experimental uremia led COHEN[1] to the assumption, that impaired liver glycogenesis is the cause of glucose intolerance in uremia. There are some additional data supporting the view that the liver plays a decisive role in this failure[2,3]. On the other hand, the decreased hyperglycemic response on glucagon administration was not proved by other authors[3,4]. Therefore the inhibiton of glycolysis was proposed as the cause of abnormal carbohydrate metabolism in the liver[3]. It was presumed worthwhile to determine liver glycogen concentration in uremic individuals to solve these contradictory findings.

Methods. Liver glycogen concentration in 9 control (i.e. patients with diseases not involving the liver or the kidney) and in 10 uremic patients was determined. The pertinent clinical data of the latter group are summarized in Table I. Glycogen concentration was determined in patients on ad libitum intake of a minimum of 150 g carbohydrate on the 3 days preceding the test. On the preceding evening the uremics as well as the controls were given 50 g glucose orally. Percutaneous liver biopsy was taken in a standard manner next morning between 08.00 and 08.30. Liver specimen was divided into 2 parts: The one was examined

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Table I. Clinical data at the time of percutaneous liver biopsy

Patient No.	Primary cause of uremia	Blood urea mg %	Standard bicarbonate mE/l	Blood erythrocytes 10^6/mm^3	Glucose tolerance test[a]
Individuals with normal glucose tolerance test					
1	Glomerulonephritis	63	18	4.24	−15
2		155	19	3.80	+ 8
3	Pyelonephritis	66	17	4.38	+ 8
4		90	17	4.08	+18
5	Amyloidosis	108	13	2.66	+10
Individuals with abnormal glucose tolerance test					
6	Glomerulonephritis	58	22	4.36	+46
7		264	20	3.02	+39
8		177	10	2.38	+58
9	Pyelonephritis	210	22	2.32	+45
10		126	17	2.40	+72

[a] Values were calculated as the difference between glycemia at 120 min and fasting glycemia. Normal values do not exceed + 20 mg %[5].