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On the Presence of Tyrosinase in the Byssus or Mucus Gland of the Bivalves

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It is well known that the cuttlefish and the other cephalopod mollusks have melanins as a suspension of black granules in the specialized anal gland or ink sac. Also, in shrimp-melanogenesis, tyrosinase¹⁾ or phenol oxidase²⁾ is involved and this enzyme has been found in shrimp and crab³⁾. However, the presence of tyrosinase in the bivalves had been unknown in any full detail. One⁴⁾ of the authors has already demonstrated that melanin-like pigments of pearl oysters are formed in the reaction mixture to which copper ions are added. Recently, Tsujii⁵⁾ has also studied the activity of the tyrosinase in the mantles of the fresh water shell-fish and pearl oysters.

In a course of studies on the formation of blackened baroque pearls, it was found that a concentration of tyrosinase was relatively high in the byssus gland of pearl oysters. Therefore, similar studies were conducted as we considered that such a fact might be found in other species of the bivalves.

The present paper describes the presence of tyrosinase in the byssus or mucus gland of some bivalves, and the results of some experiments on these enzymes.

Materials and Methods

1. Materials. Pearl oysters hanging-cultured in sea water were generally used as samples. The other species of the bivalves as fresh as possible were purchased at market. The crude enzyme solution was prepared as follows: the glands which had been separated from other tissues were ground with sand, to which were added ten times as much saline as the glands. The preparation was centrifuged for 15 minutes at 3,000 r.p.m. and the supernatant solution was used as the crude enzyme solution.

2. Tyrosinase determination. In the case of a qualitative study or a low activity of tyrosinase, the colorimetric method⁶⁾ was employed. Manometric method was used to measure only a high activity of tyrosinase.

a) **Colorimetric method.** One ml of the crude enzyme solution put into a test tube containing 3.5 ml of 0.066 M phosphate buffer solution pH 7.0 and 0.5 ml of solution with 1 mg substrate. A final volume amounted to 6.0 ml by adding a distilled water, and the reaction mixtures were incubated at 25°C. After the given

period, the rate of discoloration was observed qualitatively or determined colorimetrically. In the latter case, 1 ml of 1 N sodium hydroxyde solution was added to the reaction mixture and the reading was done by using filter of 480 m μ .

b) **Manometric method.** This method is essentially identical with that used by Horowitz and Shen.⁶⁾

Results

The byssus or mucus gland which contained a tyrosinase showed a different form from each other in the species. The byssus glands of *Pinctada martensii* and *Anadara subcrenata* were an isolate, well-defined form morphologically and a mucus gland of *Venerupis philippinarum* was readily distinguishable from the foot-tissue as shown in Fig. 1, whereas for *Meretrix lusoria* it was difficult to separate the gland from surrounding tissues.

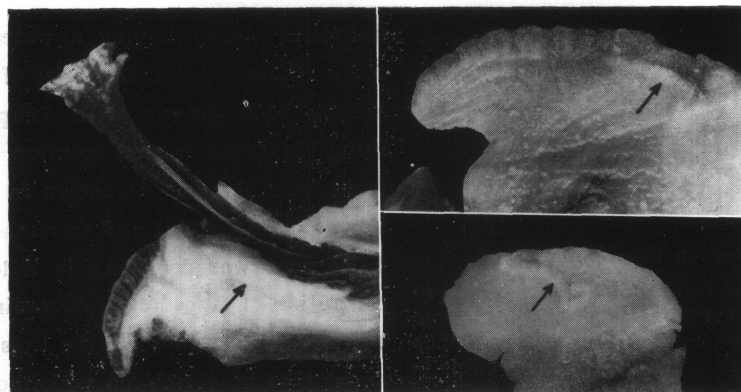


Fig. 1. Three sections of foot of the bivalves. Showing the situation of byssus gland of pearl oyster (left), mucus gland of *Anadara subcrenata* (upper) and *Venerupis philippinarum* (lower).

The formation of melanins by the crude enzyme solution is shown in Table 1. In a well-defined gland of *Pinctada martensii* or *Anadara subcrenata* a concentration of tyrosinase was very high, but in the other three species the formations of melanins did not show so remarkable as in the two species mentioned above.

Table 1. The formation of black pigments by crude enzyme solution prepared from the byssus or mucus gland of the bivalves.

Bivalves	Rate of Discoloration		
	24	48	72hr.
<i>Pinctada martensii</i>	### black *		
<i>Anadara subcrenata</i>	### black		
<i>Venerupis philippinarum</i>	+	+ violet	
<i>Meretrix lusoria</i>	+	##	### black
<i>Corbicula sp.</i>	+ violet		

* Shows the color of pigments by qualitative test.

shows black discoloration, ## intermediate and + positive.

The rates of the substrate-oxidation by tyrosinase are shown in Fig. 2. The activity of tyrosinase of *Pinctada martensii* was high at nearly pH 7.7 in the oxidation of tyrosine as a substrate in phosphate buffer solution, and in pH 8.2 in veronal buffer solution. Then, the foregoing experiments were carried out in the condition of pH 8.0. The preparation of crude enzyme from the byssus glands of pearl oysters catalyzed the oxidation of L-tyrosine, tyramine and dopa, resulting

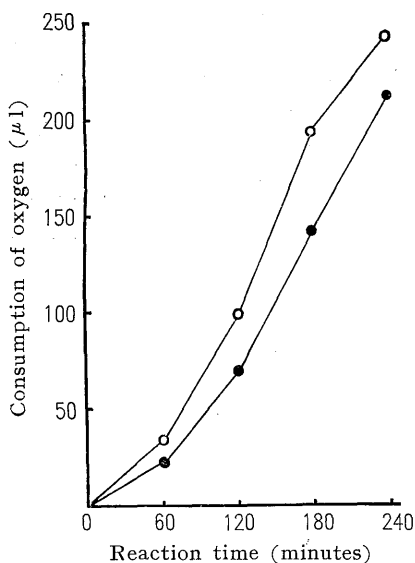


Fig. 2. The rates of oxidation of L-tyrosine or tyramine by the crude enzyme solution prepared from byssus glands of pearl oysters. The solid circle is tyramine-oxidation, the open circle L-tyrosine-oxidation.

in the formation of black pigments. But the preparation of *Andara subcrenata* showed a low activity towards the oxidation of tyramine.

In culturing pearl oysters, there are two types appearing in the condition of their feet. The one is a healthy group having a black foot, and the other an unhealthy group having a watery faded foot. Therefore, the activities of tyrosinase in both groups were compared by using the crude enzyme solution prepared from their glands. As shown in Table 2, the preparation obtained from the watery faded foot was considerably a low concentration of tyrosinase. Accordingly, the activity of tyrosinase in the byssus gland appears to be correlated with the healthiness of pearl oysters.

Table 2. A comparison of the oxidation of tyrosine between two types of feet of pearl oysters.

Type of foot	Consumption of oxygen (ml)		
	1	2	3hr.
Normal foot	48	87	174
Watery faded foot	10	20	31

Detail in text.

Next, the fact that the formation of black pigments was prolonged by an addition of the excessive amount of glycine to the substrate must be noted. This phenomenon was observed to some extent differently in the crude enzyme solution of the other test bivalves.

Summary

It was found that a concentration of tyrosinase was high in the byssus or mucus gland located in the foot tissue of bivalves. Especially, the byssus gland of pearl oysters contained a high concentration of tyrosinase.

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References

- 1) KAKIMOTO, D., and A. KANAZAWA. A study on the black discoloration of lobster II. Relation between tyrosinase and black discoloration. *Bull. J. Soc. Sic. Fish*, **22**, 476—479 (1956).
- 2) BAILEY, M. E., E. A. FIEGER, and A. F. NOVAK. Physico-chemical properties of the enzymes involved in shrimp melanogenesis. *Food Res.*, **25**, 557—564 (1960).
- 3) ————— : Phenoloxidase in shrimp and crab. *Ibid.* **25**, 565—572 (1960).
- 4) TANIGUTI, T. On the formation of melanin-like pigment by the extract solution of the internal organs of *Pinctada martensii*. This Bull., No. 9, 91—95 (1960).
- 5) TSUJII, T. Studies on the mechanism of shell-and pearl-formation. VIII. On the tyrosinase in the mantle. *Jour. Fac. Fish. Prefectural Univ Mie*, **5**, 378—383 (1963).
- 6) HAROWITZ, N. H., and SHEN, S. C. Neurospora tyrosinase. *J. Biol. Chem.*, **197**, 513—520 (1952).