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DI-PHENOLOXIDASE ACTIVITY IN THE HEMOLYMPH AND CUTICLE OF CALLIPHORA VICINA (R&D) LARVAE AND PREPUPAE

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Abstract. The development of diphenoloxidase activity in hemolymph and cuticle of *Calliphora vicina* (R & D) separately and in combination is studied in order to find the extent and role of cuticular activator.

Di-phenoloxidase (E. C. 1.10.3.1: *o*-diphenol: oxygen, oxido-reductase) is present in *Calliphora vicina* (*C. erythrocephala*) as an inactive precursor, the prophenoloxidase.[1,2] It can be activated by an activator of proteinic nature, found in the cuticle of 3rd instar larva.[3,4] Similar phenomenon has been reported for other insects also.[5–10] However, recently spontaneous development of phenoloxidase activity, without the addition of cuticular activator has been reported in silkmoth and fleshflies.[9–11]

We also observed this spontaneous development of activity in *C. vicina* in the late 3rd instar larva and prepupa. Therefore, we studied the development of diphenoloxidase activity in hemolymph and cuticle of *C. vicina*, separately and in combination, to find the extent and role of cuticular activator, in the development of diphenoloxidase activity.

Materials and Methods

Calliphora vicina larvae bred in summer season, at a controlled temperature of 22-24°, were used during experiments. The hemolymph and the cuticular extracts from the same larvae (4-8 day-old) and prepupae (on 9th day 3 hr after contraction) were prepared according to standard method.[4] We were not able to collect hemolymph in sufficient quantity from 1-3 day-old larvae. Ten grams larvae or prepupae were used in each experiment for collecting the hemolymph and the total volume was made up to 5 ml by adding 0.05M phosphate buffer, pH 6.5. Cuticles (with epidermis) of the same larvae were used for the preparation of cuticular extract, according to the standard method.[4] These partially purified preparations of hemolymph and cuticles were used for the estimation of diphenoloxidase activity but the method of estimation was slightly modified. In the standard method a preparation of cuticular extract is always added as an activator. This step was avoided to find the actual diphenoloxidase activity in hemolymph and cuticle, separately. A simultaneous comparison, using standard procedure, was also made by adding the activator preparation in another set.

The *o*-diphenoloxidase activity was measured by using 4 ml 2.4-dihydroxyphenylalanine (dopa ; 0.5 mg/ml of 0.067M phosphate buffer, pH 6.5) as substrate and 200 ml of hemolymph or cuticular preparation or both (100 ml hemolymph + 100 ml cuticular extract), according to the nature of the experiment. The colour development based on the rate of dopa oxidation, was measured in terms of O.D (absorbance) change, by using mercury filter of 492 millimicron and 20 mm quartz cells in Eppendorf spectrophotometer, at 25°. The change in absorbance was measured for 10 min by an automatic recorder and readings were converted into arbitrary dopa units (0.1 = 10 dopa units). Estimations were done at different ages in hemolymph alone, cuticle alone and the combination of both. Spontaneous activation was studied in hemolymph immediately after collection and without addition of buffer.

Results and Discussion

As evident from Table 1, the diphenoloxidase activity was very low from 4th to 7th day or rather early 8th day, in the hemolymph alone whereas in the cuticular extract alone, it was low till 6th day only. A high and significant increase was found on late 8th and 9th day in the case of hemolymph. However, in the cuticular extract it was high on 7th and late 8th day, but very low on 9th day. There was slight decrease on early 8th day which was also pointed out by Shaya and Sekeris.[12] In the case of combinations also the activity was low on 4-6 day, similar to the separate constituents. However, the combination of the two on late 8th day showed significant increase in activity as compared with the two constituents. This indicates that there are certain proteinaceous[3,4] and perhaps some non-proteinaceous constituents also, in the cuticular extract which act as a catalyst for enhancing the rate of dopa oxidation.

TABLE 1. DIPHENOLOXIDASE ACTIVITY IN TERMS OF DOPA UNITS* IN EXTRACTS AND THEIR COMBINATION.

Number of days	Diphenoloxidase activity in Hemolymph	Cuticle	Combination
4	1.5 ± 0.406	1.0 ± 0.210	1.8 ± 0.763
5	2.2 ± 0.344	3.5 ± 0.918	3.9 ± 1.014
6	2.5 ± 0.715	7.8 ± 1.054	6.2 ± 0.763
7	3.4 ± 0.611	28.5 ± 1.806	18.5 ± 0.918
8 Early	12.0 ± 1.810	18.3 ± 1.050	14.4 ± 0.632
8 Late	87.0 ± 2.405	38.4 ± 0.712	122.2 ± 1.730
9 Prepupae	147.0 ± 3.200	6.8 ± 0.725	155.8 ± 2.108

TABLE 2. DIPHENOLOXIDASE ACTIVITY IN TERMS OF DOPA UNITS* IN DIFFERENT COMBINATIONS.

Cuticle Number of days	Hemolymph Number of days 5	6	7	8	9
7	15.8 ± 0.88	14.0 ± 1.07	20.3 ± 0.28	107.4 ± 3.41	136.0 ± 2.74
8 Late	18.6 ± 1.50	20.3 ± 1.57	59.3 ± 3.24	135.6 ± 3.53	160.5 ± 2.67

*A_{492} (in 10 min), 0.100 =10 Dopa arbitrary units.

In view of the fact that high activity of the enzyme is on 7th and late 8th day in the cuticular extract, combination of these two extracts with the hemolymph of different days, was tried to investigate the presence of an inhibiting factor, present in the hemolymph, as pointed out by Thomson and Sin.[11] Table 2 indicates that the activity sufficiently increased on 8th and 9th day while up to 7th day it was quite low. In these experiments same cuticular extract was used with the hemolymph of 5-9 days larvae. If we study the Table 1, it is evident that the combination has lesser activity than the sum of two components or sometimes less than one component till early 8th day which indicates the presence of some inhibiting factor. Moreover, if we compare the activity of 7th day hemolymph + 7th day cuticle (Table 2,20.3) while only cuticle has 28.5 hemolymph 3.4 (Table 1), with 7th day hemolymph + 8th day cuticle combination (Table 2, 59.3), one can easily conclude that there is something in the cuticular extract of 8th day which enhances the enzyme activity, perhaps cuticular activator. Either it accelerates the oxidative reaction or digests the inhibitor present in the hemolymph till 7th day. The activity in the 7th day hemolymph is 3.4 while in the 8th day cuticle it is 38.4, but the combination has more activity than the sum of the two, indicating that there is a cuticular activator which might have neutralised or digested the inhibitor present in the 7th day hemo-

TABLE 3. DIPHENOLOXIDASE ACTIVITY IN THE HEMOLYMPH AT DIFFERENT AGES DEVELOPED SPONTANEOUSLY, AFTER STANDING FOR VARIOUS INTERVALS.

Number of days	Time of standing at room temperature 10 min	30 min	60 min	180 min	24 hr
4	—	—	—	—	—
5	—	—	—	—	—
6	—	—	—	—	?
7	—	—	—	—	+
8 Early	—	—	—	?	+
8 Late	+	+	+	++	+++
9 Prepupae	+	+	++	++	+++

—, nil; ?, slight; + light colour; ++ moderate colour; +++ dark colour.

lymph. The higher activity in the late 8th day and specially on 9th day hemolymph indicate that the cuticular activator has been poured into hemolymph because the activity in the cuticle is very low (Table 1). Thus it seems probable that there is an activator in the cuticle and an inhibitor in the hemolymph and the balance of the two is maintained according to the physiological needs of the insect. This is possible that the activator is produced in the cuticular cells and then secreted into the hemolymph where, it digests the inhibitor found in hemolymph, not required at that stage. This hypothesis is further strengthened by the fact that 97% activity is in the hemolymph alone on 9th day.

The spontaneous development of activity of diphenoloxidase has been reported by other workers,[11,13] at various ages. In the present case spontaneous development of activity was found only on 8th and 9th day, when the cuticular activator probably has already been secreted into the hemolymph. Therefore, we think that this spontaneous development is due to the cuticular activator. It is possible that in other insects (species) or even strains, it is secreted earlier, showing spontaneous activation at early age. It may be possible that in some species such an activator is present in the hemolymph, but not in the case of *Calliphora vicina*, we used (Table 3).

Zusammenfassung. Die Diphenoloxidase-Aktivitat wurde jeweils in der Cuticula und in der Haemolymphe von *C. vicina* untersucht, die wirksame Aktivitat in den beiden Goweben verschiedenen alter Tiere nachzuweisen. Gemische beider Extrakte wurden auch getested, in Himblick auf die Auslosung der Spontanaktivitat. Ein Hemmstoff-Aktivator-Gleichgewicht wird interpretiert.

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