THE ACTIVITY CHANGE OF ACTOMYOSIN-ADENOSINETRIPHOSPHATASE DURING INSECT METAMORPHOSIS

by

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Many insects undergo a remarkable alteration during life-span with respect to muscular activity. For example, the housefly flies very quickly, but its larva can only crawl about and its pupa possesses no motility. It is an interesting problem to pursue these apparent changes from the viewpoint of muscular biochemistry. It is well known that the adenosine triphosphate (ATP)-actomyosin-adenosinetriphosphatase (ATPase) system is closely related to muscular contraction in the rabbit muscle[1]. Insect actomyosin-ATPase is reported to have similar enzymic properties to the rabbit one, although there are some differences such as in pH optima and heat-stability[2,3].

In the present study, actomyosin was extracted from the total brei of the housefly, *Musca domestica*, in the different stages of metamorphosis, and the ATP-dephosphorylating activity was assayed in the enzyme-limiting system. In each case, ten individuals were homogenized with 3 ml cold solution containing 0.5 *M* KCl and 0.03 *M* $NaHCO_3$. The suspension was placed in the ice-box for two hours with occasional agitation, and then centrifuged. Once-washed actomyosin was dissolved in 0.5 *M* KCl and used as enzyme solution.

The reaction mixture consisted of 1 ml ATP, prepared from rabbit (150 μg labile P, K-salt), 1 ml 0.15 *M* histidine buffer, 0.1 ml 0.1 *M* $CaCl_2$, 0.1 ml 3 *M* KCl, three different concentrations of the enzyme solution and H_2O to a volume of 3.0 ml. After incubation for 5 minutes at pH 6.0 at 37° C, the mixture was deproteinized and the supernatant assayed for inorganic phosphate by the LOHMANN-JENDRASSIK method[4], using an electrophotometer. The protein content was determined by a micro-Kjeldahl procedure.

The results are summarized in Fig. 1. The actomyosin content changed in accord with the so called "U-shaped" curve during metamorphosis of the housefly. It decreased at pupation and increased again at emergence of the adult. The ATP content is reported to undergo a similar change during metamorphosis of the blowfly[5]. More striking is the activity change of the actomyosin-ATPase. The decrease of the ATPase activity parallelled the disappearance of motility of the housefly larva. Actomyosin from the immotile pupa possessed a lower ATPase activity (Q_p = 300–700). The activity rose suddenly over ten times, in a few hours, when the adult began to emerge from the puparium. The flying adult had a powerful actomyosin-ATPase activity (Q_p = 5000).

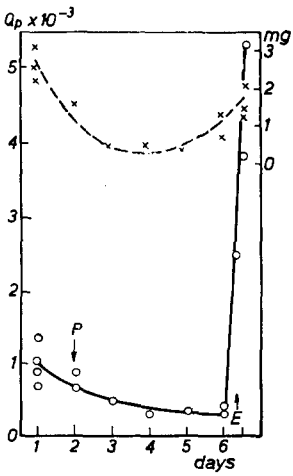

Fig. 1. Actomyosin-ATPase activity during metamorphosis of the housefly. Ordinate: Left, ATPase activity, expressed as Q_p, right, actomyosin content, given as mg protein per 10 individuals. Abscissa: Days of culture at 30° C. *P* means the time of pupation and *E* of emergence.

This tendency that ATP-dephosphorylating activity corresponds to muscular function during insect metamorphosis was first observed in the total brei of the wasp, *Vespula lewisii*[6]. The same was found at hatching of the teleost, *Orysias latipes*[7]. In the wasp thorax the dephosphorylation of ATP was due partly to the actomyosin and also to the water-extractable magnesium-activated ATPase[8]. Insect actomyosin thread rapidly shrinks on treatment with ATP[8]. Viscosity drop[2] or superprecipitation[8] with ATP was also observed. The parallelism between actomyosin ATPase activity (and also its content) and muscular function during insect metamorphosis may support the current ATP-actomyosin-ATPase theory of muscular contraction from the viewpoint of comparative biochemistry.

Here I am grateful to Dr. J. ISHIDA under whose guidance and supervision this work was carried out, and also to Dr. D. GILMOUR of Canberra for his helpful advice.

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Received March 23rd, 1954

EIN NEUES PHOSPHAT-ÜBERTRAGENDES FERMENT AUS HEFE

von

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In einer früheren Mitteilung aus dem Laboratorium der Verfasser[1] wurde postuliert, dass in der Hefe ein Ferment existiert, welches ein Gleichgewicht zwischen dem Adenylsäure-System — Adenosintriphosphat (ATP) und Adenosindiphosphat (ADP) — und dem polymeren Metaphosphat der Hefe[2] einstellt, das folgendermassen zu formulieren ist:

$$ATP + (NaPO_3)_n \rightleftharpoons ADP + (NaPO_3)_{n+1}$$

Es ist uns nunmehr mit Hilfe von radioaktiv markiertem Metaphosphat gelungen, die Existenz eines Fermentes zu beweisen, das die postulierte Reaktion zumindest von rechts nach links katalysiert, d.h. das Phosphatreste von Metaphosphat auf ADP überträgt. In einer Versuchsmischung, die ^{32}P-Metaphosphat, ADP und das Fermentpräparat enthielt, erfolgte in 30 Min. ein sehr deutlich messbarer Übergang von radioaktivem Phosphat vom Metaphosphat auf die Nucleotidfraktion. Diese wurde durch Adsorption an Norit vom Reaktionsgemisch abgetrennt und mit Pyridin eluiert. In Blindversuchen (analoge Versuchsanordnungen ohne Ferment, mit gekochtem Ferment, ohne ADP) wurde kein derartiger Übergang beobachtet.

Das pH-Optimum der Phosphatübertragung liegt bei 6.7; Adenosin-5'-phosphat (AMP) kann nicht als Akzeptor fungieren. Die Aktivität des Enzyms wird durch 0.01 *M* Fluorid komplett gehemmt.

Das Ferment konnte bisher noch nicht in Lösung gebracht werden. Bei Behandlung der Hefe mit flüssiger Luft und folgendem Zentrifugieren verbleibt es in den Zellresten. Aus technischen Gründen — Mangel an radioaktivem ATP — konnten wir bisher noch nicht überprüfen, ob die geschilderte Wirkung reversibel ist.

Seinen zurzeit bekannten Spezifitätseigenschaften zufolge wäre das Enzym nach der von einem von uns vorgeschlagenen Nomenklatur[3] als Metaphosphat —→ ADP-transphosphatase zu bezeichnen; der traditionellen Nomenklatur entspräche der Name Metaphosphat-kinase.

Eine ausführliche Beschreibung unserer Versuche wird an anderer Stelle gegeben werden.

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Eingegangen den 5. April 1954