AMP-ASE ACTIVITY IN THE WING DISK AND MESOTHERACIC LEG DISK OF SOME DIPTERAN LARVAE

by

G. D. MAJOOR

(Zoological Laboratory, University of Leiden, The Netherlands)

INTRODUCTION

In 1970 Sprey described the localization of 5'-nucleotidase in some of the imaginal disks from larvae of Calliphora erythrocephala. The enzyme could be demonstrated in the imaginal disks in a characteristic pattern. In the epithelium, the enzyme was localized mainly in two areas at the base of the future appendages.

In analogy to the contracting effect of ATP*-splitting at the periphery in cells of several types (Hoffman-Berling, 1954; Jones, 1966; Chambers et al., 1967), Sprey (1970) proposed a role for 5'-nucleotidase in the contraction of the cell membrane. This contraction, in co-operation with an intracellular "skeleton" of microtubules, should make the cells more rigid in the areas with enzyme activity. These positive areas might then be interpreted as the "skeleton" of the disks, supporting the prospective appendages.

An approach for testing the proposed hypothesis of 5'-nucleotidase activity is a comparison of the localization of the enzyme in the imaginal disks of some other dipteran species. In this paper, the results of comparative investigations on the wing disks and mesothoracic leg disks of nine other species are described.

MATERIALS AND TECHNIQUES

The diptera used for the investigations all belong to the suborder of the Cyclorrhapha. Larvae of Calliphora erythrocephala and Phormia regina were reared on horse meat at 22°C. Larvae of Musca domestica at 25°C on an artificial medium containing yeast, milkpowder, agar and aqua dest. Larvae of the Drosophilidae on a standard Drosophila medium also at 25°C. Most of the diptera were obtained from our laboratory stocks. Drosophila araias and Drosophila guaramunut were kindly provided by

* Abbreviations used: AMP = Adenosine-5'-monophosphate; dAMP = Deoxyadenosine-5'-monophosphate; ATP = Adenosine-5'-triphosphate; β-Glyc.-P. = β-Glycerophosphate.
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All reactions on enzyme activity were carried out on whole imaginal disks from the end of the third larval instar. The larvae were anaesthetized with ether after which the imaginal disks were dissected out in insect Ringer's solution (Lewerenz, 1961). The disks of the Drosophilidae were obtained by tearing the larva in the region of the imaginal disks.

The dissected disks were collected in Ringer's solution in Falcon plastic dishes, which were kept on ice. They were prefixed for 2 min in a mixture of 10 ml 40% formaldehyde and 90 ml 6% sucrose solution at 4°C. Immediately after removing the fixative, the disks were frozen for a short time to disrupt the cell membranes. After thawing they were fixed for another 10 min and rinsed in Ringer's solution at 4°C. Incubations with AMP, β-Glyc.-P. (and ATP in the case of D. melanogaster) were carried out at pH 7.2 in the presence of manganese ions, according to the method of Sprey (1970). After treatment with 2% ammonium sulphide and washing in Ringer's solution the disks were embedded in 50% glycerine/4% gelatine at 37°C.

The intensity of the brown colour of the lead sulphide at the day of incubation was taken as a measure of enzyme activity. Three categories were estimated: strongly, moderately and weakly positive. Due to large variation in thickness of disks the intensity of the reaction in the disks of different species cannot be compared.

RESULTS

The AMP-ase activity in the imaginal disks differs strongly among the species compared. In three species (D. hydei, D. funebris and D. melanogaster) no AMP-ase activity could be demonstrated. In the other species (D. araicas, D. guaramunu, D. robusta, Musca, Phormia and Zaprionus) the enzyme activity is localized in a pattern, specific for each species.

The intensity of the reaction varies slightly among the animals of the same species, although it is equal in the left and right disk of a single specimen. Sometimes in D. robusta the pattern could not be demonstrated. These differences in enzyme activity are possibly dependent on the age of the larvae. With β-Glyc.-P. as a substrate a very weak overall reaction was found in all species. Incubation without substrate also shows a very weak overall reaction in some specimen.

The result can be described as follows.

1. Drosophila funebris, D. hydei and D. melanogaster.—The imaginal disks of these species do not show any reaction. For D. melanogaster ATP as a substrate, also proved to be negative.