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MOLECULAR ENTOMOLOGY: WHAT CAN IT DO FOR US?

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INTRODUCTION

The book, "Molecular Entomology", that I edited for Alan R. Liss, Inc., in 1987 was the result of a UCLA conference held in Colorado in 1986. The organizer of those conferences, C. Fred Fox, and his coordinator, Robin Yeaton, suggested that I use the term, although it was not the first time I had heard it. In the 1960's, when the term molecular biology was applied to an emerging discipline, those of us who spent our time fishing small molecules out of insects and determining their structures occasionally called ourselves "molecular entomologists" as a joke. Now it seems that it is a term whose time has come.

As we are in the process of defining a new field, I think that we should learn from the experience of others and act with the knowledge that we have the opportunity to set an agenda for the near future. It is unfortunate that the term "molecular biology" is construed by some to be restricted to studies involving nucleic acids. The tools and the objectives of many areas of biology today, including biochemistry, biophysics, cell biology, immunobiology, molecular biology and microbiology, are so similar that it is difficult to distinguish them, much less to set up territorial boundaries between them. Leading journals make little distinction between these fields. Therefore, in defining molecular entomology, let us take the broad view and encompass any studies relating molecules and insects.

MOLECULAR INSECT SCIENCE IN TUCSON, ARIZONA

At the University of Arizona, we have recently had an opportunity to define a new format for studies related to insects in the form of a Center for Insect Science. Although many of the projects pursued through the Center have strong molecular components, ecology and behavior are also important elements. The Center was one of three Biological Centers in the United States to be awarded a grant from the National Science Fundation under its Biological Centers Program. The goals of the Center are:

a. To use the unique biology of insects to design new environmentally acceptable and selective methods for controlling insects.

b. To use the biology that insects share with other animals to design insect models for biological processes, particularly those of humans and other mammals.
c. To discover new, economically important products from insects.

These goals have provided the framework for many new interdisciplinary projects at the University and with its partners at Arizona State University in Tempe and the Carl Hayden Bee Research Laboratory in Tucson. We are also seeking international collaboration in many countries. We do this through exchange of personnel and cooperative grants to support the research.

STUDIES ON INSECT PROTEINS

Approach to the problem

The research in my laboratory and that of my collaborator, Michael Wells, is concerned primarily with insect proteins, especially those that circulate in insect hemolymph and those that find their way into insect eggs. Our approach comes from classical biochemistry—we isolate proteins and study their properties—but we have found that the techniques of molecular biology can get us information faster in some cases, and can allow us to answer different questions as well. I will describe some of our studies on several proteins in order to show you what molecular entomology has done for us.

Table 1
Properties of some major proteins of adult *Manduca sexta* hemolymph

Protein	Native molecular weight	Subunit structure	Conjugates	Comment	References
Vitellogenin	~500,000	2 ApoVg-I 2 ApoVg-II	Lipids Carbohydrate Phosphate	Female-specific major york protein precusor	Osir *et al.*, 1986
Lipophorin	~500,000	1 ApoLp-I 1 ApoLp-II 2 ApoLp-III	Lipids Carbohydrates	A high density lipoprotein (60% protein, 40% lipid)	Shapiro *et al.*, 1988
Microvitellogenin	~26,500	one polypeptide	none	Sequence of protein and gene have been determined	Wang *et al.*, 1988; 1989
Insecticyanin	88,000	4 identical 22,000 MW	biliverdin IX-γ	Sequence and crystal structure have been determined	Riley *et al.*, 1984 Holden *et al.*, 1987

Proteins under study

Table 1 lists some of the proteins from the hemolymph of *Manduca sexta* that are also found in the eggs of this species. I will discuss three of these proteins, lipophorin, insecticyanin and microvitellogenin, in order to demonstrate what we can learn from a combination of biochemistry, biophysics and molecular biology applied to insect systems.

Lipophorin

Lipophorin is the generic name (Chino *et al.*, 1981) given to a lipoprotein that has been found in the hemolymph of every insect that has been examined (Ryan *et al.*, 1984; Shapiro *et al.*, 1988). Generally, it exists as a high density lipoprotein, HDLp (Beenakkers *et al.*, 1988), that is responsible for transporting many hydrophobic materials between gut, fat body and cells. Usually, HDLp consists of two apoproteins, apoLp-I (240 kDa), and apoLp-II (80 kDa), that constitute about 60% of the lipophorin mass, and a mixture of lipids, predominantly phospholipids and diacylglycerols, that constitute about 40% of the mass (Shapiro *et al.*, 1988). In those insects capable of sustained flight (e.g., Orthoptera, Lepidoptera), the neuropeptide hormone, adipokinetic hormone or AKH, is released from the corpora cardiaca and acts upon the fat body to mobilize stored fat reserves. Fat is released to the hemolymph in the form of diacylglycerol, and is added to HDLp. This process is facilitated by a very high density lipoprotein, the lipid transfer particle, LTP (Ryan *et al.*, 1986, 1988; Van Heusden and Law, 1989). As hydrophobic material is added to HDLp, a third small apoprotein, apoLp-III, which is a major hemolymph protein, associates with the growing lipophorin. The enlarged lipophorin is a low density lipoprotein, LDLp, which may contain as many as 16 molecules of apoLp-III (Wells *et al.*, 1987). ApoLp-III appears to stabilize the expanded hydrophobic surface of LDLp, which has twice the diameter of HDLp. LDLp contains about 40% protein and 60% lipid, principally diacylglycerol (Shapiro *et al.*, 1988). This excess diacylglycerol is delivered to the flight muscle, where the fatty acids are oxidized to supply the energy for flight. LDLp is reconverted to HDLp as lipid and appoLp-III are removed (Van Heusden *et al.*, 1986). Figure 1 is a schematic diagram of these events.

ApoLp-III from *M. sexta* is a monomeric protein with a molecular weight of 18,380 (Cole *et al.*, 1987). Extensive investigation of its physical properties showed that it has an asymmetric elliptical shape, that it binds to lipid-coated surfaces and that monomolecular films of the protein at the air-water interface show an unusual phase transition on compression (Kawooya *et al.*, 1986a). This suggests that apoLp-III can unfold to cover a hydrophobic surface, a conclusion strengthened by a study of apoLp-III stoichiometry during the formation of LDLp

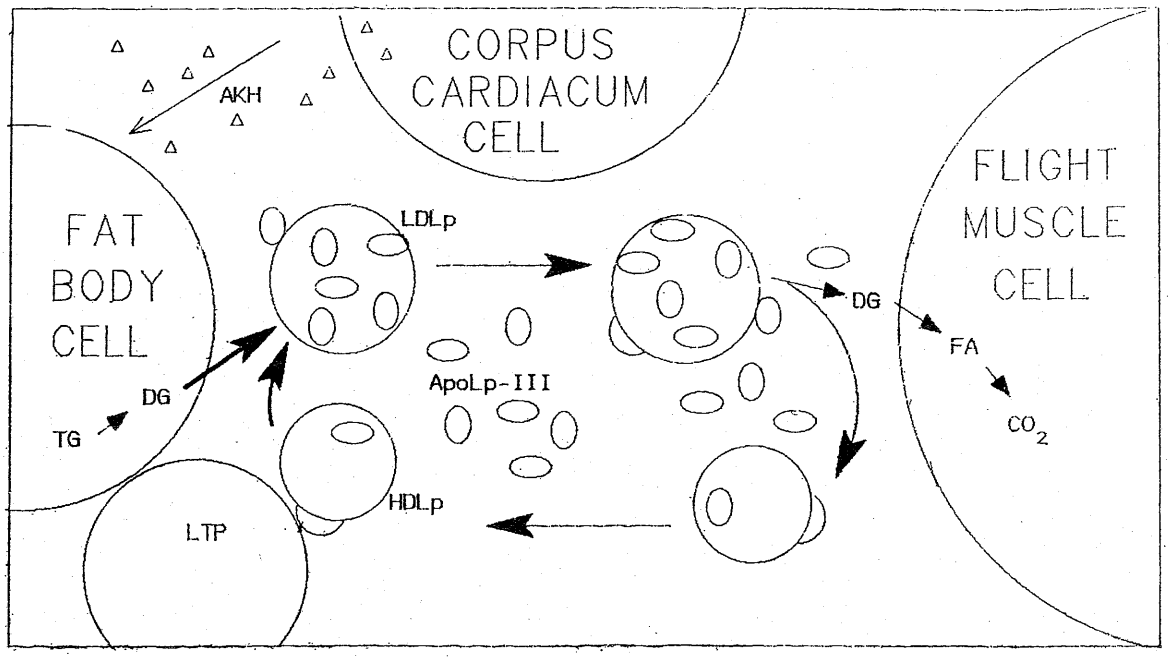

Fig. 1. Schematic representationof the transport of the transport of lipids from the fat body to the flight muscle in *Manduca sexta*. Adipokinetic hormone (AKH) released from the glandular lobes of the corpus cardiacum acts upon the fat body to stimulate the mobilization of triacylglycerol (TG) to diacylglycerol (DG). DG is released from the fat body and loaded onto high density liphorin HDLp, a process catalyzed by the lipid transfer particle, LTP. As DG is locaded onto HDLp, apolipophorin-III (apoLp-III), a protein abundant in hemolymph, associates with the expanding surface of the lipoprotein. Ultimately, a low density lipophorin, LDLP, is formed, that moves to the flight muscle, where it delivers its cargo of DG. As the lipoprotein surface contracts, apoLp-III is released into the hemolymph.

(Wells *et al.*, 1987). Given these unique properties of apoLp-III, a detailed knowledge of its structure was an important goal. We began by sequence determination, using the Edman degradation. Before we could complete that work, Ken Cole had joined us and brought the techniques of molecular biology. In a short time, he was able to clone cDNA for apoLp-III and determine its sequence (Cole *et al.*, 1987). The structure deduced from the sequence of the cDNA contains a typical signal sequence and a typical propeptide preceding the sequence of the mature peptide. Analysis of the protein structure suggests that although there is virtually no sequence homology with the human apolipoproteins, elements of the predicted secondary structure of apoLp-III are very similar to those of mammalian apolipoproteins (Cole *et al.*, 1987).

Subsequently, Kanost *et al.* (1988) succeeded in deducing the sequence of apoLp-III of the migratory locust. The protein is of a similar size to that of *M. sexta*, but is unlike it in containing covalently bound carbohydrate (Van der Horst *et al.*, 1984). The apoLp-III of *M. sexta* and *Locusta migratoria* seem to be interchangeable without loss of function, however (Van der Horst *et al.*, 1988). The

locust apoLp-III has surprisingly little sequence homology to that of *M. sexta*, but its predicted secondary structure is quite similar. The locust protein has been more amenable to crystallization in a form suitable for x-ray structural work (Holden *et al.*, 1988), and determination of its structure is close to completion. In the case of apoLp-III, molecular entomology has given us a rich array of structural information and insights into the molecular evolution of proteins.

Insecticyanin

Insecticyanin is a bright blue biliprotein found in the epidermis and hemolymph of *M. sexta* and other Lepidoptera (Cherbas, 1973). Its primary function seems to be to supply the blue component of an effective chlorophyll green camouflage coloration (Kawooya *et al.*, 1985). *M. sexta* also deposits this protein in the egg, presumably to impart a protective coloration, The yellow component of the green color is due to dietary carotene, principally lutein (Rothschild, 1975). Insecticyanin is a tetrameric protein, consisting of identical 21,378 dalton subunits, each bearing a molecule of biliverdin IX-γ (Riley *et al.*, 1984; Goodman *et al.*, 1985).

In 1984, when we had completed determination of the primary structure of *M. sexta* insecticyanin, we could find no homology with other proteins then recorded in sequence data banks. In 1987 molecular structures for insecticyanin of *Pieris brassicae* (Huber *et al.*, 1987a, b) and of *M. sexta* (Holden *et al.*, 1987) were determined by x-ray crystallography. We were surprised to find that the insecticyanin monomer had a remarkable overall structural similarity to human serum retinol binding protein and beta-lactoglobulin of bovine milk, also a carrier of retinol. A careful examination of all of the amino acid sequences then revealed small regions of high homology, although the overall sequences are quite different. More recently, it has become clear that insecticyanin is a member of a family of proteins, all of which carry hydrophobic ligadds (Pevsner *et al.*, 1988), and which have been given the generic name, lipocalins (Pervais and Brew, 1987).

Our studies on insecticyanin have taught us that overall protein structure is sometimes more important to function than is amino acid sequence. It also shows that Nature, once it invents a useful structure, can put it to a variety of uses.

Microvitellogenin

In the course of purifying apoLp-III from *M. sexta*, Dr. John Kawooya observed a protein with an apparent molecular weight of 31,000 occurring in some of his fractions. It was only observed when hemolymph from females or mixed-sex adults was used as a starting material. He was able to show that it was strictly female-specific and that it was abundant in eggs as well as hemolymph (Kawooya

and Law, 1983). In conversation with Dr. William Telfer, we realized that the *M. sexta* protein was analogous to one that occurred in *Hyalophora cecropia* that Telfer had earlier called "reluctin", (Telfer *et al.*, 1981) but now preferred to call microvitellogenin or microvitellin, because of its small size and its parallel to the major egg protein, vitellin (Telfer and Kulakosky, 1984). Up until this time it had been generally supposed that vitellogenin was the sole female-specific vitellogenic protein, so the discovery of a new protein of this class, and moreover, a small, relatively uncomplicated protein, lacking significant post-translational modification, was a cause for excitement.

Microvitellogenin was isolated from both hemolymph and eggs, and the proteins from both sources appeared to be identical in every way (Kawooya *et al.*, 1986b). For this reason, we prefer to use the name microvitellogenin for the protein, since we first isolated it from hemolymph (Kawooya and Law, 1983). The sequence of amino acids at the N-terminus was determined, and no sugars or phosphate could be detected. Antibodies were generated and used to show that microvitellogenin could not be detected in males or in larvae, but it was first present early in the development of the adult female. The site of synthesis was the fat body (Kawooya *et al.*, 1986b).

A fat body cDNA library in lambda gt 11 was screened with the microvitellogenin antibodies, and a positive clone was isolated and the 834 base pair insert was sequenced (Wang *et al.*, 1988). From the cDNA sequence it was possible to deduce that the protein was produced as a precursor with a 17 amino acid signal peptide. The mature protein has 232 amino acids, and molecular weight of 26,201 (Wang *et al.*, 1988). At the time the sequence was determined, no homology to any other proteins could be detected. Shortly before publication of our paper, however, the sequences of several "30 KD" proteins from the silkmoth, *Bombyx mori* were reported (Sakai *et al.*, 1988). The 30 KD proteins are found in the hemolymph of larvae and adults of both sexes, as well as in eggs (Izumi *et al.*, 1981). Microvitellogenin has a strong homology with the 30 KD proteins; in fact it is as similar to some of these proteins as they are to each other (Wang *et al.*, 1989).

The microvitellogenin cDNA was use to screen a genomic library from *M. sexta*, and a 3 kb insert was isolated that contained a microvitellogenin gene. The whole insert was sequenced, and it was found that the gene contained a single intron located in the 5′ non-coding region. Analysis of the 5′ upstream region of the gene revealed some areas with sequence similarity to 5′ upstream regions of the genes for vitellogenin from *L. migratoria*, and of the genes for the yolk proteins of *Drosophila melanogaster*. An area of particular interest had similarity to the steroid hormone receptor binding region of these and other

genes. This led us to look for control of the synthesis of microvitellogenin by the steroid hormone, 20-hydroxyecdysterone.

Southern blotting with the labeled cDNA probe showed that small amounts of microvitellogenin mRNA appear in the prepupal stage, even though the protein is not detectable in the hemolymph at this time. At this stage it is easy to ligate the animals behind the thorax, which isolates the hormone-producing organs in the anterior from the majority of the fat body in the posterior. One can then assay mRNA production in the fat body of the posterior of ligated animals. The production of mRNA was stimulated 100-fold over controls in animals that received 2 injections of 10 μg each of 20-hydroxyecdysone at 24-hour intervals (Wang *et al.*, 1989). In this case, molecular entomology has led us to try a classical endocrinological experiment to confirm something we have discovered in a gene structure.

I began exploring the structures of molecules from insects in 1962. I have been amply rewarded over the intervening years by discovering many new, and for me, exciting, things about insects and about biology in general. I am convinced that the constantly developing technology for exploring molecular entomology will continue to enrich us all in the future.

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