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PARTIAL CHARACTERIZATION OF THE D GROUP PROTEINS OF THE TUBULAR ACCESSORY GLANDS OF *TENEBRIO MOLITOR*

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Abstract—The tubular reproductive accessory glands (TAGs) of male mealworm beetles (*Tenebrio molitor*) produce four groups of differentiation-specific proteins. This paper concerns the D protein class which are the first of these proteins to appear in the adult TAGs. Within the D class, four peptides with apparent molecular weights of 27.7, 26.5, 25.1 and 24.0×10^3 were resolved by SDS–polyacrylamide gel electrophoresis. Each D peptide possesses a different isoelectric point. Only one or two D proteins were found in any one male. Their distribution in our laboratory population was consistent with a Hardy–Weinberg equilibrium with four alleles at one locus. In non-denaturing electrophoresis, the D proteins are separable into two bands: ND_1 which contained proteins of 26.5 and 24.0 kilodaltons and ND_2 which contained only proteins of 27.7 kd. The native D proteins may exist as dimers in their native state but we found no evidence for disulphide bonds between polypeptide chains. Amino acid analysis indicated that the two bands from non-denaturing electrophoresis are similar in composition, both to each other and to whole TAG homogenate. Both D bands are high in alanine, aspartic acid, glutamic acid and glycine and are low in methionine and tyrosine.

Key Word Index: Accessory glands, *Tenebrio molitor*, cytodifferentiation, insect reproduction

INTRODUCTION

Accessory glands of male insects produce a diverse set of products which mediate sperm maturation, transfer and storage and affect reproductive physiology (Leopold, 1976; Chen, 1984; Happ, 1984). The precise physiological roles of the secretory products of these glands are under study in many species (see references in Chen, 1984; Happ, 1984). The complexity of these secretory systems and the fact that they mature rapidly in the last pre-adult and adult stages render such accessory glands attractive material for developmental studies. The major events in their development take place during the final metamorphic transformation of the insect, in the context of well-established fluctuations in the titres of developmental hormones.

The growth of accessory glands is influenced by fluctuations in the concentrations of ecdysterone (Szöllösi and Landureau, 1977; Szopa and Happ, 1982; Szopa *et al.*, 1985). Of particular interest is the fact that many of the accessory glands of male insects are mesodermal derivatives and the impact of ecdysterone on their development may be quite different from the events which occur simultaneously in the epidermis. In *Tenebrio molitor*, the pupal ecdysterone peak appears to trigger mitotic arrest in the epidermis (Besson-Lavoignet and Delachambre, 1981) and at the same time to stimulate mitoses in the accessory glands of the male (Sroxa *et al.*, 1985). We are interested in the effect of ecdysterone on the differentiation of the male accessory glands of *T. molitor*. An analysis of the control of differentiation requires information about particular proteins which can be used as markers to score the extent of the differentiation (Kafatos *et al.*, 1977). In the present paper we describe one such class of differentiation-specific proteins.

Our model system is the reproductive accessory glands of the male mealworm beetle, *Tenebrio molitor*. There are two pairs of glands: the bean-shaped glands (BAGs) and the tubular glands (TAGs). Both sets of glands contribute proteins which are used in spermatophore formation (Black *et al.*, 1982; Happ *et al.*, 1982). The changing titres of ecdysterone are well described during the prepupal, pupal and early adult stages of this species (Delbecque *et al.*, 1978; Delbecque, 1976) and there is information on the levels of juvenile hormone which are present in the adult (Weaver *et al.*, 1980). During the early pupal period, the accessory glands grow by cell division and change in overall shape (Grimes and Happ, 1980; Happ and Happ, 1982). During the late pupal instar and shortly after ecdysis, the proteins characteristic of the reproductively mature adult are first detected (Happ *et al.*, 1982; Black *et al.*, 1982).

The TAGs consists of a secretory epithelium of one morphological cell type (Gadzama *et al.*, 1977). The glands produce four major groups of differentiation-specific proteins that differ in molecular weight and have been designated class A, B, C, and D (Happ *et al.*, 1977; Black *et al.*, 1982). The D proteins are the first to show high levels of leucine incorporation; they are heavily labelled on the day of ecdysis. These proteins range in molecular weight from 26,000 to 29,500 and also differ in isoelectric point, some are acidic (pI 4.5–5.0) while others are basic (pI 7.5–8.0) (Black *et al.*, 1982). Before the control of D protein synthesis and the function of these proteins can be analysed, this heterogenous set of molecules must be better characterized.

The present paper concerns the character of the D proteins of the TAG. The molecular weights of the various members of this class were determined. Inter- and intra-chain bonding and amino acid composition of some of the D proteins were investigated. In

addition, a population of males was examined to determine the extent of variation in D proteins within and between individuals.

MATERIALS AND METHODS

Mealworms (*Tenebrio molitor*) were obtained from a commercial supplier and from inbred laboratory colonies and reared on Purina Chick Labchow. Newly eclosed pupae were segregated, sexed and maintained at 26°C to assure uniform pupal development. Newly eclosed adults were collected, fed potato and maintained at 26°C in groups of five or less until needed.

Polyacrylamide gel electrophoresis

Solubilized TAG proteins were electrophoresed under several different gel conditions: sodium dodecyl sulphate (SDS) slabs (12%, Laemmli, 1970), slab and tube non-denaturing gels (7.5% with SDS omitted; Laemmli, 1970) and urea denaturing slab gels (7.5%). Two dimensional electrophoresis involved either non-denaturing 7.5% tube gels or pI-IEF gels (O'Farrell, 1975). The second dimension was an SDS slab gel (12% polyacrylamide).

Samples (either pooled or individual TAG glands) were prepared for SDS gels or pI gels as described by Black *et al.* (1982). The sample concentrations of β-mercaptoethanol (0–20%) and dithiothreitol (DTT, Cleland's reagent; 0–10 mM) were varied in both non-denaturing and SDS gels to investigate their effect on protein patterns. Urea concentrations in samples for urea gels varied from 0 to 7 M. Samples for non-denaturing and urea gels were homogenized in gel buffer with 10% (v/v) glycerol and 0.02% bromphenol blue. Gels were stained with 0.04% Coomassie blue R250, 50% (v/v) methanol, 10% (v/v) acetic acid and destained by diffusion in 5% (v/v) methanol and 7% acetic acid. For molecular weight determination, standards were run on each gel and the weights of the D proteins calculated by regression analysis. Molecular weight markers were obtained from Sigma Chemical Company.

Partial isolation of D proteins

Soluble TAG proteins from 10-day adult TAGs (homogenized in gel sample buffer without SDS) were separated by non-denaturing electrophoresis polyacrylamide (7.5% slabs). Two protein bands of interest (ND_1, ND_2) were excised after visualization by staining the gel's outer edges. Gel slices were homogenized in 0.005 M phosphate buffer (pH 7.4) with a Brinkman Polytron and eluted overnight on a shaker platform. After centrifugation (200 *g* 10 min, 4°C) of the slurry, the supernatant portion was lyophilized. Portions for amino acid analysis were dialysed against three changes of distilled water, hydrolysed in 6 M HCl for 24 hr at 100°C and evaporated without neutralization. Amino acid content was determined using a Varian Vista 1400 HPLC amino acid analyser. Norleucine was injected with the sample for use as an internal standard. Proline, hydroxyproline, cysteine and tryptophan were not detectable by these methods. Total protein was determined using the procedure of Lowry *et al.* (1951) with bovine serum albumin (Sigma Chemical Co.) as a standard.

Statistical methods

Statistical analysis of regression and chi-square are those described by Sokal and Rohlf (1981). Hardy–Weinbeg classes of fewer than five expected individuals are pooled together as a single error estimator.

RESULTS

According to an earlier report from this laboratory, proteins of the D class vary in molecular weight from 26,000 to 30,000 (Black *et al.*, 1982). In the present study several bands were resolved within this molecular weight range. These bands are most easily distinguished from one another when a homogenate of only one gland pair is applied to each lane of a slab gel. In our laboratory population of males, there appear to be four distinct D protein bands of the TAGs within the 27,000–29,500 dalton range on SDS gels (Fig. 1). By comparison with standards, we calculated their mean molecular weights as 27,730, 26,530, 25,090 and 23,900 (Table 1); these polypeptides were designated D27, D26, D25 and D24 in keeping with their molecular sizes. The D27 protein variant was the most frequent (at 54% of the examined alleles), followed by D26, D24 and D25 (at 4%). An additional band with an estimated molecular weight of about 22,000 was found in few individuals. We believe that this band may be yet another member of the D class.

The bean-shaped accessory gland of *T. molitor* produces a variety of products which are derived from eight distinct regions of that gland (Dailey *et al.*, 1980; Dailey and Happ, 1983). To determine whether the different D proteins were distributed among different regions of the pair of TAGs, we compared the homogenates of right and left glands and also the homogenates of distal, middle and proximal thirds of individual TAGs. No variations in protein pattern could be found among these samples. On the basis of our electrophoretic results, we have no evidence for regional specialization in the TAGs.

Not all males produce all of the D polypeptides. In any one individual, only one or two prominent D bands are seen (Fig. 1). Furthermore, not all D proteins are equally common. D27, D26 and D24 predominate. These facts suggested that the four D polypeptides might comprise a group of four alleles coded for by a single locus. If such were the case, two bands per individual represent a heterozygote, while a single band indicates either a homozygote or the presence of a null allele. If we assume all alleles are visible and that a single D band indicates a homozygote, then the allelic frequencies for each variant can be calculated for the population of 142 males (Table 2). The allelic frequencies can be used to predict the genotypic frequencies that would be expected for a single locus, four allele system in Hardy–Weinberg proportions (Table 2). These expected values were matched against the observed population values and a Chi-square analysis performed. For this Chi-square statistical test, all classes with an expected frequency of less than five individuals were pooled and used as an error estimator (Sokal and Rohlf, 1981). The Chi-square analysis is summarized in Table 2. The χ^2 value is 2.84 which corresponds to $0.9 < a < 0.5$. Thus the present set of data is not significantly different from that expected under the single locus, four allele hypothesis.

The pattern of D protein bands was strikingly different on non-denaturing gels. On gels without SDS, only two very prominent bands stained with Coomassie blue. These bands were designated ND_1 and ND_2, and they contained the D proteins. Each of these bands was cut from a preparative gel and eluted. The eluates were solubilized with SDS-sample buffer and run on an SDS-containing gel. The ND_2 band yielded only proteins co-migrating with D27 while the

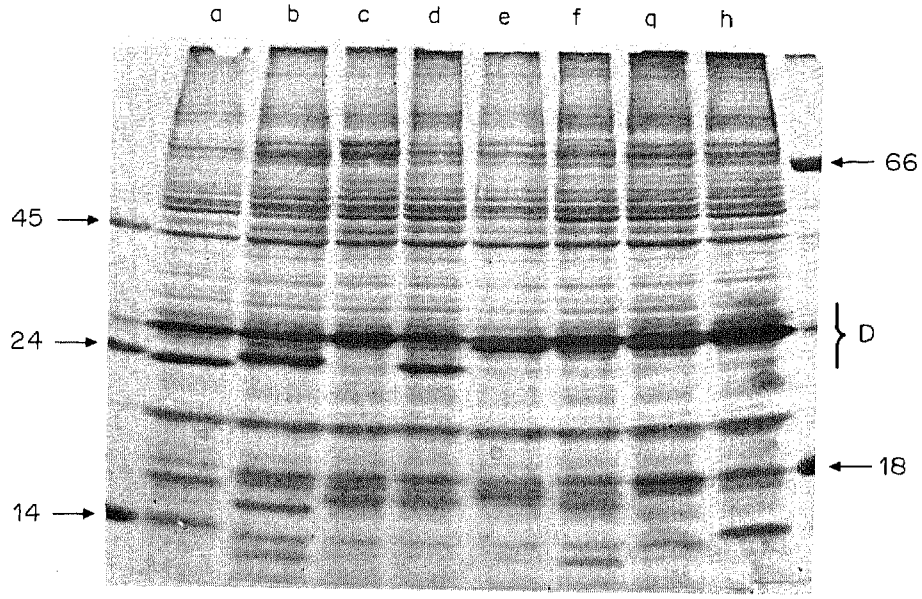

Fig. 1. Polyacrylamide gel electrophoresis of 8-day adult TAG homogenates on an SDS containing 12% gel. D protein variants are as indicated; (a) D27:24, (b) D27:25, (c) D27:27, (d) D27:24, (e) D27:26, (f) D27:27, (g) D27:26, (h) D27:27. Molecular sizes are in kilodaltons. Coomassie blue stained.

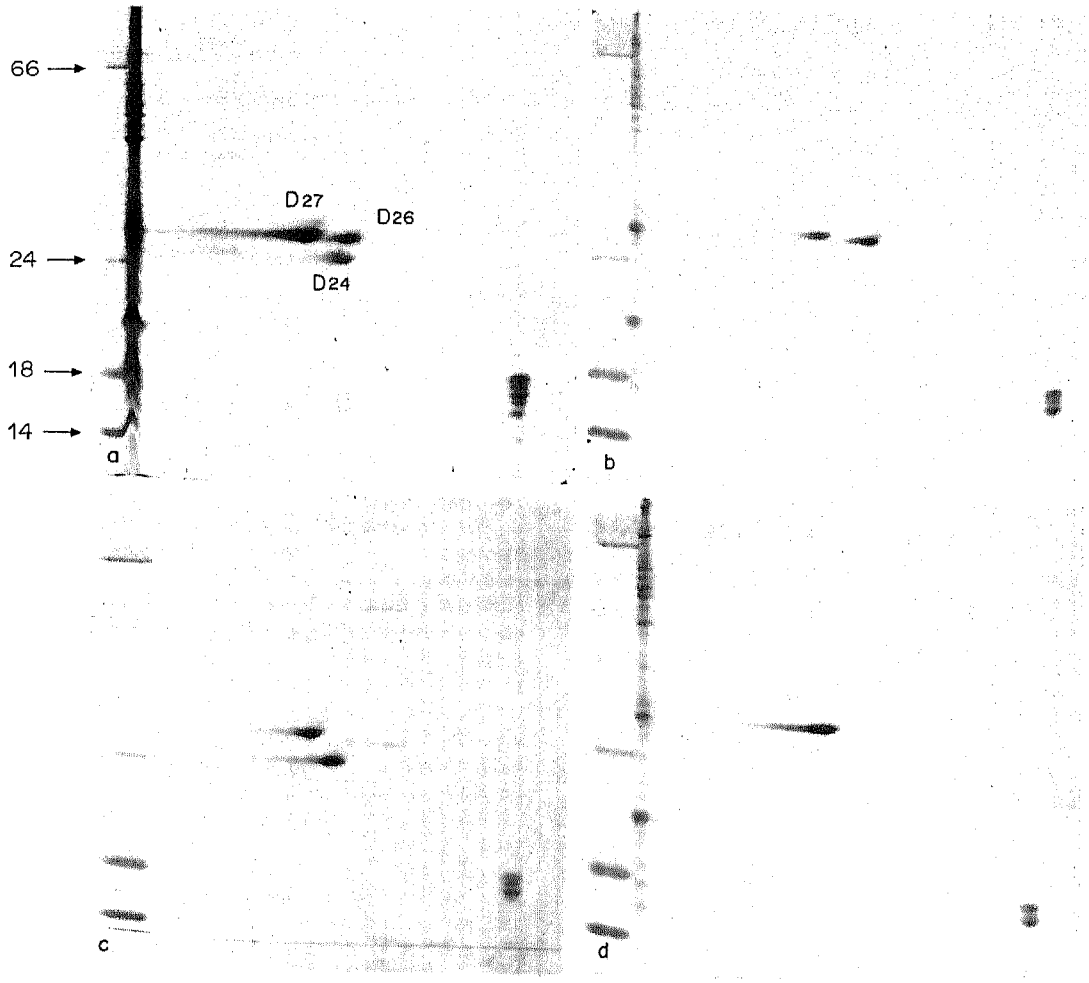

Fig. 2. Two dimensional polyacrylamide gel electrophoresis of 8-day adult TAG proteins. First dimension (horizontal) non-denaturing 7.5% gel pH 8.9, second dimension (vertical) SDS, 12% gel; (a) pooled TAGs (3) showing D27, D26 and D24, (b) D27:26, (c) D27:24, (d) D27:27. Molecular sizes are in kilodaltons. Coomassie blue stained.

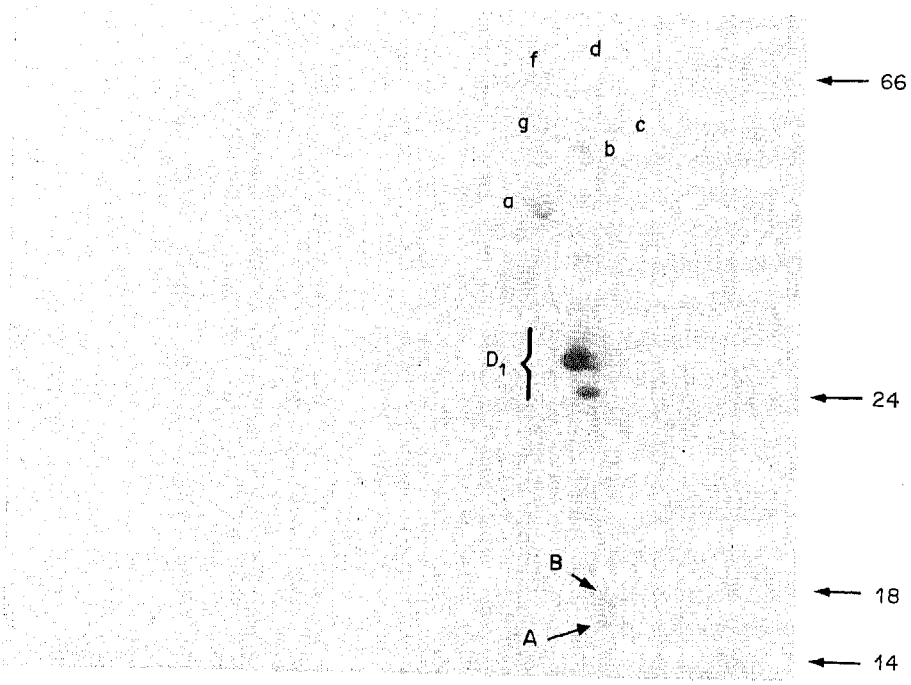

Fig. 3. Two dimensional pI-SDS 12% gel of TAG proteins from 8-day adults: (A) class A proteins; (B) class B proteins; (D_1) acidic class D proteins; small letters refer to reference proteins described in Black *et al* (1982); pH gradient from 8.0 (left) to 4.0 (right). Coomassie blue stained.

Table 1. Molecular weights of D protein variants in the TAG glands of *T. molitor* (molecular weights × 10^{-3})

Band	*n*	Mean mol. wt	±SE
D27	44	27.73	±0.10
D26	17	26.53	±0.15
D25	3	25.09	±0.69
D24	17	23.9	±0.22

Table 2. Analysis of D proteins of *Tenebrio* TAGs: calculation of allelic frequencies,* Hardy–Weinberg ratios† and Chi-square values in a population of 142 males

Variant genotype	Observed number	Expected number	Expected proportion	$(o-e)^2$ e
$D_{27:27}$	51	54.0	0.38	0.17
$D_{27:26}$	38	34.1	0.24	0.45
$D_{27:25}$	9	7.1	0.05	0.51
$D_{27:24}$	28	27.0	0.19	0.04
$D_{26:26}$	2	4.3	0.03	
$D_{26:25}$	1	2.8	0.02	
$D_{26:24}$	9	8.5	0.06	0.03
$D_{25:25}$	0	0.3	0.002	0.01
$D_{25:24}$	2	1.4	0.01	
$D_{24:24}$	2	2.8	0.02	
Remainder	7	11.3	—	1.64
Total			$\chi^2 = 2.84$	
			$df = 1$, $\alpha > 0.05$	

* allelic frequencies = [2(homozygotes) + heterozygotes]/total number of alleles. $D_{27} = 0.62$, $D_{26} = 0.19$, $D_{25} = 0.04$, $D_{24} = 0.15$.
† All classes with expected values of less than 5 were pooled.

ND_1 band contained equal amounts of proteins indistinguishable from D26 and D24.

The relationships between the bands in the non-denaturing gels and those on SDS gels were also examined in two dimensional gels. The first (horizontal) dimension was a non-denaturing tube gel, without SDS and the second (vertical) dimension was an SDS-containing polyacrylamide slab. When three TAGs were pooled before electrophoresis, spots corresponding to D27, D26 and D24 were clearly visible (Fig. 2).

We also ran experiments with glands from individual males. One gland of each pair was first run on one-dimensional SDS gels while the other gland was frozen for two-dimensional analysis. From the one-dimensional gels, we established the phenotype of the D proteins in each male. Then the second gland of known D phenotype was run on the two-dimensional system. Each two-dimensional gel from an individual beetle showed only one or two of the three common D variants (Fig. 2). When D27 was present in the one-dimensional gel, there was a single spot derived from ND_2 on the two dimensional gel. When D24 and/or D26 were present on the one-dimensional gel, there were the corresponding one or two spots derived from ND_1 on the two-dimensional gel. The components of ND_1 (D26 and D24) appear more negatively charged (running closer to the anode in the first dimension) than is the major component of ND_1 (D27). These results raise the possibility that ND_1 and ND_2 are dimers of D proteins. Alternatively, similarity of overall charge is as likely an explanation for the fact that D26 and D24 run together in ND_1. The rare variant (D25) was never seen in samples run on two dimensional (non-denaturing, SDS) gels and therefore its contribution to the ND_1 and ND_2 system is unknown at the present time.

Two dimensional pI-SDS gels of leucine incorporation into TAG proteins show two groups of D proteins, an acidic group (pI 4.5–5.0) and a basic group (pI 7.5–8.0) (Black *et al.*, 1982). In the present study, pI-SDS gels were run on three pooled TAGs and stained with Coomassie blue. Only the acidic subgroup of D proteins was seen (Fig. 3).

To investigate the nature of inter- and intra-chain bonding between the polypeptides in native D proteins, we varied the concentration of β-mercaptoethanol (0–20%, v/v) or dithiothreitol (0–10 mM) in the sample preparation mixture for the SDS gels. Mobility was unaffected by increasing concentrations of these reagents which are known to disrupt S–S linkages between peptides in other systems (Massagué and Czech, 1982). Interchain hydrogen binding should be masked by the presence of high concentrations of urea. We added 7 M urea to the non-denaturing gel mixture to disrupt hydrogen bonds. There was no apparent effect on the mobility of ND_1 or ND_2.

We explored the similarities among the major D polypeptides by determining the amino acid contents of ND_1 and ND_2. Samples of these partially purified proteins were electrophoresed on SDS and on non-denaturing gels and consisted of one (ND_2) or two (ND_1) protein bands. Rigorous purification was not attempted. Levels of 15 amino acids were quantified (Table 3). The similarities between ND_1 (which is a mixture of D24 and D26) and ND_2 (which is composed mostly of the single polypeptide, D27) are marked. Alanine, aspartic acid, glutamic acid and glycine accounted for over 60% of the total residues in both isolates. However, ND_1 is higher in acidic amino acids and lower in methionine than is ND_2. Traces of amino sugars were detected in ND_2 but not in ND_1. The amino acid content of the TAG homogenate was lower in aspartic acid and alanine than were the D proteins.

Amino acids are frequently grouped according to the characteristics of their side chains (Table 4). Comparison of amino acid groups in ND_1 and ND_2 show at least one difference between these D protein variants. Acidic amino acid residues were higher and basic amino acids were lower in ND_1 than in ND_2. If this difference in acidic residues represents the real predominance of carbonyl groups in ND_1, and not the corresponding neutral amide groups hydrolysed to

Table 3. Amino acid composition of ND_1, ND_2 and TAG of *T. molitor* (residues/1000)

Amino acid	ND_1	ND_2	TAG
Aspartic acid	185.0	174.6	136.8
Threonine	47.5	41.7	68.8
Serine	65.0	59.8	69.0
Glutamic acid	125.8	107.0	135.4
Proline	ND	ND	ND
Glycine	95.0	104.3	87.5
Alanine	206.0	188.0	108.4
Valine	31.2	36.3	68.3
Methionine	TR	12.4	0.0
Isoleucine	25.8	34.0	48.7
Leucine	32.3	51.0	69.2
Tyrosine	TR	TR	7.2
Phenylalanine	24.2	24.9	37.0
Lysine	61.7	63.2	79.4
Histidine	30.4	27.3	29.3
Arginine	70.1	75.5	55.0

ND = not determined by this method. TR = trace amounts present.

Table 4. Comparison of amino acid composition by classes of side chains (residues/1000 total residues)

Class of amino acid	*Drosophila* PS-1*	*T. molitor* ND_1	*T. molitor* ND_2	*T. molitor* TAG	*T. molitor* BAG	*T. molitor* Spermatophore†	Moth spermatophore‡	Pseudoscorpion spermatophore§
Non-polar	448	414	438	419	473	531	647	552
Small	426	366	351	265	274	264	238	474
Basic	71	162	166	164	123	107	36	74
Acidic	351	311	282	272	237	223	116	198
Charged	422	473	448	436	360	330	152	272
Glycine	0	95	104	88	89	91	92	240
Proline	76	ND	ND	ND	71	134	317	5
Tyrosine	0	TR	TR	7	38	35	21	47
Hydroxylated	129	122	101	145	151	151	147	158

ND = not determined. TR = trace amounts present. Classes of amino acids (by side chain): Non-polar: glycine, alanine, valine, leucine, isoleucine, phenylalanine, proline. Small: glycine, alanine, serine. Basic: lysine, histidine, arginine. Acidic: aspartic and glutamic acid. Charged: acidic and basic goups. Hydroxylated: serine, threonine, tyrosine, hydroxyproline. *References:* *Baumann (1974). †Frenk and Happ (1976). ‡Navon *et al.* (1983). §Hunt and Legg (1971).

the acid form by the analytical procedure, then ND_1 could have a greater negative charge than ND_2 during electrophoresis at pH 8.9. This difference is consistent with the greater mobility of ND_1 in non-denaturing gels.

DISCUSSION

Amino acid composition of accessory gland products

On the basis of physical properties and functions, the secretions of the accessory glands of male insects can be divided into two general classes, the soluble proteins which usually comprise the seminal plasma and the insoluble proteins which form spermatophores or mating plugs. In *T. molitor* as in moths (*Anagasta kuehniella*), crickets (*Acheta domesticus*) and flies (*Glossina morsitans*), the spermatophores are assembled from morphologically heterogenous secretory products which are stored in the lumina of the male reproductive tract (Dailey *et al.*, 1980; Riemann and Thorson, 1979; Kaulenas, 1976; Kokwaro, 1982). However, few detailed biochemical analyses have been performed on soluble or insoluble products secreted by male accessory glands.

The most complete study on a soluble protein product of an accessory gland is the analysis of PS-1 (Baumann, 1974), which is made in the paragonia of *Drosophila funebris*. PS-1 is a chain of 27 amino acids, of which alanine, aspartic acid, glutamine and serine are the most abundant. In the D proteins and in the homogenate of TAGs of *T. molitor*, the most common acids are also alanine, aspartic acid and glutamic acid (Table 3). When amino acids are grouped according to their side chains (Table 4), similarities are still apparent. However, PS-1 is a much smaller protein and is completely devoid of several amino acids, including glycine and tyrosine.

Little is known about intraspecific variation in proteins produced by male accessory glands. The five variants of the D class in *T. molitor* constitute an unusual system. All of the D variants described in the present paper have similar isoelectric points, roughly in the range from pH 4.5 to 5.0. An even greater apparent diversity in isoelectric points is seen in leucine incorporation studies. Two dimensional pI-SDS gels show that in the molecular weight range from 26,400 to 30,000, there are two distinct clusters of proteins, one of which is acidic (pI 4.5–5.0) and the other of which is basic (pI 7.5-8.0) (Black *et al.*, 1982). However, on two dimensional pI-SDS gels stained with Coomassie blue, only the acidic group was seen. We suspect that the basic group represent the primary translation products which subsequently give rise to the acidic group via some kind of post-translational modification. Such post-translational modification, including proteolytic cleavage, addition of lipid or sugar groups, and addition of phosphate or sulphate, have been reported in many systems, including those of insects (e.g. Leopold, 1976; Beckendorf and Kafatos, 1976; Chen *et al.*, 1978; Brennan and Mahowald, 1982; Fargnoli and Waring, 1982).

The wall of the spermatophore of *T. molitor* contains insoluble protein components. Amino acid contents of the BAG and of the spermatophore of *T. molitor* have been reported previously (Frenk and Happ, 1976) and are included in Table 4. The amino acid compositions of the BAG and the spermatophore are rather similar: both contain high levels of proline and tyrosine and relatively low content of charged amino acids. Tyrosine is frequently involved in cross-linking of structural proteins of cuticle and eggshells (for examples see Petri *et al.*, 1976; Fargnoli and Waring, 1982). In comparison to the spermatophore and the BAGs, the D proteins and the TAG homogenates are much higher in charged residues and much lower in tyrosine.

Amino acid analyses are available for the spermatophores of only three species of arthropods (Table 4). In all three, non-polar amino acids are prominent, accounting for 53% or more of the amino acids in spermatophores of *T. molitor, Spodoptera littoralis* and *Chthonius ischnocheles*. High levels of tyrosine and proline are found in the spermatophore of the moth, *Spodoptera* (Navon *et al.*, 1983) and tyrosine is also relatively high in the spermatophore of the pseudoscorpion, *C. ischnocheles* (Hunt and Legg, 1971). In addition, alanine and glycine, each account for 10% or more of the residues in the spermatophore of *T. molitor* (Frenk and Happ, 1976) and *S. littoralis* (Navon *et al.*, 1983). This admittedly sparse evidence suggests that there may be general similarities among the structural proteins of arthropod spermatophores.

The function of the D proteins

Unlike proteins of the A, B and C classes, those of the D class are not seen in SDS gels of the spermatophore (Happ *et al.*, 1982; Black, 1983) and thus they do not appear to be major components of the seminal plasma or of the spermatophore wall. The soluble

proteins in *D. funebris*, PS-1 and PS-2, act on the female fly to increase the rate of egg production and to reduce her responsiveness to courting males (Baumann, 1974). The accessory glands of male *Acanthocelides obtectus*, a bruchid beetle, produce a secretion which accelerates egg deposition in females (Quesneau-Thierry *et al.*, 1975). Male accessory glands contain an oviposition stimulant for female *Melanoplus sanguinipes* (Friedel and Gillott, 1976). In *T. molitor*, it is possible that small amounts of the D proteins are transferred to the female and influence her physiology.

Several D variants were described in this paper. The heterogeneity of these polypeptides and their variable distribution among the males argues against their role as a signal acting on the female. As noted above, no more than two D protein variants are found in any one male and their distribution through our population of males is consistent with a Hardy–Weinberg equilibrium assuming four or five alleles at one locus. Final proof of allelic variation at one locus will depend upon genetic studies involving specific crosses and analysis of offspring. The presence of null alleles (which code for no visible protein product) or epistatic interactions between several loci could also explain our results. However, the observed variation in D proteins argues against the role of D proteins as simple (conserved) triggers which act on the reproductive physiology of the female. *A priori*, one would expect natural selection toward constancy among males to remove ambiguity in a signal system. Selection may act on the male accessory gland esterase (EST-6) of *D. melanogaster*. This esterase appears either to mediate sperm release motility or utilization by the female (Gilbert, 1981) or degrade a lipid in the female reproductive tract to an anti-aphrodisiac which reduces the chance of further mating (Mane *et al.*, 1983). In either case, males with less efficient forms of the enzyme may be at a reproductive disadvantage. In *T. molitor*, an esterase is present in both TAG and spermatophore, but its characteristics and function are unknown. The D proteins of *T. molitor* TAGs may have some enzymatic function which is advantageous in reproduction.

The D proteins could also be required for the assembly, stabilization, or ejection of the spermatophore. It is also possible that during the process of spermatophore formation, the D proteins are modified by proteolysis or covalent cross-linking such that their characteristic mobilities on SDS gels are changed and we simply fail to detect them on our gels. Work in progress in our laboratory involves specific antibodies which will be used to trace the fate of these D proteins.

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