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ECDYSTERONE-INDUCED MODIFICATIONS OF PROTEIN SYNTHESIS IN A *DROSOPHILA MELANOGASTER* CULTURED CELL LINE

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SUMMARY

Changes in the rate of synthesis of Kc 0 % *Drosophila melanogaster* cell proteins in response to the insect moulting hormone, ecdysterone, have been examined by two-dimensional gel electrophoresis. There was no global alteration of protein synthesis in induced cells. Approximately 4 % of more than 350 polypeptides separated by this technique were qualitatively or quantitatively different in treated cells. Three polypeptides were newly induced, two were repressed and six had an increased rate of synthesis. Two spots identified as actins II and III were more intensely labeled in treated cells suggesting that actin synthesis was implicated in morphological changes observed after ecdysterone treatment. It is concluded that ecdysterone can bring about discrete and specific modifications after short time of treatment and can repress as well as induce polypeptide synthesis.

Steroid hormones play an essential role in gene regulation in multicellular organisms by eliciting specific responses from target cells. Ecdysterone is an hormonal steroid of critical importance in the development and differenciation of insects (1). It induces puffs in *Drosophila* salivary gland chromosomes (2). Changes in the mRNA composition have been shown in imaginal discs and fat body (3).

Morphologically, *Drosophila melanogaster* cultured cells undergo, following ecdysterone addition, a dramatic transformation involving cell elongation, extension of extremely thin processes and finally an arrest of cell division (4, 14). In addition, active cellular aggregation takes place. Changes in cell surface texture are noted in scanning electron-micrographs (5). These transformations are accompanied by the induction of eserine-sensitive acetyl-cholinesterase activity (6, 7) and β-galactosidase activity (8).

In the present work, high resolution two dimensional polyacrylamide gel electrophoresis technique of O'Farrell (9) has been used to verify whether a modification in protein synthesis is involved in the transformation of an established cell line of *Drosophila melanogaster* (Kc 0 %), under ecdysterone effect, and, if so, at what time after the administration of the hormone such transformation can be detected.

We find that ecdysterone gives a rapid, very limited and a highly specific response in Kc 0 % cells. Of over 350 polypeptides detected, fewer than 4 %

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show modifications in synthesis : three are newly induced, two are repressed and six have increased rate of synthesis. Actins II and III belong to this last class of hormone controlled proteins. The first modifications were observed between 4 and 7 hrs of treatment.

MATERIALS AND METHODS

- Kc 0 % *Drosophila melanogaster* cells were derived from the lines established by Echalier and Ohanessian (10). They were cultured at 23°C at a concentration of 5 to 40 x 10^6 cells per ml in D22 medium without serum. The doubling time was 24 to 26 hrs under these conditions. When required, ecdysterone (SIMES - Milan) was added to the cultures at a final concentration of 0.1 µM.

- D22 medium was removed from bottles of exponentially growing cells, and replaced with the same quantity of methionine free culture medium containing 50 µCi/ml ^{35}S-methionine (specific activity 400 mCi/mMole, CEA) with or without ecdysterone during 3 hrs. Then cells were harvested and washed twice with saline buffer (NaCl 25 mM, EDTA 24 mM, Tris 10 mM, $CaCl_2$ 3 mM and $MgCl_2$ 2 mM, pH 8.0).

- Proteins were extracted from cells in two different ways, depending on the type of gel electrophoresis used. When proteins were analysed by one dimensional electrophoresis, cells were lysed directly in the "final sample buffer" as described by Laemmli (11). For two-dimensional electrophoresis, the method of O'Farrell (9) was used for protein preparation and for isoelectrofocusing. Ultrapure urea (Schwarz-Mann) was used in all samples. For the first dimension, the sample was added to the low pH end of the isoelectrofocusing gel. The focusing time was 6800 v.h.. The second dimension gels were 12 % acrylamide (11) with an acrylamide-bisacrylamide ratio of 100 : 2.7. Equal amounts of trichloracetic acid-precipitable radioactivity were loaded on to each isoelectric focusing gels. Slab gels were fixed, stained, dried and exposed to Kodirex film (Kodak).

RESULTS

In a first set of experiments, total proteins from Kc *Drosophila* cells with and without ecdysterone treatment were analyzed by one dimensional electrophoresis. Whatever the time of hormone treatment (from 0 to 52 hrs), no modifications were observed in the protein pattern when examined either by staining (no shown) or autoradiography (Fig. 1). This suggests that ecdysterone effect on protein synthesis is not drastic and, if changes occured after hormone treatment, they only affect a small number of specific proteins.

Since two dimensional electrophoresis affords greatly improved resolution, we undertook an analysis of proteins from control and hormone treated cells by this method. Figure 2 shows two dimensional gel patterns of total proteins from control and ecdysterone treated cells. In both, cells were labelled with ^{35}S-methionine during three hours before harvesting. Figures 2 A and B represent the Coomassie blue stained gels of control and ecdysterone treated extracts which were subsequently autoradiogrammed (Fig. 2 C and D). Approximately 350 polypeptides were resolved on the gels. The majority of labelled spots were common to both the control and the hormone treated cells and also corresponded

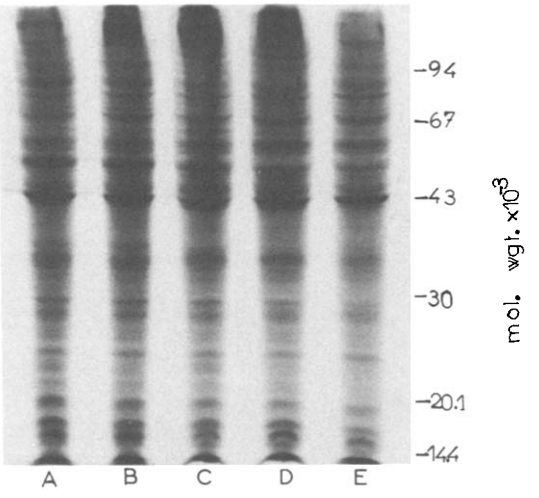


Figure 1. Autoradiogram of a slab gel of total proteins of Kc 0 % cells. Control cells (I) ; cells treated with 0.1 µM ecdysterone for 4 hrs (B), 7 hrs (C), 24 hrs (D), 52 hrs (E). Proteins were electrophoresed in 12 % acrylamide, 0.1 % SDS. Protein standards coelectrophoresed were phosphorylase b (94.000), Albumin (67.000), Ovalbumin (43.000), Carbonic anhydrase (30.000), Trypsin inhibitor (20.000), α-Lactalbumin (14.400) and are indicated at the right of the autoradiogram.

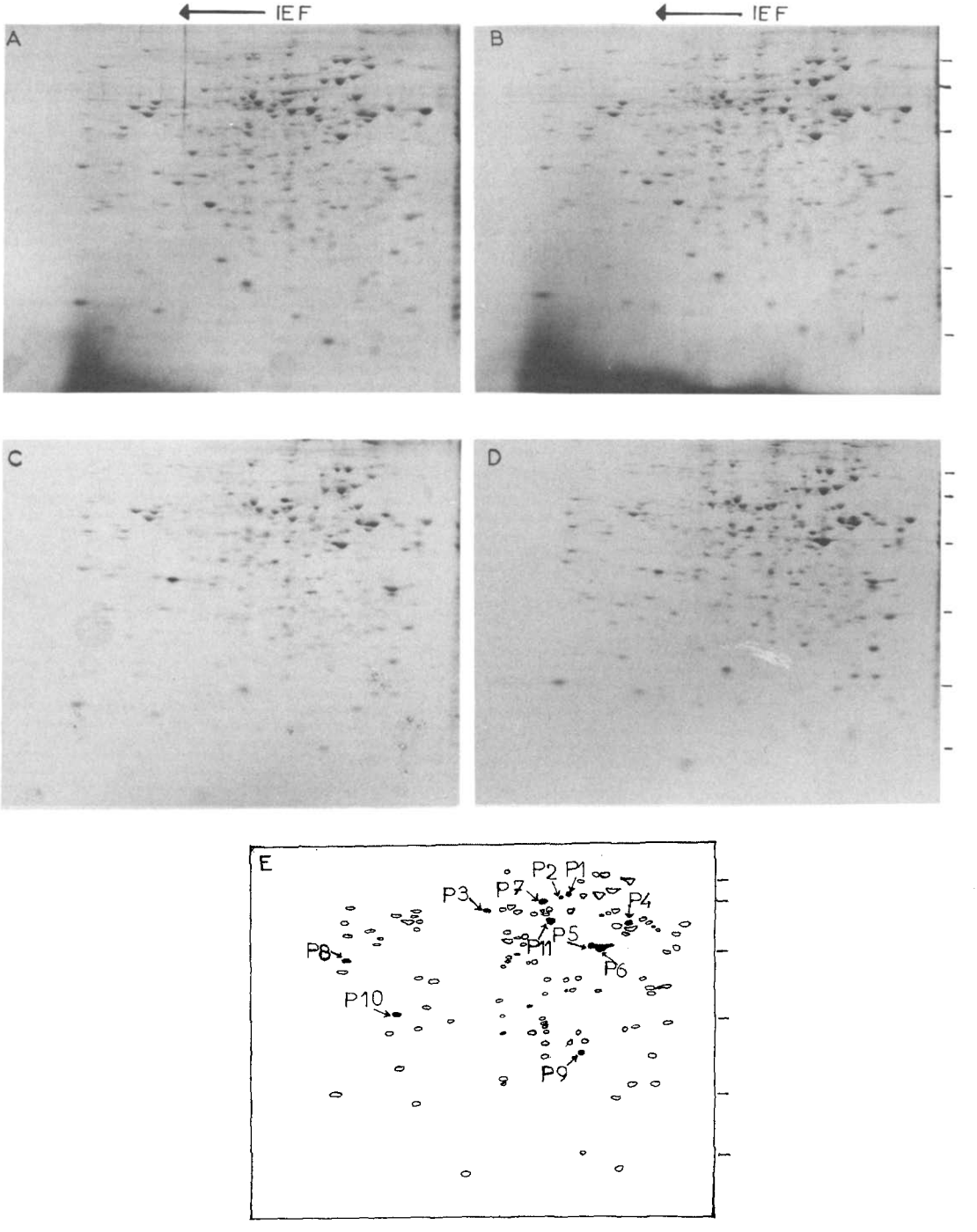
to the steady-state protein population as revealed by coomassie blue staining. However, close inspection of autoradiograms revealed discrete qualitative and quantitative changes in several polypeptides.

Visualisation of protein maps by staining and by autoradiography permits certain conclusions to be drawn on the nature of cellular polypeptides. Coomassie blue stained gels show polypeptides that are quantitatively important either because they are synthesized at a high rate or they accumulate as a result of reduced catabolism. Autoradiogram of pulse labelled cell proteins, on the other hand, permit detection of polypeptides that have arisen as a result of new synthesis. The intensity of spots on the autoradiogram can be assumed to be proportional to the quantity of protein newly synthesized.

The results of gel electrophoresis (Fig. 2) are summarized in Table I.

Spots P4, P9, P10 (group A) : these were not detectable in the control gels whether visualised by staining or by autoradiography indicating that these polypeptides were not normally synthesized by the cells either before or during labelling with ^{35}S. However, when the cells were pretreated 24 hrs with ecdysterone, all these three spots appeared on the autoradiogram indicating that their synthesis was hormone induced. The synthesis of polypeptide P4, in the presence of the hormone, was apparently great enough to become detectable even by staining.

Spots P1, P2, P3, P5, P8 (group B) : these were present in normal cells at levels which could be barely detected when tested by staining (0 or + intensity).


IEF
A
IEF
B
C
D
E
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11

However, under labelling with ^{35}S, the same spots revealed a faint intensity suggesting that the polypeptides implicated were characterized by a high turnover and consequently did not accumulate in the cell and became detectable under pulse labelling. Administration of ecdysterone had the effect of enhancing the synthesis of these polypeptides, P1 and P2 becoming detectable even by staining.

Spots P7, P11 (group C) : these were major spots of control cells ; they completely disappeared on autoradiogram of hormone treated cells suggesting that their synthesis was inhibited by ecdysterone.

In order to investigate the kinetics of ecdysterone effect on the labelling of polypeptides, the following experiment was designed. Cells were treated with ecdysterone 1, 4, 21, 48 hrs before the addition of ^{35}S-methionine. Incubation was continued in the presence of ecdysterone and ^{35}S-methionine for another 3 hours. Control cells were also incubated with ^{35}S-methionine for three hours but without previous ecdysterone treatment.

Up to 4 hrs of treatment, the hormone produced no difference in protein pattern either on stained gels or on autoradiograms. Labelling of spots P1, P2, P3, P4, P9 was increased after only 7 hrs of treatment and enhanced progressively by 24 hrs and 52 hrs of treatment (Fig. 3). Intensity differences due to ecdysterone treatment for the spots P5, P8, P10 were not detectable after 7 hrs of treatment but became evident after 24 hrs and more marked after 52 hrs. The polypeptides of spots P7 and P11 were present in control cells, at 4 hrs and 7 hrs but had disappeared at 24 hrs and 52 hrs of treatment as revealed by autoradiography. However no detectable differences were noted for these polypeptides in Coomassie blue stained gels (not shown). This would indicate that the disappearance of polypeptides P7 and P11 following ecdysterone treatment was probably not due to a post-translational modification of the protein into a product with changed electrophoretic migration.

Spots P5 and P6 have been further characterized. They have the same molecular weight of 44 000 daltons, an isoelectric point similar to that of actin purified from adult chick muscle (5.72), with P6 slightly more acidic than P5, and a high affinity for DNase I immobilized on agarose. These two spots probably belong to the class of actins. They appeared as radioactive spots in autoradiograms (Fig. 2 lower panels), but, only P6 could be visualized on Coomassie blue stained gels (Fig. 2 upper panels) suggesting that P5 is a transient form which

Figure 2. Two dimensional gel electrophoresis of proteins from control and ecdysterone treated (0.1 µM, 24 hrs) Kc 0 % cultured cells. (A and B), stained gels for control and 24 hrs treated cells respectively ; (C and D) autoradiograms of gels A and B respectively ; (E) diagram of the positions of unchanged polypeptides (unfilled spots) and of ecdysterone controlled polypeptides (filled spots). Molecular weight standards shown on the right of the gel are the same as those in the one dimensional gel (Figure 1).

TABLE I

Changes in protein pattern of Drosophila melanogaster Kc 0 % cells as a result of ecdysterone treatment

Polypeptide Groups	Polypeptide Spots	Approximate Molecular weight	As revealed by staining		As revealed by autoradiography		Time of treatment when hormone effect was detected
			control	treated	control	treated	
A	P4	52 000	0	+	0	++++	7 hrs
	P9	21 500	0	0	0	++	7 hrs
	P10	32 000	0	0	0	++	24 hrs
B	P1	70 000	0	+	0/+	+++	7 hrs
	P2	68 000	0	+	0/+	+++	7 hrs
	P3	58 000	+	+	+	+++	7 hrs
	P5	44 000	0	0	++	+++	24 hrs
	P8	40 000	0	0	+	++	24 hrs
C	P7	62 000	++	++	+++	+	24 hrs
	P11	51 000	++	++	+++	+	24 hrs

The symbols 0, +, ++, +++, ++++ are employed here to indicate a semiquantitative measure of spot intensity as detected visually, 0 being classed undetectable and ++++ being the most abundant class. This evaluation was based on visual examination of gel electrophoregrams in Fig. 2.

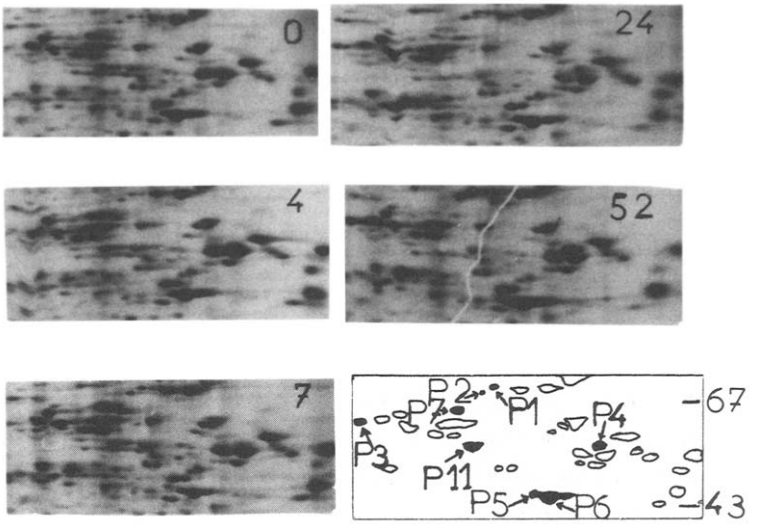


Figure 3. Relevent parts of two dimensional gel autoradiograms of control (0) and ecdysterone treated Kc 0 % cells. Ecdysterone treatment (0.1 µM was carried out for 4, 7, 24 and 52 hrs as indicated on the photographs.

does not accumulate. Indeed, on autoradiograms, relative intensity of P5 and P6 were the same in control, 4 hr and 7 hr ecdysterone treated cells, but were enhanced after 24 hrs for P5 and after 52 hrs for both (Fig. 3).

DISCUSSION

Whatever their precise origin, several *Drosophila* permanent cell lines have been shown to exhibit marked changes in morphology, behaviour and growth after ecdysterone treatment. Best-Belpomme et al. (13) have reported the appearance of one new band of protein when cells were treated with ecdysterone. However their analysis was carried out in gels under nondenaturing conditions. In the present work, we describe the specificity and the timing of labelling of a number of proteins in Kc 0 % cells. Of over 350 polypeptides detected by two dimensional electrophoresis, changes were observed in fewer than 4 %, after 24 hrs of hormone treatment. The earliest effect of the hormone was observed between 4 and 7 hrs after treatment and continued until 52 hrs at least. Clearly, the vast majority of Kc 0 % genes are unresponsive to the hormone, indicating a very limited and therefore, highly specific action.

We were able to distinguish three types of response to ecdysterone : a) new synthesis of polypeptides which were not present or present below detection in the controls. b) increased synthesis of those polypeptides which were clearly present in the control cells at low levels. c) inhibition of synthesis of those polypeptides which were present in easily detectable amounts in control cells.

According to three usual criteria, molecular weight, isoelectric point and affinity for DNase I immobilized on agarose, polypeptides P5, P6 were identified as actins II and III. P6 and not P5 could be visualized on stained gels. Under the labelling conditions, P5 appeared as a smaller radioactive spot than P6. This result is in agreement with Berger (12) who described actin III as a precursor of actin II in cultured *Drosophila cells*. In our experiments, relative intensity of the two radioactive spots was the same in control cells as in treated cells for 4 and 7 hrs. This intensity was enhanced after 24 hrs or longer of treatment. This suggests that the synthesis of actin III and perhaps its conversion to actin II are enhanced in ecdysterone treated cells.

We were unable to detect the presence of actin I in our gel analysis either in control or ecdysterone treated cells. Actin I, unlike actin II and III, is a constituent of muscle cell only, and occurs both in myogenic culture and *in vivo*. This suggests that the Kc 0 % cells, whose origin is unknown, is at least not related to myogenic cells. Increased synthesis of actin due to ecdysterone could be related to morphological changes which have been previously observed after more than 24 hrs of treatment with this hormone (4). Six actin genes have recently been localized in *Drosophila* (14, 15). It will be interesting to know if the same actin genes are functional in control and hormone treated cells.

Our results show that action of ecdysterone is less drastic than heat shock effect. Differences in protein synthesis after ecdysterone treatment with physiological concentration of hormone (0.1 µM) can be observed in a short period of time. As early as 7 hrs, when changes in the pattern of protein synthesis are observed, treated cells cannot be distinguished morphologically from untreated cells. Recently, it has been reported (16) that at least 3 polypeptides are induced after 4 hrs of treatment with 1 µM of ecdysterone in a Kc cell line. Other authors have also reported (17) that only a small number of proteins is ever affected by ecdysterone (1 µM) after three days of treatment in four different cell lines. Only six proteins whose synthesis is increased by ecdysterone are common in all the cell lines examined, including the two cytoplasmic actins and at least one of the tubulins. All these results are in agreement with our data obtained with Kc 0 % cells treated by 0.1 µM of ecdysterone. This concentration dependance of the Kc cell response, the limited changes in macromolecular synthesis promoted by ecdysterone and the fact that ecdysterone can induce as well as repress polypeptides synthesis are similar to those described for other *Drosophila* tissues. Transient stimulation of RNA synthesis between 3 hrs and 20 hrs of treatment (18) described for this cell line could also be related to our results. In cultured cell line, the primary action of ecdysteroïd hormones could occur, in part, at the transcriptional level as described in a number of *Drosophila* steroid responsive tissues such as imaginal discs or fat body (3). Whether the changes in pro-

tein synthesis observed in the present work result from synthesis of new messengers or from selective translation of a constant pool of mRNA in under investigation.

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