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Note

Effect of endogenous protease activity on sodium dodecyl sulphate electrophoresis of homogenates of peach-potato aphids (*Myzus persicae*, Sulz)

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We studied the esterases and other proteins of *Myzus persicae* by electrophoresis[1,2] as part of a project to characterise the biochemical nature of resistance of these insects to organophosphorus and carbamate insecticides. The non-denaturing buffers of Davis[3] and Williams and Reisfeld[4] gave clearly-defined enzyme[1] and protein[2] bands on polyacrylamide gels from homogenates of single aphids containing 30–40 μg total protein. However, when run on sodium dodecyl sulphate (SDS) gels in the buffer system of Laemmli[5], nearly all the material staining with Coomassie brilliant blue appeared at the front with the marker dye indicating that the proteins had been degraded to low-molecular-weight peptides.

Comparable degradation was not apparent when aphid haemolymph samples were characterised by SDS electrophoresis[6], but collecting sufficient haemolymph for routine analysis of many populations is not feasible and information about only one fraction of the total insect proteins is obtained. For example, in that work, only 12 protein bands were detected, whereas we have found more than 60 bands from homogenates of whole aphids.

We describe a simple procedure to avoid protein degradation in whole aphid homogenates. This might be applicable to other organisms, especially those which are homogenised *in toto* with the consequent risk of releasing potent proteases.

EXPERIMENTAL

Aphids

The origin and rearing of the *M. persicae* clones were described previously[7].

Chemicals

The protease inhibitors, diisopropyl phosphorofluoridate (DFP), phenylmethylsulfonyl fluoride (PMSF) and soybean trypsin inhibitor (STI), and the protein mol. wt. standards (SDS-6H) were obtained from Sigma. Appropriate precautions were taken when handling DFP which is volatile and extremely toxic.

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Electrophoresis

In the standard procedure, ten aphids (approximately 3 mg) were homogenised with a glass rod in distilled water (10 μl) in a 400-μl polyethylene microcentrifuge tube. Loading buffer (10 μl), containing bromocresol purple as tracking dye, was added to give the final concentrations, SDS (2%), 2-mercaptoethanol (5%), urea (4.25 *M*) and Tris–HCl (62.5 m*M*, pH 6.8); the tube was then heated in a boiling water bath for 2 min. After centrifuging for 3 min at approximately 9,000 *g*, the whole supernatant was run on a 3 mm thick polyacrylamide gel slab with 3% stacking and 10% running gels in the buffer system of Laemmli[5]. Proteins were located by staining with Coomassie brilliant blue R.

The standard procedure was varied as described below to confirm and then overcome the protein degradation suspected from preliminary experiments.

RESULTS AND DISCUSSION

Fig. 1a shows a gel stained for protein after running an aphid homogenate prepared by the standard procedure. Bands of higher intensity were expected since approximately 300 μg protein were applied to the gel and much less than this (100 μg) gives good bands when run on the same gels in non-denaturing buffers. However

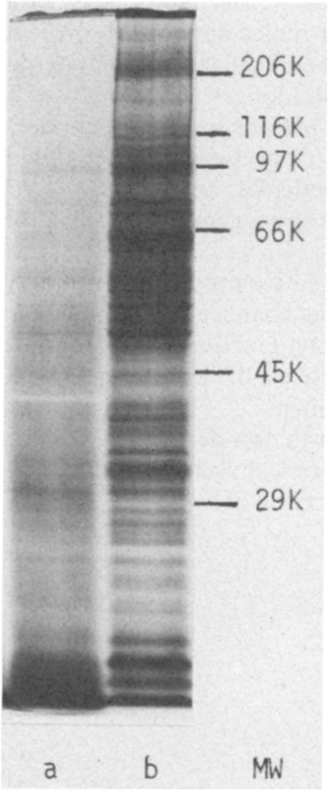

Fig. 1. SDS electrophoresis gel stained for protein after running homogenates of 3 mg aphids (clone G6) prepared (a) by the standard procedure (see text) and (b) by immersing the intact aphids in 10 μl water and heating at 100°C for 10 min before homogenising and then treating as in (a). MW indicates the positions of the standards myosin, β-galactosidase, phosphorylase B, albumin, ovalbumin and carbonic anhydrase, in order of decreasing molecular weight.

the presence of a dark band at the front, corresponding to peptides of mol.wt. less than 10,000, suggested that extensive protein degradation had occurred.

To confirm this, approximately 5 μg of a protein (carboxylesterase E4) purified (see ref. 2) from resistant aphids were added to the aphid homogenates at various stages of the standard procedure. This amount of E4 gives an intense band on SDS gels[2] corresponding to a mol.wt. of 65,000. When denatured separately, by boiling for 2 min in SDS, mercaptoethanol and urea, and then added to a similarly denatured aphid homogenate, the E4 band was as intense as when run alone. However, if E4 was mixed with the homogenate immediately before denaturing, no such band was detected. This suggested that the aphid homogenate had very strong protease activity resistant to high temperature since the exogenous E4 was degraded in less than 2 min in a boiling water bath.

We therefore tried to prevent this degradation by incorporating protease inhibitors in the buffer and incubating the homogenates at room temperature for 10 min before denaturing at 100°C. STI (up to 100 μg per homogenate), PMSF (10 m*M*) and DFP (0.1 m*M*) alone or in combination had no significant effect on the result.

Similarly, boiling the homogenates for 10 min before adding SDS, mercaptoethanol and urea and reboiling only partially prevented the protein degradation. However, when the aphids (3 mg) were immersed in water (10 μl) and placed in a boiling water bath *before* homogenising, many higher-molecular-weight protein bands were apparent on the gel (Fig. 1b) and little low-molecular-weight material was present. The optimum duration of boiling was 10 min; longer periods caused a large amount of protein to remain on the origin, shorter periods led to protein degradation. Fig. 1 shows results obtained with aphids of clone G6; all other aphid clones examined, whether susceptible or resistant to insecticides, behaved similarly. Preboiling the intact aphids also preserved the integrity of exogenous E4 added at any stage before chemical denaturation.

The protease(s) thus appears to be inactive against native proteins, but rapidly hydrolyses them when they are partially denatured, whether chemically or by boiling. Other proteases are known to behave similarly[8]. Proteolytic activity persists for a short time when homogenates are boiled even in the presence of denaturing chemicals, but after such treatment for 2 min no activity remains.

It should be stressed that proteolysis does not occur in non-denaturing buffers, and aphid homogenates can be characterised electrophoretically under such conditions with no apparent protein degradation or loss of enzyme activity.

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