The effect of conventional and artificial diet on esterase band pattern in *Myzus persicae* (Sulzer)

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Summary. The esterase band pattern on a conventional potato leaf diet differs markedly from that in aphids of the same strain culture on an artificial diet. Possible reasons for these differences are discussed.

The influence of diet on aphid biology is considerable. The fecundity, size and longevity all vary depending on the food source[3]. In this paper we report changes in esterase band pattern as visualized by polyacrylamide gel electrophoresis which have occurred in a strain kept on artificial diet[4] as compared to a culture of the same strain kept on potato leaves.

The effect of diet on esterase band pattern has become important because of the involvement of an esterase (E4) in organophosphate insecticide resistance in this species. This enzyme has been shown to break down paraoxon[5] and is the predominant basis of organophosphate resistance in *M. persicae*. Clearly if diet can affect levels of certain esterases, then plant varieties might affect the level of susceptibility to organophosphate insecticides of field populations of aphids. The work here merely demonstrates beyond doubt that diet can have a profound effect on esterase band pattern.

Materials and methods. It has not been possible to adapt any insecticide resistant strains of *M. persicae* to artificial diet but one susceptible strain, Y5, has been reared successfully on artificial diet for over 2 years. This strain originated from the departmental glasshouse and was a low esterase susceptible strain. A few individuals of this strain were taken and recultured on potato leaves (var. Chara). The strain did not culture well on the plant material initially and it took several generations to reach normal size. The artificial diet used was that of Dadd and Mittler[4] and consists essentially of a 15% sucrose solution mixed with 21 amino acids, some vitamins, salts and EDTA in appropriate concentrations.

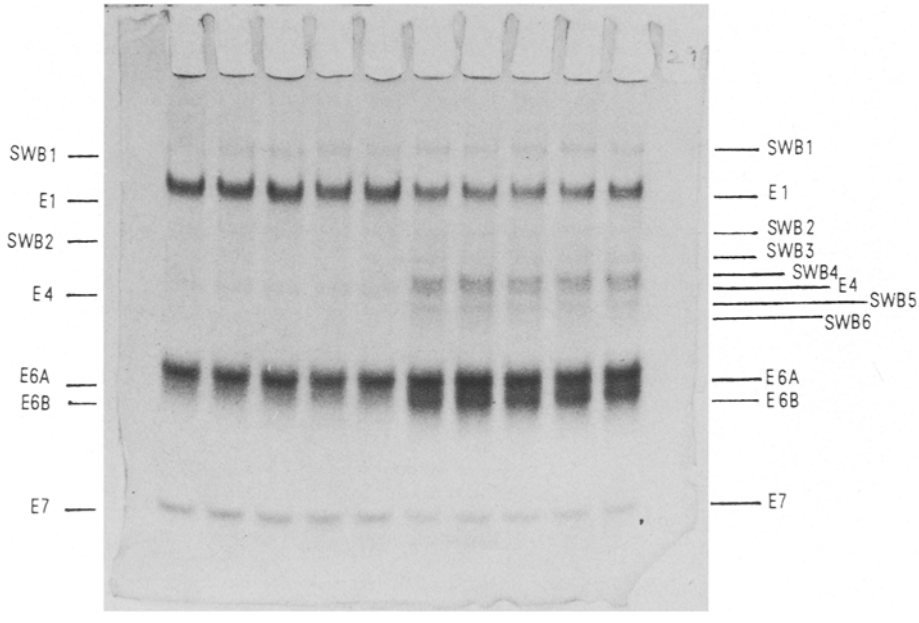
Apterae from both food sources were harvested and a bulk homogenate at 60 µg aphid/µl was made up in a solution of 10% sucrose, 0.5% Triton X-100 and coloured with bromophenol blue marker dye. This was loaded onto 7.5% polyacrylamide vertical slabs on a tris-borate-EDTA continuous buffer system and stained for esterases. The technique has been described previously[6].

Results. The results show that 2 types of effect occur. The first involves changes in band intensity and the second involves the presence of different bands (figure). Band SWB1 and E1 are constant in both treatments whilst E4 and E6B are both stronger staining in the diet culture. What is more unusual is that the culture on artificial diet shows 4 extra bands, 2 on either side of the E4 band - these are marked SWB3-6.

Conclusions and discussion. It is clear that the food source can effect the esterase band pattern considerably. There could be a number of explanations for these results. One explanation could be that the type of symbiont growing within an aphid may be selected on the artificial diet for different characteristics including the type of esterase produced. Esterase production by symbionts has been found by Nur[7] in Coccids and it is possible that parthenogenetic organisms rely more on symbiotic microorganisms as a source of variation.

Alternatively induction of aphid esterases that are present in a plant-reared aphid, perhaps in small quantities, but are not fully expressed normally could be the answer. Esterases may be needed for metabolising nutrients. The nutrients of the artificial diet may require special esterases for metabolism and this need may result in enzyme induction. The change in E4 and E6B levels with this treatment lends some support to this idea.

Finally the extra bands could be due to strong selection on artificial diet for loci in the heterozygous state whilst potato leaves may select for homozygotes. For example if the E4 locus was a tetramer then 4 extra bands would be produced by a heterozygote. Alternatively 2 duplicate E4 loci in the heterozygote state with different E4 alleles at each locus



5 esterase-stained samples of strain Y5 from potato leaves (5 left pockets) together with 5 samples of the same strain on artificial diet (5 right pockets) run on a vertical acrylamide gel. There are 4 extra bands on the right (artificial diet). E1, E4, E6 and E7 labelling is after Devonshire[5], SWB1-SWB6 are less well defined bands some of which are unique to our gels.

would give the same result if E4 was a dimer. This latter suggestion is rather unlikely in a parthenogenetic system and although we have reported heritable changes within a strain previously[6], the system dealt with there was almost certainly an exceptional case.

The final answer awaits further work but such diet induced changes in enzyme polymorphism indicate it is important to think carefully in interpreting findings of variations in natural populations on which so much of modern evolution theory is based.

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Collagen treated with (+)-catechin becomes resistant to the action of mammalian collagenase[1]

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Summary. Treatment of radioactively labeled guinea-pig skin soluble collagen or calf skin collagen with the flavonoid (+)-catechin makes the collagen resistant to the action of mammalian collagenase but not to the action of bacterial collagenase. Complete resistance to the action of the mammalian enzyme may be achieved by incubating 0.6 mg of collagen (dry weight) with 0.1 mM (+)-catechin, followed by dialysis to remove the unbound flavonoid. Since incubation of the mammalian enzyme with (+)-catechin does not inhibit its activity, it is postulated that (+)-catechin binds tightly to collagen and modifies its structure sufficiently to make it resistant to enzyme degradation.

The flavonoid (+)-catechin has been proposed as a collagen stabilizer because of its postulated ability to form hydrogen bonds and possibly cross-links among different collagen chains[2]. Because of this claim, in the past years we have repeatedly added (+)-catechin to the culture medium of fibroblasts derived from patients with various inherited diseases considered to involve the structure and function of collagen. When the collagen synthesized by the cultured fibroblasts of those patients was excessively soluble, the addition of (+)-catechin to their culture medium decreased its abnormal solubility[3]. In other studies, administration of (+)-catechin to experimental animals treated with β-aminopropionitrile or 3′,3′-iminodipropionitrile has produced similar, beneficial changes in collagen solubility and has decreased the extent of lesions[4,5].

Despite these practical results, no evidence has been provided thus far for a physical interaction between collagen and (+)-catechin. In the present communication we demonstrate that incubation of soluble collagen with (+)-catechin results in the formation of a tightly-bound complex which becomes resistant to the action of mammalian collagenase.

Materials and methods. Human skin fibroblasts, obtained from patients by punch skin biopsies after obtaining informed consent, were cultured in minimum essential medium containing Earle's base and 10% fetal calf serum, as described previously[6]. In order to harvest the mammalian collagenase produced by these fibroblasts, the cells were cultured in serum-free medium for 2 days, as described by Bauer et al.[7]. Thereafter, the medium was dialyzed exhaustively at 4 °C against 0.01 M Tris-HCl buffer, pH 7.5, containing 0.1 mM calcium chloride. The retentate was concentrated to $^1/_{10}$ of its volume by lyophilization and aliquots of this crude enzyme preparation, containing 200 µg protein/ml, were used as described.

The activity of the enzyme was measured using either ^{14}C-acetylated collagen or [^{14}C]glycine-labeled guinea-pig skin soluble collagen. The former was prepared by treating acid-soluble collagen from calf skin with [1-^{14}C]acetic anhydride (New England Nuclear, Boston, MA) according to the method of Gisslow and McBride[8]. The ^{14}C-glycine-labeled guinea-pig skin soluble collagen was a gift of Dr M.H. Dresden of this department.

The incubation mixture consisted of acetylated collagen, 0.6 mg, 1800 cpm, in 0.3 ml of 0.01 M Tris-HCl buffer pH 7.8 containing 0.4 M sodium chloride; crude enzyme preparation, 40 µg protein, in 0.2 ml of 0.1 M Tris-HCl buffer pH 7.5; 0.5 M Tris-HCl buffer pH 7.5, 70 µl; 1.0 mM calcium chloride 70 µl and water, 60 µl, to a final volume of 0.7 ml. Incubation was performed at 37 °C for 5 h. Thereafter, 1 mg of bovine serum albumin in 0.1 ml of water was added as a carrier and proteins were precipitated with trichloroacetic acid (5% final concentration) at 4 °C for 20 min. The bulk of the precipitate was removed by centrifugation at 700 × g for 15 min and the supernatant collected was centrifuged again at 15,000 × g for 2 h at 4 °C. A 0.6-ml aliquot of the clear supernatant was added to 10 ml of Aquasol (New England Nuclear) and counted in a Packard Tri-Carb spectrometer.

When the ^{14}C-glycine-labeled substrate was employed, 0.2 mg, 3300 cpm, in 50 µl Tris-HCl buffer, were added to 0.2 ml of enzyme solution in presence of 0.1 mM calcium

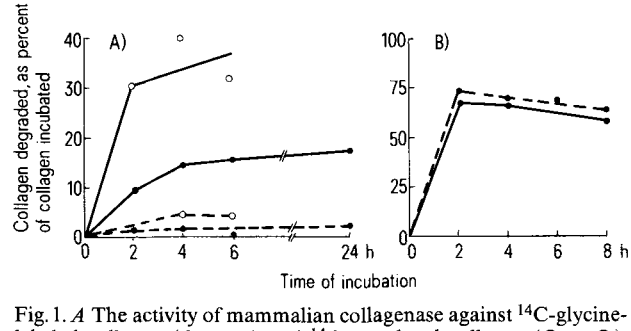

Fig. 1. *A* The activity of mammalian collagenase against ^{14}C-glycine-labeled collagen (○——○) and ^{14}C-acetylated collagen (●——●). The broken lines indicate the activity of the enzyme against the same substrates pretreated with (+)-catechin. *B* The activity of bacterial collagenase against ^{14}C-acetylated collagen (●—●) and against the same substrate pretreated with (+)-catechin (●– –●).