Degradation of the Dispersion-Inducing Substances in the
Alimentary Canal of the German Cockroach,
Blattella germanica (LINNÉ) (Blattaria: Blattellidae)

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The dispersion-inducing repellent activity in the alimentary canal of the German cockroach was studied. Aqueous extracts of the oral secretion and of the foregut and midgut contents of the German cockroach had certain degrees of repellent activity to first instar nymphs of the same species when applied to preference tests. By gel filtration column chromatography, the repellent activity in the foregut extract was eluted into higher and lower molecular weight fractions (HMF and LMF), representing the same elution pattern observed in the oral secretion. These were supposed to be identical with the dispersion-inducing substances secreted in the saliva. In the midgut extract, only the LMF had repellent activity, and in the hindgut the activity in both the HMF and LMF was insignificant. On the other hand, aggregation activity was observed in the MeOH extract of the rectum and feces. Therefore, it is considered that the dispersion-inducing substances, or pheromones, are secreted in the saliva, and when swallowed they are degraded in the alimentary canal. It is supposed that they do not interfere with the role of aggregation pheromone secreted from the rectal pads.

INTRODUCTION

In overcrowded condition, the German cockroaches secrete dispersion-inducing substances (SUTO and KUMADA, 1981; NAKAYAMA et al., 1984; ROSS and TIGNOR, 1985, 1986). The substances are contained in the saliva and are soluble in water. They were fractionated into at least two fractions of different molecular weights on Sephadex column chromatographies (NAKAYAMA et al., 1984). The repellent activity was found also in the crude extract of the crop (SUTO and KUMADA, 1981; NAKAYAMA et al., 1984).

The German cockroaches also secrete an aggregation pheromone from the rectal pad cells into the lumen (ISHII and KUWAHARA, 1967, 1968). This is discharged with the feces.

In this paper, we report changes in the repellent activity in the alimentary canal of the German cockroach.

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MATERIALS AND METHODS

German cockroaches, *Blattella germanica* (L.), reared in our laboratory for several generations at 25°C and 70% relative humidity with water and mouse food (Nippon Clea Co. Ltd.) were used.

The oral secretion was collected by confining 100 adults of both sexes in a 200 ml glass beaker as described previously (SUTO and KUMADA, 1981; NAKAYAMA et al., 1984). Aqueous extracts were prepared from the crops, midguts (including caeca), and hindguts together with their contents dissected from 50 adult females. Fifty each of the same parts were homogenized in 3 ml of saline solution in a glass homogenizer, to which 9 ml of ethyl ether was added; it was shaken vigorously, then centrifuged at 1,500 rpm for 5 min to separate the ether extract. This procedure was repeated three times before the aqueous extract was collected. The extracts were used for preference tests and gel filtration analyses.

In one experiment, the tissues and contents of the foregut, midgut and hindgut were separated with forceps, and each of them was extracted with saline solution and then with ether as described above.

In another experiment, the hindgut was further divided into the ileum-colon and the rectum. Extraction from these parts and 0.2 mg of the fresh feces was first made with methyl alcohol according to ISHII and KUWAHARA (1967), then with saline solution.

Gel filtration was performed by Sephadex G-100 column chromatography (1 × 40 cm). Three milliliters of the eluate was collected in each fraction tube. Absorbance at 280 nm was measured for protein content, then the preference tests were made with the appropriate fractions.

Evaluation of the preference tests was made as described previously (SUTO and KUMADA, 1980; NAKAYAMA et al., 1984).

RESULTS

The results of preference tests with first instar nymphs of the German cockroach and the extracts prepared from the foreguts, midguts and hindguts together with their contents are shown in Table 1-I. The repellent activity in the oral secretion and crop extract (NAKAYAMA et al., 1984) was confirmed for reference. The aqueous extract of the midgut had repellent activity, whereas that of the hindgut showed only insignificant activity. Comparison of the repellent activity of the tissues to the contents is shown in Table 1-II. Significant repellent activity was observed in the aqueous extracts of the foregut- and midgut contents, but the activity was insignificant in the extract of the hindgut contents, while the aqueous extracts from the tissues of the foregut, midgut and hindgut did not show any significant activity. These results indicate that the repellent substance(s) exists in the contents of the foregut and midgut.

Neither aqueous nor MeOH extracts of the ileum-colon attracted the tested nymphs, but the MeOH extract of the rectum caused aggregation. Further, the aggregation activity was more significant in the MeOH extract of the feces (Table 2).

Results of column-chromatographic analysis of the extracts of the oral secretion, foregut, midgut, hindgut and feces are shown in Fig. 1. The repellent fractions from the oral secretion were obtained at around tube number 6 (tentatively designated as the

Table 1. Repellent responses of the first instar nymphs of German cockroach to aqueous extracts prepared from oral secretion and dissections of alimentary canal of adults of the same species

Samples ^a	No. of test	Number of nymphs aggregated on filter paper		Aggregation index ^c (mean±S.E.)
		Impregnated	Control	
I				
Oral secretion	10	12	81	−0.803±0.223
Foregut ^b	10	15	75	−0.753±0.221
Midgut ^b	8	9	67	−0.719±0.247
Hindgut ^b	10	20	61	−0.381±0.261
II				
Foregut				
Tissue	10	54	52	−0.051±0.269
Contents	10	18	66	−0.557±0.232
Midgut				
Tissue	10	29	51	−0.269±0.299
Contents	10	3	89	−0.933±0.034
Hindgut				
Tissue	10	40	26	+0.245±0.244
Contents	10	33	48	−0.368±0.169

^a A sheet of filter paper (2 $\times$ 5 cm) was impregnated with the aqueous extracts obtained from ten cockroaches.

^b Sample was extracted from the tissue and the contents together.

^c Negative values indicate repellency, whereas positive values show aggregation.

Table 2. Preference tests with aqueous and methanol extracts from hindgut portions and feces of adult German cockroach for the first instar nymphs of the same species

Samples ^a	No. of tests	Number of nymphs aggregated on filter paper		Aggregation index ^b (mean $\pm$ S.E.)
		Impregnated	Control	
Ileum-Colon				
Aqueous	10	33	29	-0.052 ± 0.199
MeOH	10	66	36	$+0.289 \pm 0.215$
Rectum				
Aqueous	9	27	39	-0.233 ± 0.289
MeOH	10	86	21	$+0.579 \pm 0.180$
Feces				
Aqueous	10	63	31	$+0.360 \pm 0.139$
MeOH	10	81	6	$+0.842 \pm 0.068$

^a Aqueous and MeOH extracts were prepared from the ileum-colon or rectum together with their contents and from the fresh feces as described in MATERIALS AND METHODS.

^b Positive values indicate aggregation on the impregnated paper, whereas negative values show repellency.

higher molecular weight fraction, HMF) and tube number 12 (the lower molecular weight fraction, LMF) (Fig. 1a). Their filtration patterns were quite similar to the Sephadex elution pattern of the extract of the oral secretion reported previously (NAKAYAMA et al., 1984).

Fig. 1. Sephadex G-100 elution patterns of the aqueous extracts of *B. germanica* and repellent activity. Samples: a) oral secretion, b) foregut, c) midgut, d) hindgut and e) feces. Column size: 1×40 cm. Fraction size: 3.0 ml/tube. Bioassay: 0.2 ml of the solution was applied to a piece of filter paper (2×5 cm) and bioassayed for the first instar nymphs of *B. germanica*. Aggregation index: mean values of 10 replications are shown. Negative values indicate repellent activity, whereas positive values show aggregation. ● represents mean values of significant repellency, ○ indicates mean values of insignificant activity.

In the foregut extract, the gel filtration patterns of the repellent fractions were similar to those in the oral secretion, being filtrated into two fractions, HMF and LMF (Fig. 1b). However, the gel filtration pattern of the midgut extract differed to some extent; the repellent activity in HMF was reduced and only LMF had a significant repellent activity (Fig. 1c). Except for the slight repellency observed in the HMF of the hindgut extract, none of the fractions of the hindgut and feces showed any significant repellent activity (Fig. 1d and e).

DISCUSSION

Some cockroach pheromones are produced by the glandular organs associated with the digestive system and passed in the feces (ISHII and KUWAHARA, 1967; BODENSTEIN, 1970; TAKAHASHI and KITAMURA, 1976). The dispersion-inducing substances, or repellent-active pheromones, are also contained in the saliva and crop of the German cockroach (SUTO and KUMADA, 1981; NAKAYAMA et al., 1984).

The present study was undertaken to see whether the repellent activity in the saliva was changed while passing through the alimentary canal. The foregut, midgut

and hindgut were dissected from the German cockroaches and their extracts were examined for the repellent activity. The activity was found in the aqueous extracts of the foregut and midgut (Table 1-I), particularly in the extracts from the contents of these portions (Table 1-II). In the hindgut, however, no significant repellent activity was observed; instead, aggregation pheromone activity was found from the rectum extract (Table 2). By gel filtration column chromatography on Sephadex G-100, the repellent activity in the foregut extract was recognized in both the HMF and LMF, with the elution pattern quite similar to that of the oral secretion; however, in the midgut extract, only LMF showed the repellent activity (Fig. 1 a-c). In the extracts of the hindgut and feces, the repellent activity diminished in both fractions (Fig. 1 d and e).

The salivary secretion of the German cockroach is a clear, non-viscous fluid containing various substances such as amylase, cellulase, maltase, mucous component, and dispersion-inducing substances. The foregut of the cockroach has a thick continuous epicuticle that provides for the low permeability of aqueous fluids. Except for the possible absorption of lipids, it plays no role in the secretion or absorption (BIGNELL, 1981). Digestion occurs in the foregut by the saliva swallowed with food and by some digestive enzymes passed forward from the midgut (CORNWELL, 1968). Because the aqueous extract of the mouse food, which was used for rearing the cockroaches, has no repellent activity to the first instar nymphs of the German cockroach, and because of the similarity of the oral secretion and foregut in the gel filtration patterns of their repellent activity, the substances responsible for the activity in the foregut are supposed to be derived from the saliva.

In the midgut, many kinds of digestive enzymes such as amylase, invertase, maltase, lipase, esterase and proteases are secreted, and it is here that the main absorption of digested products takes place (BIGNELL, 1981). Since the repellent activity in HMF of the midgut extract was reduced to some extent, it is supposed that the dispersion-inducing substance in the HMF was inactivated through digestion or absorbed into the body or both.

The principal function of the hindgut is to reabsorb water or certain ions from the excreta (WALL, 1977). Another unique function of the hindgut is to secrete the aggregation pheromone from the rectal pads (ISHII and KUWAHARA, 1967, 1968) as confirmed in the MeOH extract of the rectum (Table 2). The reduction of the repellent activity in the hindgut was not attributed to masking by the aggregation pheromone, because the ileum-colon, the fore portion of the hindgut, showed neither repellent nor aggregation activity (Table 2).

In conclusion, it is hypothesized that the dispersion-inducing substances secreted in the saliva are swallowed and gradually degraded in the alimentary canal, consequently not interfering in the aggregation pheromone secreted from the rectal pads.

More detailed biochemical characters of the dispersion-inducing substances and their ecological significance in the cockroach behavior are being studied.

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