Biophysical properties of the surface of desiccation-tolerant mutants and parental strain of the entomopathogenic nematode *Heterorhabditis megidis* (strain UK211)

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SUMMARY

Entomopathogenic nematodes (EPN) are useful biological control agents of insect pests. However, the infective juvenile (IJ) stage which is the only stage to occur outside the host is susceptible to environmental extremes such as desiccation. We have isolated desiccation-tolerant strains of the EPN *Heterorhabditis megidis*. In this paper we describe the surface properties of these desiccation-tolerant mutants. Heterorhabditid IJs retain the sheath of the previous larval stage. The mutant lines possess alterations in the surface properties of the sheath. Differences were observed in fluorescent lipid analogue insertion into the surface of the sheath. Furthermore, cationized ferritin-binding studies demonstrated that the mutant lines possessed an increase in net negative surface charge. Removal of the surface layer of the sheath resulted in the loss of the mutant phenotype and in a reduction in the desiccation tolerance of the parental strain. Therefore, the negatively charged 'surface coat' appears to play an important role in the desiccation tolerance of *Heterorhabditis* species.

Key words: desiccation, *Heterorhabditis megidis*, cuticle, sheath.

INTRODUCTION

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are important biological control agents of insect pests. The infective juvenile (IJ) is the only stage to occur free in the soil and is the most environmentally resistant stage. Its function is to locate and infect a new insect larva host. However, EPN IJs are susceptible to environmental extremes (Gaugler, 1988; Kaya, 1990) so their commercial exploitation is limited at present to small-scale agricultural markets such as protected horticulture. The genetic improvement of EPNs has, therefore, been considered as a method of improving the environmental tolerances of EPN IJs, thus broadening the agricultural field in which they can be marketed (reviewed by Burnell & Dowds, 1996).

We have reported the isolation of mutant lines of *Heterorhabditis megidis* strain UK211 that have increased tolerance to desiccation at low humidities (O'Leary & Burnell, 1997). We demonstrated that IJs of the mutant strains had a lower rate of water loss than the parental strain at 0% relative humidity (RH). The mutant strains also possessed an altered tolerance to detergent action, being more susceptible than the parental strain to treatment with anionic detergents while being more tolerant than the parental strain to treatment with cationic detergents. We hypothesized that the change responsible for the mutant phenotype occurred in the outer cuticle of the IJs.

The nematode cuticle acts as an exoskeleton but is also an important barrier between the nematode and the external environment. During their developmental cycle nematodes moult and form a new cuticle at each juvenile stage. However, many nematode juvenile stages such as the IJs of both families of entomopathogenic nematodes (*Heterorhabditis* spp. and *Steinernema* spp.) can also retain the cuticle of the previous juvenile stage (Poinar, 1979). This retained cuticle or sheath is more tightly associated with heterorhabditid IJs than steinernematid IJs, which suggests that the sheath may play a more important role in the survival of IJs of *Heterorhabditis* spp. This is further supported by the work of Campbell & Gaugler (1991*a*) who demonstrated that the sheath of heterorhabditid IJs protected them from desiccation but that the presence of the sheath on steinernematid IJs did not affect their desiccation tolerance.

The nematode cuticle is covered by a predominantly lipid epicuticle (reviewed by Wright, 1987; Maizels, Blaxter & Selkirk, 1993; Roberts &

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Modha, 1997). The epicuticle frequently displays a trilaminate (dark-light-dark) pattern similar to that of plasma membranes; however, the epicuticle is not homologous to plasma membrane since the lateral mobility of surface lipid is highly restricted (Kennedy *et al*. 1987) and the lipid surface of the epicuticle differs in the length and nature of the lipophilic probes which will insert into it (Kennedy *et al*. 1987; Kennedy & Proudfoot, 1993; Proudfoot *et al*. 1993). Reports have shown that the outermost layer of the epicuticle in the anhydrobiotic juveniles of *Anguina tritici* (Bird & Buttrose, 1974) and the J4 stage of *Ditylenchus dipsaci* (Wharton & Barrett, 1985) undergoes alterations in response to desiccation. Furthermore, it has been suggested that the permeability barrier of the cuticle of nematodes such as *A. agrostis* (Preston & Bird, 1987; Bird & Zuckerman, 1989) and *D. dipsaci* (Wharton *et al*. 1988) lies in the epicuticle and is lipoprotein in nature. The alterations in the response of the desiccation-tolerant mutant lines isolated in our laboratory to detergents suggested that the mutant phenotype was, most likely, expressed in the external layer of the sheath. We have, therefore, investigated the biophysical properties of the surface of the sheath of IJs in 2 of the mutant lines (14 and 16) and parental strain using fluorescent probes, and measured their mobility within the plane of the surface using Fluorescent Recovery After Photobleaching (FRAP).

Many stages and species of nematode possess a glycoprotein surface coat which lies above the epicuticle and bears a net negative charge to which cationized ferritin has been shown to bind (Himmelhoch & Zuckerman, 1978; Murrel, Graham & McGreevy, 1983; Blaxter *et al*. 1992). Murrel *et al*. (1983) suggested that an increase in surface negative charge of free-living nematodes or free-living stages of parasitic nematodes may play a role in the desiccation tolerance of these nematodes, since the presence of a strongly ionized surface could facilitate the maintenance of a film of water on the nematode surface. We have therefore investigated this possibility in the parental strain and desiccation tolerant mutant lines of *H. megidis*.

Very little information exists on the sheath of *Heterorhabditis* at the structural and biochemical level. This is the first attempt to study the sheath of *Heterorhabiditis* using fluorescent lipid probes and cationized ferritin. The binding of these probes which have been used extensively in cell membrane (McKay, Kusel & Wilkinson, 1991; Ladha, Mackie & Clarke, 1994) and animal parasite studies (Foley *et al*. 1986; Kennedy *et al*. 1987; Proudfoot *et al*. 1993; Redman & Kusel, 1995) provides the first information on the composition and properties of the surface of the sheath of *Heterorhabditis*.

MATERIALS AND METHODS

Nematode culture

The parental strain and desiccation tolerant mutant lines (14 and 16) of *Heterorhabditis megidis* were cultured in *Tenebrio molitor* larvae at 20 °C. For each line, all IJs that emerged from cadavers were pooled. Experiments were carried out on samples taken from the total number of IJs to emerge to reduce batch variance (O'Leary *et al*. 1998). IJs were stored at 15 °C and were less than 10 days old for all experiments.

Removal of sheath

The sheaths of the mutants and parental strain were removed by treating the IJs with 0·5 % sodium hypochlorite (NaOCl) for 20 min (Campbell & Gaugler, 1991*b*). The IJs were then washed 5 times with tap water to remove the sodium hypochlorite and the loose sheaths.

Assessment of cuticle damage

Two samples each containing approximately 100 IJs were treated for 20 min, one with distilled water alone and the other with distilled water containing 0·5 % NaOCl. The samples were washed 3 times with distilled water and then incubated with 10 μg/ml Hoechst 33258 (Bisbenzimide 33258, Sigma) for 15 min (Modha *et al*. 1997). IJs were then mounted on a microscope slide in water and checked for fluorescence.

Desiccation protocol

A 1 ml suspension of ensheathed IJs in water (conc. 200 IJs/ml) was vacuum-filtered onto a 2·5 cm Whatman No. 1 filter paper disc using a Sartorious funnel and a vacuum flask attached to an electric pump. The discs were then transferred to 3 cm Petri dishes which were then placed in 30 cm diameter glass desiccators containing the appropriate concentration of sulphuric acid for a relative humidity of 57 % to be created (Solomon, 1951). After the desiccation period, the Petri dishes were removed and the IJs were rehydrated with tap water and allowed to recover at 20 °C for 24 h. Percentage survival was assessed by microscopical observation and response to probing.

Fluorescent lipid probes

Two of the lipid analogues used were based on aminofluorescein (AF) fluorochromes i.e. 5-(N-octadecanoyl) amino fluorescein (C18-AF) and 5-(N-dodecanoyl) amino fluorescein (C12-AF). C12-

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AF and C18-AF differ in aliphatic chain length, containing 12 carbon atoms and 18 carbon atoms respectively. These two probes and the third lipid analogue octadecyl rhodamine B (RhC18) were obtained from Molecular Probes Inc., Junction City, OR, USA. IJs were labelled with fluorescent lipid analogues by incubating them for 15 min at room temperature in 1 ml of tap water to which 5 μl of ethanol containing 10 μg of probe had been added. IJs were then washed 5 times in water and mounted on a microscope slide in water containing 50 mg/ml of the paralysing agent carbamylcholine chloride (BDH).

Cationized ferritin labelling

IJs were labelled with 35 μg/ml cationized ferritin labelled with fluorescein isothiocyanate (Molecular Probes Inc.) for 20 min at room temp. IJs were then washed 5 times in water and mounted on a microscope slide in water containing 50 mg/ml of carbamylcholine chloride. For double labelling experiments with lipid analogues and cationized ferritin IJs were first labelled with the lipid analogue, washed 5 times and then labelled with the appropriate concentration of cationized ferritin (FITC labelled), washed 5 times and then mounted on a microscope slide in water containing 50 mg/ml of carbamylcholine chloride.

Fluorescence quantitation

A Leitz Orthoplan UV microscope with an NPL Fluotar objective lens and a Leitz MPV compact quantitative photometry system were used for fluorescent measurements. Fluorescence was normalized by subtracting a background reading from a fluorescent area on the labelled nematode surface. Thus the fluorescence values obtained are relative between groups within an experiment but data from experiments performed at other times are not directly comparable. For each determination, 5 measurements were taken from an approximate area of 20 μm^2 at the following intervals: the tail region (i.e. within 50 μm of the tail end); half way between the tail and the middle of the IJ (i.e. ca. 200 μm from the posterior); the middle of the IJ (i.e. ca. 400 μm from the posterior); half way between the middle and the head of the IJ (i.e. ca. 600 μm from the posterior) and finally, the head of the IJ (i.e. within 50 μm of the tip) along the lengths of 10 worms except for the results shown in Fig. 2 where readings were taken from 30 worms. In all cases the mean ($\pm$standard error) reading is presented.

The extent to which the lipid probes had inserted into the sheath was measured by incubating the fluorescently labelled IJs with the membrane impermeant molecule trypan blue (0·25 % w/v for 1 min at room temperature) which can quench fluorescence by resonance energy transfer (Stryer, 1978; Foley *et al*. 1986; Kennedy *et al*. 1987). For all fluorescent values autofluorescence measured from unlabelled IJs was subtracted from the fluorescent values obtained from labelled IJs.

Fluorescence Recovery After Photobleaching (*FRAP*)

FRAP measures the rate of lateral diffusion of membrane components. A small area of the labelled surface is photobleached with a laser and the rate and extent of diffusion of surrounding probe into the photobleached area is measured. The extent of diffusion into the photobleached area is expressed as percentage recovery (% R) of spot fluorescence after the bleaching pulse, extrapolated to infinite time. The rate of lateral diffusion is expressed by a coefficient of that fraction (D_L). Preliminary experiments suggested that laser pulsing at 96 Hz was the most suitable frequency for measuring fluorescence, as higher frequencies bleached the fluorescence of the labelled surface while measurements were being taken.

The small area (spot radius 1·2 μm) was bleached with an argon ion laser (LEXEL model 85). The diffusion of lipid probe into the spot was recorded on an oscilloscope (Digital Storage model 4035) and a print-out was obtained of the trace. The percentage recovery values were calculated from these traces. Due to the high autofluorescence of the centre region of the IJs, measurements were taken from the head regions (i.e. within 50 μm of the tip of the IJ) of 10 IJs from each strain labelled with C18-AF. Measurements were also taken from the head region of 10 IJs of each strain labelled with C18-AF and cationized ferritin (350 μg/ml).

Statistical analysis

Data are presented as the mean $\pm$ S.E. ANOVA was performed and the significance of difference was tested using Duncan's multiple range test at $P < 0{\cdot}05$ significance level. Proportional data were normalized for ANOVA using an arcsine transformation. The Spearman rank correlation coefficient was calculated using the Multistat statistical package (Day, 1988).

RESULTS

Effect of sheath loss on the desiccation tolerance of H. megidis

Removal of the sheath of IJs of *H. megidis* with NaOCl resulted in a decrease in their rapid desiccation (57 % RH) tolerance (Fig. 1). Furthermore, the increased desiccation tolerance of the mutant lines 14 and 16 was no longer apparent in the desheathed IJs, suggesting that the physiological change responsible for the mutant phenotype lay in the sheath of the IJs.

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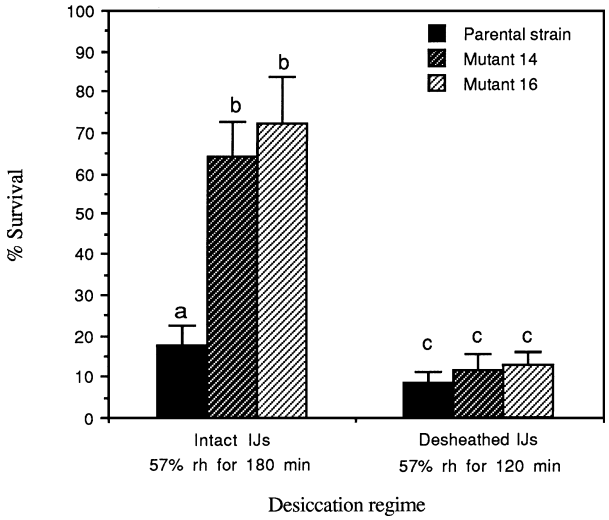

Fig. 1. The effect of desheathment on the desiccation tolerance of IJs of the parental strain and desiccation-tolerant mutant lines of *Heterorhabditis megidis* (Strain UK211) during rapid desiccation at 57% r.h. Means followed by the same letter are not significantly different (Duncan's multiple range test, $P < 0{\cdot}05$).

Effect of desheathment with sodium hypochlorite

Damaged parasites can be detected by the uptake of membrane impermeable compounds through the damaged surface (Lima *et al*. 1994; Modha *et al*. 1997). The damaged areas of the parasite are observed to give a blue fluorescence with the DNA binding compound Hoechst 33258. No evidence of any diffusion of Hoechst 33258 into the IJs ex-sheathed with 0·5% NaOCl nor into untreated IJs was found.

Probe binding to the surface of heterorhabditid IJs

Two fluorophore-conjugated fatty acid analogues C18-AF, and C12-AF bound to the sheath of *H. megidis*. These probes bear a net negative charge and typically insert into the polar–non-polar interface of a lipid surface with the charged fluorophore head group exposed at the surface (Loew, 1988). Another non-aliphatic phenoxazine lipophilic probe Nile Red (Greenspan, Mayer & Fowler, 1985; Sackett & Wolff, 1987) also bound to the sheath, albeit to a lesser extent, indicating that probe binding was not merely a characteristic of probes with aliphatic chains (results not shown).

Binding of C18-AF and C12-AF (Fig. 2) to the sheath of the parental strain demonstrated that the lipid surface of the sheath was not uniform for the entire length of IJs of the parental strain. Elevated levels of C18-AF binding occurred at the middle of the nematode, and the extent of probe binding decreased towards the head and tail region of the sheath. The reverse situation was detected for the C12-AF probe i.e. the extent of probe binding was greater at the head and tail region of the nematode

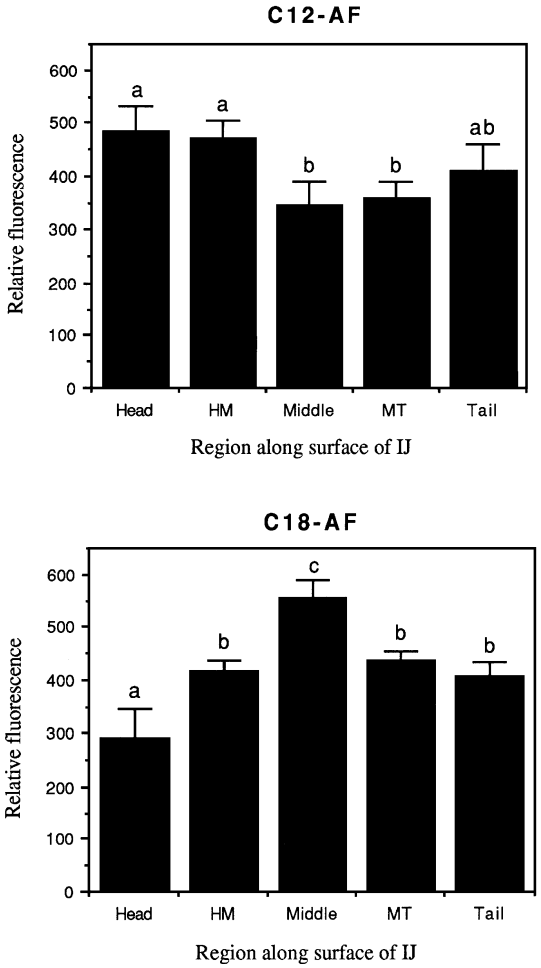

Fig. 2. Binding of lipid analogues C18-AF and C12-AF to the surface of IJs of *Heterorhabditis megidis* (Strain UK211, parental strain). Fluorescence measurements were taken at 5 different regions: the head; the region between the head and the middle of the IJ (HM); the middle of the IJ; the region between the tail and the middle of the IJ (MT); and the tail region of the IJ (see Materials and Methods for further details). Data for each lipid analogue were statistically analysed separately. For each lipid analogue means followed by the same letter are not significantly different (Duncan's multiple range test, $P < 0{\cdot}05$).

sheath than in the middle region. This was confirmed by a correlation analysis (Spearman rank correlation $= -0{\cdot}9$; $P < 0{\cdot}05$) and is further supported by results obtained from binding of these probes to sheaths of the parental strain and desiccation tolerant mutant lines. The mutant lines (14 and 16) have increased C18-AF binding but decreased C12-AF binding (Fig. 3). This not only indicates that C18-AF binding is negatively correlated with C12-AF binding, but shows that the lipophilic surface of the sheath is different in the mutant lines. IJs of the mutant lines and parental strain, however, bound equal quantities of the lipid analogue RhC18, a positively charged lipid analogue with an 18 carbon aliphatic tail. The binding of cationized ferritin to the surface of *H. megidis* indicates that a net negative

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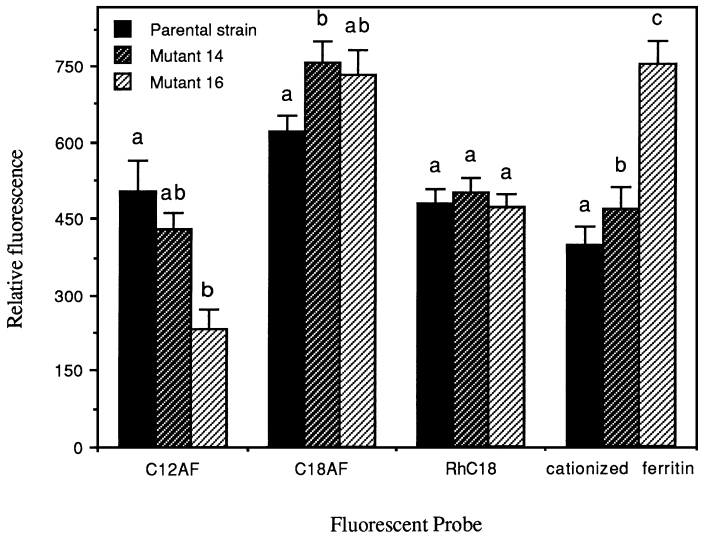

Fig. 3. Binding of the fluorescent probes C12-AF, C18-AF, RhC18 and cationized ferritin (35 μg/ml) to the surface of intact IJs of the desiccation-tolerant mutant lines and parental strain of *Heterorhabditis megidis*. Data for each fluorescent probe were statistically analysed separately. For each fluorescent probe means followed by the same letter are not significantly different (Duncan's multiple range test, $P < 0{\cdot}05$).

Table 1. Mean percentage quenching ± S.E. of fluorescent lipid probes with trypan blue from the outer cuticle of IJs of the mutant lines and parental strain of *Heterorhabditis megidis* strain UK211

(Means followed by the same letter are not significantly different (Duncan's multiple range test, $P < 0{\cdot}05$)).

	Fluorescent probe	
	C18-AF	C12-AF
Parental strain	100 (a)	71·7 ± 2·3 (b)
Mutant 14	100 (a)	70·1 ± 4·9 (b)
Mutant 16	100 (a)	72·9 ± 6·2 (b)

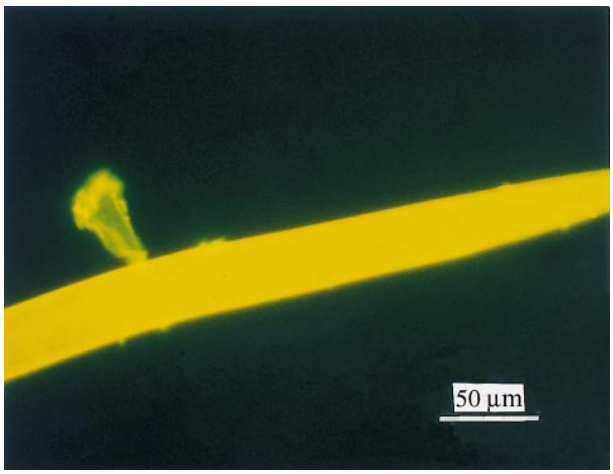

Fig. 4. Binding of cationized ferritin (35 μg/ml) and the resultant shedding of surface material from IJs of *Heterorhabditis megidis*.

surface charge is present on the IJs. Furthermore IJs of the mutant strains 14 and 16 displayed increased binding of cationized ferritin to their surface indicating that an increased net negative surface charge exists on IJs of the mutant lines.

Quenching of probes

The percentage quenching of fluorescence of IJs labelled with C12-AF and C18-AF by 0·25 % trypan is shown in Table 1. Quenching occurs by Foster Resonance Energy Transfer and since 2 molecules must be less than 4 nm apart for Forster Resonance Energy Transfer to occur (Foley *et al*. 1986), the data outlined in Table 1 demonstrate that both C12-AF and C18-AF were restricted to the surface of the sheath. Quenching values did not differ between the mutant lines and parental strain. Typical values for quenching of C18-AF and C12-AF were 100 % and 71·7 % (± 2·34 %) respectively implying that C12-AF inserts further into the surface of the sheath, possibly due to its shorter aliphatic chain length. Due to the high fully internalized autofluorescence observed with the rhodamine filter it was difficult to quantify quenching of the probe RhC18. Double labelling of IJs with C12-AF and RhC18 lead to complete quenching of C12-AF fluorescence by Forster Resonance Energy Transfer, indicating that these 2 probes lie in the same layer of the sheath.

Net charge of IJ surface

The binding of cationized ferritin (35 μg/ml) to the surface of the mutant lines 14 and 16 caused shedding of surface material from the sheath (Fig. 4). This concentration of cationized ferritin did not cause

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Table 2. Mean relative fluorescence values ± S.E. from the surface of IJs of the parental strain and desiccation-tolerant mutant lines (14 and 16) of *Heterorhabditis megidis* double labelled with the lipid analogue RhC18 and with a range of concentrations of cationized ferritin (i.e. 35, 350 and 700 μg/ml)

(Cationized ferritin (c.f.) fluorescence was measured through the fluorescein filter while RhC18 fluorescence from the same specimen was measured through the rhodamine filter. Means followed by the same letter are not significantly different (Duncan's multiple range test, $P < 0{\cdot}05$)).

	Strain		
	Parental strain	Mutant 14	Mutant 16
RhC18	223·7 ± 24·8 (a)	241·2 ± 29·5 (a)	229·3 ± 22·1 (a)
35 μg/ml c.f.	241·3 ± 28·5 (a)	229·6 ± 18·8 (a)	226·4 ± 21·7 (a)
RhC18	234·9 ± 21·6 (a)	232·7 ± 41·3 (a)	226·8 ± 19·6 (a)
350 μg/ml c.f.	434·1 ± 36·8 (b)	536·2 ± 35·8 (c)	782·6 ± 58·2 (d)
RhC18	237·1 ± 26·8 (a)	238·4 ± 23·6 (a)	246·9 ± 28·4 (a)
700 μg/ml c.f.	62·5 ± 11·2 (e)	54·3 ± 13·8 (e)	79·1 ± 5·6 (e)

Table 3. Percentage recovery (%R ± S.E.) of the fluorescent lipid probe C18-AF after photobleaching the sheaths of IJs of the parental strain and mutant lines of *Heterorhabditis megidis* in the presence and absence of FITC-labelled cationized ferritin (c.f.) (700 μg/ml)

(No significant difference was found between any of the treatments (Duncan's multiple range test, $P < 0{\cdot}05$)).

	Fluorescent probe	
	%R C18-AF	%R C18-AF with c.f.
Parental strain	14·14 ± 1·12	15·41 ± 1·62
Mutant 14	14·94 ± 0·94	15·70 ± 1·65
Mutant 16	14·58 ± 0·97	13·82 ± 1·30
Isolated sheaths (parental strain)	13·98 ± 1·65	14·24 ± 1·40

shedding when the IJs were pre-labelled with the lipid analogue RhC18 (Table 2). This dye was used, rather than C12-AF or C18-AF, since it can be visualized in the rhodamine filter, while FITC-labelled cationized ferritin could be visualized on the same specimen using the FITC filter. However, increasing the concentration of cationized ferritin to 700 μg/ml results in the shedding of surface material from RhC18-labelled IJs. This can be observed from microscopical evidence and is also indicated by the presence of low fluorescence values for this concentration of cationized ferritin in Table 2. These fluorescence values are low because the FITC-labelled cationized ferritin has been shed from the surface of the cuticle. The fluorescence levels due to binding of RhC18 to the surface of the IJs remained constant despite the loss of surface material caused by the highest concentration of cationized ferritin (700 μg/ml). This indicates that the shedding of this surface material from the IJs did not affect the underlying lipid region. The lowest concentration of cationized ferritin (35 μg/ml) does not show significantly greater binding of cationized ferritin to the RhC18 pre-labelled mutant lines as compared with the RhC18 pre-labelled parental strain. This low concentration of cationized ferritin may not have been sufficient to saturate the cationized ferritin binding sites on the surface of the parental strain. The intermediate concentration of cationized ferritin (350 μg/ml) bound to the mutant lines to a greater extent. However, increasing the concentration of cationized ferritin to 750 μg/ml caused substantial shedding of surface material from the sheath, resulting in the loss of fluorescence from the surface of the IJs. Cationized ferritin-induced shedding of surface material also occurred from isolated sheaths and from the sheaths of dead IJs.

Fluorescence Recovery After Photobleaching (*FRAP*)

FRAP experiments were performed to determine if the lipid surface components of the sheaths of the mutant lines differed in lateral mobility. The percentage recovery values of C18-AF for the parental strain, the 2 mutant strains and for isolated sheaths are shown in Table 3. Percentage recovery was low indicating that lateral mobility of surface lipid was restricted. FRAP was also performed on the parental strain and mutants labelled with C18-AF and 700 μg/ml cationized ferritin which caused shedding of the surface material from the sheath. Binding of labelled cationized ferritin and the resultant shed-

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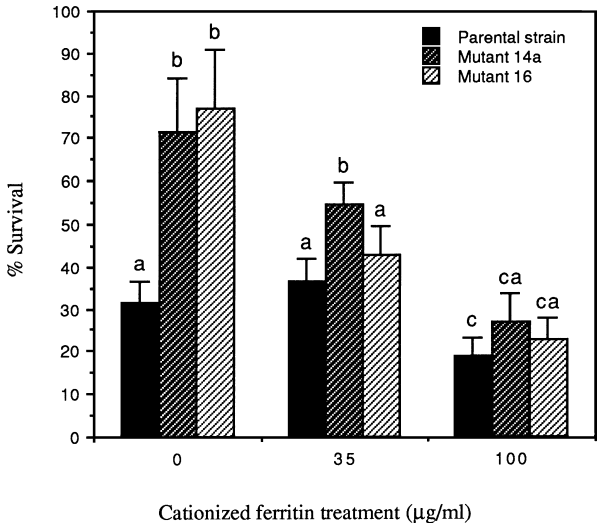

Fig. 5. The effect of pre-treating IJs of the desiccation-tolerant mutant lines and parental strain of *Heterorhabditis megidis* with different concentrations of cationized ferritin on their desiccation tolerance at 57 % r.h. for 160 min. The 3 concentrations of cationized ferritin were: 0 μg/ml, i.e. control; 35 μg/ml and 100 μg/ml. Means followed by the same letter are not significantly different (Duncan's multiple range test, $P < 0{\cdot}05$).

ding did not alter the percentage recovery of fluorescence in the bleached spot for C18-AF. Percentage recovery was found to be low, with an average value of between 14 and 15 % for all strains regardless of whether cationized ferritin was, or was not, included in the experiment (Table 3). Lateral diffusion (D_L) values were not calculated due to the low percentage recovery values. These results also indicate that the fluidity of the lipid surface of the sheath is independent of the presence of the living nematode since the surface of isolated sheaths show the same level of lateral diffusion as do the lipid surface of sheaths surrounding nematodes.

The effect of neutralizing IJ net surface charge on the desiccation tolerance of the mutant lines and parental strain of H. megidis

Binding of unlabelled cationized ferritin (35 μg/ml, Sigma Chemical Co.) to the surface of ensheathed IJs of the mutant lines and parental strain of *H. megidis* resulted in the complete loss of the mutant phenotype from mutant 16 and partial loss of the mutant phenotype from mutant 14 (Fig. 5). However, incubating IJs of the mutant 14 with a greater concentration of cationized ferritin (100 μg/ml) caused complete loss of the mutant phenotype. In addition, binding of cationized ferritin (100 μg/ml) to IJs of the parental strain resulted in a decrease in their desiccation tolerance, thus reflecting the importance of a negative surface coat in desiccation tolerance.

DISCUSSION

Desheathing the IJs of the parental strain and desiccation-tolerant mutants of *H. megidis* with 0·5 % NaOCl resulted in the loss of the mutant phenotype, indicating that the change responsible for the mutant phenotype occurred in the sheath. Actively removing the sheaths of IJs with NaOCl may damage the surface of the underlying 'living' cuticle (Timper & Kaya, 1989; Davey & Sommerville, 1982). Campbell & Gaugler (1991*b*) suggested that sheath removal with NaOCl should only be used for comparison with an active exsheathing method. However, since no other method exists for successfully and rapidly removing the sheaths of heterorhabditids, NaOCl was considered to be the only available option. Desheathing of the IJs with NaOCl results not only in the loss of the mutant phenotype, but it also decreases the desiccation tolerance of wild-type *H. megidis*. This decrease observed for the parental strain and the loss of the increase in desiccation tolerance observed in the mutants could be partially due to cuticle damage caused by the desheathing process. The fact that *H. megidis* IJs exsheathed with 0·5 % NaOCl do not take in the membrane impermeable compound Hoechst 33258, however, suggests that the underlying cuticle is not damaged by exsheathment with NaOCl. The critical nature of the sheath in the improved desiccation tolerance of the mutants is also demonstrated by the cationized ferritin binding studies reported in this paper.

Although fluorescence quantitation of IJs labelled with lipid analogues yielded significant differences in probe binding for the mutants and parental strain with C12-AF and C18-AF, it is unclear how this is related to the improved desiccation tolerance of the mutants. It is unlikely that these differences in lipid insertion are related to the greater negative surface charge of the mutants because of the increased binding of the negatively charged lipid probe C18-AF to the surface of the mutants.

Incubating IJs in 700 μg/ml cationized ferritin caused shedding of material from the surface of the IJs. This shed material may be part of a surface coat on the outer surface of the sheath of heterorhabditids. This loss of material was not due to an active process because it occurred to the same extent in isolated sheaths and in dead IJs. Loss of this coat may have resulted from a charge-dependent reaction occurring between the positively charged ferritin and the negatively charged surface coat. Flooding and exposure of the surface of the IJs with an excess of positive charge in the form of cationized ferritin may aggregate the negatively charged molecules in the surface coat. This aggregation may break non-covalent bonds between surface coat and epicuticle, leading to the removal of the surface coat from the sheath of the IJs. The loss of the surface coat does not apparently affect the integrity of the underlying

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lipid. The loss of a surface coat without affecting probe insertion into the lipophilic layer has also been observed for *Toxocara canis* juveniles using rhodamine-conjugated anti-surface coat antibody and the C18-AF probe (Kennedy & Proudfoot, 1993). Binding of the lipid analogue RhC18 to the IJ surface was unaffected by the removal of the surface coat, and cationized ferritin did not affect membrane fluidity.

The fluidity of the surface lipid of *H. megidis* is restricted in the presence or absence of the surface coat. Rigidity of epicuticular lipids has been observed in other nematodes (Kennedy *et al*. 1987; Proudfoot *et al*. 1993; Kennedy & Proudfoot, 1993) and may be due to the fact that the lipids exist in a gel phase, or are restricted by other layers of the cuticle or that these lipids may exist in an unusual phase similar to that observed at the surface of the infective larvae of *Trichinella spiralis* (Lee, Wright & Shivers, 1984). Whatever the reason for this rigidity of surface lipid it is clear that it is unrelated to the improved desiccation tolerance of these mutants, although it may play some role in the desiccation tolerance of wild type strains of *Heterorhabditis*.

The increased binding of cationized ferritin to the mutant lines found in this study suggests that the surface of the mutant lines is more negatively charged than the surface of the parental strain. IJs of mutant line 16 bound significantly more cationized ferritin than did the IJs of mutant line 14; however, the desiccation tolerance of IJs from line 16 was not significantly greater than that of line 14 IJs (O'Leary & Burnell, 1997). O'Leary & Burnell (1997) found that these desiccation-tolerant mutant lines were more susceptible to anionic detergents while being more tolerant to cationic detergents and suggested that the surface of the mutant lines may be less negatively charged than the surface of the parental strain. The detergent effects which O'Leary & Burnell (1997) observed may not be related simply to the charge of the surface coat since IJ survival was measured following detergent treatment. Detergents can solubilize the lipid epicuticle (reviewed by Blaxter *et al*. 1992) and SDS (to which the mutants were more sensitive than the parental strain) also solubilizes the underlying collagen cuticle (Cox, Kusch & Edgar, 1981). Thus the altered tolerance of the mutant lines to detergents may reflect phenotypic changes that are not located at the surface of the sheath.

Murrel *et al*. (1983) have suggested that the strongly negative epicuticular charge of *Strongyloides ratii* juveniles and in *Caenorhabditis briggsae* may be related to desiccation tolerance. The presence of a strongly ionized or polar coat on the surface of free-living or free-living stages of nematodes could facilitate the maintenance of a film of water over the surface of the cuticle. The increased net negative charge of the desiccation-tolerant mutants of *H. megidis* appears to be strongly associated with their improved desiccation tolerance. Neutralizing the charge difference between the mutants and parental strain results in the loss of the observed increase in desiccation tolerance of the mutants. It also leads to a decline in the desiccation tolerance of the parental strain, indicating the importance of surface negative charge in wild-type populations of *H. megidis*.

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