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J. Ravaux · M. Zbinden · M. F. Voss-Foucart
P. Compère · G. Goffinet · F. Gaill

Comparative degradation rates of chitinous exoskeletons from deep-sea environments

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Abstract Hydrothermal vent environments, particularly those associated with the vestimentiferan *Riftia pachyptila,* are believed to be among the highest chitin-producing systems. In order to elucidate the chitin cycle in these environments, we estimate the in situ chitin degradation rates of tube-worm exoskeletons. Our in situ experiments show that the tubes of *Riftia* are highly stable structures. Comparative measurements of the degradation rates of *Riftia* tubes and crab shells immersed at deep-sea vents show that the tubes would be degraded within 2.5 years, whereas the time for the total degradation of the vent crab (*Bythograea thermydron*) carapaces would not exceed 36 days. The importance of the microbial participation in this degradation was estimated for *Riftia* tubes. Based on previous work, we calculated chitin production by a population of *Riftia* tubes of about 750 g m^{-2} $year^{-1}$ (763). From our in situ experiments, we estimated a microbial chitinolysis rate of about 500 g m^{-2} $year^{-1}$ (496) (65% of the chitin produced).Exoskeletons containing β-chitin appear more stable in natural environments than those containing α-chitin and would thus be less available as carbon and nitrogen sources. In contrast, isolated β-chitin was hydrolysed faster than α-chitin during in vitro degradation experiments; for instance, *Riftia* β-chitin was degraded about 3- to 4-fold faster than *Bythograea* α-chitin. A stabilization process by disulfide bonds of the proteins-chitin link, rather than the crystalline form of the chitin (α/β), accounts for the resistance of *Riftia* tubes to enzymatic attacks.

Communicated by S.A. Poulet, Roscoff J. Ravaux and M. Zbinden contributed equally to this work

J. Ravaux · M. Zbinden · F. Gaill (✉)
"Adaptation et Evolution en Milieux Extrêmes", FRE 2634
CNRS, UPMC, 7 Quai St-Bernard, 75252 Paris Cedex 05, France
E-mail: Francoise.Gaill@snv.jussieu.fr
Fax: +33-1-44275250

M. Zbinden · M. F. Voss-Foucart · P. Compère · G. Goffinet
Institut de Zoologie, Université de Liège, 22 Quai Ed Van Beneden,
4020 Liège, Belgium

Introduction

Chitin is one of the most abundant polysaccharides in nature, and is distributed throughout all kingdoms as it is a crucial component of the cell walls of moulds, yeasts, fungi and certain green algae, and is a major component of the cuticles and exoskeletons of worms, mollusks and arthropods (Jeuniaux 1982). From an ecological point of view, chitin plays a key role in the biogeochemical cycles of both carbon (C) and nitrogen (N), and the rates of chitin production and degradation affect C and N pools and availability (Poulicek et al. 1998). The annual chitin production in the ocean exceeds 2×10^9 tonnes (review in Goffinet 1996), and the highest production recorded in marine environments is that associated with the hydrothermal vent vestimentiferan *Riftia pachyptila* (Gaill et al. 1997). Previous studies by Shillito et al. (1999) showed that the quantity of chitin generated by *Riftia* for building its tube could reach 100 times that produced by other marine species. How such high levels of chitin are secreted is partially understood (Shillito et al. 1995), but the rates of degradation and the actors responsible for the removal of these huge amounts of chitin are still unknown.

Riftia tube was previously shown to be very resistant to in vitro aggressive chemical and physical treatments (Gaill and Hunt 1986). Moreover, empty tubes were often observed in situ after an animal's death (Fustec et al. 1987; Roux et al. 1989). However, the rates of degradation, and the processes involved in the tube longevity remain to be determined. The tube differs from chitinous structures of other organisms by several original characteristics; in particular, it is composed of giant β-chitin crystallites (Gaill et al. 1991). Our initial hypothesis was that tube stability could be due to the presence of these rare β-chitin microfibrils in *Riftia* tubes. In order to test this hypothesis, we first compared

in situ biodegradation of *Riftia* tube to that of other matrices containing either α- or β-chitin. Furthermore, the stabilization processes (i.e. hardening by tanning and/or mineralization or disulfide bonds (S–S bonds) in the chitin-associated proteins) and their influence on chitin degradation rate were investigated in vitro on different α- and β-chitin-containing matrices.

A second question was the identification of actors involved in degradation processes. It has been shown that microorganisms like chitinolytic bacteria, which were found to be ubiquitous in the marine environment, play a major role in chitin recycling processes in the ocean (Poulicek et al. 1998). *Riftia* tubes were shown to be suitable colonization surfaces for microorganisms (Gaill and Hunt 1991), and some bacteria that are known to degrade long polymers like chitin were found associated with the tubes (Lopez-Garcia et al. 2002). Our experimental approach allowed us, for the first time, to quantify the chitinolysis rate by microbial populations, and to estimate their importance in the chitin cycle at deep-sea vents.

Materials and methods

Animal collection

Riftia pachyptila, *Bythograea thermydron,* and *Tevnia jerichonana* specimens were collected at the 13°N site (Elsa) of the East Pacific Rise during the Hero 1 and 2 cruises by the Nautile and the Alvin submersibles. *Lamellibrachia* sp. and *Escarpia* sp. specimens were collected at various cold seeps in the Gulf of Mexico. *Rimicaris exoculata* were collected during the Diva cruise (Mid-Atlantic Ridge). *Aphrodita aculeata* specimens were collected at Banyuls-sur-mer (France), *Carcinus maenas* specimens were collected at Roscoff (France), and *Loligo* sp. were commercially purchased.

In situ experimentation

Hydrothermal vents

In situ experiments were made during the Hero cruises (13°N site, Elsa). Once collected on board, pieces of *Riftia pachyptila* tubes (35–60 mg of dry weight) and *B. thermydron* exoskeletons (500 mg to 1 g of dry weight) were dissected, air dried, weighed and then put down in the vicinity of their original location (Fig. 1). Reference samples were also weighed and used for biochemical analysis. The samples were recovered after 7, 12 and 180 days, air dried and then further analysed (see Sample composition) in the laboratory.

Calvi Bay

For comparison, our data were made complete by performing in situ parallel experiments in a coastal environment, i.e. Calvi Bay (Corsica) in the Mediterranean Sea, using more species. Dried samples of vestimentiferan tubes and crab carapaces were placed on the bottom and recovered after 6, 7, 20, 26, 120 and 180 days. In parallel, *A. aculeata* setae (β-chitin) and *Loligo* sp. pen (α-chitin) were also placed on the bottom in Calvi Bay and recovered after 7, 26 and 120 days.

For each material, several samples were placed in situ, and similar results were obtained whatever their initial weight. Hence, in order to compare the different materials, the results were expressed as percent of the initial dry weight.

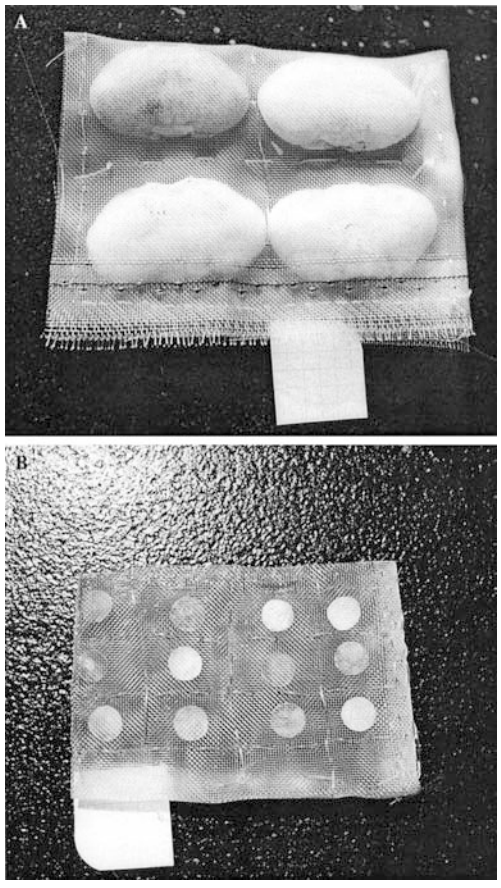


Fig. 1A, B In situ degradation experiments. The samples were packed in a net before immersion. **A** *Bythograea thermydron* cephalothorax carapaces; **B** circular sections (2.5 cm diameter) of *Riftia pachyptila* tube samples

Sample composition

Calcium, protein and chitin content

Pieces of dried samples were submitted to a 24-h treatment with 0.5 M HCl at room temperature, washed with distilled water, dried and weighed. Calcium was assayed in the HCl extracts with the Calcospectral reaction (Spectroquant 14815, Merck). One to five milligrams of the HCl treatment residues, that is, organic matrix dried weights (odw), were subsequently subjected to two successive 3-h treatments with 0.5 M NaOH at 100°C in order to set chitin free from glycoproteic complexes. Proteins were assayed in these NaOH extracts according to Lowry et al. (1951). Some NaOH extracted residues were subjected to a subsequent formic acid treatment (samples were incubated for 1 h at room temperature in a mixture of formic acid/10% H_2O_2). Chitin was estimated in the residual material from HCl and NaOH treatments using an enzymatic method (Jeuniaux 1963) with repeated chitinase incubations.

Histochemistry

The silver methenamine reaction (Hmt-Ag; Locke and Huie 1980) was used to reveal reducing groups as SH groups and polyphenols, and for indirect demonstration of SS bonds. It was applied on semithin sections (1–2 μm) of unfixed, epoxy-embedded tube fragments of *Escarpia* sp. and *R. pachyptila*. Tanned and polyphenol-containing materials, i.e. *A. aculeata* setae and *C. maenas* carapace (Goffinet and Jeuniaux 1994), were used as controls. Several

controls of the reaction were used, such as SS-bond reduction by thioglycolate, and SH-group alkylation by iodoacetic acid/boric acid treatment.

In vitro degradation experiments

Two series of experiments were performed using methods adapted from Jeuniaux (1963). In the first series, the time needed for enzymatic degradation of chitin from samples containing the α or β form was tested. The residual material from HCl and NaOH extraction of the samples was suspended in chitinase solution (Sigma C 6137, 1 mg ml^{-1}) and incubated at 37°C. The reaction mixture contained an excess of chitinase, so that the rate of the reaction would not depend on the initial weight of the samples (from 5 to 10 mg of decalcified dry weight). An aliquot of the supernatant of the reaction mixture was pipetted every 2 h until 8 h, every 4 h until 32 h and every 8 h until 96 h, and incubated with chitobiase solution. After centrifugation, the supernatant of chitobiase treatment was recovered for N-acetylglucosamine assay. In the second series, we applied the same procedure to test the effect of a previous performic acid treatment (1 h at room temperature), known to break disulfide bonds, on the enzymatic degradation of chitin from samples containing the α or β form.

Results

Four types of chitin-containing materials were studied: carapace (crustacean), tube (vestimentiferan), setae (annelid) and pen (cephalopod). These samples were obtained from species living in hydrothermal vents (EPR, MAR), cold seeps (Gulf of Mexico) and coastal environments (Calvi Bay) (Table 1).

Sample composition

Among the nine structures studied, only the crustacean exoskeletons are mineralized, with a mineralization percentage ranging from about 20% for the shrimp *Rimicaris exoculata* to 70% for both crabs (*Bythograea thermydron* and *Carcinus maenas*) (Table 1). The mineralized fraction is dominated by calcium carbonate ($68.9 \pm 3.0\%$ $CaCO_3$ of dry weight) for the crab *B. thermydron*. The organic fraction is composed of chitin and proteins.

The chitin content of the different exoskeletons, assayed with conventional methods, ranged from $30.0 \pm 1.6\%$ for *Escarpia* sp. to $64.3 \pm 2.7\%$ for *B. thermydron* (in percent of odw) (Table 1). The protein content in the NaOH extract from the residue of decalcification ranged from $16.0 \pm 2.8\%$ for *C. maenas* to $75.3 \pm 3.4\%$ for *Lamellibrachia* sp. (Table 1). The protein content of the vestimentiferan tubes varies between species, ranging from 33.5% for *Tevnia jerichonana* to 75.3% for *Lamellibrachia* sp., and within species, as the standard deviation of the protein content values of *Riftia pachyptila* and *Escarpia* sp. was 10.3% and 10.5%, respectively.

The investigation of SH groups and SS bonds in the two vestimentiferan tubes (*R. pachyptila* and *Escarpia* sp.), in *C. maenas* exoskeleton and in *Aphrodita aculeata* setae demonstrates the presence of disulfide bonds in both vestimentiferan tubes (while free SH groups were absent or poorly represented) and the absence of other strong reducing groups as polyphenols. Indeed, a weak positive result was observed with the silver methenamine reaction (Hmt-Ag) that strongly increased when the SS bonds were reduced by thioglycolate (TGY), and was abolished by SH-group blocking agents (iodoacetic or boric acid) (Table 2).

The tanned *Aphroditida* setae and the crab carapace produced a positive result to the Hmt-Ag reaction for all conditions tested. This confirms the presence of reducing polyphenols (responsible for the staining), the absence of SH groups (no decrease of staining after iodoacetic or boric acid treatments) and SS bonds (no increase of staining after TGY) in these samples (Table 2).

In situ degradation experiments

In situ experiments consisted of following the degradation processes of *Riftia* tube and *Bythograea* carapace samples during several months of residence either in their natural environment, or in a coastal environment. In the latter environment, two samples from non-vent species (*Aphrodita* setae and *Loligo* pen) were used as references of β-chitin containing structures.

Table 1 Chemical composition of different extracellular matrices (in percent of initial dry weight). Each value is the mean of *n* measurements and is given with standard deviation. The chitin and protein contents are expressed as percent of the organic fraction. Only the NaOH-soluble proteins were considered here

Species	Environment	Chitin type	Calcium carbonate content	Residue after decalcification	Chitin content	Protein content
Carcinus maenas	Shallow seawater	α	71.6 ± 5.1 ($n=3$)	≈ 15	49.9 ± 3.6 ($n=3$)	16.0 ± 2.8 ($n=3$)
Rimicaris exoculata	Hydrothermal vents (Atlantic)	α	17.8 ± 1.9 ($n=5$)	≈ 50	48.9 ± 1.7 ($n=3$)	33.1 ± 3.0 ($n=5$)
Bythograea thermydron	Hydrothermal vents (Pacific)	α	68.9 ± 3 ($n=4$)	≈ 10	64.3 ± 2.7 ($n=3$)	16.6 ± 1.0 ($n=8$)
Loligo sp.	Shallow seawater	β	–	100	35.4 ± 4.0 ($n=3$)	65.8 ± 5.9 ($n=6$)
Aphrodita aculeata	Shallow seawater	β	–	100	31.9 ± 2.3 ($n=3$)	73.6 ± 3.3 ($n=2$)
Lamellibrachia sp.	Cold seeps	β	–	100	32.0 ± 0.2 ($n=3$)	75.3 ± 3.4 ($n=6$)
Escarpia sp.	Cold seeps	β	–	100	30.0 ± 1.6 ($n=3$)	63.4 ± 10.5 ($n=6$)
Tevnia jerichonana	Hydrothermal vents (Pacific)	β	–	100	48.7 ± 2.9 ($n=3$)	33.5 ± 3.6 ($n=9$)
Riftia pachyptila	Hydrothermal vents (Pacific)	β	–	100	31.6 ± 0.7 ($n=3$)	46.0 ± 10.3 ($n=16$)

Table 2 Demonstration of polyphenols, SH groups and disulfide bonds (SS bonds) in vestimentiferan tubes, annelid setae and crab carapace by histochemistry using the silver methenamine reaction (Hmt-Ag). A positive reaction is indicated by +, and negative reaction by −. for iodoacetic acid (*IAc*), thioglycolate (*TGY*) and boric acid (*Bo*)

Species	Experimental condition	SH-group blocking agents	Reducing agent	Results
		(IAc/Bo)	(TGY)	(Hmt-Ag)
Riftia pachyptila	1			weak
	2		+	+
	3	+		−
Escarpia sp.	1			weak
	2		+	+
	3	+		−
Carcinus maenas[a]	1			+
	2		+	+
	3	+		+
Aphrodita acculeata	1			+
	2		+	+
	3	+		+

[a]Results obtained with epicuticle

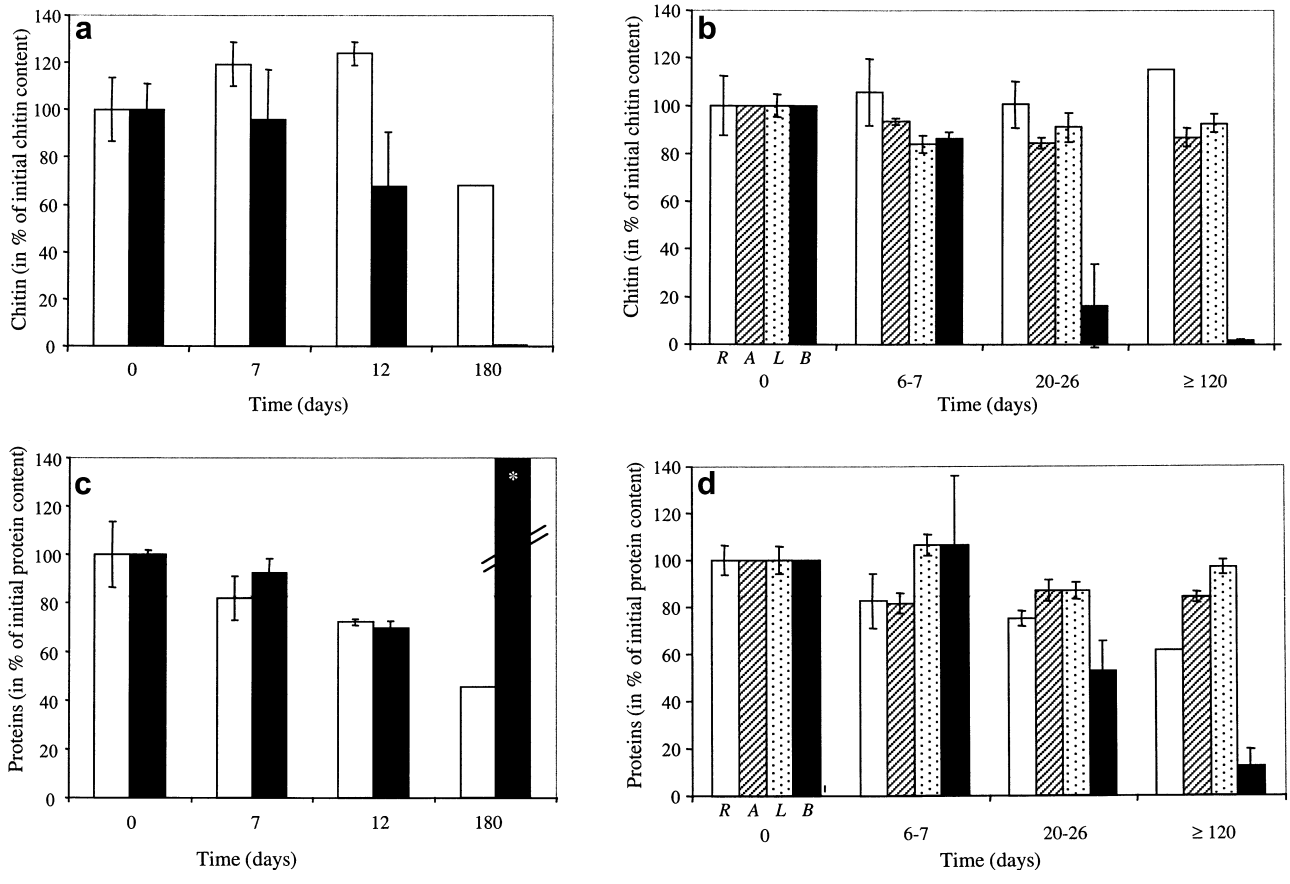
Experiments at hydrothermal vents

R. pachyptila tube appeared to be more stable than *B. thermydron* carapace. During the first 12 days of the experiment, the crab cuticle had lost 33% of its organic matrix (in percent of the initial odw). *Riftia* tube was only slightly degraded, as 20% of its HCl insoluble fraction had disappeared after 180 days (in percent of the initial odw).

Chitin was also shown to be more stable in *Riftia* tube than in the crab carapace. After 180 days of experimentation, about all of the chitin (i.e. 99.6% of the initial chitin content) from the crab carapace was degraded, whereas only 30% (32.1) of the *Riftia* tube chitin was lost (Fig. 2a). On a short time scale, chitin from *Riftia* tube stayed intact for 12 days, while about 30% (32.5) of the crab carapace chitin was degraded.

Initially, the degradation of the NaOH-soluble proteins proceeded similarly in the vestimentiferan tube and the crab carapace (Fig. 2b) (i.e. for both materials the protein content decreased about 30% after 12 days). When the samples were recovered after 180 days, about 50% of the proteins were lost in *Riftia* tube, but the crab carapace was contaminated by microorganisms which increased the protein content (i.e. an apparent increase of 135% of the protein quantity was recorded between 12 and 180 day samples).

Fig. 2a–d In situ degradation experiments. **a, b** In hydrothermal vent (13°N, EPR); **c, d)** in Calvi Bay (Mediterranean Sea). Some values of chitin and protein contents are presented with their standard deviation ($n=3$). *Biased value of weight due to a huge bacterial contamination. *White bars* = *Riftia pachyptila* tube (R); *hatched bars* = *Aphrodita aculeata* setae (**a**); *spotted bars* = *Loligo* sp. pen (L); *black bars* = *Bythograea thermydron* cuticle (**b**)



Experiments in Calvi Bay

In the shallow seawater environment, the vent crab carapace is the fastest exoskeleton to be degraded. After 120 days of in situ exposure, about 80% of the crab carapace organic matrix was degraded, while the other samples lost about 15% of their HCl-insoluble fraction. The results of experiments with the vent crab exoskeleton in both environments are similar: a rapid and significant decrease of 30% of the organic matrix content occurs after 12 days in the vent site, and a decrease of 20% occurs after 20 days in Calvi Bay.

Material containing β-chitin was much more stable than that composed of α-chitin. Indeed, while chitin stayed almost intact in the three β-chitin-containing samples after 120 days of the experiment, there was only 16% (percent of the initial chitin content) left after 20 days, and next to nothing after 120 days, in the crab carapace (Fig. 2c).

The protein degradation profiles differed in the four samples (Fig. 2d). The NaOH-soluble protein content of *Aphrodita* setae and *Loligo* pen was stable throughout all the experiments. By contrast, after only 20 days, half of the protein in the crab carapace and a quarter of the proteins in *Riftia* tube were degraded. When the samples were recovered, about 90% of the proteins were lost in the *Bythograea* carapace (120-day experiment) and about 40% in the *Riftia* tube (180-day experiment).

In vitro degradation experiments

Chitin crystallites were extracted with conventional methods (see Materials and methods) from the different samples containing either the α or β form of chitin. The maximal time needed for the total in vitro enzymatic degradation of the chitin crystallites is illustrated in Fig. 3. The β-chitin appeared to be degraded faster than the α-chitin. Degradation was obtained within 20–64 h for β-chitin samples, and within 64–96 h for the α-chitin samples.

A performic acid pre-treatment, used to oxidize the cystine residues of the chitinoproteic complexes, had no effect on the crab carapace α-chitin degradation rate as shown in Fig. 3 for *Bythograea*. In contrast, the degradation rate of the vestimentiferan β chitin was highly modified (i.e. a total degradation was observed within 20–32 h instead of 36–64 h) (Fig. 3). The breakage of disulfide bonds seemed to increase the accessibility of the chitin to enzymatic hydrolysis, as shown in Fig. 3 for *Riftia*.

Discussion

Hydrothermal vent environments, and particularly those occupied by the endemic vestimentiferan *Riftia pachyptila,* are considered to be one of the highest chitin-producing marine ecosystems (Gaill et al. 1997). Based on previous work by Shillito et al. (1999), the production of chitinous tubes of *Riftia* can be estimated at about 7.6 kg m^{-2} $year^{-1}$ (for densely packed clusters of 700 ind m^{-2}; Sarradin et al. 1998). On the other hand, direct in situ observations (Fustec et al. 1987; Roux et al. 1989) revealed that vestimentiferan tubes can still be observed after the death of animals, whereas crab carapace remains are never observed. We hypothesized that the giant β-chitin crystallites that compose the tube could be responsible for its stability.

The present investigations, aiming to assess the ability of *Riftia* tube to withstand in situ biological attacks, confirmed that *Riftia* tubes are highly stable structures. They demonstrated that after about 6 months exposure the tube samples appeared slightly altered, in contrast to exoskeleton fragments of the vent crab *Bythograea thermydron*. The estimated degradation rate of *Riftia* tube organic material is less than 4% $month^{-1}$ (3.3) (40% $year^{-1}$, when extrapolated from the results

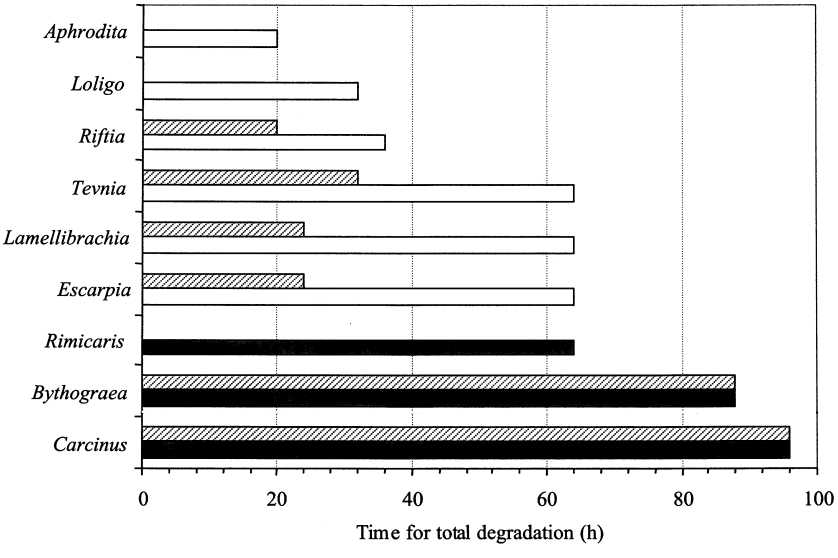


Fig. 3 Time for in vitro total degradation of chitin. Chitin crystallites were purified from different chitinous matrices and enzymatically degraded by a commercial chitinase (*Streptomyces griseus*, Sigma) in vitro. The reaction mixture contained an excess of chitinase, so that the rate of the reaction does not depend on the initial weight of the samples (from 5 to 10 mg of decalcified dry weight). *White bars* β-Chitin; *grey bars* α-chitin; *hached bars* with performic acid treatment

obtained after 180 days), whereas it reaches more than 80% month^{-1} (82.5) in the crab carapace (extrapolated from 12 days exposure). These data suggest that *Riftia* tubes would be degraded within 2.5 years, while carapaces would be degraded in about 1 month (36 days).

With regard to chitin, its average degradation rate at the vent site does not exceed 6% month^{-1} (about 65% year^{-1}, when expressed in percent of initial chitin content; extrapolated from 180-day exposure) for *Riftia*, and about 80% month^{-1} (extrapolated from 12-day exposure) for *Bythograea* (Table 3). Reference experiments performed at shallow depths in Calvi Bay confirmed these results, indicating that the crab carapace and the *Riftia* tube exhibit different degradation profiles (Fig. 2). In *Riftia* tube, the loss of organic matter is mainly due to the protein fraction. The loss of organic material in the vent crab results mainly from a decrease in the chitin content, while the amount of alkalo soluble proteins decreased more slowly, as was observed in previous experiments on carapace samples of the shore crab *Carcinus maenas* in Calvi Bay (Poulicek et al. 1985; Voss-Foucart et al. 1984). Considering the rate of chitin degradation, the calculated residence time of chitin at deep-sea vents would be 1.5 years (565 days) for β-chitin (*Riftia* tubes) and 36 days for α-chitin (crab exoskeletons) (Table 3). By comparison, residence time of chitin in the open sea ranges from 103 to 140 days (see review in Poulicek et al. 1998). However, our histological and chemical data clearly revealed that the crystallographic β form of the chitin is not responsible for the stability of the vestimentiferan tubes.

Table 3 In situ biodegradation experiments of *Riftia* and *Bythograea* exoskeleton. For the crustacean carapace, only the organic fraction was considered for the calculations. The estimated degradation rate of *Riftia* tube organic material is 3.3% month^{-1} (about 40% year^{-1} when extrapolated from the results obtained after 180 days), and 82.5% month^{-1} in the crab carapace (extrapolated from 12-day exposure), whatever the initial weight of the sample. These data suggest that *Riftia* tubes would be degraded within 2.5 years (909 days), while carapaces would be degraded in 36 days. Based on previous work by Shillito et al. (1999), the production of chitin by *Riftia* can be estimated at 763 g m^{-2} year^{-1} (calculations based on a chitin content in fresh-secreted tube of about 10% of the total dry weight; Ravaux et al. 1998; for densely packed clusters of 700 ind m^{-2}; Sarradin et al. 1998). The average degradation rate of chitin at the vent site is 5.4% month^{-1} (about 65% year^{-1}, when expressed in percent of the initial chitin content; extrapolated from 180-day exposure) for *Riftia* and 81.2% month^{-1} (extrapolated from 12-day exposure) for *Bythograea*. This leads to a calculated residence time of chitin at deep-sea vents of 565 days for β-chitin (*Riftia* tubes) and 36 days for α-chitin (crab exoskeletons)

	Riftia pachyptila	*Bythograea thermydron*
Exoskeleton		
Degradation rate: microbial tube consumed[a]	3.3% month^{-1} or 3 kg m^{-2} year^{-1}	82.5% month^{-1}
Time for total degradation	909 days	36 days
Chitin		
Production	763 g m^{-2} year^{-1}	
Degradation rate: microbial chitin consumed[a]	5.4% month^{-1} or 496 g m^{-2} year^{-1}	81.2% month^{-1}
Residence time	565 days	36 days

[a]The samples were enclosed in nets that only allowed access to microorganisms, which means that the estimated tube and chitin degradation rates are essentially a measurement of the microbial tube lysis and chitinolysis rates

Histochemical studies on *Riftia*, *Escarpia* and *Lamellibrachia* tubes demonstrate the presence of disulfide bonds in vestimentiferan tube proteins. The lack of free SH groups suggests that all the SH groups are involved in disulfide bonds. These results are quite consistent with the results of Gaill and Hunt (1986), who reported a high cystein content (10% of the amino acids) in *Riftia* tubes. In contrast, no disulfide bonds were found in *Aphrodita* setae, another β-containing structure which was shown to be stabilized by quinonic bonds (Goffinet and Jeuniaux 1994), and confirmed here by revealing the presence of phenol groups. A preliminary oxidation of disulfide bonds significantly increases the rate of in vitro degradation of vestimentiferan chitin but has no effect on *Bythograea* or *Carcinus* carapaces. The breakage of disulfide bonds seems to increase the accessibility of the chitin polymers to enzymatic hydrolysis (see Fig. 3 for *Riftia*). This leads to the conclusion that the stability of the vestimentiferan tubes is not due to the cristallographic form of the chitin, but results rather from some properties of its associated proteic fraction.

From an ecological standpoint, when considering a calculated 40% year^{-1} rate for tube degradation in *Riftia* (extrapolated from the results obtained after 180 days) and a tube production of 7.6 kg m^{-2} year^{-1}, a tube degradation rate of 3 kg m^{-2} year^{-1} is yielded (Fig. 4). The balance of exoskeleton production and degradation is positive, leading to an accumulation of 4.6 kg m^{-2} year^{-1}. Expressed in terms of chitin, this leads to a chitin production in *Riftia* populations of 763 g m^{-2} year^{-1} (based on chitin content in fresh-secreted tube of about 10% of the total dry weight; Ravaux et al. 1998), and a chitin biodegradation rate of about 496 g m^{-2} year^{-1} (based on a chitin degradation rate of 65% year^{-1}, in percent of the initial content). Assuming chitin production to be 763 g m^{-2} year^{-1} and chitin degradation 496 g m^{-2} year^{-1}, the populations of *Riftia* would be responsible for a net production of 267 g m^{-2} year^{-1} of chitin. Our protocol of in situ experiments can partly account for this apparent storage of tube material. It must be stressed that the samples were enclosed in nets that only allowed access by microorganisms. At first, this means that the estimated tube and chitin degradation rates are essentially a measurement of the microbial tube lysis and chitinolysis rates (i.e. the microorganisms consume about 40% of the tube and 65% of the chitin produced) (Table 3 and Fig. 4, pathway 3). Whether these microorganisms are associated with the tubes, either at the tube surface or within the tube-wall, remains to be determined (Fig. 4, pathways 4 and 5). However, recent studies have

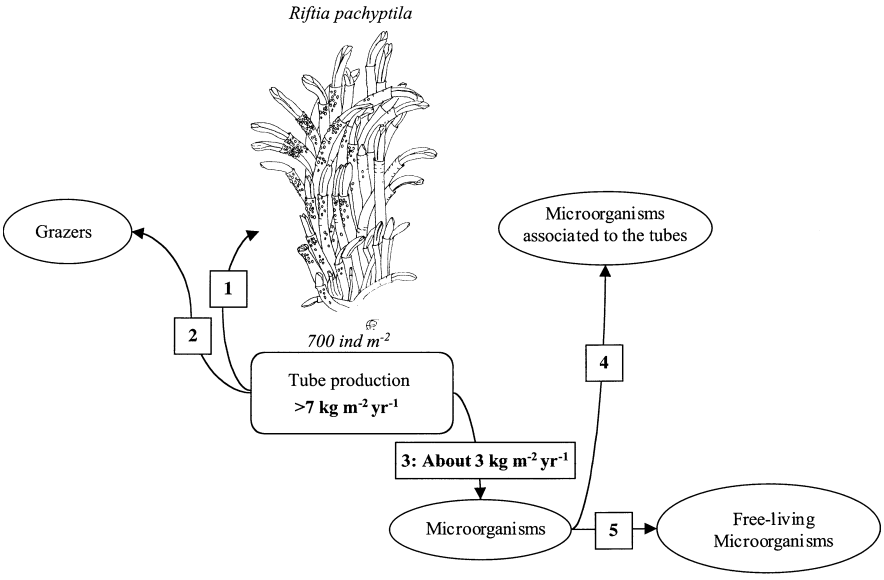


Fig. 4 The different pathways of tube biodegradation by hydrothermal fauna. The potential biodegraders are represented. We provide here an estimated tube production by a population of *Riftia* (with a density of 700 individuals m^2; Sarradin et al. 1998), and the rate of tube degradation by microorganisms (pathway 3). The contribution of the different types of microorganisms (pathways 4 and 5), as well as the involvement of the other organisms (pathways 1 and 2) are to be further elucidated. See text for more explanations. Drawing of *Riftia* clump by F. Pradillon

identified bacteria lineages from the Cytophagales and the Verrumicrobia phylum in *Riftia* tube samples (Lopez-Garcia et al. 2002), and colonial-like bacterial islands were observed within the tube (Lechaire et al. 2002), which could use the tube as a source of nutrients. Finally, we recognize that there are other potential biodegraders not considered in our calculations. These biodegraders remain to be identified, but some are suspected to have the ability to hydrolyse the tube, i.e. *R. pachyptila* itself (Fig. 4, pathway 1) and the limpets (Fig. 4, pathway 2), which are very abundant on *Riftia* tubes (Sadosky et al. 2002). An enzymatic activity reponsible for chitin degradation (chitinase) was found in tissues of *Riftia* adult individuals (Ravaux et al. 1998). In limpets that colonize *Riftia* tubes, the stomach contained excised pieces of *Riftia* tubes (Fretter 1988). The involvement of these species in tube material recycling deserves further study.

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