Cuticle structure and composition of two invertebrates of hydrothermal vents: *Alvinella pompejana* and *Riftia pachyptila*

Hydrothermal vents
Collagen
Cuticle
Liquid crystals
Invertebrates

Sources hydrothermales
Cuticule
Collagène
Invertébrés
Cristaux liquides

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ABSTRACT

The structure of the cuticle from the "Pompeii worm" *Alvinella pompejana* and *Riftia pachyptila* is presented. The cuticle structure of *Alvinella* is similar to that of other annelid species, whereas that of *Riftia* presents patterns which are observed in biological analogues of cholesteric liquid crystals. The amino acid composition of a vestimentiferan cuticle is presented for the first time and compared with that of other invertebrates.

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RÉSUMÉ

Structure et composition de la cuticule de deux invertébrés des sources hydrothermales : *Alvinella pompejana* et *Riftia pachyptila*

La structure des cuticules d'*Alvinella pompejana* et de *Riftia pachyptila* est présentée. La structure de la cuticule d'*Alvinella* est similaire à celle des autres espèces de polychètes, tandis que celle de *Riftia* présente des aspects originaux comparables à ceux que l'on observe dans les analogues biologiques des cristaux liquides cholestériques. Pour la première fois, la composition en acides aminés de la cuticule d'un vestimentifère est présentée : les résultats obtenus sur *Riftia pachyptila* sont comparés à la composition en acides aminés de la cuticule d'autres invertébrés.

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INTRODUCTION

The polychaetous worm, *Alvinella pompejana* (annelid), and the so-called tube worm *Riftia pachyptila* (vestimentiferan), are the most conspicuous members of vent communities. These species live in an area where the vent fluid mixes with the surrounding sea water.
They inhabit a biotope showing a high temperature range when compared with the surrounding water temperature (2°C): 7-15°C for *Riftia pachyptila* and 20-40°C for *Alvinella pompejana* (Desbruyères *et al.*, 1982). *Alvinella pompejana* is surrounded by an acidic fluid with little dissolved oxygen and a high concentration of reducing gases and metal species (Desbruyères *et al.*, 1985). The presence of such an environment incited the authors to study the cuticle, which has a self-evidently protective function in the annelids (*see* Richards, 1984). We present here the data obtained on cuticle of *Alvinella pompejana* and *Riftia pachyptila*. The cuticle is thought to be collagenous in annelids (Murray, Tanzer, 1985) and in pogonophora (Gupta, Little, 1970 ; Southward, 1984) which are close to vestimentifera (Jones, 1985).

This is the first time that the amino acid composition of a vestimentiferan cuticle is described.

MATERIALS AND METHODS

Alvinella pompejana and *Riftia pachyptila* were collected in the East Pacific Rise near 13°N with the submersible Cyana during the *Biocyarise* cruise (2600 m depth, April 1984).

Ultrastructural studies were made on pieces of worm fixed with 0.4 M cacodylate-buffered glutaraldehyde (3 % final concentration) at pH 7.2 and then post-fixed with osmium tetroxyde (1 % final concentration) and embedded in Durcupan. Thin sections were stained with aqueous uranyl acetate and lead citrate and examined using a Philips EM 201 TEM (Centre de Biologie Cellulaire, CNRS, Ivry-sur-Seine, France).

Biochemical analyses were performed on cuticles separated from the body wall of frozen worms and stored in 90 % ethanol or air-dried.

The amino acid composition is given after hydrolysis by 6 N HC1 for 24 hr at 105°C with an automatic amino acid analyzer JEOL JLC 5 AH. The hydroxyproline content was controlled by a specific analysis using the method of Stegemann (1958).

RESULTS AND DISCUSSION

Cuticle structure

The structure of *Alvinella pompejana* and *Riftia pachyptila* cuticles is illustrated in Figure 1. Cuticles are essentially fibrous. Each fibril is actually a bundle of microfibrils.

The cuticle of *Alvinella pompejana* is composed of superimposed layers of fibrils with only two fibril directions (Fig. 1 *a*).

The structure of the "Pompeii worm" cuticle is analogous to that of other polychaete studied so far (Richards, 1984). This structure is similar to a pseudo-orthogonal plywood. Some fibrils present a sinuous aspect in pseudo-longitudinal sections (Fig. 1 *a*). This aspect has been considered as an indication of the helicity of cuticle fibrils (Gaill, Bouligand, 1985), a feature which differentiates *Alvinella pompejana* from other annelid species.

Cuticle fibrils of pogonophora are throught to be collagenous (Gupta, Little, 1970) as in polychaetes (Murray, Tanzer, 1985), though not cross-striated. No striation is observed in the cuticle fibrils of *Riftia pachyptila.*

The fibrils of the vestimentifera *Riftia pachyptila* cuticle present more than two directions. In this species, the distribution of bundles varies from the base to the cuticle surface : series of nested arcs are well observed in the inner part of the cuticle and disordered patterns prevail in the outer part just below the epicuticle (Fig. 1 *b*).

The structure of *Riftia pachyptila* cuticle is quite different from the organization of pogonophoran cuticles (Gupta, Little, 1970 ; Southward, 1984). For the first time, parallel series of nested arcs are observed in vestimentifera and it is well known that these patterns are observed in biological analogues of cholesteric liquid crystals (Bouligand, 1972). The structure consists of thin layers of parallel fibrils which are regularly superimposed : fibrils in two successive layers differ by an angle which ranges from 30° to 60°. The twist is strongly discontinuous. Most invertebrate cuticles with such twisted arrangements of fibrils are much more continuous (Bouligand, Giraud-Guille, 1985).

Cuticle composition

Alvinella pompejana and *Riftia pachyptila* cuticles contain approximately 60 to 70 % dry weight of protein. Their amino acid composition is very similar (Tab.). The low content of glycine (140 and 152 res/1000), of proline (55 and 57 res/1000) and of hydroxyproline (33 and 28 res/1000) demonstrates a high content of non-collagenous proteins in these cuticles. As the exact amino acid composition of the collagenous fraction is as yet unknown, it is not possible to give their exact collagen content. However, if one considers that these collagens are similar to the vertebrate Type I collagen (containing 94 res/1000 of hydroxyproline), then the collagen amount would account for approximately 15 % of the cuticle dry weight. But, if these collagens are similar to annelid cuticle collagens with 150 res/1000 of hydroxyproline (Tab.), then the collagen content would be as low as 10 % of the dry weight. An exact comparison between these two collagens and with the annelid and nematod cuticle collagens already described, requires their isolation and purification. A difference is worth noting from now: as for the annelid and nematode cuticle collagens (Tab.), the *Alvinella pompejana* cuticle collagen does not contain hydroxylysine, whereas the *Riftia pachyptila* cuticle collagen contains a high amount of hydroxylysine (7 res/1000 in the whole cuticle).

The collagen percentage of the cuticular proteins of *Alvinella pompejana* and *Riftia pachyptila* seems to be very low (10 to 15 % of cuticle dry weight) when compared with that of other invertebrates.

The collagen content of the sipunculid cuticle analyzed by Voss-Foucart *et al.* (1978) is about 50 %. It represents more than 60 % of the nematode cuticle of *Caenorhabditis elegans* (Ouazana, Herbage, 1981) and about 90 % of the cuticle proteins of annelids such as *Nereis japonica* (Kimura, Tanzer, 1977) or *Lumbricus terrestris* (Muir, Lee, 1970).

In conclusion, although the structures of *Alvinella pompejana* and *Riftia pachyptila* cuticles are dissimilar, their protein contents are very similar, with a high percentage of non-collagenous proteins. Further purification of their cuticle collagen would be necessary for exact comparison with other invertebrate cuticular collagens.

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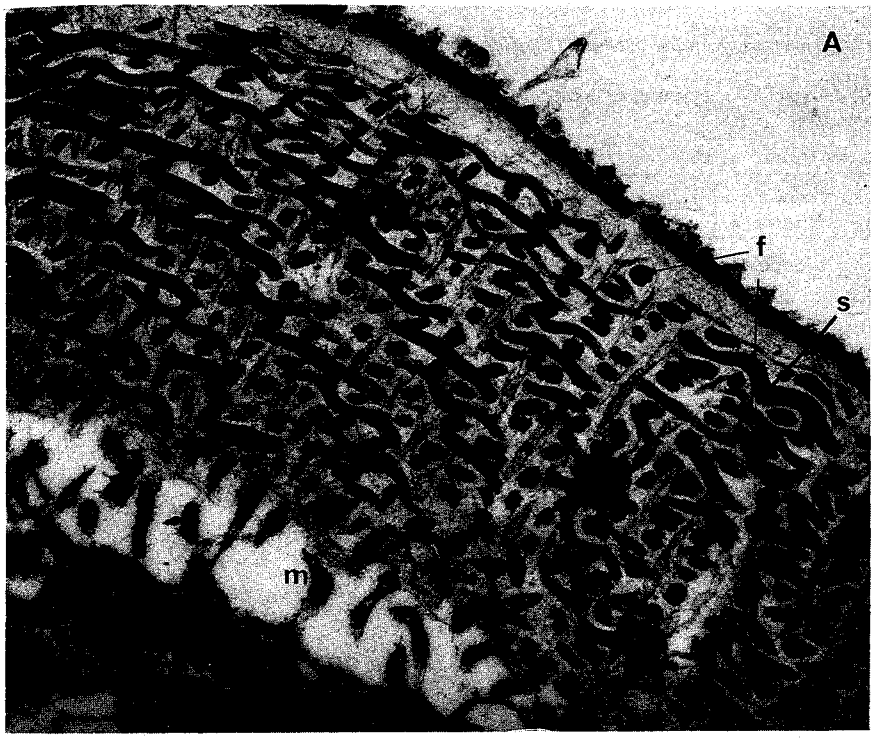

Figure 1*a*
Ultrastructural aspect of the "Pompeii worm" cuticle (Alvinella pompejana) *: the cuticle is observed in transverse section. Fibrils cut in longitudinal (L) and cross sections (C). They form a pseudo-orthogonal plywood (x 40 000 ; f : fibrils ; m : microvilli ; s : sinusoidal aspect of a fibril section).*
Aspect ultrastructural de la cuticule du "ver de Pompéi" *(Alvinella pompejana)* : la cuticule est observée en section transversale. Les fibres sont coupées longitudinalement (L) et transversalement (C). Elles forment un contreplaqué pseudo-orthogonal (x 40 000 ; f : fibres ; m : microvillosités ; s : aspect sinusoïdal de la section fibrillaire).

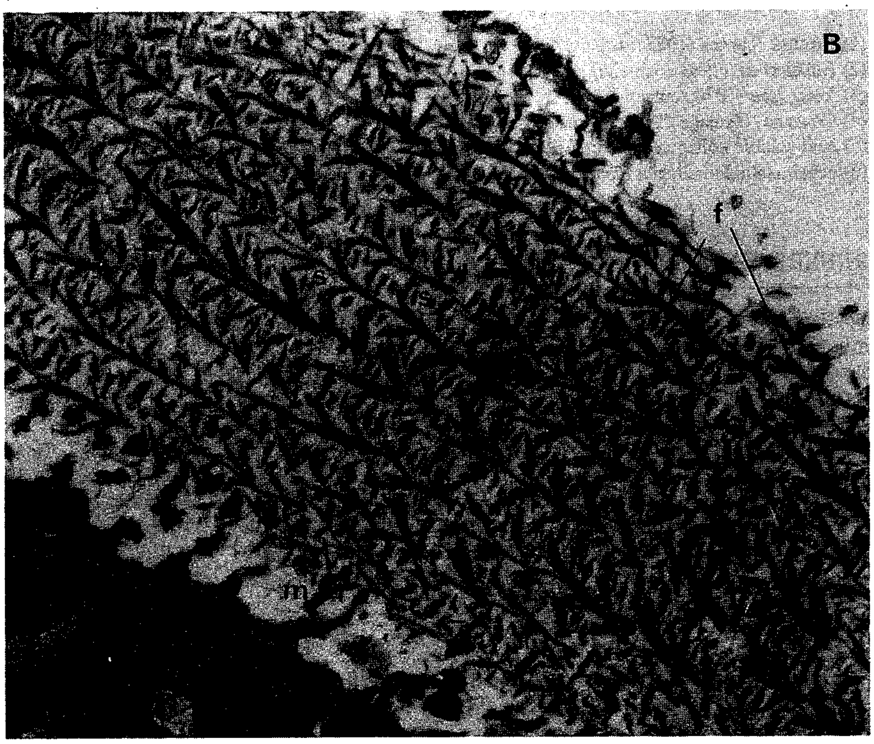

Figure 1*b*
Ultrastructural aspect of the tube worm cuticle (Riftia pachyptila) *: the cuticle is observed in transverse section. Bundles of fibrils present different orientations. Their organization varies through the cuticle thickness where series of nested arcs (s) are observed (x 40 000 ; f : fibrils ; m : microvilli).*
Aspect ultrastructural de la cuticule de *Riftia pachyptila :* la cuticule est observée en section transversale. Les groupes de fibres présentent différentes orientations. Leur organisation varie le long de l'épaisseur de la cuticule, où des séries d'arceaux sont observées (x 40 000 ; f : fibres ; m : microvillosités).

Table
Amino acid composition (per 100 residues) of Alvinella pompejana and Riftia pachyptila cuticles compared with other invertebrate cuticular collagen and vertebrate collagen (types I and II).
Composition en acides aminés (par résidus pour 1000) d'Alvinella pompejana et de la cuticule de Riftia pachyptila comparée avec celle du collagène cuticulaire d'autres invertébrés et du collagène de vertébrés. (type I et II)

Amino acid	*Alvinella pompejana*	*Riftia pachyptila*	*Nereis virens (1)*	*Pomatoceros lamarkii (2)*	*Lumbricus terrestris (3)*		*Caenorhabditis elegans (4)*		*Vertebrate collagen*	
	cuticle	cuticle	cuticle collagen	cuticle	cuticle	soluble proteins	cuticle	soluble proteins	Type I (5)	Type II (6)
Ohpro	33	28	147	-	159	160	82	103	94	96
Pro	55	57	51	82	10	10	114	117	121	121
Gly	140	152	347	224	320	320	251	268	330	326
Ala	76	83	88	57	18	117	131	112	119	103
Asp	102	102	10	85	61	62	79	85	45	48
Thr	67	40	43	68	52	49	30	24	18	23
Ser	70	55	69	71	89	94	44	41	33	26
Glu	113	118	133	108	75	76	97	96	75	93
Halfcys	-	-	-	-	-	-	25	29	-	-
Val	44	45	7	4	25	17	31	21	21	18
Met	-	6	0.8	1	tr	tr	2.1	2.5	6	8
Ileu	38	33	5	14	21	15	15	11	11	9
Leu	73	64	18	14	30	30	21	16	23	26
Tyr	20	16	0.4	9	4	-	10	3.8	3	1
Phe	30	25	2.2	15	9	-	11	9.7	13	13
Ohlys	-	7	-	-	-	-	-	-	7	21
Lys	68	80	0.6	21	21	15	25	26	26	17
His	17	9	0.3	6	1	1	9	10	5	2
Arg	54	80	76	57	23	22	23	25	20	21

(1) Kimura, Tanzer (1977 : annelid)
(2) Bubel *et al.* (1983 : annelid)
(3) Muir, Lee (1970 : annelid)
(4) Ouazana, Herbage (1981 : nematod)
(5) acid-soluble collagen from calf skin (Herbage *et al.*, 1977 ; vertebrate)
(6) pepsin-soluble collagen from calf-articular cartilage (Herbage *et al.*, 1977 ; vertebrate)

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