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# Biochemical characteristics of collagens from marine invertebrates with special emphasis on Polychaeta and Vestimentifera from deep-sea hydrothermal vents

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**Abstract:** The amino acid compositions of the cuticular collagens of five polychaete species (*Arenicola marina, Nereis diversicolor, Alvinella pompejana, Alvinella caudata, Paralvinella grasslei*) were compared using the Matsamura method revised by Murray et al. (1982). These results show the biochemical diversity of their cuticular collagens. We did not find any correlation between the amino acid composition of the cuticular collagens and the taxonomic position of the species investigated. Furthermore, there is no correlation between this composition and the type of habitat of the animals. In contrast with the cuticular collagens, all the interstitial collagens from polychaete species cluster in the same expected area. Thus, together these data indicate that vent annelid collagens do not exhibit any original biochemical characteristics when compared to that of coastal species. The cuticular collagen of the vestimentiferan *Riftia pachyptila* cannot be separated from the whole annelid cuticular collagen area when the Matsamura analysis is done starting with the constituent chain amino acid composition. The same is true for the vestimentiferan interstitial collagen when the method is applied to the primary sequence of the α chain. These results are in favor of the close phylogenetical relationship between polychaete and vestimentiferan collagens hypothetised by Sicot et al. (1997a).

**Résumé** : *Caractéristiques biochimiques des collagènes d'invertébrés marins, en particulier des Polychaeta et Vestimentifera des sources hydrothermales profondes.*
Les compositions en acides aminés des collagènes cuticulaires de cinq espèces de polychètes (*Arenicola marina, Nereis diversicolor, Alvinella pompejana, Alvinella caudata, Paralvinella grasslei*) sont comparées en utilisant la méthode de Matsumura revue par Murray et al. (1982). Ces résultats montrent la diversité biochimique de ces collagènes cuticulaires. Nous n'avons pas trouvé de corrélation entre la composition en acides aminés des collagènes cuticulaires et la position taxonomique des espèces étudiées. De plus, il n'y a pas de corrélation entre cette composition et le type d'habitat des animaux. Contrairement aux collagènes cuticulaires, tous les collagènes interstitiels des espèces de polychètes se regroupent dans la même aire des collagènes fibrillaires. Ainsi, l'ensemble de ces données indique que les collagènes des annélides des sources hydrothermales profondes ne présentent aucune caractéristique biochimique originale par rapport à celles des collagènes d'espèces côtières. Le collagène du vestimentifère *Riftia pachyptila* ne peut être séparé de l'aire de tous les collagènes cuticulaires des annélides quand l'analyse de Matsumura est faite à partir de la composition en acides aminés de la chaîne constituante. La même chose est observée pour le collagène interstitiel du vestimentifère quand la méthode est appliquée à la séquence primaire de la chaine α. Ces résultats sont en faveur d'une étroite relation phylogénétique entre les collagènes des polychètes et des vestimentifères suggérée par Sicot et al. (1997a).

*Keywords* : extracellular matrix, collagen, annelid, vestimentiferan, marine invertebrates, hydrothermal vents.

## Introduction

Invertebrates extracellular matrix (ECM) characteristics are still poorly known (Har-El & Tanzer, 1993; Engel, 1997)

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when compared to the knowlegde obtained on vertebrates ECM (review in van der Rest & Garrone, 1991; van der Rest & Bruckner, 1993). Even if results on these ECM begin to increase with new data on specific models, such as *Drosophila melanogaster* (see Fessler et al., 1994), *Coenorhabditis elegans* (see Kramer, 1994) or sea urchins (Exposito et al., 1995), the biochemical characteristics of their main components, the fibrillar collagens, are still poorly known. This is especially true for the ECM from marine organisms where most of the data have been obtained on deep sea-hydrothermal vent animals (review in Gaill & Hunt, 1991; Gaill, 1993; Sicot et al., 1997a).

Collagens constitute a large and diverse family of multidomain proteins of the extracellular matrix (review invan der Rest & Garrone, 1991; van der Rest & Bruckner, 1993). The collagen molecule is a triple helix which results from the assembly of three identical or similar polypeptides, named α chains. These chains are assembled from Gly-X-Y sequence repeats, in which X and Y are frequently respectively proline and hydroxyproline. Up to now 19 vertebrate collagen types have been described. The fibrillar collagens form the most homogenous subfamily.

For annelids and vestimentiferans, two types of collagens have been described (Gaill et al., 1991; Mann et al., 1996), an interstitial collagen and a cuticular one. The interstitial collagens are similar to fibrillar collagens since they form striated fibrils (Gaill et al., 1994). Cuticular collagens differ by their localisation (surface of the animal), their length (2,4 µm) and their globular structure at one end (Gaill et al., 1991).

In order to characterize the specificity of these collagens, we have used a method previously helpful to classify different types of invertebrate collagens. This method, created by Matsumura (1972) and revised by Murray et al. (1982), is based on the analysis of the collagen amino acid composition, and more precisely on the relative ratios of polar, hydroxylic and hydrophobic amino acids of the molecules. The results obtained by several studies (Matsumura, 1972, Murray et al., 1982, Murray & Tanzer, 1985), indicate the existence of five different invertebrate collagen groups: the interstitial collagen, the basement membrane collagen, the cuticular collagen from nematods, polychaetous and oligochaetous annelids, forming three additional groups. Previous studies (Hamraoui, 1994) have shown the original location of the cuticular collagen from *Arenicola marina* (Linnaeus) in the Matsamura diagram. This collagen was surprizingly located in between the polychaete and the oligochaete areas, differing thus from what was previously predicted (Murray et al., 1982). Similar analysis were performed on a larger set of data in order to confirm these results. The amino acid composition of the cuticular collagen of five polychaete species (*Arenicola marina, Nereis diversicolor* O.-F. Muller, *Alvinella pompejana* Desbruyères & Laubier, *Alvinella caudata* Desbruyères & Laubier *Paralvinella grasslei* Desbruyères & Laubier) were compared with that of the vestimentiferan *Riftia pachyptila* Jones.

The second goal of this study was to determine if the collagens from deep-sea hydrothermal vent organisms exhibited some specific biochemical features, which were not shared by coastal species from the same phyla. Concerning this, a similar analysis was performed on a second type of collagen, the interstitial collagen, widely distributed among the animal kingdom and recognized as belonging to the fibrillar collagen family (Gaill et al., 1994; Sicot et al., 1997a). In order to clarify the overall meaning of the results obtained on the annelid and vestimentiferan collagens, additional comparison were done with similar data obtained on the interstitial collagens from marine invertebrates such as echinoderms (Matsumura, 1973; Pucci-Minafra et al., 1978; Shimizu et al., 1990; Trotter et al., 1994; Trotter et al., 1995) and cnidarian tissues (Tillet-Barret et al., 1992).

## Material and methods

### I. Data set of amino acid compositions

The data on cuticular and interstitial collagens of polychaetes were obtained from the body wall of different species, using the same extraction and purification methods (Gaill et al. 1995). Table 1 give the informations relative to the data we used and the corresponding species. These data were obtained from the amino acid composition of the triple helix (native molecule).

Additional data being available on the vestimentiferan *Riftia pachyptila*, the amino acid composition of the native collagen molecule was compared with that of the purified constituent chains in the case of the cuticular collagen (Mann et al., 1996) and with the deduced composition from the α chain primary sequence (Mann et al., 1992) in the case of the interstitial collagen (Table 1).

Analyses performed on the echinoderm and cnidarian collagens (Tillet-Barret et al. 1992 and Miura & Kimura, 1985) are given in Table 1.

### II. Matsumura's method

This method (Matsumura, 1972) consists in calculating the ratios of polar ($aa_P$), hydroxylic ($aa_{OH}$) and hydrophobic ($aa_\phi$) amino acid to total amino acids, for each collagen, and graphing the resulting ratios, R values, onto a triangular coordinate space (Fig. 1A). To calculate the R values (see Table 2 and Table 3), we used the amino acid analyses expressed as residues/1000 with:

$$R_P = aa_P \times 1000/aa_P + aa_{OH} + aa_\phi$$

**Table 1.** Data set concerning the origin of amino acid compositions of collagens studied in this work.
**Tableau 1.** Données concernant l'origine des compositions en acides aminés des collagènes étudiés dans ce travail.

| Animal | Tissue source | Collagen type | Origin of amino acid composition | References |
|---|---|---|---|---|
| **Polychaetes**: | | | | |
| *Arenicola marina* | body wall | IC/CC | triple helix | Gaill et al., 1995 |
| *Nereis diversicolor* | body wall | IC/CC | triple helix | Gaill et al., 1995 |
| *Alvinella pompejana** | body wall | IC/CC | triple helix | Gaill et al., 1995 |
| *Alvinella caudata** | body wall | IC/CC | triple helix | Gaill et al., 1995 |
| *Paralvinella grasslei** | body wall | IC/CC | triple helix | Gaill et al., 1995 |
| **Vestimentiferans**: | | | | |
| *Riftia pachyptila** | body wall | IC | triple helix | Gaill et al., 1991 |
| | body wall | IC | primary sequence | Mann et al., 1992 |
| | plume region | CC | triple helix | Gaill et al., 1991 |
| | plume region | CC | constituent chains | Mann et al., 1996 |
| **Echinoderms**: | | | | |
| *Paracentrotus lividus* | Aristotle's lantern | IC | triple helix | Pucci-Minafra et al., 1978 |
| *Asthenosoma ijimai* | test | IC | triple helix | Shimizu et al., 1990 |
| *Eucidaris tribuloides* | spine ligament | IC | triple helix | Trotter et al., 1994 |
| *Asterias amurensis* | body wall | IC | triple helix | Kimura et al., 1993 |
| *Stichopus japonicus* | body wall fibril | IC | triple helix | Matsumura, 1973 |
| *Cucumaria frondosa* | dermis fibril | IC | triple helix | Trotter et al., 1995 |
| **Cnidarians**: | | | | |
| *Veretillum cynomorium* | mesoglea | IC | constituent chains | Tillet-Barret et al., 1992 |
| *Stomolophus nomurai* | mesoglea | IC | constituent chains | Miura & Kimura, 1985 |

* Deep-sea hydrothermal vent species.
CC: Cuticular collagen.
IC: Interstitial collagen.

$R_{OH} = aa_{OH} \times 1000/aa_P + aa_{OH} + aa\phi$
$R_\phi = aa_\phi \times 1000/aa_P + aa_{OH} + aa_\phi$
where
$aa_P$ = Asp + Glu + Hyl + Lys + His + Arg
$aa_{OH}$ = Hyp + Thr + Ser + Tyr + Hyl
$aa_\phi$ = Val + Met + Leu + Ile + Tyr + Phe.

## Results

I. Cuticular collagen of polychaetes and vestimentiferans

The cuticular collagen of *A. marina* and *N. diversicolor*, for wich the R values have been calculated from the whole molecule, are located close to the area defined by Murray et al. (1982) for this collagen type (Fig. 1A). Collagens from both theses species have similar ratio ($R_\phi$) of hydrophobic amino acids. They differ in their hydroxylic ($R_{OH}$) and polar ($R_P$) amino acids ratios (Table 2). However the collagen from *N. diversicolor* is located outside the expected area (P), whereas that of *A. marina* is located in between the P and O areas (Fig. 1A).

The cuticular collagen from the hydrothermal vent species *A. pompejana, A. caudata* and *P. grasslei* have similar ratios of hydrophobic amino acids and differ in their hydroxylic and polar amino acid ratios. That of *P. grasslei* is located close to the point representing the *N. diversicolor* collagen. The *A. caudata* collagen is located close to the *A. marina* one. All these collagens are located in a band which is parallel to the hydroxylic amino acid axis (Fig. 1A). Cuticular collagen from two species belonging to the same family have not the same localization: that of *A. pompejana* is located close to the polychaetes area (P) whereas the collagen of *A. caudata* is close the oligochaetes one (O).

The cuticular collagen of the vestimentiferan *Riftia pachyptila* exhibits a singular position in the Matsumura triangular coordinate space (Fig. 1A), as already shown by Hamraoui (1994), when the amino acid composition of the homotrimeric molecule is considered. However, this

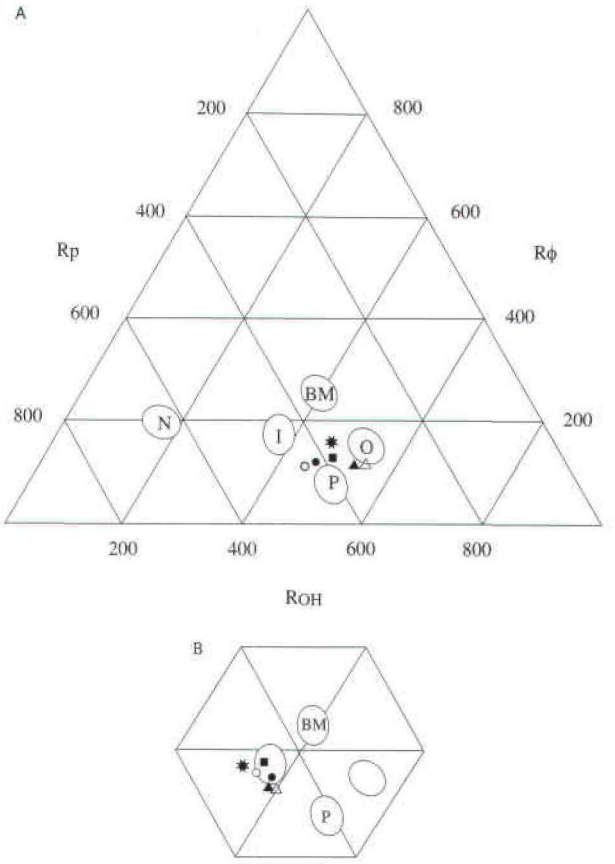



**Figure 1.** Collagens of polychaetes and vestimentiferans: location on the Matsumura diagram, revised by Murray et al. (1982). (A) Cuticular collagens. (B) Interstitial collagens (see Table 1 for references). Only the middle basal part of the diagram is presented in B. Capital letterings indicate areas defined for different collagen types by Murray et al. (1982): BM basement membrane collagens, I interstitial collagens, N cuticular collagens of nematods, O cuticular collagens of oligochaetes, P cuticular collagens of polychaetes. (■) *Alvinella pompejana*, (▲) *Alvinella caudata*, (●) *Paralvinella grasslei*, (o) *Nereis diversicolor*, (△) *Arenicola marina*, (✱) *Riftia pachyptila*.

**Figure 1**. Collagènes de polychètes et de vestimentifères : localisation sur le diagramme de Matsumura, adapté par Murray et al. (1982). (A) Collagènes cuticulaires. (B) Collagènes interstitiels (voir tableau 1 pour références). Seule la partie basale du diagramme est représentée en B. Les lettres en majuscule indiquent les aires définies pour les différents types de collagènes par Murray et al., 1982 : BM collagènes de membranes basales, I collagènes interstitiels, N collagènes cuticulaires des nématodes, O collagènes cuticulaires des oligochètes et P collagènes cuticulaires des polychètes. (■) *Alvinella pompejana*, (▲) *Alvinella caudata*, (●) *Paralvinella grasslei*, (o) *Nereis diversicolor*, (△) *Arenicola marina*, (✱) *Riftia pachyptila*.

**Table 2.** Values of $R_\phi$, $R_{OH}$ and $R_p$ representing respectively the ratios of hydrophobic ($aa_\phi$), hydroxylic ($aa_{OH}$) and polar ($aa_p$) amino acids to total amino acids of interstitial and cuticular collagens from deep-sea hydrothermal vent polychaetes and vestimentiferans, and from littoral polychaetes (see Table 1 for references).

**Tableau 2.** Valeurs de $R_\phi$, $R_{OH}$ et $R_p$ représentant respectivement les rapports des acides aminés hydrophobes ($aa_\phi$), hydroxylés ($aa_{OH}$) et polaires ($aa_p$) sur les acides aminés totaux des collagènes interstitiels et cuticulaires de polychètes et vestimentifères des sources hydrothermales profondes et de polychètes littorales (voir Tableau 1 pour les références).

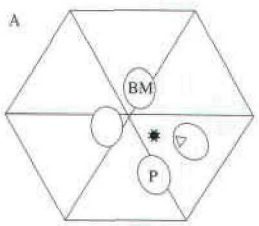
| Animal | | Symbol | Interstitial Collagen | | | Cuticular Collagen | | |
|---|---|---|---|---|---|---|---|---|
| | | | $R_\phi$ | $R_{OH}$ | $R_p$ | $R_\phi$ | $R_{OH}$ | $R_p$ |
| **Polychaeta:** | | | | | | | | |
| *Arenicola marina* | | △ | 125 | 404 | 471 | 115 | 545 | 340 |
| *Nereis diversicolor* | | o | 145 | 354 | 500 | 108 | 449 | 444 |
| *Alvinella pompejana** | | ■ | 171 | 355 | 474 | 124 | 486 | 389 |
| *Alvinella caudata** | | ▲ | 129 | 404 | 465 | 108 | 527 | 365 |
| *Paralvinella grasslei** | | ● | 141 | 399 | 460 | 118 | 456 | 426 |
| **Vestimentifera:** | | | | | | | | |
| | primary sequence | ▼ | 188 | 402 | 410 | | | |
| *Riftia pachyptila** | triple helix | ✱ | 164 | 331 | 506 | 161 | 465 | 372 |
| | constituent chains | ▽ | | | | 152 | 514 | 333 |

* Deep-sea hydrothermal vent species.

**Table 3.** Values of $R_\phi$, $R_{OH}$ and $R_p$ representing respectively the ratios of hydrophobic ($aa_\phi$), hydroxylic ($aa_{OH}$) and polar ($aa_p$) amino acids to total amino acids of interstitial collagens from echinoderms and cnidarians (see Table 1 for references).

**Tableau 3.** Valeurs de $R_\phi$, $R_{OH}$ et $R_p$ représentant respectivement les rapports des acides aminés hydrophobes ($aa_\phi$), hydroxylés ($aa_{OH}$) et polaires ($aa_p$) sur les acides aminés totaux des collagènes interstitiels d'échinodermes et de cnidaires (voir tableau 1 pour les références).

| Animal | Symbol | Interstitial Collagen | | |
|---|---|---|---|---|
| | | $R_\phi$ | $R_{OH}$ | $R_p$ |
| **Echinodermata:** | | | | |
| *Paracentrotus lividus* | ① | 198 | 445 | 357 |
| *Asthenosoma ijimai* | ② | 174 | 369 | 496 |
| *Eucidaris tribuloides* | ③ | 172 | 394 | 430 |
| *Asterias amurensis* | ④ | 160 | 359 | 481 |
| *Stichopus japonicus* | ⑤ | 168 | 339 | 493 |
| *Cucumaria frondosa* | ⑥ | 215 | 340 | 444 |
| **Cnidaria:** | | | | |
| *Veretillum cynomorium* | | | | |
| α1 | ⓐ | 156 | 284 | 560 |
| α2 | ⓑ | 117 | 350 | 534 |
| *Stomolophus nomurai* | | | | |
| α1 | ⓒ | 166 | 316 | 518 |
| α2 | ⓓ | 170 | 339 | 491 |
| α3 | ⓔ | 146 | 305 | 550 |



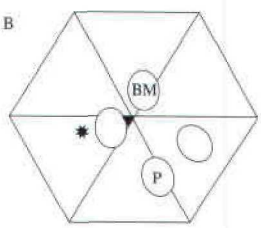
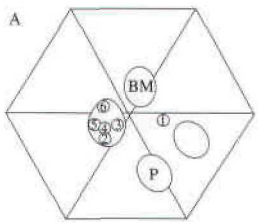




**Figure 2.** Collagens of *Riftia pachyptila*: location on the Matsumura diagram (see Table 1 for references). (A) Cuticular collagen: (✱) triple helix, (▽) constituent chains. (B) Interstitial collagen (✱) triple helix, (▼) α chain primary sequence.

**Figure 2.** Collagènes de *Riftia pachyptila*: localisation sur le diagramme de Matsumura (voir tableau 1 pour références). (A) Collagène cuticulaire : (✱) triple hélice, (▽) chaînes constituantes. (B) Collagène interstitiel : (✱) triple hélice, (▼) séquence primaire de la chaîne α.



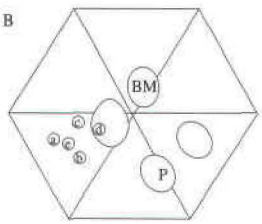




**Figure 3.** Echinoderms and cnidarians collagens: location on the Matsumura diagram (see Table 1 for references). (A) Echinoderms (triple helix) ① *Paracentrotus lividus,* ② *Asthenosoma ijimai,* ③ *Eucidaris tribuloides,* ④ *Asterias amurensis,* ⑤ *Stichopus japonicus,* ⑥ *Cucumaria frondosa.* (B) Cnidarians (α constituent chains) From *Veretillum cynomorium*: ⓐ $\alpha_1$, ⓐ $\alpha_2$ and from *Stomolophus nomurai:* ⓒ $\alpha_1$, ⓓ $\alpha_2$, ⓔ $\alpha_3$.

**Figure 3.** Collagènes d'échinodermes et de cnidaires : localisation sur le diagramme de Matsumura (voir tableau 1 pour références). (A) Echinodermes (triple hélice) : ① *Paracentrotus lividus,* ② *Asthenosoma ijimai,* ③ *Eucidaris tribuloides,* ④ *Asterias amurensis,* ⑤ *Stichopus japonicus,* ⑥ *Cucumaria frondosa.* (B) Cnidaires (chaînes constituantes α) de *Veretillum cynomorium* : ⓐ $\alpha_1$, ⓑ $\alpha_2$ et de *Stomolophus nomurai* : ⓒ $\alpha_1$, ⓓ $\alpha_2$, ⓒ $\alpha_3$.

singularity disappears when the R value is calculated from the purified constituent chain composition. In this case, the vestimentiferan collagen is shifted towards the oligochaete area (O) as illustrated in Fig. 2A.

II. Interstitial collagens from polychaetes and vestimentiferans: comparison with echinoderms and cnidarians fibrillar collagens

All the interstitial collagens of the five polychaetes species (Table 2) are located, as expected, in the corresponding area labelled (I) (Fig. 1B). The *R. pachyptila* interstitial collagen is observed outside this area when we consider the amino acid composition of the triple helical molecule (Fig. 2B). However this vestimentiferan collagen falls down inside the (I) area (Fig. 2B) when we consider the deduced composition from the complete primary sequence of this chain (Table 2).

Interstitial collagens from various tissues of echinoderm species (Table 1, 3) are located in the same fibrillar collagen area (I) except in the case of the Aristotle's lantern of *Paracentrotus lividus* (Fig. 3A). These data were obtained

from the amino acid composition of the native molecules. By contrast the location of most of the cnidarian a chain collagens (Table 1, 3) are located outside this area (Fig. 3B).

## Discussion

The cuticular collagens of polychaetes have specific biochemical characteristics as indicated by our results and their location in the triangular coordinate space of Matsumura (1972). They are characterized by a high ratio of hydroxylic amino acids and a small hydrophobic amino acid ratio, differing thus from the interstitial collagen family as shown by Murray et al. (1982). The observed variability of the biochemical characteristics of the cuticular collagens of polychaetes is quite surprising when compared to previous results (Murray & Tanzer, 1985). Our data do not corroborate the discontinuity between the two groups of polychaetes and oligochaetes, but confirm the biochemical diversity of the cuticular collagen of polychaetes already hypothetized by Hamraoui (1994).

We did not find any correlation between the amino acid composition of the cuticular collagens and the taxonomic position of the species investigated. Thus two closely related species such as *Alvinella pompejana* and *A. caudata* have different positions in the diagram of Matsumura (Fig. 1A). Moreover, two species from different families *Nereis diversicolor* and *Paralvinella grasslei* are located, according to the biochemical composition of their cuticular collagen, in the same area. Furthermore, there is no correlation between the amino acid composition of these collagens and the type of habitat of the polychaetes species. Thus, species living in the littoral zone (*A. marina* and *N. diversicolor*) have different positions in the diagram of Matsumura; while polychaetes living in quite different environments, deep-sea hydrothermal vents (*P. grasslei*) and littoral zone (*N. diversicolor*) have a similar location in the diagram.

In contrast with the cuticular collagens, all the interstitial collagens from polychaete species cluster in the same expected (I) area (Fig. 1B). These data indicate that vent polychaete collagens do not exhibit any original biochemical characteristics when compared to that of coastal species.

It must be noted that the thermal stability is the only specific characteristic of the collagens from vent species. Collagens from *A. pompejana* have the highest thermal stability (45°C) known, up to now, in marine invertebrates (Gaill et al., 1995) whereas those from *A. marina*, which live in colder areas, have a lower thermal stability (28°C). This indicates specific adaptations of their collagens properties to environmental temperatures (Sicot et al., 1997b).

Our results do not corroborate the singularity of the *Riftia pachyptila* collagens in the Matsumura diagram (Hamraoui, 1994; Hamraoui & Gaill, 1997). The cuticular collagen of R. *pachyptila* cannot be separated from the whole annelid collagen area previously defined (P and O, Fig. 2A) when the R values (Table 2) are calculated from the amino acid composition of the constituent chain. The same is true for the interstitial collagen when the Matsumura method is applied to the primary sequence of the α chain (Fig. 2B). Even though vestimentiferan phylogenetical specificities have been shown in different studies (Jones, 1985, Williams et al., 1993, Young et al., 1996), our results confirm the close phylogenetical relationship between polychaete and vestimentiferan collagens (Sicot et al., 1997a). Complementary analyses including the knowledge of the collagen primary sequence of polychaetes (Sicot et al., 1997b) and of other species of vestimentiferans would help to confirm these results.

The differences observed between the *Riftia pachyptila* collagen samples (Table 2, Fig. 2) indicate the importance of the purification process in the resulting data. The position obtained in the Matsumura diagram vary with the data set considered (triple helical molecule, α chain composition or primary sequence).

The interstitial collagen of different tissues extracts from different echinoderm species displays an homogeneity similar to that observed in the polychaetes, except in one case (Fig. 3A). In contrast, the cnidarian collagen chains are located outside the (I) area (Fig. 3B), a fact which may also result from the similarity of these molecules with the V and XI collagen types (Tillet-Barret et al., 1992; Tillet et al., 1996). Moreover, recent data pointed out the diversity of the collagen types in marine organisms (Coyne et al., 1997; Engel, 1997; Garza & Torre-Blanco, 1997) and complementary analyses are needed to clarify the molecular taxonomy and phylogeny of invertebrate collagens.

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