DISTRIBUTION AND QUANTITATIVE IMPORTANCE OF CHITIN IN ANIMALS

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ABSTRACT

Using a specific and quantitative enzymatic-identification procedure, the polysaccharide chitin has been found in a wide range of animal species. It is used by protozoans, mainly ciliates, to build up cyst walls. It also constitutes the bulk of the stalks or stems of most hydrozoan colonies, but it is rarely produced by Scyphozoa (jellyfishes) and Anthozoa, and is absent in sponges.

Chitin is the main structural polysaccharide of most invertebrates belonging to the Protostomia. Arthropods are the best known and most important chitin-producing animals; the dry organic matter of their cuticles can contain up to 80% chitin. Besides the arthropods, relatively large amounts of chitin may be found in the setae of annelids (from 20 to 38% of dry weight), in the skeleton of the colonies of Bryozoa and in the shells and other structures (jaws, radulae, gastric shield) of many mollusc species (up to 7% of the dry organic matter in gastropods and bivalve shells, and up to 26% in cephalopods). Chitin is only absent in free and parasitic flatworms (Platyhelminthes), nemerteans, sipunculids and leeches. In some other groups, such as nematodes and rotifers, chitin is present only in the egg envelopes.

Chitin synthesis has never been observed in echinoderms or vertebrates, but the tubes of some Pogonophora contain 33% chitin, while tunicates secrete a chitinous peritrophic membrane.

From an ecological point of view, besides crustaceans and molluscs, marine benthic animals are a rich source of chitin. Despite their small size, bryozoan and hydrozoan colonies yield a large biomass with relatively substantial amounts of chitin. Some bryozoans play a role in the epuration of fresh water and produce a considerable biomass of chitin-containing colonies as well.

The proportion of "free" chitin, i.e., not bound to other organic molecules, is generally low (less than 10%), although it can account for 80% of the total chitin in mollusc shells.

INTRODUCTION

The first comprehensive studies of chitin distribution in animals (9, 10,20,26,28) were based on histochemical methods, such as the chitosan test by Campbell (5). These methods lacked specificity, however, and were sometimes unreliable, especially with small animals or when the amount of chitin was low (23,25). The more recent x-ray diffraction method (27) gives accurate but only qualitative results.

In order to obtain both qualitative and quantitative data on chitin

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occurrence and localization, an enzymatic method based on the use of purified chitinases was developed (13,14). Owing to the specificity of chitinases (Enzyme Nomenclature:3.2.1.14) for the β-1,4 glucosidic linkages in N-acetylglucosamine polymers, this enzymatic method is highly specific for chitin, provided the purified chitinase preparation is devoid of other hydrolase. Moreover, this method enables us to discriminate the "free" chitin from the chitin chemically bound to other substances (14).

This distribution of chitin biosynthesis in animals has already been discussed from an evolutionary point of view (13,14,16). The aim of the present paper is to try to summarize the numerous data so far obtained with regard to the main ecological features of chitin-containing animals.

METHODS

Chitin was identified and estimated by the enzymatic method of Jeuniaux (13,14). After desiccation under vacuum, the material was weighed, treated with 0.5 N HCl at room temperature, washed, weighed, then treated with 0.5 N NaOH for 6 hours at 100°C. After washing, the residual material was suspended in a buffer (citric acid 0.2 M - Na_2HPO_4 0.4M) at pH 5.2 and incubated for 4-8 hours at 37°C with 1 ml purified chitinase (0.9 mg/ml for 0.02-2 mg chitin), using thymol as an antiseptic. After centrifugation, an aliquot of the supernatant was incubated with chitobiase (lobster serum 10 times diluted with distilled water) at pH 5.2 at 37°C for 2 hours. The liberated N-acetylglucosamine was determined by the method of Reissig et al. (24). The results are expressed as mg of chitin per 100 mg dry organic matter (chitin %).

"Free" chitin is estimated by the same procedure, omitting any previous treatment with NaOH.

The enzymatic method was also used for the qualitative detection of chitin in small animals (3,13,15). After treatment with HCl and NaOH, the washed residues were stained with Congo Red, then incubated with chitinase and observed under the light microscope.

Chitinase was purified from submerged culture filtrates of <u>Streptomyces antibioticus</u> (11) following the procedure described by Jeuniaux (12,13,15).

RESULTS

<u>Micro- and meiofauna</u>

Among protozoa, chitin is used by most ciliates to build cyst walls (14 of 22 species so far studied) (4), or sheaths in the case of the sessile species (Folliculina) that can sometimes be abundant on marine substrates.

The mesopsammic meiofauna pluricellular species living in soft sediments are mainly Turbellaria, nemerteans, nematodes, rotifers and gastrotrichs, which are devoid of chitinous structures. The eggs of nematodes, rotifers and gastrotrichs are, however, often provided with chitinoproteic envelopes (14.6% chitin with respect to total dry weight of the amictic eggs of <u>Brachionus leydigii</u>) (6,17).

Kinorhynchs, small and scarce mesopsammic marine animals, are covered with a chitinous cuticle. Tardigrades, which are often more common, also possess(contrary to the opinion of some authors) a relatively thick cuticle made of chitin, probably bound to proteins (3).

Endoparasites

Chitin was found neither in parasitic flatworms (Cestoda, Trematoda) nor in round worms (Nematoda), except in the egg envelopes of the latter (16.6% in ascarid eggs) (13).

Terrestrial invertebrates

Chitin is the structural polysaccharide of the cuticle of insects, arachnids and myriapods (20 to 80%). Terrestrial tardigrades (3) and onychophorans (27) also possess a chitinous cuticle. The setae of earthworms (Oligochaeta) are also typical chitinous structures (27). In the pulmonate gastropods, snail shells contain small amounts (3%) of chitin (13), mainly as "free" chitin.

Planktonic and pelagic animals

The marine zooplankton are a rich source of chitin, being mainly formed by small holoplanktonic crustaceans and, in certain periods, by the meroplanktonic larvae of pelagic or benthic crustaceans. Their cuticular organic matter contains about 60-80% chitin.

Planktonic and pelagic coelenterates (Hydrozoa, Scyphozoa, Ctenophora) are devoid of chitin, with the exception of the pelagic colonies of Chondrophoridae such as Velella, the floating apparatus of which is a chitinous perisarc, as previously stated (10,22), containing about 48% chitin, almost entirely as "free" chitin.

The most important chitin-producing pelagic animals are the cephalopods, mainly cuttlefishes, the shells of which sometimes accumulate on the beaches with the tide. Chitin accounts for 26% of the organic matter of Sepia officinalis shells, and for 17.9% of squid (Loligo vulgaris) pens, mainly as "free" chitin.

Benthic marine fauna

Most benthic marine invertebrates produce chitinous structures, with the exception of sponges, flatworms (Turbellaria), nemerteans, echinoderms, sipunculids, pterobranchs and enteropneusts (7,13,27). The thick mantle, or tunic, of the sessile tunicates (sea squirts) is devoid of chitin, but these animals secrete a chitinous peritrophic membrane (21).

In crustaceans, the proportion of chitin in the cuticle is about 65-85% of the dry organic matter.

In molluscs, chitin was found in variable amounts in a wide variety of morphological structures (8,13,23), mainly as "free" chitin. The proportion of chitin varied from 0.1 to 7.3% in the periostracum, traces to 0.2% in prisms, 0.1 to 1.2% in mother of pearl, and from 0.2 to 8.3% in the calcitostracum of bivalve shells, the higher values being found in burrowing species such as Glycymeris, Venus, Pholas, Zyrphaea and Mya. The gastric shield of bivalves is also made of chitin (27.7% in Zyrphaea crispata [1]). In marine gastropods, the proportion of chitin was 3.0 to 7.0% in mother of pearl of 3 species of Prosobranchia, 19.7% in the radulae, and 36.8% in the gizzard teeth of an opisthobranch. In the shell plates of Acanthochites discrepans (Polyplacophora), chitin amounted to 12%.

In different kinds of setae of marine worms (Polychaeta), chitin accounts for 20 to 38% of the dry organic matter and is mainly bound to quinone-tanned proteins. The tubes secreted by tubicolous worms do not contain any chitin.

Besides this macrofauna, benthic communities of the continental shelf also include the encrusting or erected colonies of hydrozoans and bryozoans (Ectoprocta). In both cases, the cuticular organic matrix of these colonies is made of chitin more or less bound to proteins, calcified in many species. In hydrozoans, the amount of chitin varied from 3.2 to 30.3% (13). In whole colonies of bryozoans (Flustra, Scrupocellaria, Cellaria, Crisia), the amount of chitin was 1.6 to 6.4%. Despite their small size, hydrozoan and bryozoan colonies may comprise an important element in epifaunal communities and may thus represent a large biomass of chitinous organic matter.

Chitin was also found in the stalk cuticle of Endoprocta, in the tubes of Phoronidea (13.5%), and in those of Pogonophora (33%) (2,7). The shells of brachiopods seemed to be devoid of chitin, with the exception of those of some Inarticulata (Lingula, Discinisca) (9), in which chitin amounted to 29% of the dry organic matter (13). The cuticle of the pedicle was chitinous in all the brachiopods so far studied (9,13,27).

Benthic freshwater invertebrates

Besides insects and crustaceans, a few bryozoan species may develop important colonies in some semi-polluted waters and give rise to the production of a large biomass (19) mainly made up of a chitinoproteic exoskeleton (ectocyst) (9,13). The biomass of Plumatella fungosa colonies in a pond was estimated to be 15.6 tons/ha (fresh weight) and the annual production to be 112 Kg nitrogen/ha/year. These colonies play a prominent part in the process of water purification (18).

CONCLUSIONS

If chitin is mainly secreted by cells of epidermal origin, the endoderm layer is also able to synthesize this polysaccharide, not only in arthropods, annelids and even tunicates (peritrophic membranes), but also in molluscs (gastric shields and gizzard plates).

The biosynthesis of chitin is a very old property of the animal cell, already present in Protozoa. This property was retained by most invertebrate animals of those groups belonging to the protostomian evolutionary lineage. At the top of this lineage, arthropods have exploited to a maximum the ability to use chitin as a structural polysaccharide, chitin often constituting, indeed, more than 50% of the cuticular organic matter. However, chitin may also be found in appreciable amounts in annelids, molluscs and in hydrozoan and bryozoan colonies, which form an important part of the marine benthic biomass.

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