

Chapter 2

Chitin: Structure, Chemistry and Biology



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Abstract Chitin is a linear polysaccharide of the amino sugar N-acetyl glucosamine. It is present in the extracellular matrix of a variety of invertebrates including sponges, molluscs, nematodes and arthropods and fungi. Generally, it is an important component of protective or supportive extracellular matrices that cover the tissue that produces it or the whole body of the organism. Chitin fibres associate with each other adopting one of three possible crystalline organisations, i.e. α -, β - or γ -chitin. Usually, chitin fibre bundles interact with chitin-binding proteins forming higher order structures. Chitin laminae, which are two-dimensional sheets of α -chitin crystals with antiparallel running chitin fibres in association with β -folded proteins, are primary constituents of the arthropod cuticle and the fibrous extracellular matrix in sponges. A tri-dimensional composite material of proteins coacervates and β -chitin constitute hard biomaterials such as the squid beak. The molecular composition of γ -chitin-based structures that contribute to the physical barrier found in insect cocoons is less well studied. In principle, chitin is a versatile extracellular polysaccharide that in association with proteins defines the mechanical properties of tissues and organisms.

Keywords Extracellular matrix · Cuticle · Body shape · Evolution · Barrier

2.1 Introduction

In his main work about animal biology *Historia animalium* (Greek: Τῶν περὶ τὰ ζῷα ἱστοριῶν, *Ton peri ta zoia historion*), Aristotle (384–322 BC) named the casted case of the larvae of a moth he found in clothes, probably the common clothes moth, *Tineola bisselliella*, (Hummel 1823), *χιτῶν* (*kithon*), which means ‘sheath’ in Greek (Fig. 2.1a). In his description of the integument of other insect species, he used the terms ‘skin’ and ‘clothing’. Thus, the term *χιτῶν* does not seem to specifically assign

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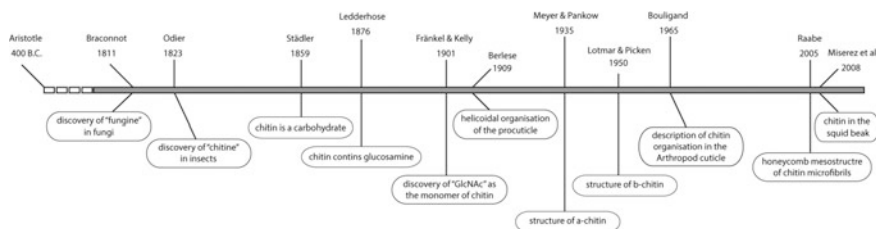


Fig. 2.1 *History of chitin.* The term chitin was coined by Aristotle more than three centuries BC. It took 18 centuries until the first scientific experiments were done to start unravelling chitin chemistry that was achieved with Fränkel and Kelly's work in 1901. Thereafter, the second era of chitin research focussed on chitin structure. The third era of chitin research, starting with Berlese in 1909, dealt with chitin organisation at the ultrastructural level especially in Arthropods

the insect cuticle in Aristotle's work but is rather loosely defining any structure that surrounds an insect. In 1822, the French naturalist Auguste Odier nevertheless used this term to denote a substance he extracted from the elytral cuticle of the scarab beetle, probably *Melolontha melolontha*: *Chitine* (Odier 1823). Initially, incubation of the elytra in water or warm alcohol did not change the appearance of the tissue. In the next step, Odier extracted chitin by cooking the elytra in potash. He found that the obtained transparent substance represented 25% of the initial weight of the elytra. He mentioned that chitin contains carbon but only very little nitrogen as compared to other animal substances like hairs and horn.

Odier was probably unaware of the work by another French scientist namely Henri Braconnot, who more than a decade before Odier's discovery, extracted a substance, probably chitin, from the cell wall of fungi that he had termed *fungine* (Braconnot 1811). In this case, fungi including *Agaricus* species were boiled in alkali to obtain fungine (detailed protocols of this extraction and of the following experiments were not reported in the respective article). Fungine was subsequently treated with potash, ammoniac and ether to show that fungine is composed of carbon, nitrogen, hydrogen and acetate. A few decades later, Georg Städler proposed in 1859 that chitin from crabs is a carbohydrate (Städler 1859). In the last quarter of the nineteenth century, Strasbourg, a city now in France, at that time in Germany, became a centre of chitin chemistry research. There, in 1876, the German scientist Georg Ledderhose identified the monomers of chitin to be a sweet sugar that can be consumed by yeast as an energy source (Ledderhose 1876). Subsequently, in the laboratory of Felix Hoppe-Seyler, chitin was defined as poly- or oligosaccharide containing nitrogen and acetic acid. In the late nineteenth century, the polysaccharide in fungi (*Boletus edulis*, *Agaricus campsetris*, *Morchella esculenta*) was named *Pilzcellulose* (Engl. fungal cellulose) (Winterstein 1893–95). In Strasbourg, it was soon discovered that *Pilzcellulose* and the arthropod chitin are the same molecule (Hoppe-Seyler 1894). In this work, Hoppe-Seyler also identified a deacetylated form of chitin he named chitosan. At the beginning of the next century, in 1901, Fraenkel and Kelly published two important findings on chitin. First, after extraction with sulphuric acid and acetone followed by neutralisation

with barite, alkalisation and precipitation with alcohol, they determined the composition of chitin demonstrating that it consists of an acetylated chitosamine (glucosamine) with the acetyl group bond to a nitrogen yielding N-acetyl-chitosamine (Fränkel and Kelly 1901). Second, they proposed for the first time that chitin is a polysaccharide. However, for more than a decade, the constitution of chitin, especially its nitrogen content was still under debate (Morgulis 1916). In 1926, finally, the structure proposed by Gonell (1926), confirmed in 1935 by Meyer and Pankow (1935) was widely accepted. Meyer and Pankow used X-ray diffraction to analyse chitin organisation in the spiny lobster *Palinurus vulgaris* (or *elephas*), an organisation that later was named α -chitin with antiparallel chitin fibres (Fig. 2.2a).

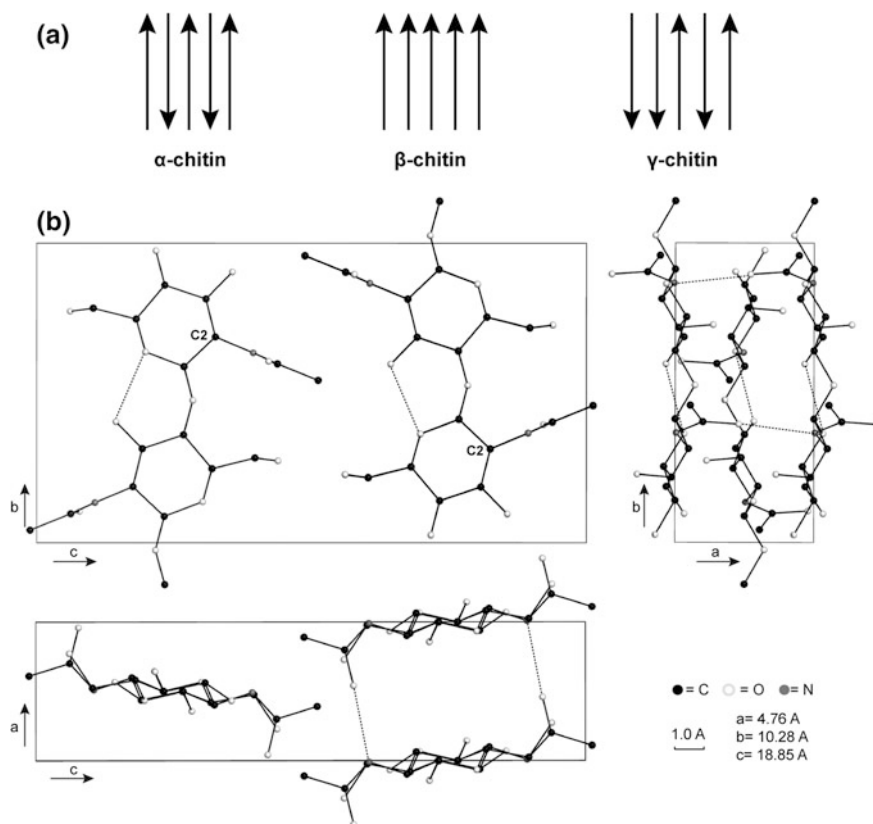


Fig. 2.2 *Chitin crystalline structure.* Commonly, chitin fibres are arranged in parallel. Three different possible organisation types with respect to the orientation of the fibre can be distinguished, α , β or γ (a). Here, the chitin crystallite according to Carlstrom (1957) in the α -form is shown (b). This model is detailed in the CIF file (from The Cambridge Crystallographic Data Centre (CCDC), Deposition Number: 1264751). We used the PyMOL software to draw the three projections ab, bc and ac of the crystallite. Hydrogen bonds are represented by dotted lines. The carbon number 2 (C2) is shown in the bc projection. Please note that the molecular and cellular mechanisms of chitin crystallite production are unknown

Besides α -chitin, two other types of chitin organisation were described, namely β -chitin with parallel running chitin fibres and γ -chitin with alternating parallel and antiparallel aligned chitin fibres (Fig. 2.2a). The organisation of β -chitin was unravelled in 1950 by Lotmar and Picken analysing chitin crystals by X-ray diffraction in bristles of the polychaete *Aphrodite aculeate* (Lotmar and Picken 1950), while γ -chitin was first described in 1962 or 1963 in Loligo, a squid genus (Rudall 1963).

Discovery of chitin in different phyla occurred at different dates and was often under debate. It was, for instance, ultimately in 2007, when chitin was discovered as a component of the skeletal fibres in marine sponges (Ehrlich et al. 2007a, b; Brunner et al. 2009) after some confusion as to whether chitin in sponges may derive from exogenous sources of endobionts (Dauby and Jeuniaux 1986). It should, however, be noted that Städeler in 1859 isolated a substance by stepwise extraction, which he named *spongine* that could have been chitin (Städeler 1859). In the recent works, chitin was detected in skeletal pieces of marine sponges of the order Verongida using Calcofluor White and a chitin-specific antibody. In addition, by Fourier-transform infrared (FTIR) and Raman spectroscopy and X-ray analyses, it was shown that sponge chitin adopts the α -conformation. The authors also reported the identification of transcripts and genomic DNA coding for a chitin synthase that shows homologies to chitin synthases in Arthropods and fungi suggesting that these enzymes are monophyletic (Zakrzewski et al. 2014; Goncalves et al. 2016). In 1972, chitin was confirmed unambiguously as a component of mollusc shells after a decade of debate (Peters 1972). The squid beak also consists of a chitin-based composite matrix. In this case, chitin adopts the organisation of β -chitin and interacts with specific proteins. In nematodes, chitin is present in the eggshell and in the pharynx (Wharton 1980; Zhang et al. 2005), but absent in the body wall (Watson 1965). To our knowledge, the crystalline structure of chitin in nematodes has not been directly determined. However, higher order organisation of chitin in the eggshell of *Trichuris suis* (Wharton and Jenkins 1978) resembles the organisation of chitin in insect cuticles (see below) suggesting that also here chitin preferably adopts the α -chitin crystalline structure. Finally, chitin oligosaccharides are found in vertebrates where they are involved in the production of hyaluronic acid, a polysaccharide related to chitin (Semino et al. 1996; Semino and Allende 2000). The presence of *bona fide* chitin has been reported in the gut of fish by histology and Fourier-transform infrared spectrometry (FTIR) (Tang et al. 2015). In the same work, genes coding for putative enzymes distantly related to invertebrate and fungal chitin synthases were mentioned. Considering that the presence of chitin in vertebrates would be a paradigm shift from chitin being confined to invertebrates, biochemical and X-ray diffraction experiments should be performed to corroborate these findings.

Commonly, chitin is found exclusively in the extracellular space as a protective and supporting component of complex extracellular matrices like the cell wall of fungi, the shell of molluscs or the cuticle of arthropods. Correlating with its requirement in different contexts, chitin organisation varies. In this chapter, we summarise the chemistry and structural organisation of chitin that together accounts

for its versatile use in the majority of living organisms. Besides in nature, chitin and its derivative chitosan are used in biomedicine and biotechnology (Crini et al. 2007). This chapter, however, will not address this issue.

2.2 Chemistry

Chitin is a linear polymer of the amino sugar *N*-acetyl-D-glucosamine (GlcNAc). The β -glycosidic bonding between GlcNAc residues entails a repetition of di-saccharides with respect to the position of the *N*-acetyl group (Fig. 2.2). Chitin ($C_8H_{13}O_5N$)ⁿ, hence, can be considered as polymer of two GlcNAcs, i.e. chitobiose. Despite its charges especially at the acetyl group, chitin is insoluble in aqueous and non-polar solutions.

A number of attempts were undertaken to assess the molecular organisation of chitin fibre. Three types of crystalline structure of chitin, namely α -, β - and γ -chitin (Fig. 2.2), have been observed in especially protostome animals and fungi (Wester 1909; Jeuniaux 1982). In 1957, by X-ray diffraction Diego Carlström analysed in a very comprehensive way the structure of α -chitin from the lobster (*Homarus americanus*) cuticle (Carlstrom 1957). According to his data, a chitin crystallite unit cell is composed of two antiparallel oriented celluloses. The axes are $a = 4.76 \text{ \AA}$, $b = 10.28 \text{ \AA}$ and $c = 18.85 \text{ \AA}$. This orthorhombic conformation is stabilised by hydrostatic bonds in the a and b direction, whereas binding forces in the c direction are rather weak. In principle, this structure has been confirmed repeatedly with only minor modifications. Minke and Blackwell by analysing X-ray diffractions determined the dimensions of the α -chitin unit cell as $a = 4.74 \text{ \AA}$, $b = 18.86 \text{ \AA}$ (corresponding to c according to Carlström) and $c = 10.32 \text{ \AA}$ (corresponding to b according to Carlström) (Minke and Blackwell 1978). In 2009, Sikorski et al. described the axes of the α -chitin unit cell as $a = 4.72 \text{ \AA}$, $b = 18.89 \text{ \AA}$ (corresponding to c according to Carlström) and $c = 10.30 \text{ \AA}$ (corresponding to b according to Carlström), again by X-ray diffraction analyses (Sikorski et al. 2009). In a rather recent work, using Fourier-transform infrared (FTIR) and solid state cross-polarising/magic-angle spinning (CP-MAS) ^{13}C NMR spectrophotometers, the crystalline structure of α -, β - and γ -chitin were characterised in detail using crab shell, squid pen and ‘*Lucainade*’ (probably *Lucanidae* beetles) as sources, respectively (Jang et al. 2004). It was confirmed that α - and γ -chitin have two types of hydrogen bonding, which stabilise the crystal unit in two directions, i.e. within the sheet of chitin fibres (intrasheet, between the carbonyl group of amide I and the amide II) and between sheets (intersheet, between the CH_2OH side chain and the carbonyl group). β -chitin, by contrast, has only the intrasheet hydrogen bonds. As determined by Differential Scanning Calorimetry (DSC), the evaporation of bound water occurs between 50 and 150 °C in all three types of chitin (endothermic peak). The maximum decomposition temperature of the crystalline structure (exothermic peak) depends on hydrogen bonding and is, therefore, highest in α -chitin (330 °C), lowest in β -chitin (230 °C) and intermediate in γ -chitin (310 °C). In extensive

X-ray diffraction assays, these authors also found that the crystalline structures of all three types of chitin are stable in the range of 25–250 °C.

The structural stability of chitin is illustrated by its occurrence in fossils. Palynological investigations identified Lepidopteran wing scales in sediments in the Trias more than 200 million years ago (mya) (van Eldijk et al. 2018; Zhang et al. 2018). These findings suggest that Lepidoptera are evolutionarily older than previously assumed, refuting the hypothesis of the co-evolution of Lepidoptera and flowering plants that rose maximum 160 mya in the Jurassic period (Katz 2018; Nepi et al. 2018). The oldest fossil containing chitin has been isolated in Burgess Shale in Canada. It is an artefact of the basal demosponge *Vauxia gracilentia* as old as 505 million years, the Middle Cambrian period (Ehrlich et al. 2013). Classical and modern methods were applied to detect fossilised chitin. Using Calcofluor White (CFW) that fluoresces upon binding to β -(1,4) and β -(1,3)-linked polysaccharides (Herth and Schnepf 1980), Ehrlich and colleagues identified chitin fibres in fossilised material.

Concise information on chitin fibre or microfibril length is scarce. After demineralisation, depigmentation and deproteinisation of the lobster (*Homarus americanus*) cuticle, chitin nanofibres with lengths around 1.000 nm (maximum 3.000 nm) and the average width of 4.0 nm were isolated (Mushi et al. 2014).

Chitin fibres are synthesised by chitin synthases, which are membrane-inserted glycosyltransferases with multiple transmembrane domains (see Chap. 3). At the cytoplasmic face of this enzyme, the activated chitin monomer UDP-GlcNAc—produced in the cytoplasm by the Leloir pathway (Merzendorfer and Zimoch 2003; Araujo et al. 2005; Schimmelpfeng et al. 2006; Tønning et al. 2006; Moussian 2008, 2013)—enters the active site of the enzyme, that offers an UDP-less GlcNAc as an acceptor. The second UDP-GlcNAc is bound to the first one releasing the UDP moiety. In a comprehensible model, the initial two GlcNAcs rotate by 180° thereby exposing the acceptor C4 of the second residue for the next reaction (Dorfmueller et al. 2014). In the following, rotation of every other GlcNAc residue allows stereotypic continuation of polymerisation. Thus, the presentation of NAC moieties on C2 alternates (Fig. 2.2).

Nonsense and missense mutations in chitin synthase coding genes cause a chitin-less cuticle in the fruit fly *Drosophila melanogaster* (Moussian et al. 2005). Likewise, downregulation of chitin synthase transcripts by RNA interference provokes a reduction in cuticular chitin in various insects (Arakane et al. 2005, 2008; Zhang et al. 2010). The cuticle without or with reduced chitin is, in all cases, lethal. Inhibition of chitin synthase activity by insecticides like Nikkomycin Z reduces the amounts of chitin at the same time interfering with the organisation of the chitin-containing extracellular matrix and the structure of the respective organ (Schonitzer and Weiss 2007; Gangishetti et al. 2009). This observation indicates that the chitin structure and organisation depend on chitin amounts.

Both in fungi and in arthropods, pure chitin does not seem to prevail. Some acetyl groups are removed during cell wall or cuticle differentiation by chitin deacetylases thereby converting chitin at least partially to chitosan (Neville 1975). This issue is dealt with in Chap. 5 of this book.

2.3 Chemistry to Structure

Chitin is present in most invertebrate animals including arthropods, molluscs, nematodes and Porifera, but also in fungi (Zhang et al. 2005; Ehrlich et al. 2007a, b). In Porifera, like in the Arthropod cuticle, the matrix consists of α -chitin, while in mollusc beaks β -chitin prevails and γ -chitin is found in insect cocoons.

Chitin is the major scaffolding component of the insect cuticle accounting for 20–50% of the weight of the cuticle (Chapman 2013). Generally, in terrestrial insects chitin amounts scale isometrically with body size (Lease and Wolf 2010). This suggests an optimised ratio between cuticle thickness and body volume. The chemistry of chitin, a polymer of an amino sugar, defines its physical properties. The asymmetry of the chitin crystallite, in any case, entails that stiffness or elasticity along the three axes a, b or c differs. The highest stiffness value is observed on the b-axis (after Carlström) along the chain of covalently bound sugar monomers. The two other axes that are stabilised by hydrogen bonds are probably more elastic.

In a series of simulation experiments, for a α -chitin unit consisting of four pairs of antiparallel chains with a diameter of $2 \times 2 \text{ nm}^2$ and a length of 5–35 nm an elastic modulus of 92.26 GPa was determined (Yu and Lau 2015). Addition of proteins preferably with rich on β -folds and water, decreased the elastic modulus to 36.39 GPa. This is probably due to the decrease of hydrogen bonds in chitin–chitin interactions. In the model, ductility increased with the length of fibres but reached a plateau longer than 20 nm. Another work, applying X-ray diffraction, measured the elastic modulus of α -chitin parallel to the fibre axis to be 41 GPa (Nishino et al. 1999). These values are clearly below the estimated stiffness of 150 GPa for chitin nanofibres (Vincent and Wegst 2004), which is markedly higher than the value of pure copper (124 GPa) and 50 GPa lower than the value of steel (200 GPa) (Callister and Rethwisch 2013). Experimentally determined values for chitin-containing cuticle types largely vary depending on the prevalent component (Vincent and Wegst 2004). The Young modulus of a Resilin-rich cuticle is about 1 MPa (Resilin is a chitin-binding protein rich in elastic cuticles), of soft cuticles between 1 kPa and 50 MPa, of sclerotised cuticles between 1 and 20 GPa. Thus, in nature, the mechanical properties of chitin-containing structures differ due to the association of chitin with proteins and organic molecules (sclerotisation). These differences do not distinguish only different types of cuticles, but may also prevail within the same cuticle. In the American lobster, for instance the stiffness of the outer procuticle, i.e. exocuticle is around nine GPa, whereas in the inner endocuticle it is around 4 GPa (Raabe et al. 2005a, b).

In nature, according to Neville (Neville 1975), around 17 antiparallel running chitin fibres are bundled forming chitin microfibrils with a diameter of around 3 nm and a length ranging from few nanometres to micrometres. Usually, these microfibrils are arranged in parallel constituting two-dimensional horizontal sheets, the laminae. This arrangement is stabilised by hydrostatic interactions within a fibre and between fibres. The laminae, in turn, are stacked to establish the procuticle. Yves Bouligand showed in a series of ultrastructural experiments that stacking of

these sheets is helicoidal in the cuticle of some crustaceans (Bouligand 1965). Subsequently, Neville and Luke showed that this organisation occurs also in insects (Neville and Luke 1969). Bouligand's model of chitin microfibril organisation ('twisted plywood') has been unanimously accepted to date. The helicoidal organisation of the procuticle, however, was already observed by the Italian entomologist Antonio Berlese in 1909 (Berlese 1909). In his book 'Gli Insetti', in Fig. 516 on page 468, he drew cuticle laminae that are stacked helicoidally. The underlying microscopic method of this scheme is unclear. Bouligand did not seem to know this work and did not cite it in his seminal article in 1965. Besides a helicoidal arrangement, in certain types of cuticles like the beetle elytral cuticle, laminae may also be stacked in a preferred direction. In any case, to what extent chitin microfibrils interact with each other in the vertical direction, is not known. The helicoidal organisation of the chitin laminae in the (pro)-cuticle according to Berlese and Bouligand is also found in Oenychophores but not in Tardigrades (Harrison and Rice 1993). This indicates that the molecular mechanisms of chitin laminae organisation have been evolved very early in arthropod evolution, but after the separation of Tardigrades from Arthropods and Oenychophores.

2.4 Structure to Biology

Chitin as a constituent of the cuticle plays an important role in defining the physical or mechanical properties of the arthropod cuticle (Fig. 2.3). In the arthropod cuticle, chitin is associated with proteins and adopts higher order organisation that is crucial for its function. Commonly, as revealed by genome sequences, arthropod species have hundreds of chitin-binding proteins (Cornman et al. 2008; Cornman and Willis 2008; Futahashi et al. 2008; Cornman 2009; Cornman and Willis 2009; Cornman 2010; Rosenfeld et al. 2016). These proteins evolve fast as the number of chitin-binding proteins differs considerably within insect orders. According to Hamodrakas and colleagues, chitin may be recognised and bound by especially proteins with antiparallel β -sheet half-barrel structure (Hamodrakas et al. 2002).

In some specialised cuticle, like in the head skeleton of dipteran (e.g. *Drosophila melanogaster*) larvae that is used for food grinding, it is not possible to see chitin microfibrils at the ultrastructural level. By contrast, the body cuticle of *D. melanogaster* larvae, which is soft and flexible, chitin microfibrils are well visible by electron microscopy. Assuming, therefore, that chitin fibres are rather short (unstructured) in the head skeleton but long (visible) in the body cuticle, we propose that short chitin microfibrils are associated with hard cuticles, whereas long chitin microfibrils are present in soft cuticles. These observations may also suggest that chitin microfibril length negatively correlates with stiffness or softness of the cuticle in arthropods for forces perpendicular to the microfibril axis.

The structure and the mechanical properties of the chitin-based cuticle have been extensively studied in the lobster *Homarus americanus* (Raabe et al. 2005a, b, 2006, 2007). The main finding is that the b-axis of the α -chitin unit cell (the c-axis

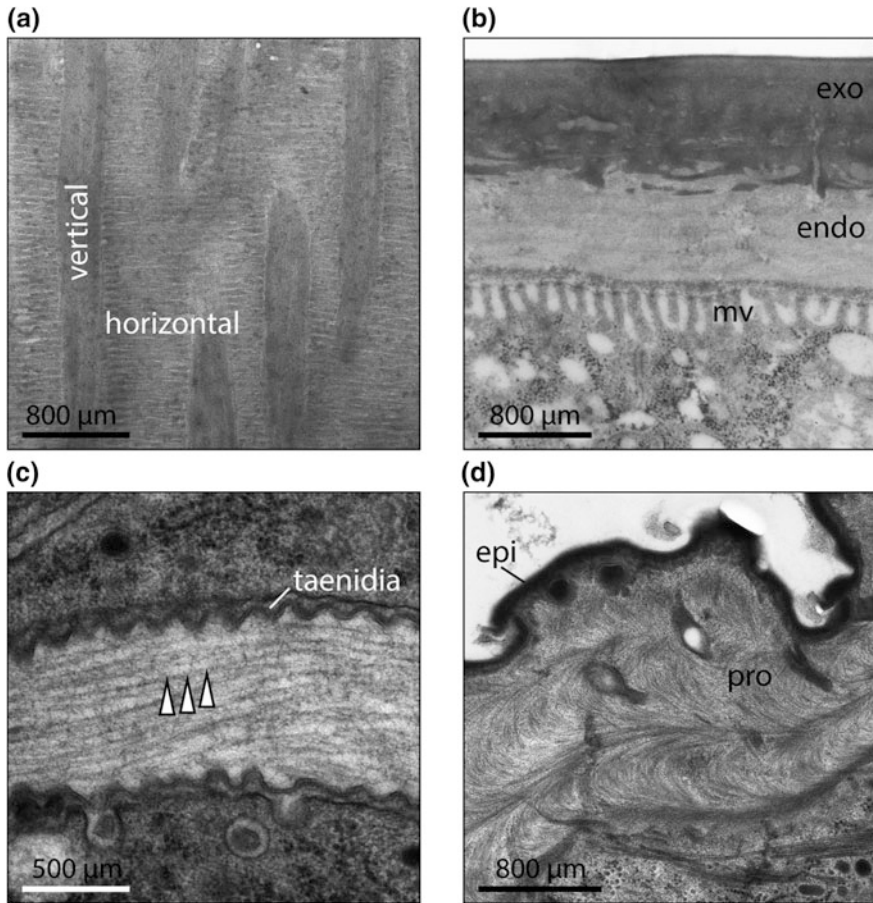


Fig. 2.3 Higher organisation of chitin. The cuticle of the beetle elytra (*Tribolium castaneum*) consists of vertical and horizontal chitin–protein matrices. **b** The procuticle of the *Tribolium castaneum* larval cuticle is subdivided into two regions with highly cross-linked (exocuticle, *exo*) and weakly cross-linked (endocuticle, *endo*) chitin adjacent to the microvilli (*mv*) that are involved in cuticle secretion. **c** The tracheal lumen of developing *Drosophila melanogaster* embryos contains a chitinous matrix (triangles) needed for tracheal morphogenesis. **d** Oblique sections through the procuticle (here a *Parhyale hawaiiensis* juvenile) reveals the illusion arc-like chitin fibres indicative of a helicoidal arrangement of chitin laminae (see Chap. 9)

according to Carlström), has a preferred fibre texture normal (with some 10° of inclination) to the surface of the exoskeleton at any position of the body. The crystallographic texture of hard mineralised regions, as opposed to the texture of soft membranous regions, has, however, an additional albeit infrequent fibre orientation perpendicular to the normal. These fibres may represent chitin in the pore canals that run from the epidermal cells to the cuticle surface. Moreover, a twisted

honeycomb structure has been described to stabilise the lobster cuticle counteracting cracking by forces applied (Raabe et al. 2005a, b).

In the beak of the Humboldt squid *Dosidicus gigas* β -chitin associates with proteins, which in turn are cross-linked by a variety of organic molecules that especially associate with histidines, a process named sclerotisation (Miserez et al. 2010). An opposite gradient of water and sclerotisation defines beak material stiffness and elasticity with the stiffest region with 5 GPa being at the beak tip and the softest region with 0.05 GPa being at the margin of the so-called wing (Miserez et al. 2008).

Chitin is not only a structural element of extracellular matrices but may also be implied in organ shape during development. In *D. melanogaster*, it was demonstrated that tracheal tube diameter and length regulation depend on a luminal chitin matrix (Fig. 2.3) (Tonning et al. 2005; Luschnig et al. 2006; Moussian et al. 2006). This matrix was already discovered in 1966 by M. Locke, who, however, did not recognise it as a chitinous matrix (Locke 1966). Formation and organisation of this matrix involve many of the proteins and enzymes that are also active during epidermal cuticle formation. The specificity remains to be investigated.

2.5 Concluding Remarks

In summary, the chemistry and biophysics of chitin alone, a versatile polysaccharide target for modifications in length and by deacetylation, are not sufficient to explain its use in a variety of tissues and organisms. Rather, associations of chitin with proteins, organic molecules and water are important modulators of mechanical and biophysical properties of the chitin matrix. The versatility of chitin is best illustrated by its presence in most animal species ranging from sponges, nematodes to insects and also in fungi. Additionally, different body parts with different functions are equipped with different, optimised types of chitin matrices. We can imagine that, with modern omics tools in hand, the molecular ecology of these structures will characterise the fourth era of chitin research.

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References

- Arakane Y, Muthukrishnan S, Kramer KJ, Specht CA, Tomoyasu Y, Lorenzen MD, Kanost M, Beeman RW (2005) The *Tribolium* chitin synthase genes TcCHS1 and TcCHS2 are specialized for synthesis of epidermal cuticle and midgut peritrophic matrix. *Insect Mol Biol* 14(5):453–463

- Arakane Y, Specht CA, Kramer KJ, Muthukrishnan S, Beeman RW (2008) Chitin synthases are required for survival, fecundity and egg hatch in the red flour beetle, *Tribolium castaneum*. *Insect Biochem Mol Biol* 38(10):959–962
- Araujo SJ, Aslam H, Tear G, Casanova J (2005) Mummy/cystic encodes an enzyme required for chitin and glycan synthesis, involved in trachea, embryonic cuticle and CNS development—analysis of its role in *Drosophila* tracheal morphogenesis. *Dev Biol* 288(1):179–193
- Berlese A (1909) *Gli insetti – loro organizzazione, sviluppo, abitudini e rapporti coll'uomo*. Milano, Società Editrice Libreria
- Bouligand Y (1965) On a twisted fibrillar arrangement common to several biologic structures. *C R Acad Sci Hebd Seances Acad Sci D* 261(22):4864–4867
- Braconnot H (1811) Sur la nature des champignons. *Annales de chimie ou recueil de mémoires concernant la chimie et les arts qui en dépendent et spécialement la pharmacie* 79:265–304
- Brunner E, Ehrlich H, Schupp P, Hedrich R, Hunoldt S, Kammer M, Machill S, Paasch S, Bazhenov VV, Kurek DV, Arnold T, Brockmann S, Ruhnnow M, Born R (2009) Chitin-based scaffolds are an integral part of the skeleton of the marine demosponge *Ianthella basta*. *J Struct Biol* 168(3):539–547
- Callister WD, Rethwisch DG (2013) *Materials science and engineering: an introduction*. Wiley, New York
- Carlstrom D (1957) The crystal structure of alpha-chitin (poly-N-acetyl-D-glucosamine). *J Biophys Biochem Cytol* 3(5):669–683
- Chapman RF (2013) *The Insects*. Cambridge, Cambridge University Press, Structure and Function
- Comman RS (2009) Molecular evolution of *Drosophila* cuticular protein genes. *PLoS ONE* 4(12): e8345
- Comman RS (2010) The distribution of GYR- and YLP-like motifs in *Drosophila* suggests a general role in cuticle assembly and other protein-protein interactions. *PLoS One* 5(9)
- Comman RS, Togawa T, Dunn WA, He N, Emmons AC, Willis JH (2008) Annotation and analysis of a large cuticular protein family with the R&R Consensus in *Anopheles gambiae*. *BMC Genom* 9:22
- Comman RS, Willis JH (2008) Extensive gene amplification and concerted evolution within the CPR family of cuticular proteins in mosquitoes. *Insect Biochem Mol Biol* 38(6):661–676
- Comman RS, Willis JH (2009) Annotation and analysis of low-complexity protein families of *Anopheles gambiae* that are associated with cuticle. *Insect Mol Biol* 18(5):607–622
- Crini G, Badot P-M, Guibal E (2007) *Chitine et chitosane – Du biopolymère à l'application*, Presse universitaire de Franche-Comté
- Dauby P, Jeuniaux C (1986) Origine exogène de la chitine décelée chez les Spongiaires. *Cah Biol Mar* 28:121–129
- Dorfmueller HC, Ferenbach AT, Borodkin VS, van Aalten DM (2014) A structural and biochemical model of processive chitin synthesis. *J Biol Chem* 289(33):23020–23028
- Ehrlich H, Krautter M, Hanke T, Simon P, Knieb C, Heinemann S, Worch H (2007a) First evidence of the presence of chitin in skeletons of marine sponges. Part II. Glass sponges (Hexactinellida: Porifera). *J Exp Zool B Mol Dev Evol* 308(4):473–483
- Ehrlich H, Maldonado M, Spindler KD, Eckert C, Hanke T, Born R, Goebel C, Simon P, Heinemann S, Worch H (2007b) First evidence of chitin as a component of the skeletal fibers of marine sponges. Part I. Verongidae (demospongia: Porifera). *J Exp Zool B Mol Dev Evol* 308(4):347–356
- Ehrlich H, Rigby JK, Botting JP, Tsurkan MV, Werner C, Schwille P, Petrasek Z, Pisera A, Simon P, Sivkov VN, Vyalikh DV, Molodtsov SL, Kurek D, Kammer M, Hunoldt S, Born R, Stawski D, Steinhof A, Bazhenov VV, Geisler T (2013) Discovery of 505-million-year old chitin in the basal demosponge *Vauxia gracilentia*. *Sci Rep* 3:3497
- Fränkel S, Kelly A (1901) Beiträge zur Constitution des Chitins. *Monatsh Chem* 23(2):123–132
- Futahashi R, Okamoto S, Kawasaki H, Zhong YS, Iwanaga M, Mita K, Fujiwara H (2008) Genome-wide identification of cuticular protein genes in the silkworm, *Bombyx mori*. *Insect Biochem Mol Biol* 38(12):1138–1146

- Gangishetti U, Breitenbach S, Zander M, Saheb SK, Muller U, Schwarz H, Moussian B (2009) Effects of benzoylphenylurea on chitin synthesis and orientation in the cuticle of the *Drosophila* larva. *Eur J Cell Biol* 88(3):167–180
- Goncalves IR, Brouillet S, Soulie MC, Gribaldo S, Sirven C, Charron N, Boccara M, Choquer M (2016) Genome-wide analyses of chitin synthases identify horizontal gene transfers towards bacteria and allow a robust and unifying classification into fungi. *BMC Evol Biol* 16(1):252
- Gonell HW (1926) Rontgenographische Studien an Chitin. *Hoppe-Seyler's Zeitschrift fuer Physiologische Chemie Berlin* 152:18–30
- Hamodrakas SJ, Willis JH, Iconomidou VA (2002) A structural model of the chitin-binding domain of cuticle proteins. *Insect Biochem Mol Biol* 32(11):1577–1583
- Harrison FW, Rice ME (1993) Onychophora, chilopoda, and lesser protostomata. John Wiley, New York
- Herth W, Schnepf E (1980) The fluorochrome, calcofluor white, binds oriented to structural polysaccharide fibrils. *Protoplasma* 105(1–2):129–133
- Jang MK, Kong BG, Jeong YI, Lee CH, Nah JW (2004) Physicochemical characterization of alpha-chitin, beta-chitin, and gamma-chitin separated from natural resources. *J Polym Sci Part A Polym Chem* 42(14):3423–3432
- Jeuniaux C (1982) La chitine dans le regne animal. *Bulletin de la Societe Zoologique de France* 107(3):363–386
- Katz O (2018) Extending the scope of Darwin's 'abominable mystery': integrative approaches to understanding angiosperm origins and species richness. *Ann Bot* 121(1):1–8
- Lease HM, Wolf BO (2010) Exoskeletal chitin scales isometrically with body size in terrestrial insects. *J Morphol* 271(6):759–768
- Ledderhose G (1876) Über salzsaures Glucosamin." *Berichte der deutschen chemischen Gesellschaft*: 1200–1201
- Locke M (1966) The structure and formation of the cuticulin layer in the epicuticle of an insect, *Calpodex ethlius* (Lepidoptera, Hesperidae). *J Morphol* 118(4):461–494
- Lotmar W, Picken LER (1950) A new crystallographic modification of chitin and its distribution. *Experientia* 6(2):58–59
- Luschnig S, Batz T, Armbruster K, Krasnow MA (2006) serpentine and vermiform encode matrix proteins with chitin binding and deacetylation domains that limit tracheal tube length in *Drosophila*. *Curr Biol* 16(2):186–194
- Merzendorfer H, Zimoch L (2003) Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. *J Exp Biol* 206(Pt 24):4393–4412
- Meyer KH, Pankow G (1935) Sur la constitution et la structure de la chitine. *Helv Chim Acta* 18 (1):589–598
- Minke R, Blackwell J (1978) The structure of alpha-chitin. *J Mol Biol* 120(2):167–181
- Miserez A, Rubin D, Waite JH (2010) Cross-linking chemistry of squid beak. *J Biol Chem* 285 (49):38115–38124
- Miserez A, Schneberk T, Sun C, Zok FW, Waite JH (2008) The transition from stiff to compliant materials in squid beaks. *Science* 319(5871):1816–1819
- Morgulis S (1916) The Chemical Constitution of Chitin. *Science* 44(1146):866–867
- Moussian B (2008) The role of GlcNAc in formation and function of extracellular matrices. *Comp Biochem Physiol B Biochem Mol Biol* 149(2):215–226
- Moussian B (2013) The Arthropod Cuticle. In: Minelli A, Boxshall G, Fusco G (eds) *Arthropod biology and evolution*. Springer, Berlin, Heidelberg, pp 171–196
- Moussian B, Schwarz H, Bartoszewski S, Nusslein-Volhard C (2005) Involvement of chitin in exoskeleton morphogenesis in *Drosophila melanogaster*. *J Morphol* 264(1):117–130
- Moussian B, Tang E, Tonning A, Helms S, Schwarz H, Nusslein-Volhard C, Uv AE (2006) *Drosophila* Knickkopf and Retroactive are needed for epithelial tube growth and cuticle differentiation through their specific requirement for chitin filament organization. *Development* 133(1):163–171
- Mushi NE, Butchosa N, Salajkova M, Zhou Q, Berglund LA (2014) Nanostructured membranes based on native chitin nanofibers prepared by mild process. *Carbohydr Polym* 112:255–263

- Nepi M, Grasso DA, Mancuso S (2018) Nectar in Plant-Insect Mutualistic Relationships: From Food Reward to Partner Manipulation. *Front Plant Sci* 9:1063
- Neville AC (1975) *Biology of the arthropod cuticle*. Springer Verlag, Berlin Heidelberg New York
- Neville AC, Luke BM (1969) A Two-system model for chitin-protein complexes in insect cuticles. *Tissue Cell* 1(4):689–707
- Nishino T, Matsui R, Nakamae K (1999) Elastic modulus of the crystalline regions of chitin and chitosan. *J Polym Sci B: Polym Phys* 37(11):1191–1196
- Odier A (1823) Mémoires sur la composition chimique des parties cornées des insectes. Mémoires de la Société d'Histoire Naturelle de Paris 1:29–42
- Peters W (1972) Occurrence of chitin in Mollusca. *Comparat Biochem Physiol B* 41((3)):541–550
- Raabe D, Al-Sawalmih A, Yi SB, Fabritius H (2007) Preferred crystallographic texture of alpha-chitin as a microscopic and macroscopic design principle of the exoskeleton of the lobster *Homarus americanus*. *Acta Biomater* 3(6):882–895
- Raabe D, Romano P, Sachs C, Al-Sawalmih A, Brokmeier H-G, Yi S-B, Servos G, Hartwig HG (2005a) Discovery of a honeycomb structure in the twisted plywood patterns of fibrous biological nanocomposite tissue. *J Cryst Growth* 283(1–2):1–7
- Raabe D, Romano P, Sachs C, Fabritius H, Al-Sawalmih A, Yi S, Servos G, Hartwig HG (2006) Microstructure and crystallographic texture of the chitin-protein network in the biological composite material of the exoskeleton of the lobster *Homarus americanus*. *Materials Science and Engineering a-Structural Materials Properties Microstructure and Processing* 421(1–2):143–153
- Raabe D, Sachs C, Romano P (2005b) The crustacean exoskeleton as an example of a structurally and mechanically graded biological nanocomposite material. *Acta Mater* 53(15):4281–4292
- Rosenfeld JA, Reeves D, Brugler MR, Narechania A, Simon S, Durrett R, Foox J, Shianna K, Schatz MC, Gandara J, Afshinnekoo E, Lam ET, Hastie AR, Chan S, Cao H, Saghbini M, Kentsis A, Planet PJ, Kholodovych V, Tessler M, Baker R, DeSalle R, Sorkin LN, Kolokotronis SO, Siddall ME, Amato G, Mason CE (2016) Genome assembly and geospatial phylogenomics of the bed bug *Cimex lectularius*. *Nat Commun* 7:10164
- Rudall KM (1963) The chitin/protein complexes of insect cuticles. *Treherne & Wiggles-worth*, vol 1, Beament, Treherne & Wiggles-worth, vol 1, pp 257–313, 24 figs, pp 257–313
- Schimmelpfeng K, Strunk M, Stork T, Klambt C (2006) Mummy encodes an UDP-N-acetylglucosamine-diphosphorylase and is required during *Drosophila* dorsal closure and nervous system development. *Mech Dev* 123(6):487–499
- Schonitzer V, Weiss IM (2007) The structure of mollusc larval shells formed in the presence of the chitin synthase inhibitor Nikkomycin Z. *BMC Struct Biol* 7:71
- Semino CE, Allende ML (2000) Chitin oligosaccharides as candidate patterning agents in zebrafish embryogenesis. *Int J Dev Biol* 44(2):183–193
- Semino CE, Specht CA, Raimondi A, Robbins PW (1996) Homologs of the *Xenopus* developmental gene DG42 are present in zebrafish and mouse and are involved in the synthesis of Nod-like chitin oligosaccharides during early embryogenesis. *Proc Natl Acad Sci U S A* 93(10):4548–4553
- Sikorski P, Hori R, Wada M (2009) Revisit of alpha-chitin crystal structure using high resolution X-ray diffraction data. *Biomacromol* 10(5):1100–1105
- Städeler G (1859) Untersuchungen über das Fibroin, Spongine und Chitin, nebst Bemerkungen über den thierischen Schleim. *Justus Liebig Annalen der Chemie* 111(1):12–28
- Tang WJ, Fernandez J, Sohn JJ, Amemiya CT (2015) Chitin is endogenously produced in vertebrates. *Curr Biol* 25(7):897–900
- Tonning A, Helms S, Schwarz H, Uv AE, Moussian B (2006) Hormonal regulation of mummy is needed for apical extracellular matrix formation and epithelial morphogenesis in *Drosophila*. *Development* 133(2):331–341
- Tonning A, Hemphala J, Tang E, Nannmark U, Samakovlis C, Uv A (2005) A transient luminal chitinous matrix is required to model epithelial tube diameter in the *Drosophila* trachea. *Dev Cell* 9(3):423–430

- van Eldijk TJB, Wappler T, Strother PK, van der Weijst CMH, Rajaei H, Visscher H, van de Schootbrugge B (2018) A Triassic-Jurassic window into the evolution of Lepidoptera. *Sci Adv* 4(1):e1701568
- Vincent JF, Wegst UG (2004) Design and mechanical properties of insect cuticle. *Arthropod Struct Dev* 33(3):187–199
- Watson BD (1965) The fine structure of the body-wall in a free-living nematode, *Euchromadora vulgaris*. *Quart. J. micr. Sci.* 106(1):75–81
- Wester DH (1909) “Über die Verbreitung und Lokalisation des Chitins im Tierreich “ *Zool. Jahrb. Abt. Syst.* 28:531–558
- Wharton D (1980) Nematode egg-shells. *Parasitology* 81(2):447–463
- Wharton DA, Jenkins T (1978) Structure and chemistry of the egg-shell of a nematode (*Trichuris suis*). *Tissue Cell* 10(3):427–440
- Yu Z, Lau D (2015) molecular dynamics study on stiffness and ductility in chitin-protein composite. *J Mater Sci* 50:7149–7157
- Zakrzewski AC, Weigert A, Helm C, Adamski M, Adamska M, Bleidorn C, Raible F, Hausen H (2014) Early divergence, broad distribution, and high diversity of animal chitin synthases. *Genome Biol Evol* 6(2):316–325
- Zhang J, Liu X, Zhang J, Li D, Sun Y, Guo Y, Ma E, Zhu KY (2010) Silencing of two alternative splicing-derived mRNA variants of chitin synthase 1 gene by RNAi is lethal to the oriental migratory locust, *Locusta migratoria manilensis* (Meyen). *Insect Biochem Mol Biol* 40 (11):824–833
- Zhang Q, Mey W, Ansorge J, Starkey TA, McDonald LT, McNamara ME, Jarzembowski EA, Wichard W, Kelly R, Ren X, Chen J, Zhang H, Wang B (2018) Fossil scales illuminate the early evolution of lepidopterans and structural colors. *Sci Adv* 4(4):e1700988
- Zhang Y, Foster JM, Nelson LS, Ma D, Carlow CK (2005) The chitin synthase genes *chs-1* and *chs-2* are essential for *C. elegans* development and responsible for chitin deposition in the eggshell and pharynx, respectively. *Dev Biol* 285(2):330–339