Carbohydrate Polymers

Volume 252, 15 January 2021, 117204

Review

Progress in chitin analytics

Mikhail V. Tsurkan [a], Alona Voronkina [b], Yuliya Khrunyk [c d], Marcin Wysokowski [e f], Iaroslav Petrenko [f], Hermann Ehrlich [f g]

Show more

https://doi.org/10.1016/j.carbpol.2020.117204

Get rights and content

Highlights

- Physicochemical, biochemical, and immunochemical methods for chitin identification.
- Benefits and limitations of analytical techniques for chitin identification.
- Challenges and future directions of chitin analytics.

Article preview

Abstract

Introduction

Section snippets

References (321)

Cited by (170)

Abstract

Chitin is the second most abundant biopolymer and functions as the main structural component in a variety of living organisms. In nature, chitin rarely occurs in a pure form, but rather as nanoorganized chitin-proteins, chitin-pigments, or chitin-mineral composite biomaterials. Although chitin has a long history of scientific studies, it is still extensively investigated for practical applications in medicine, biotechnology, and biomimetics. The complexity of chitin has required the development of highly sensitive analytical methods for its identification. These methods are crucial for furthering disease diagnostics as well as advancing modern chitin-related technologies. Here we provide a summary of chitin identification by spectroscopic (NEXAFS, FTIR, Raman, NMR, colorimetry), chromatographic (TLC, GC, HPLC), electrophoretic (HPCE), and diffraction methods (XRD, WAXS, SAXS, HRTEM-SAED). Biochemical and immunochemical (ELISA, immunostaining) methods are described with respect to their medical application. This review outlines the history as well as the current progress in the analytical methods for chitin identification.

Introduction

Chitin is an ancient and abundant structural amino polysaccharide that functions as the main nanostructured component in a broad assortment of skeletal constructs of uni-and multicellular organisms. Studies of chitin biosynthesis in diverse phyla, as well as its practical application in biomedicine, technology, and biomimetics are still trending more than 200 years after its discovery. In nature, chitin does not occur in a pure form, but as nanoorganized chitin-proteins, chitin-pigments, or chitin-mineral composite biomaterials, which complicates its identification. Consequently, the development of highly sensitive analytical methods for its identification was crucial to advance modern chitin-related technology and disease diagnostics. This review is dedicated to the critical analysis of experimental reports on the identification of chitin starting in the 19th century until present with respect to structural, physicochemical, and biochemical methods.

Chitin is one of the fundamental structural biopolymers in the animal world with a long history beginning with the first chitin-producing ancient fungi that arose on Earth (Bengtson et al., 2017; Loron et al., 2019). The ancient origin of chitin was demonstrated in the fossil filaments of eukaryotic organisms found in 810 to 715 million years (Myr) old dolomitic shale (Bonneville et al., 2020) as well as in 505 Myr old fossil remnants of the basal demosponge *Vauxia gracilenta* (Ehrlich, Rigby et al., 2013; Ehrlich, Kaluzhnaya, Brunner et al., 2013; Ehrlich, Kaluzhnaya, Tsurkan et al., 2013). Historically, chitin was the first described in fungi (1811) and arthropods (1826) (see for historical overview by Crini (Crini, 2019)), which follow by its discovery in diverse phyla including protists, diatoms, coralline algae, sponges, corals, worms, pogonophorans, bryozoans, mollusks, insects, spiders as well as crustaceans). During evolution, chitin disappeared in the phylogenetic tree in Deuterostomia, and it is not still be found in Echinodermata (2019, Brunet & Carlisle, 1958; Ehrlich, 2010; Kammer et al., 2010; Rudall & Kenchington, 1973; Żółtowska-Aksamitowska, Shaala et al., 2018; Żółtowska-Aksamitowska, Tsurkan et al., 2018). There are a few reports of the presence of chitin in membrane-like structures in fish and amphibians (Tang, Fernandez, Sohn, & Amemiya, 2015; Wagner, Lo, Laine, & Almeder, 1993), but its origin is still questionable as it could be species-specific mycosis.

The driving force of chitin discoveries in different organisms was the appearance of new analytical tools and approaches since its first description in 1811. From the beginning, the chitin detection was problematic because its natural occurrence as a compound firmly bound to other substances such as polysaccharides, lipids, proteins, pigments, as well as biominerals ($CaCO_3$, or silica) localized in diverse skeletal structures (Ehrlich, Krautter et al., 2007; Ehrlich, Maldonado et al., 2016; Finke, 2007; Kombrink, Sánchez-Vallet, & Thomma, 2011; Muzzarelli, 2011; Politi et al., 2012; Rahman & Halfar, 2014; Rudall, 1963; Whipps & Lewis, 1980; Wysokowski et al., 2014). The main method of chitin identification was chemical or enzymatic removal of these hindering substances, thus purifying chitin (Klinger et al., 2019; Pighinelli, 2019). There is a general agreement that chitin, $(C_8H_{13}0_4O_5N)n$, is a straight-chain polymer of N-acetyl-D-glucosamine (N-acetyl-2-amino-2-deoxy-D-glucose) units, joined to each other by 1,4-ß-glycosidic bonds with a small amount of deacetylated monomer units (2-amino-2-deoxy-D-glucose). The first chemical structure of chitin was proposed by Meyer and Mark in 1928 (Meyer & Mark, 1928). Subsequent examination of its structure revealed the existence of three different polymorphic forms of chitin (alpha, beta, and gamma) that differ in the arrangement of polymeric chains, as shown in Fig. 1 (Anitha et al., 2014; Jang, Kong, Jeong, Lee, & Nah, 2004; Kaya et al., 2017; Rinaudo, 2006). These polymorphic forms are unique to chitin and are responsible for the difference of some of its physical and chemical properties that disappear after deacetylation to chitosan, which usually is amorphous, however can be transformed into crystalline phase by hydrolysis in HCl (for details see Cartier, Domard, & Chanzy, 1990; Okuyama, Noguchi, Miyazawa, Yui, & Ogawa, 1997; Osorio-Madrazo et al., 2010, 2011). The deacetylation degree of chitin is used as a measure of chitosan formation, which is considered successful with deacetylation levels greater than 50 percent. The resulting change of the structural characteristics is often ignored, though its complexity could be compared to the secondary (Fig. 1d–f), tertiary (Fig. 1g), and quaternary (Fig. 1h) structures of proteins. The alpha and beta polymorphic forms of chitin are well studied, while only a few original reports describe its polymorphic gamma form (Jang et al., 2004; Kaya et al., 2017). There is some debate that the gamma form is a fine mixture of alpha and beta chitin that appears during metamorphosis, but this discussion is out of the scope of this review, and, therefore, we will follow the established abbreviations.

One key property of pure chitin is its strong resistance to alkaline treatment. Chitin can keep its structure even under harsh chemical conditions (1 M NaOH) while other biopolymers degrade. As a result, the traditional way to determine the chitin amount in a sample is the gravimetrical method, which is performed by weighing chitin that is collected after alkaline treatment (Black & Schwartz, 1950; Bradić, Novak, & Likozar, 2020; Finke, 2007). Strong alkaline (>1 M NaOH and heating) or acetic (>3 M HCl and heating) conditions transform chitin into chitosan by de-acetylation and partial fragmentation. This feature was utilized in three general methods of chitin detection traditionally used until 1950 (Richards, 1947, 1951). The first is the isolation and identification of glucosamine (dGlcN) crystals; the second is chitosan color reactions and sphalerite formation and the third is color reactions by diaphanol (50 percent solution of glacial acetic acid saturated with chlorine dioxide). The chronology of these analytical methods development and application could be easily followed in scientific publications until 1950, as seen in Tables S1 (19th century) and S2 (the first half of the 20th century).

Since 1950, new structural, spectroscopic, and chromatographic methods of analyses were developed, and a large number of analytical papers on chitin identification were subsequently published. The majority of these publications were focused on the development of quantitative and qualitative analyses of chitin (Hamed, Özogul, & Regenstein, 2016; Lopes, Antelo, Franco-Uría, Alonso, & Pérez-Martín, 2018) for industrial applications. Chitin identification was also a significant part of food research for quality assurance and contamination measures. Only a limited amount of studies focused on the detection of chitin in fossils and new biological sources. Nevertheless, despite nearly 200 years of chitin identification studies, these works still bring unexpected scientific results, such as the discovery of chitin in sponges in 2007 (Ehrlich, Krautter et al., 2007).

Recently, chitin identification studies have brought new perspectives to the medical diagnosis of diverse parasitic and immunological diseases (Bueter, Specht, & Levitz, 2013; Elieh Ali Komi, Sharma, & Dela Cruz, 2018). For example, Sendid et al. (2008) suggested a link between Crohn's disease and invasive *Candida albicans* infection, as both of these conditions correlated with a significantly increased level of chitin antibodies. Additionally, chitin was detected in the central nervous system tissue samples from patients with Alzheimer's disease. This discovery allowed for the suggestion of mycoses as a possible reason for dementia and Alzheimer's diseases (Pisa, Alonso, Rábano, Horst, & Carrasco, 2016). Together, chitin identification remains an essential instrument in anthropological, biotechnological, and medical applications (Fig. 2).

There are numerous books (Gupta, 2010; Jeuniaux, 1982; Khor, 2002; Muzzarelli, 1977; Roberts, 1992; Van den Broek, Boeriu, & Stevens, 2020) and valuable reviews (Abo Elsoud & El Kady, 2019; Anitha et al., 2014; Arnold, Brück, Garbe, & Brück, 2020; Boissière, 1967; Cabib, 1987; Casadidio et al., 2019; Cauchie, 2002; Chen & Peng, 2019; Cohen, 1987, 2010; Duan, Huang, Lu, & Zhang, 2018; Jayakumar et al., 2011; Jones, Kujundzic, John, & Bismarck, 2020; Kadokawa, 2019; Kasaai, 2009; Liu, Cooper et al., 2019; Liu, Zhang, & Zhu, 2019; Merzendorfer, 2006; Moussian, 2019; Peter, 2005; Rinaudo, 2006; Shamshina, 2019; Shamshina, Berton, & Rogers, 2019; Steinfeld, Vafaei, Rösner, & Merzendorfer, 2019; Varlamov, Il'ina, Shagdarova, Lunkov, & Mysyakina, 2020; Yang & Zhang, 2019; Yeul & Rayalu, 2013; Younes & Rinaudo, 2015) that detaily describe chitin biosynthesis, distribution, characterization, properties, and applications. Surprisingly, to the best of our knowledge, there are no publications that outline the current state of the art of the methods of chitin identification. In this review, we aim to present the progress of analytical methods in chitin identification reported up today.

Section snippets

Structural and spectroscopic methods

The molecular feature of chitin, which is the only polysaccharide built up exclusively of N-acetyl-D-glucosamine units (dGlcNAc), underlines the identification strategy of this polymer using diverse diffraction, scattering, and spectroscopic methods. IR, Raman, and NEXAFS spectroscopy provide information about the molecular moieties of the analyzed sample and thus allow distinguishing chitin from other biopolymers, but could be confusing in complex sample analysis. X-ray and electron ...

Chromatographic and electrophoretic methods

Chitin is an insoluble polymer and, therefore, cannot be directly investigated in chromatographic methods of analysis. Nevertheless, chromatographic and electrophoretic methods can be used for the analysis of dGlcN, which is the only product of chitin's acetic hydrolysis. These methods could not discriminate between chitin and chitosan, but they are a speedy and easy method to indicate and quantify their presence in the sample. One of the first attempts was reported in 1955 by the use of paper …

Chemical methods

Chemical methods of analysis are based on the ability of chitin to react with many low weight molecules of inorganic or organic (dyes) origin and to be visualized due to the development of the corresponding color. Here, we discuss the traditional "chitosan-iodine test" and "Chlor-zinc iodide test" as well as modern chitin staining techniques, which include the application of special fluorochromes. These chemical methods are inexpensive and straightforward but have a large (above nanograms) …

Biochemical and immunochemical methods

Biochemical methods of analysis include enzymatic methods for chitin identification, which are based on the specificity of chitolytic enzymes (chitinase, yatalase) to digest only chitin. It also includes the chitin-binding protein methods, which utilize proteins of non-immune origin with high selective chitin-binding properties. The development and application of immunochemical detection of chitin using specific antibodies are also described. This immunochemical technique is a very sensitive …

Genome-wide analyses of chitin synthases for chitin identification

What is the identification of chitin using modern genomics? There are a lot of publications concerning the identification of chitin synthases genes in diverse organisms (see for overview (Durkin, Mock, & Armbrust, 2009; Li et al., 2016; Merzendorfer, 2006; Wang et al., 2019). Nevertheless, the presence of chitin synthases genes does not reveal where and on which stage of the organism's development chitin appears. For example, chitin synthase genes have been confirmed in the freshwater sponge …

Summary

The study of chitin expands our understanding of the origin and evolution of polysaccharides in uni- and multicellular organisms. In fundamental science, it has stimulated research on possible molecular mechanisms of chitin formation and its structural diversity on nano- and microlevels, as well as enabled direct comparison between physicochemical and biochemical records of chitins produced in various organisms under diverse environmental conditions. In applied science, chitin represents an …

Acknowledgments

This work was partially supported by the DFG Project HE 394/3, SMWK Project no. 02010311 (Germany), Ministry of Science and Higher Education (Poland); Yuliya Khrunyk is supported by the Russian Science Foundation (grant no. 18-13-00220). …

References (321)

A. Anitha *et al.*
Chitin and chitosan in selected biomedical applications
Progress in Polymer Science (2014)

G. Ashwell *et al.*
A colorimetric procedure for the determination of N-acetylated-3-amino hexoses
Archives of Biochemistry and Biophysics (1965)

E.D.T. Atkins *et al.*
Electron diffraction and electron microscopy of crystalline α-chitin from the grasping spines of the marine worm Sagitta
International Journal of Biological Macromolecules (1979)

S. Bartnicki-Garcia *et al.*
An electron microscope and electron diffraction study of the effect of Calcofluor and Congo Red on the biosynthesis of chitin in vitro
Archives of Biochemistry and Biophysics (1994)

R.L. Benson

Assay for 2-amino-2-deoxy-D-glucose in the presence of other primary amines

Carbohydrate Research (1975)

V.P. Bhavanandan *et al.*

The interaction of wheat germ agglutinin with sialoglycoproteins. The role of sialic acid

The Journal of Biological Chemistry (1979)

F. Biancalana *et al.*

A protocol for the simultaneous identification of chitin-containing particles and their associated bacteria

Systematic and Applied Microbiology (2017)

M. Bo *et al.*

Isolation and identification of chitin in the black coral *Parantipathes larix* (Anthozoa: Cnidaria)

International Journal of Biological Macromolecules (2012)

N. Boden *et al.*

Demonstration and characterization of chitinases in the Drosophila Kc cell line

Insect Biochemistry (1985)

O.I. Bogdanova *et al.*

Structure of β-chitin from *Berryteuthis magister* and its transformation during whisker preparation and polymerization filling

Carbohydrate Polymers (2016)

View more references

Cited by (170)

Xanthan gum in aqueous solutions: Fundamentals and applications

2022, International Journal of Biological Macromolecules

Show abstract

Chitin and derivative chitosan-based structures — Preparation strategies aided by deep eutectic solvents: A review

2022, Carbohydrate Polymers

Show abstract

Biomaterials from the sea: Future building blocks for biomedical applications

2021, Bioactive Materials

Show abstract

Structural basis for directional chitin biosynthesis ↗

2022, Nature

Show abstract

Chitin and Chitosan: Prospective Biomedical Applications in Drug Delivery, Cancer Treatment, and Wound Healing ↗

2022, Marine Drugs

Chitin and Chitosan Derivatives as Biomaterial Resources for Biological and Biomedical Applications ↗

2020, Molecules

View all citing articles on Scopus ↗

View full text