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Low temperature thermal behaviour of chitins and chitin–glucans

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Abstract

In chitin, chitin–glucans and some chitosans, cooling to temperatures between −13 and −31°C apparently reduces or breaks linkages between the sheets of chains in their structures. When additionally an alkaline medium of NaOH, KOH or LiCl/amide exists, this tendency to break is strengthened, reaching in all cases solvation and subsequent dissolution. In chitin, the solubilized fractions correspond to the crystalline β-form or to amorphous materials. In chitin–glucans, the solubilized fraction is a disorganized material in which the chitin remains firmly bound to glucan.

In a DSC study, the thermal effect that appears at around −22°C is derived from the phase transition between the distorted structure poor in intersheet hydrogen bonding and the undistorted structure rich in intersheet bonding. In chitin, it seems that the phase transition occurs from the β- to the α-form, although a change from a bent to a straightened configuration could also explain it.

INTRODUCTION

In the course of fungal cell wall polysaccharide extraction procedures, when the residue from the treatment of the cell wall material with 1 M NaOH at 25°C was left overnight at −20°C, a β-glucan–chitin complex was dissolved [1–3].

Chitin occurs naturally in several crystalline forms in which the poly(*N*-acetyl-D-glucosamine) chain adopts a 2_1 screw axis, with two residues repeating in 10.3–10.4 Å as in cellulose [4–6]. The polymorphs differ in the packing and polarities of the adjacent chains (Table 1 and Figs. 1, 2), although the form-arrangement associations such as β-chitin

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TABLE 1

Crystallographic data of chitin forms

	α-Chitin	β-Chitin
Polarities of neighbouring chains	Antiparallel (⇅)	Parallel (↑↑)
Crystallographic system	Orthorhombic	Monoclinic
Unit cell dimensions	$a = 4.74$ Å $b = 18.86$ Å $c = 10.32$ Å $\alpha = \beta = \gamma = 90°$	$a = 4.85$ Å $b = 9.26$ Å $c = 10.38$ Å $\alpha = \gamma = 90°$ $\beta = 97.5°$
Space group	$P2_12_12_1$	$P2_1$

(parallel chains) or α-chitin (antiparallel chains) are not rigidly defined. In the most common and stable chitin α-form, the fact that the possibility of a $P2_1$ symmetry alternative to $P2_12_12_1$ exists [6], requires consideration of both parallel and antiparallel chain systems.

There are crystal structures which contain inherent degrees of freedom which are activated when the external conditions of the crystal are changed. At sufficiently low temperatures, these structures tend to have low symmetry with substantial distortions/disorder that disappear with temperature increases and reappear sometimes only if the materials are

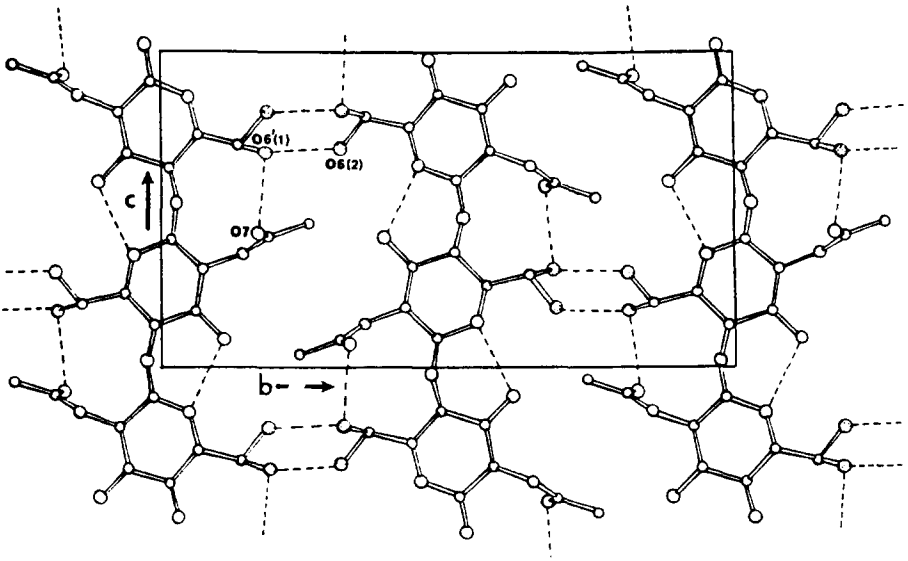


Fig. 1. The structure of α-chitin; *bc* projection. The CH_2OH group on the corner chain forms an intramolecular O6′(1)–H · · · O7 bond and the CH_2OH of the centre chain forms an intermolecular O6(2)–H · · · O6′(1) bond.

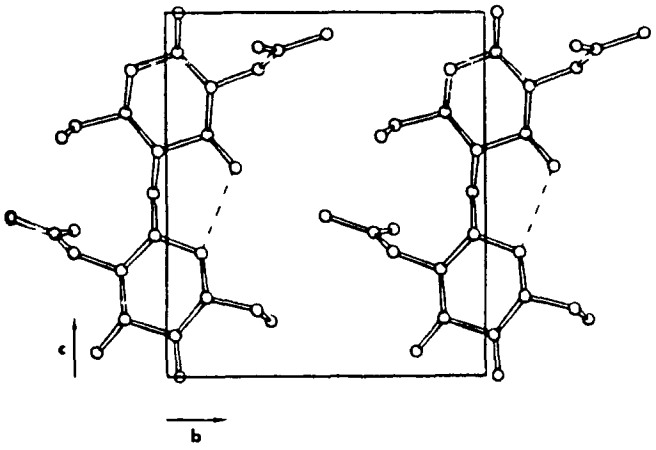


Fig. 2. The structure of β-chitin; *bc* projection.

recooled to their former conditions. It is therefore convenient to consider the distorted structure and the undistorted structure as two distinct phases. The transition between them, as generated by temperature, pressure, or any other thermodynamic variable, is called a structural phase transition.

One of the simplest examples of this behaviour is the perovskite structure and its derivatives [7, 8].

In this work we report the thermal behaviour at low temperatures of chitin and chitin–glucans obtained from fungal cell walls and other sources. Models having different symmetry types and/or conformations were used in an attempt to identify the structures involved in the phase transition.

EXPERIMENTAL

Samples

Specimens of chitin and chitin–glucans from fungi were isolated according to Leal et al. [1] and Gómez-Miranda and co-workers [2, 3]. β-Chitin was obtained from *Loligo* pen. α-Chitin from shells of crab was purchased from Sigma (C 3641). Samples of chitosan, hyaluronic acid, *N*-acetyl-D-glucosamine and cellulose have also been obtained from Sigma (references C 3646, H 1751, A 8625 and C 6288, respectively). Mixed α-/β-chitins were obtained from shrimps. The chitin content in chitinous materials was determined by Svennerholm's method, as reported by Chen and Johnson [9].

Apparatus

DSC curves were recorded in a Perkin-Elmer DSC apparatus, in dynamic N_2 ($20\,cm^3\,min^{-1}$), at a heating rate of $10°C\,min^{-1}$, and with sealed capsules of aluminium as sample containers.

X-Ray powder patterns were performed with a Philips PW 1710 diffractometer using Cu Kα radiation.

IR spectra were obtained by the KBr technique on a Perkin-Elmer 457 IR spectrophotometer.

RESULTS

DSC data

Figures 3a–3l show the low temperature DSC curves of *N*-acetyl-D-glucosamine, chitin–glucans, chitins and chitosan.

In Table 2 it can be observed that the studied phase transition is a function of the chitin content in the material. Chitin–glucans with low chitin content show onset and peak temperatures and enthalpy change values lower than those of chitin–glucans with high chitin content, those of the latter being lower than those of highly pure chitins. Chitosans obtained by partial deacetylation of chitin (with chitin content higher than 20%) also show the transition phenomenon, at the temperatures and enthalpy changes expected.

In spite of the above assertion, other factors in addition to chitin content influence the low temperature transition processes. In fact, highly pure chitins (with low amounts of other polysaccharides) from different sources show different thermal behaviour (Table 2) and even, occasionally, the lack of appearance of effects in DSC. Since it has been stated [10] that in any one given chitinous material the two reported crystallographic forms of chitin can coexist (in contrast to cellulose, where manifestations are in only one phase) and such forms show very different physical properties, the phase transition effect will only be evidenced to the degree that the "thermally active" form is present.

However, it is necessary to indicate that the phase transition phenomenon is irreversible, and for its regeneration a new treatment of the material with 1 M NaOH or KOH at low temperatures is essential. Such irreversibility is associated with the DSC exothermic effect that appears at temperatures immediately above that of transition in a few cases (chitin–glucan from *Aphanoascus terreus*) during the first heating (Fig. 3e), and in general, in a second heating (Fig. 3a).

Another feature observed is that the in-cooling extraction phenomenon is not unlimited: the yield of soluble fraction from the parent material

TABLE 2

Temperatures and enthalpy change of the phase transition for various chitin related compounds of different origin

Polysaccharide	Source	Temperatures		Enthalpy change, ΔH ($J\,g^{-1}$)	Chitin content (%)
		Onset (°C)	Peak (°C)		
N-acetyl-D-glucosamine		−44.6	−31.0	20.3	
Chitin–glucans	*Eupenicillium pinetorum*	−54.8	−31.2	34.7	30.8
	Penicillium verruculosum F4I-S	−44.2	−30.1		30.5
	Talaromyces helicus F4	−40.3	−21.7		43.0
	Aphanoascus terreus F4I	−34.4	−21.6	42.0	44.6
	Penicillium ochro-salmoneum F4I-S	−40.5	−18.3	39.2	34.7
	Aphanoascus verrucosus F4	−34.7	−19.1	34.6	49.2
Chitins	Crab shells	−32.0	−20.0	78.1	>90
	Shrimps	−28.8	−18.5	44.5	>72
	Penicillium verruculosum F4I-2	−24.2	−16.6	31.1	~30
	Other fungi	−25.9	−12.9	94.1	>90
Chitosan		−41.7	−24.9	36.4	<30

decreases from approximately 10% in the first extraction, to even lower yields and subsequent exhaustion. The exhausted material gives no phase transitions.

In chitin–glucans, the in-cooling extraction with NaOH leads to a soluble material and to a residue, both with similar chitin percentages, but with thermal effect temperatures which do not coincide: only the soluble fractions (F4I-S) obey the general trend reflected in Table 2; the insoluble fractions (F4I-R) behave as if they had higher chitin content.

Whether the preliminary treatment with NAOH leading to the purification of chitin from natural sources has been performed in heating or at room temperature, the solubilities of the resulting materials are almost the same when they are later submitted to cold extraction. Likewise, no significant change in solubility occurs between a material boiled in water (in the absence of NaOH) and an untreated material.

X-Ray diffraction data

Figures 4 and 5 show the X-ray powder patterns of the soluble and insoluble fractions that result from the NaOH treatment at −25°C of shrimp chitin. The indexation of these diffraction diagrams permits us to

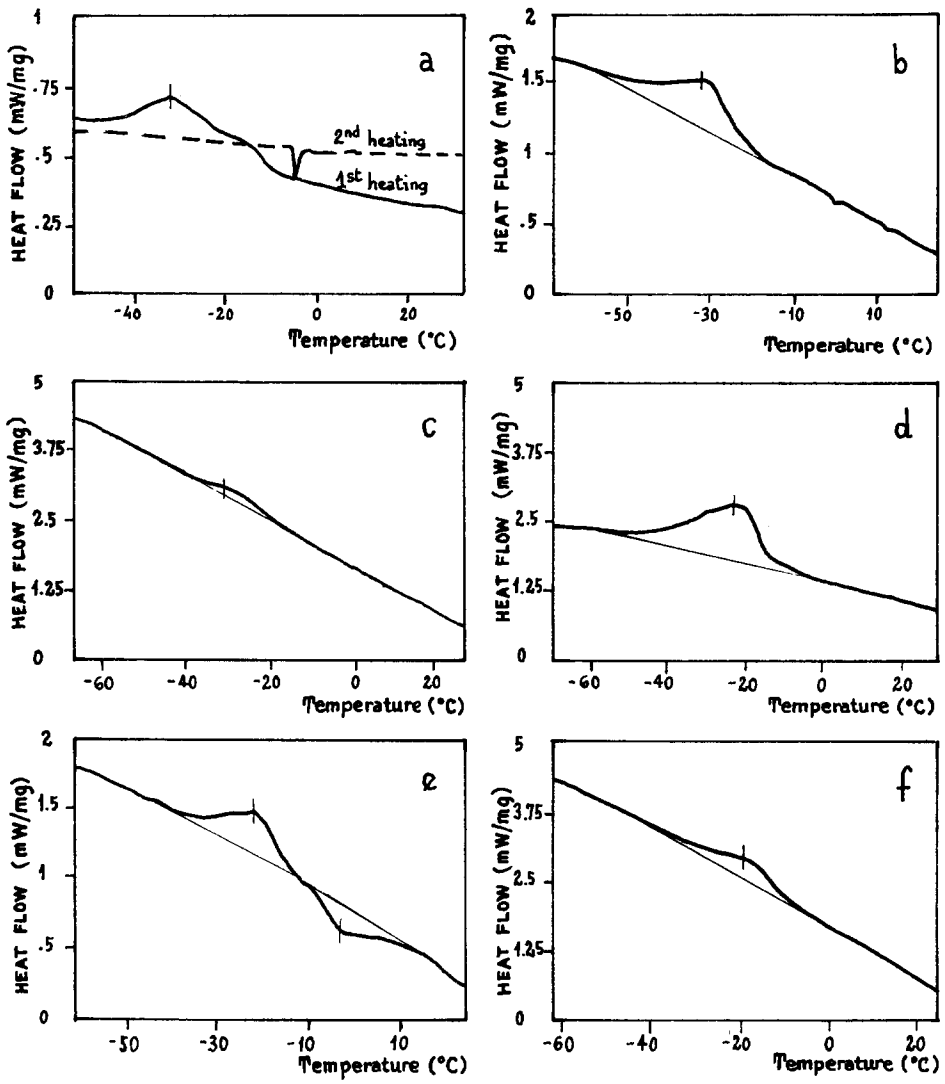


Fig. 3. DSC curves for (a) *N*-acetyl-D-glucosamine; (b) chitin from *Eupenicillium pinetorum*; (c) chitin from *Penicillium verruculosum* F4I-S; (d) chitin from *Talaromyces helicus*; (e) chitin from *Aphanoascus terreus*; (f) chitin from *Penicillium ochro-salmoneum* F4I-S; (g) chitin from *Aph. verrucosus* F4; (h) chitin from crab shells; (i) chitin from shrimps; (j) chitin from *Penicillium verruculosum* F4I-R; (k) chitin from other fungi; (l) chitosan from partially deacetylated chitin.

assign lattice parameters, which in the case of the soluble fraction correspond to those of the β-form, and in the case of the insoluble fraction to those of the α-form.

Figure 6 presents the diffraction pattern from a pool of soluble fractions obtained by repeated treatment of the former insoluble fraction with NaOH in the cold (more than 10 steps). Its indexation leads to a different

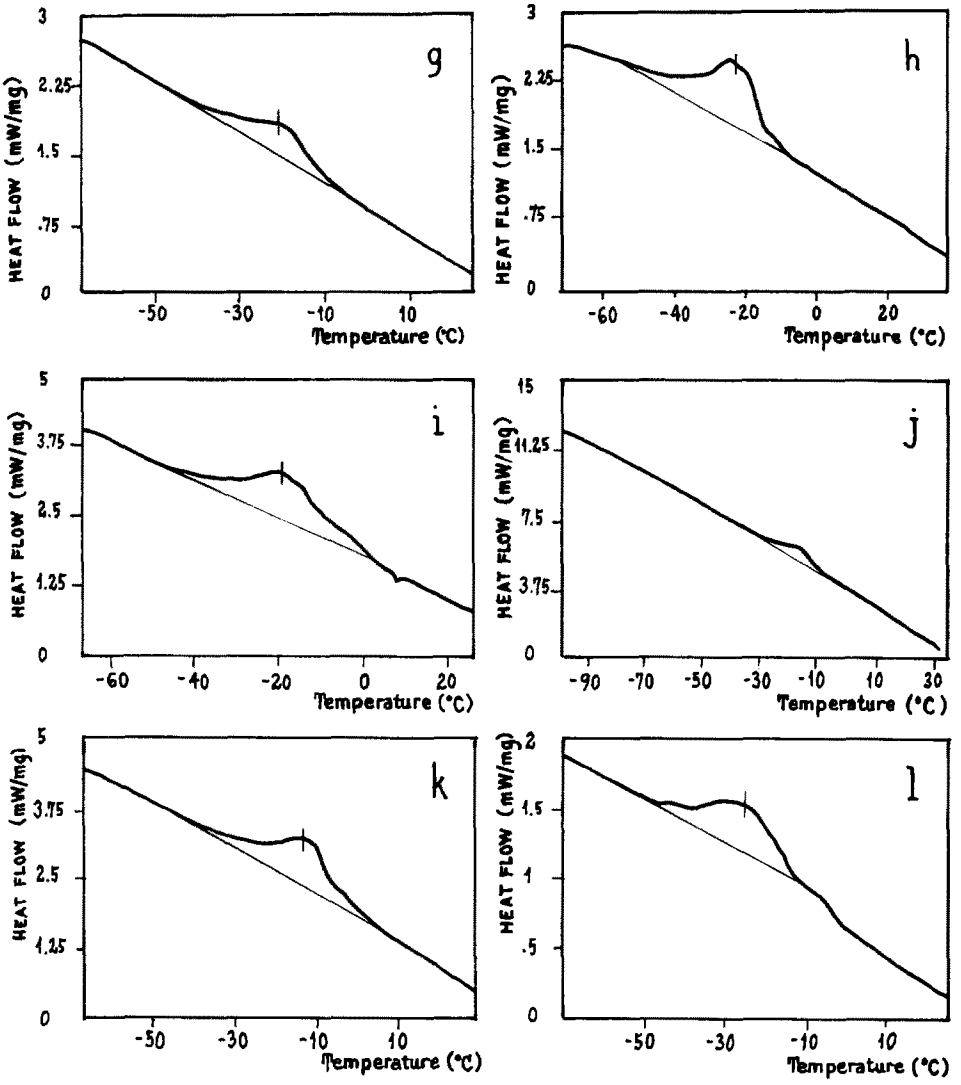


Fig. 3. (continued)

monoclinic unit cell which was neither from α- nor β-forms ($a = 4.89$ Å, $b = 9.06$ Å, $c = 7.4$ Å, $\alpha = \gamma = 90°$; $\beta = 97°$) although this unit cell can be considered as being derived from that of β-chitin given that it conserves the dimensions of two of its parameters (a and b).

Our interpretation of these results is that the treatment of chitin with NaOH at low temperature leads, in a first step, to a release of the β-form content of the parent material and, in the following steps, to a disorganized (amorphous) chitin.

For chitin–glucans, the only X-ray diffraction pattern registered (Fig. 7)

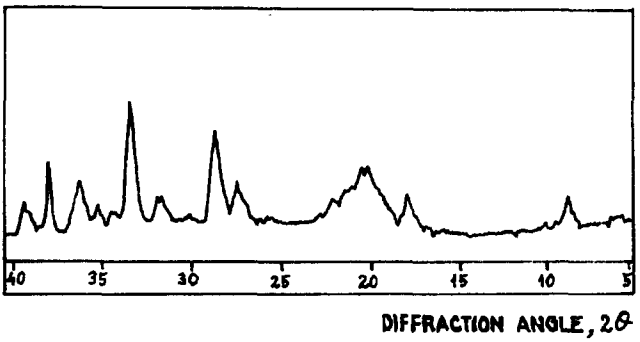


Fig. 4. X-Ray diffraction pattern of the soluble fraction that results from NaOH treatment at −25°C of shrimp chitin.

is that of the soluble fraction from *Penicillium ochro-salmoneum*. Its indexation provided characteristic parameters ($a = 4.87$ Å; $b = 17.15$ Å; $c = 7.02$ Å; $\alpha = \beta = \gamma = 90°$) that do not agree well will those of the α- or β-chitin forms. This result is in accord with the consideration that in fungi the nature of the interaction between chitin and glucan is very strong.

IR spectra

The IR spectra of crab shells chitin (α-form) and those chitin–glucans rich in chitin have shown an absorption band of 3120 cm^{-1} (Figs. 8a, 8b)

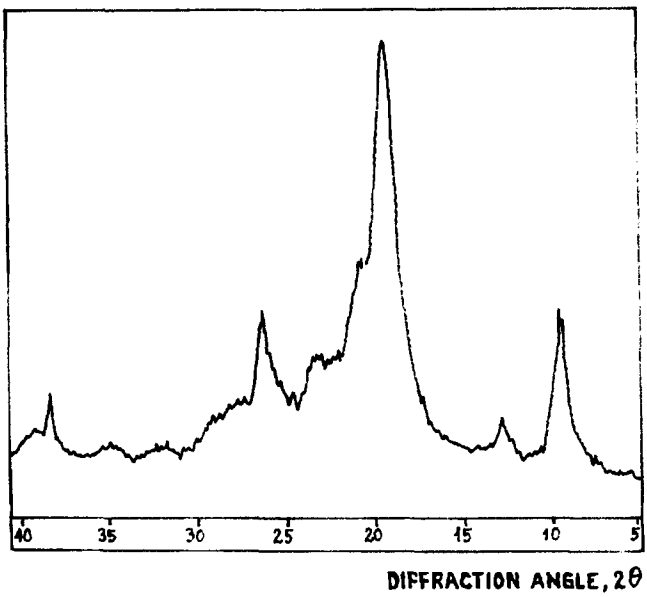


Fig. 5. X-Ray diffraction pattern of the insoluble fraction that results from NaOH treatment at −25°C of shrimp chitin.

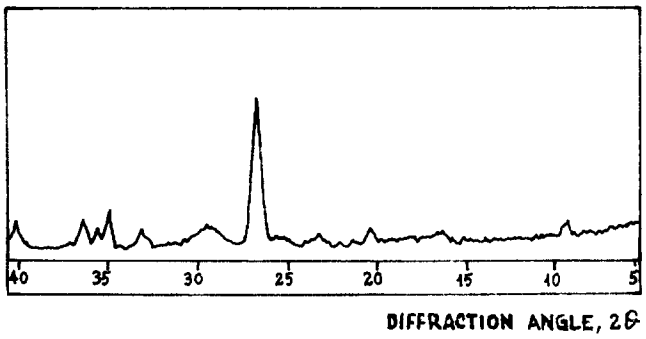


Fig. 6. X-Ray diffraction pattern of a pool of soluble fractions obtained by repeated treatment with NaOH in cold (>10 steps) of the above insoluble fraction.

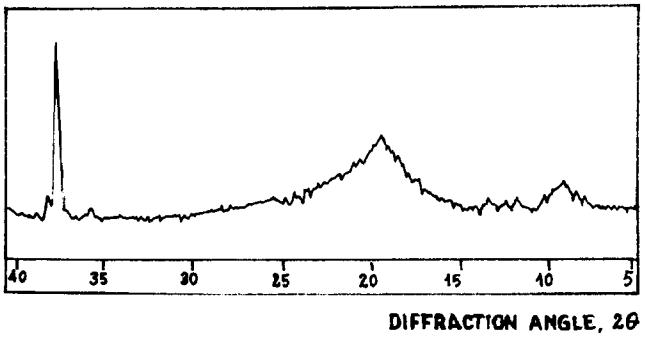


Fig. 7. X-ray diffraction pattern of the soluble fraction that results from the NaOH treatment at −25°C of chitin from *Penicillium ochro-salmoneum.*

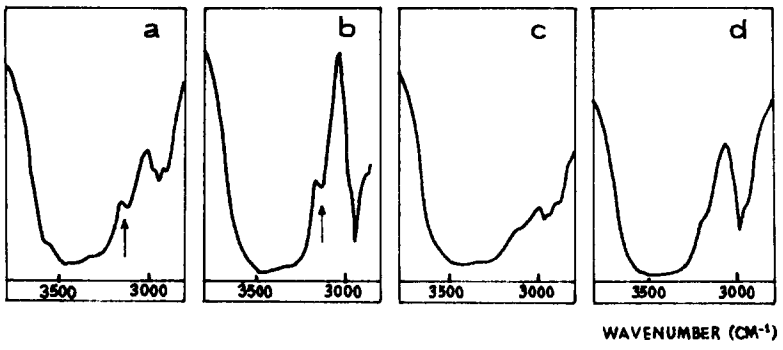


Fig. 8. IR spectra of (a) chitin from crab shells, (b) chitin from *Aphanoascus verrucosum*, (c) β-chitin from *Loligo* pen, (d) chitin from *Penicillium verruculosum.*

attributed to O–H str $\perp$ and O_6–H $\cdots$ O_6 inter. This band does not appear in either the IR spectra of chitin from *Loligo* pen (β-form) or chitin–glucans poor in chitin (Figs. 8c, 8d).

Thus, the IR band at 3120 cm^{-1} can be used as a marker of the intermolecular hydrogen bonding content and to recognize different crystalline forms or chitin richness in chitinous materials.

DISCUSSION

The observation that highly deacetylated (>69%) chitosan does not yield any thermal effect whereas *N*-acetylglucosamine does with extreme values for the phase transition studied (Table 2) led us in a first hypothesis to postulate the involvement of the *N*-acetyl groups as protagonists of such effects. In 1972, Phelps [11] stated that the function of *N*-acetyl substituent in chitin is a mystery and that perhaps the nitrogen atom affords more favourable hydrogen bond facilities than those existing in cellulose. Therefore, the possibility of breaking/formation of hydrogen bonds, mediated by *N*-acetyl groups, was suggested as responsible for the phase transition phenomenon.

Nevertheless, subsequent observations have not entirely supported this hypothesis: the transition thermal effect has also been observed in fungal acid exopolysaccharides with *O*-acyl groups [12, 13] whereas, against prognosis, the hyaluronic acid (a heteropolymer with the sugar derivatives *N*-acetylglucosamine and D-glucuronic acid and which has super-hydrogen bonding in its structure) produces no observable DSC effects at low temperatures.

If, theoretically, we ignore the *N*-acetyl substituents as being responsible for the phase transition phenomenon, we are forced to consider the rest of the polymer as such. Examining the structures of chitin and cellulose reveals that both are remarkably similar and this is surprising for chitin because the *N*-acetyl group on C2 is very bulky and might be imagined as offering a greater degree of rigidity to the polysaccharide backbone than that existing in cellulose. From this similar structural behaviour, a similar thermal behaviour and analogous physical properties should be derived: in practice, cellulose at the beginning does not give the expected thermal effect, although it certainly is dissolved in 1 M NaOH in cooling, and only after activation by this treatment shows the predicted endothermic effect ($T_{onset} = -38°C$; $T_{peak} = -26.7°C$; $\Delta H = 37.8\ J\ g^{-1}$).

The activation can be interpreted as the change in alkaline media of the hydroxyl groups to alkoxide anions, this latter being stabilized by sodium or other cations (the solubilization of chitin in the amide/LiCl non-aqueous system can be interpreted in a similar manner).

What has been said above leads us to state that the phase transition

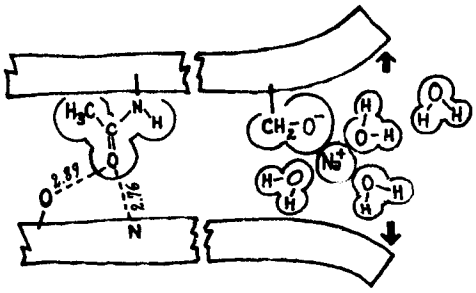


Fig. 9. First step in the solvation–dissolution of a chitin/cellulose-type polysaccharide showing the partial entry of the solvent molecules between the sheets of chains.

effect under consideration is associated, in general, with structures having an ordered packing of chains determined by an almost complete scheme of intermolecular hydrogen bonding. In particular it is associated with polysaccharides having a cellulose-type backbone with bulk substituents, such as *N*-/*O*-acetyl groups, or alkoxide anions (these latter, electrically neutralized with Li^+, Na^+ or K^+ cations) which assure an adequate separation between the structural sheets of chains. It is likely that these substituents facilitate for the overall structure a similar hydrogen bonding conformation (ribbon-type), strong in ordinary conditions but weak under cooling, allowing in this second case the partial entry of the solvent between the sheets of chains and the subsequent solvation–dissolution (Fig. 9).

The absence of the phase transition in chitosan can be justified by the lower volume of the NH_2 substituents and their very low reactivity with NaOH (the activation and following solubilization could only be possible in dilute acid media by formation of the entity NH_3^+). In hyaluronic acid the presence of *N*-acetyl substituents does not confer the expected thermal activity because the basic backbone is not of cellulose/chitin-type (the insertion of β-(1→3) linked glucuronic acid residues changes the overall structure from ribbon to random coil). On the contrary, the *O*-acylated polysaccharides (β-glucans with (1→6) or (1→3) linkages and hollow helices as expected conformational type) show the reported phase transition, possibly by change to a ribbon family when they function as extracellular framework materials [11].

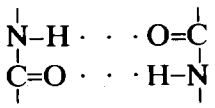
Mechanism of the phase transition

In chitin, the β-form is associated with a parallel ($\uparrow\uparrow$) chain structure, with the existence of an intermolecular hydrogen bond and with the absence of hydrogen bonding between the sheets, which explains the ease with which it swells in water to produce hydrates. The α-form exhibits a change in chain polarity ($\uparrow\downarrow$) that results in additional interchain bonding

between the sheets and thus accounts for their insolubility. The coexistence of both forms in the same material can explain their partial solubility. Since the $\beta \rightarrow \alpha$ transformation is favoured by heating, vigorous thermal treatments can justify the insolubility of certain chitinous materials. On the contrary, an $\alpha \rightarrow \beta$ transition by cooling is forbidden and it cannot thus explain the solubilization. Nevertheless, if we admit a symmetry $P2_12_12_1$ (only ⇅ forms) at high temperatures and a $P2_1$ reduced symmetry (⇅ and ↑↑ forms) at low temperatures, it is equivalent to admitting the possibility of the solubilization (in a solvent) at low temperatures and to justifying the DSC effect as a partial transformation ↑↑ to ⇅, in other words, by a relative gain of intermolecular versus intramolecular hydrogen bonding.

Another possibility could be to accept Carlstrom's model [4] (polymeric chains in "bent" configuration) for the low temperature phase, and a "straight" configuration for the high temperature phase. A bent structure, formally equivalent to two-edge dislocations in the intrachain hydrogen-bond network (Fig. 10), causes a disruption in crystalline order and its propagation could allow chains to slide over one another (chain sliding could occur by breaking only two hydrogen bonds at a time; this process would require far smaller forces than would be required to slide chains over each other bodily). Upon cooling this disruptive effect could occur relatively easily in chitin structures but less easily in cellulose, where each chain is hydrogen bonded to four near neighbours.

It has been observed in cellulose, and extended to chitin [14], that the crystalline regions are surrounded by paracrystalline areas. For these latter, it has been postulated that CO and NH bonds from *N*-acetylglucosamine side groups on disordered chitin chains can occur.



Independently of whether the above proposed hydrogen bonds or the weaker van der Waals' interactions between chitin side chains are involved in the paracrystalline state (presumably representative in the low temperature phase), a rise of temperature must lead to a breaking of the earlier bonds in favour of the stronger interchain $O_6\text{–}H \cdots O_6$, in the high temperature phase.

CONCLUSIONS

The cooling between −13 and −31°C apparently reduces or breaks crystalline linkages (intermolecular hydrogen bonds and van der Waals'

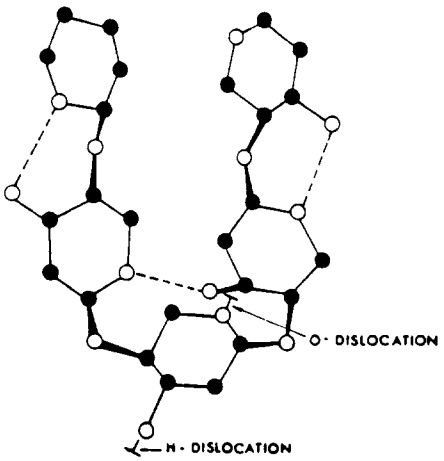


Fig. 10. A bent structure in a polysaccharide chain is formally equivalent to two-edge dislocations.

interactions) between the sheets of chains in chitin, chitin–glucans and in chitosans from incompletely deacetylated chitin. When a NaOH, KOH or LiCl/amide medium exists, this possibility of breaking is enhanced (even in the case of cellulose), leading in all cases to the phenomenon of solvation and subsequent dissolution. In chitin, the solubilized fractions correspond to the less crystalline β-form or to amorphous materials. In chitin–glucans, the solubilized fraction is a disorganized material in which the chitin remains firmly bound to glucan.

In DSC, the thermal effect that appears for the studied materials around −22°C (in a heating programme from −70°C) is determined by the phase transition between the distorted structure poor in intersheet hydrogen bonding and the undistorted structure rich in intersheet bonding. In chitin, it seems that the phase transition occurs from the β- to the α-form, although a bent-straightened configuration change has also been considered.

ACKNOWLEDGEMENTS

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