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X-ray fibre diffraction studies of chitosan and chitosan gels

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ABSTRACT

Fibres obtained from freshly prepared films of ungelled chitosan yielded X-ray diffraction patterns indicative of high crystallinity and characteristic of 8_5 helices arranged in a monoclinic unit cell ($a = 1.38$, $b = 1.63$, $c = 4.07$ nm, $\alpha = 96.46°$). This is the first report of an 8-fold helical structure for chitosan. Exposure of these fibres to high humidity led to a progressive change from an 8-fold to a 2-fold helical structure arranged in the more orthodox orthorhombic unit cell. Gels were prepared by dissolving chitosan in acetic acid and then inducing gelation by (*a*) raising the pH of the solution, (*b*) *N*-acetylation, or (*c*) *N*- and *O*-acetylation. The gels formed in (*a*) were non-crystalline, and those in (*b*) and (*c*) gave diffraction patterns similar to those for chitin gels.

INTRODUCTION

Chitin, the major structural component of the exoskeleton of invertebrates and the cell walls of fungi, is a (1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucan. In the cuticles, apodemes, and chaetae of animals, chitin is associated with a considerable proportion of protein as a 2-phase block-copolymeric system (chitin–protein complex).

Treatment of chitin with alkali leads to *N*-deacetylation and the formation of chitosan. The terms "chitin" and "chitosan" refer to families of partially substituted polysaccharides.

An orthorhombic unit cell [$a = 0.89$, $b = 1.70$, c (fibre axis) = 1.025 nm] has been proposed[1] for chitosan, and subsequent X-ray studies[2–5] of chitosan and chitosan–metal complexes have reported a 2-fold helical structure, similar to those

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of chitin and cellulose. Recent X-ray diffraction studies[6] revealed that the chloride, fluoride, and sulphate salts of chitosan crystallise in a monoclinic unit cell with a helical repeat of 4.073 nm. The chitosan backbone in these salts is believed[6] to adopt either a left-handed (8_5) or right-handed (8_3) helix, the first reported structures which were not 2-fold helices. We now report on an 8-fold helical structure for chitosan.

Solutions of chitosan (NAc 17%) in aqueous acetic acid can be gelled by (*a*) raising the pH of the solution, (*b*) mixed *N*- and *O*-acetylation, and (*c*) selective *N*-acetylation. We now report X-ray diffraction studies of aligned fibres prepared from such gels.

EXPERIMENTAL

Three chitosans with various degrees of acetylation and viscosities (9% and 718 cps, 17% and 1060 cps, and 21% and 1400 cps) were a gift from Dr. P. Sandford (Protan Laboratories). Chitin (68% *N*-acetylated) was purchased from Protan Laboratories.

X-ray diffraction studies were carried out on aligned fibres prepared from chitin and chitosan gels, and from films cast from ungelled chitosan. Strips were cut from the chitin and chitosan gels, and stretched up to 100% under conditions of controlled humidity in sealed vessels in order to produce aligned fibres. A solution of ungelled chitosan in aq 2% acetic acid was cast on to a polytetrafluoroethylene support and allowed to dry in order to form a film. Strips were cut from the film and stretched up to 100% as described above. The X-ray wavelength was 0.154 nm and the interior of the flat-plate camera was flushed continuously with He in order to reduce air scatter. All fibres were dusted with calcite for calibration purposes.

Chitin gels were prepared as follows. Chitin (0.1 g) was added to a 5% solution of LiCl in *N*,*N*-dimethylacetamide. After 48 h, the bulk-solution viscosity had increased markedly. The viscous solution was decanted from the swollen, undissolved chitin, and poured onto a glass slide. Gelation occurred and was accompanied by shrinkage of the sample. Gels were dialysed against distilled water for 72 h and used to prepare fibres for X-ray studies.

Chitosan gels were prepared as follows.

(*a*) *N-Acetylation*[4,7]. To a solution of 1060 cps chitosan (1 g) in aq 10% acetic acid (20 mL) was added MeOH (80 mL), and the mixture was stirred for 60 min. To an aliquot (50 mL) was added acetic anhydride (1.2 mL), and the slurry was stirred until clear, then poured onto glass slides, and allowed to set. The resulting gels were washed with distilled water for 72 h. This chitosan was 60.6% *N*-acetylated.

(*b*) *Partial* O- *and* N-*acetylation*[7,8]. Chitosan 1060 cps (1 g) was dissolved in aq 10% acetic acid (50 mL). To an aliquot (25 mL) was added acetic anhydride (12.5 mL), the mixture was stirred until clear, and slabs of gel were prepared as in (*a*). The combined *N*- and *O*-acetylation of this chitosan was 55.4%.

(*c*) *Treatment with alkali*[9,10]. A solution of chitosan 1060 cps (1 g) in a mixture of glacial acetic acid (2%), glycerol (75%), and water (23%) was dialysed against M NaOH for 24 h. The resulting opaque gel was then dialysed against distilled water for 72 h.

The degree and type of acetylation in chitosan gels prepared in (*a*) and (*b*) were assessed by IR spectroscopy[11] on thin films that had been washed in aq ammonia–MeOH.

RESULTS AND DISCUSSION

Oriented fibres prepared from ungelled chitosan films yielded diffraction patterns (Fig. 1a) indicative of high crystallinity, which were consistent with the monoclinic unit cell ($a = 1.38$, $b = 1.63$, $c = 4.07$ nm, $\alpha = 96.48°$) reported[6] for chitosan salts (chloride, fluoride, and sulphate). The first strong meridional reflection lies on the 8th layer line, which is consistent with an 8-fold single helical structure having an axial rise per monosaccharide repeat unit of 0.51 nm.

The linked-atom least-squares refinement system[12] was used to produce stereochemically satisfactory helical models of the polysaccharide, which were consistent with the observed pitch and helical symmetry. Left- and right-handed 8-fold single helical structures were examined. The bond lengths, bond angles, and chair conformations were fixed at standard values[13] and the glycosidic bond angles were used as variable parameters. Both 8_5 and 8_3 single helical structures were found to be allowable stereochemically and to pack into the observed unit cell (Fig. 2). Preliminary modelling studies[14] favoured the 8_5 helical structure.

This is the first reported observation of chitosan in a conformation other than a 2-fold helix. Exposure of chitosan fibres to a high (98%) humidity overnight produced an irreversible change from the 8_5 helical structure to the more-conventional 2-fold helical form crystallised in the orthorhombic unit cell. Storage of the fibres over silica gel appeared to increase the stability of the 8_5 helix such that

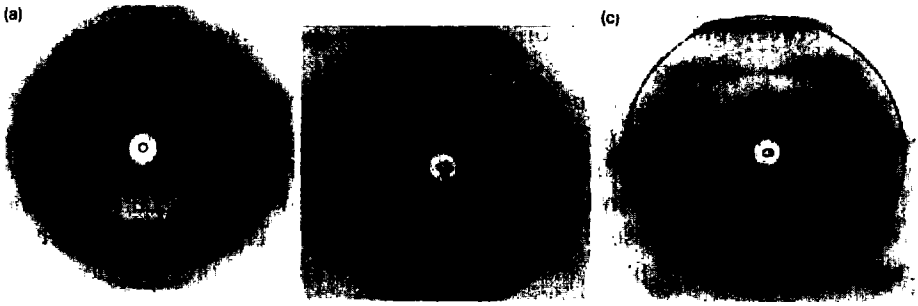

Fig. 1. X-ray fibre diffraction patterns obtained for (a) freshly prepared chitosan showing the 8_5 helical conformation, (b) partially aged chitosan (~ 8 h, 98% relative humidity), (c) fully-aged (~ 12 h, 98% relative humidity) showing the 2-fold helical structure.

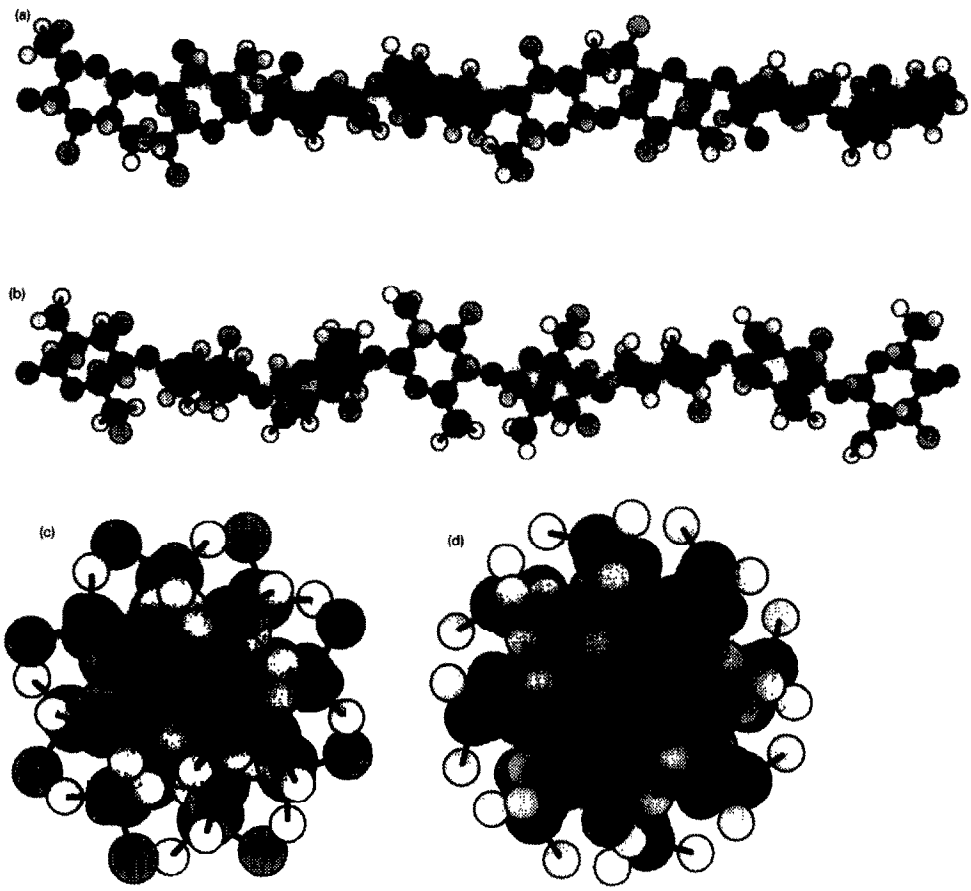

Fig. 2. Stereochemically acceptable molecular models of chitosan: (a) 8_3 helix, (b) 8_5 helix, and end projections of (c) the 8_3 helix and (d) the 8_5 helix.

prolonged exposure to 98% relative humidity was required to cause the transition to the 2-fold helix. The fibre-diffraction pattern in Fig. 1b shows chitosan in transition between the 8_5 helical form (Fig. 1a) and the 2-fold helical form (Fig. 1c).

X-ray diffraction patterns of unoriented freshly prepared chitosan films showed spacings equivalent to those for the 8_5 helical structure crystallised in the monoclinic unit cell. Aging and hydration of these films led to diffraction patterns that showed spacings equivalent to those for the 2-fold helical form crystallised in the orthorhombic unit cell. These studies showed that the 8_5 helix was not an artifact induced by stretching the films. Films were prepared after dissolving chitosan (*a*) by heating without stirring and (*b*) without heating or stirring. The diffraction patterns obtained for these unoriented films corresponded to those for the 8_5 helical structure in the monoclinic unit cell. These studies showed that the 8_5 helix

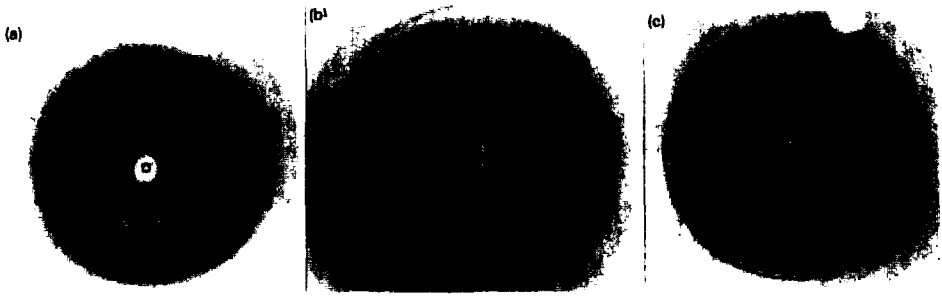

Fig. 3. X-ray fibre diffraction patterns for (a) chitosan gel prepared by *N*-acetylation, (b) chitosan gel prepared by mixed *N*- and *O*-acetylation, and (c) chitin gel.

was not an artifact of the preparation procedure. It is possible that the 8_5 helix could be a stable conformation for the molecules in solution and that, under the right conditions, this structure can become entrapped when films are formed. However, once the chitosan forms the 2-fold helical structure, the 8_5 helix cannot be regenerated without dissolving the chitosan and re-forming a new film.

Chitosan gels prepared by increasing the pH of chitosan solutions in dilute acetic acid are opaque. The X-ray fibre diffraction patterns are of poor quality and indicate non-crystallinity. It seems likely that gelation arises due to the localised precipitation of the chitosan and the formation of an amorphous interconnected network.

Both *N*- and *N*,*O*-acetylation of chitosan yields transparent gels which give high-quality fibre diffraction patterns indicative of high crystallinity (Figs. 3a and b). The patterns obtained are the same for gels prepared by either of the two methods and are identical to those for oriented fibres prepared from chitin gels (Fig. 3c). Each method of acetylation appears to produce "chitin-like" junction zones within the gels. These observations agree with the model for gelation suggested by Moore and Roberts[11] who investigated the role of *N*-acetylation upon the gelation of chitosan. It was suggested that sufficient *N*-acetylation would overcome the electrostatic repulsion between protonated amine groups along the polysaccharide chain, leading to aggregation between chain segments as a result of intermolecular hydrogen bonding between *N*-acetylated regions. In effect, *N*-acetylation introduces insoluble "chitin-like" regions within the polysaccharide chain. Thus, the junction zones observed in the gel, as observed experimentally, should be the same as those in a chitin gel.

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