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Structural investigation of porcine stomach mucin by X-ray fiber diffraction and homology modeling

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ABSTRACT

The basic understanding of the three dimensional structure of mucin is essential to understand its physiological function. Technology has been developed to achieve orientated porcine stomach mucin molecules. X-ray fiber diffraction of partially orientated porcine stomach mucin molecules show *d*-spacing signals at 2.99, 4.06, 4.22, 4.7, 5.37 and 6.5 Å. The high intense *d*-spacing signal at 4.22 Å is attributed to the antiparallel β-sheet structure identified in the fraction of the homology modeled mucin molecule (amino acid residues 800–980) using Nidogen–Laminin complex structure as a template. The X-ray fiber diffraction signal at 6.5 Å reveals partial organization of oligosaccharides in porcine stomach mucin. This partial structure of mucin will be helpful in establishing a three dimensional structure for the whole mucin molecule.

1. Introduction

Mucins are high molecular weight macromolecules whose extensive components are carbohydrates. The oligosaccharides play a main role in maintaining the structural architecture and conformation of mucins. The mucins are of two types, secretary and membrane bound. Based on the bound carbohydrate side chains, the mucins are further classified into different types. The mucins are encoded by 12 different mucin genes viz. MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC9, MUC11 and MUC12 [1]. For example, MUC1 is localized in the epithelial cells lining in the respiratory, reproductive and gastrointestinal tracts [2]. The biological role of mucins is to provide biolubrication in many biological fluids including saliva, tears, sweat, gastric secretions and genetic tracts. An understanding of the functioning and malfunctioning of this type of proteoglycans is important for the development of treatments for a number of diseases such as cystic fibrosis (bronchial mucin), arthritis (hyaluronates associated with chondroitin sulfate) and stomach ulcers (stomach mucin) [3,4]. Stomach mucins are also of interest to the pharmaceutical industry since they form a barrier towards drug adsorption. The structural studies of mucin remains a challenging task because of its high molecular weight, viscousness, non-crystallinity and chemical heterogeneity with high degree of glycosylation.

Fibrous structures that provide low resolution X-ray diffraction data are effectively used to propose the three dimensional structural models for fibrous molecules [5,6]. Several models were proposed for mucins based on experimental investigation. A few of them to mention here are bottle brush type structures [7], pearl necklace model [8], daisy chain morphology in dumb bell configuration [9] and double-globular comb structure [10]. In this paper, X-ray fiber diffraction coupled with homology molecular modeling is used to propose the structural model for mucin.

2. Materials and methods

2.1. Mucin fiber preparation

Type III partially purified porcine stomach mucin was purchased from Sigma Biochemicals (Sigma Aldrich, M1778). 0.5 g of porcine mucin was dissolved in 25 ml of distilled water with continuous stirring for about 24 h and the resulting turbid solution was centrifuged at 5000 rpm for 15 min at 18 °C. The supernatant of the solution was poured on a Teflon sheet and dried in the sunlight for a day which yields a thin film material of brittle nature. The dried mucin film was subjected to stretching which was carried out by placing one end of the film to the micro clip and made to hang on a stand and the other end to another micro clip which acts as a stretching weight. This set up was placed inside the desiccator for three days in which 75% humidity was maintained by keeping the saturated sodium chloride solution inside the chamber. The film was stretched three times that of its original length in the longitudinal direction. This fiber was then used for the X-ray diffraction experiment. In the annealing process, the mucin was stretched in the presence of humidity inside the hot oven which was maintained at 60 °C. It was found that annealing the

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film at 60 °C for two days noticeably improved the degree of crystallinity. This fiber is used for recording another X-ray fiber diffraction pattern.

2.2. Fiber diffraction pattern

The X-ray patterns were recorded in a Bruker Microstar rotating anode generator equipped with Mar345 dtb image plate recording system. The incident wavelength used was 1.5418 Å (Cu Kα). The diffraction patterns were collected at room temperature with the source to detector distance as 150 mm. The exposure times are varied in the range of 10–60 min.

2.3. Homology modeling

The sequence of porcine stomach mucin obtained from Swissprot (Accession number: Q0MSH8) is used as an input to find out suitable template structure in the SWISSPDB VIEWER and the template structure obtained thus is used for homology modeling in SWISS-MODEL.

3. Results and discussion

The structure of porcine stomach mucin was investigated using X-ray fiber diffraction and molecular modeling. This is the first time an X-ray fiber diffraction for partially oriented molecules of porcine mucin was recorded. The X-ray fiber diffraction pattern of porcine mucin (fiber prepared under 75% humidity) is shown in Fig. 1. This pattern is highly diffuse and indicates minimal ordering of mucin molecules. However, improved diffraction pattern was obtained when the fiber was stretched in hot oven maintained at 60 °C in the presence of 75% humidity and is shown in Fig. 2. The

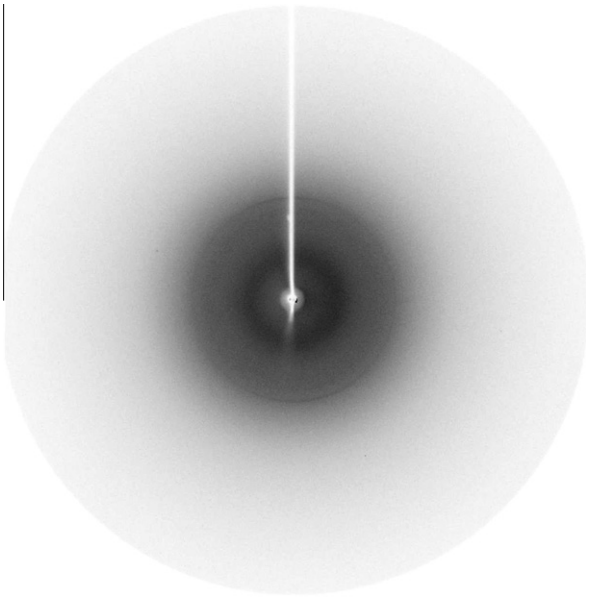

Fig. 1. The X- ray fiber diffraction pattern of the mucin fiber (fiber stretched at room temperature, 75% humidity).

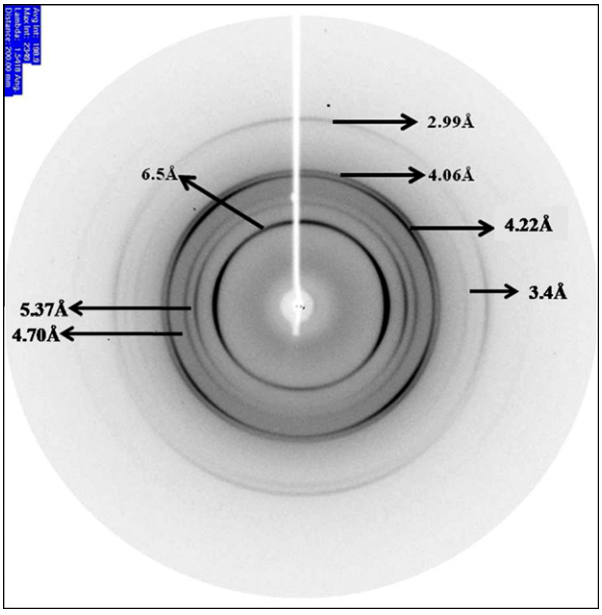

Fig. 2. The X-ray fiber diffraction pattern of the mucin fiber showing diffraction signals at the *d*- spacing of 6.5, 5.37, 4.70, 4.22, 4.06 and 2.99 Å. (Fiber stretched at 60 °C, 75% humidity).

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CLUSTAL 2.0.12 multiple sequence alignment

Porcine     ----------------SFFNCWNKTCPENYCYNQGRCYISQSLTCEPACTCPPAFMED-- 838
1NPE_A      GTHLLFAQTGKIERLPLERNTMKKTEAKAFLHIPAKVIIGLAFDCVDKVVYWTDISEPSI 60
                              *  :** .: : :  .:  *. :: *    .  . : *

Porcine     TRCFLAGKNFCPTILPELPPR------------LIQLLLSEKENASQADVNATVAYRLG 885
1NPE_A      GRASLHGGEPCTIIRQDLGSPEGIALDHLGRTIFWTDSQLDRIEVAKMDGTQRRVLFDTG 120
             *. * * : *. *  :* .                 :  *.. * *.  ..:  * :  *

Porcine     NLDVRAFLR------------NRQVERVELPAIPASG------------NFLQYWKVISE 916
1NPE_A      LVNPRGIVTDPVRGNLYWTDWNRDNPKIETSHMDGTNRRILAQDNIGLPNGLTFDAFSSQ 180
             :: *.::             **:  ::* . : .:.           * * :  . *:

Porcine     FQYRPGGP-VCDFLN------TRLLDAVVEAFLSQASQRRWKRSEEPRNNVVFQPISRRD 974
1NPE_A      LCWVDAGTHRAECLNPAQPGRRKVLEGLQYPFAVTSYGKNLYYTDWKTNSVIAMDLAISK 240
            : :  .*.   : **       ::*:.:  .*   :  :.   ::   *.*:   ::  .

Porcine     VLSVMA---------------------- 980
1NPE_A      EMDTFHPHKQCRLYGITIALSQCPQGH 267
             :..:
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Fig. 3. Sequence alignment of porcine stomach mucin and Nidogen–Laminin complex (1NPE_A) with ClustalW. '*' indicates the residues in that column are identical in the alignment, ':' indicates conserved substitutions, '.' indicates semi-conserved substitutions. Red- (Small, Hydrophobic, Aromatic (not-Y)), Blue- (Acidic), Magenta- (Basic), Green- (Hydroxyl, Amine, Basic-Q). (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)