Structure and Function of Chitosan (V). Conformations of Ethylene Glycol Derivatives of Chitin and Chitosan

Bull. Univ, Osaka Pref., Ser. B, Vol. 48,
大阪府立大学紀要, 農学・生命科学, 1996

Structure and Function of Chitosan (V). Conformations of Ethylene Glycol Derivatives of Chitin and Chitosan

Toshifumi YUI, Kunihiko NAKATA and Kozo OGAWA,

Faculty of Engineering, Miyazaki University
Department of Applied Biochemistry, College of Agriculture
Division of Biological Materials and Bioengineering,
Research Institute for Advanced Science and Technology

(Receved October 31, 1995)

Abstract

Molecular structures of ethylene glycol derivatives of chitin and chitosan, where 0-6 of chitin chain was etherified and both 0-3 and 0-6 of chitosan were substituted, were studied by X-ray fiber diffraction methods coupled with conformational analyses. The extended two-fold helical conformations of both chitin and chitosan chains were retained even by the etherifications. Possible molecular conformations of these derivatives were proposed.

Introduction

Chitin and chitosan have been under intensive investigations for their industrial usage. Komiyama *et al.* have reported that several water soluble derivatives of chitin and chitosan form respective complexes with a streptococcal α-glucan which is produced by *Streptcoccus mutans* and is related to dental plaque formation on human tooth; as a result these derivatives are expected to prevent the occurrence of dental caries[1]. Among them, ethylene glycol derivatives of chitin and chitosan showed higher rate of the complex formation than others[1]. Furthermore, the ethylene glycol chitin quite effectively inhibited the adsorption of *S. mutans* onto a hydroxyapatite, a model compound for human tooth[2]. Chemical structure of the α-glucan has been analyzed to consist of a backbone (1→3)-α-D-glucan chain with a few short side chains of (1→6)-α-linked D-glucose residues attached on the 0-6 of the backbone glucan[3]. The X-ray diffraction study on the polymorphic behavior of the backbone glucan revealed that even when the relative humidity changed from 0 to 100% the glucan always keeps dehydrated polymorph which may be required to form dental plaque[4]. In this paper we propose the molecular conformations of the ethylene glycol derivatives of chitin and chitosan, which may give an idea how they form the respective complexes with the streptococcal α-glucan.

Materials and Methods

Ethylene glycol derivatives of chitin (PEGT) and chitosan (PEGS), in which the hydroxyl

groups attached to C-3 and/or C-6 of each monomer residue (*N*-acetyl glucosamine for PEGT and glucosamine for PEGS) were etherified by ethylene glycol, were supplied by Lion Co., Tokyo. Sano *et al.* suggested that only C-6 position of each monomer residue of chitin was etherified by ethylene glycol[2]. Whereas, the degrees of substitution of PEGS was 1.92[5]. The possibility of etherification of the free amino group ($-NH_2$) of chitosan was ignored since a colloidal titration method using potassium polyvinyl sulfate solution to measure the content of the free amino group[6] indicated that the degrees of non-substitution to the group of PEGS was 98%. So that, all the hydroxyl groups at both C-3 and C-6 were regarded to be substituted by ethylene glycol in PEGS. The aqueous solutions of both PEGT and PEGS (2.5g/100ml each) were cast and dried in air, respectively. In order to get a fiber diffraction pattern, a strip of the resultant PEGT film was stretched 1.8 times of the original length in 50% aqueous isopropyl alcohol solution, annealed in 75% aqueous isopropyl alcohol solution at 180℃ in a sealed bomb, and washed with isopropyl alcohol and methyl alcohol followed by air drying. In the case of the chitosan derivative, PEGS, a strip of the film was stretched on a hot bench at 280℃.

X-ray diffraction patterns were recorded at 100% relative humidity under a helium atmosphere in a flat-film camera with a Rigaku Geigerflex X-ray diffractometer using Ni-filtered Cu-$K\alpha$ radiation at 40kV and 15mA. Conformational analysis was done by the aid of computer programs, MM2(77)[7] and PS79[8]. All calculations for the conformational analysis were carried out on a FACOM-M1600 computer at Miyazaki University Computer Center.

Results and Discussion

A stretched film of the ethylene glycol chitin (PEGT) showed two strong reflections on the equator having *d*-spacings of 1.26 and 0.45nm, one (d=0.512nm) on the 2nd layer, a broad reflection (0.336nm) on the 3rd layer, and a weak spot (0.257nm) on the 4th layer lines (Fig. 1 left).

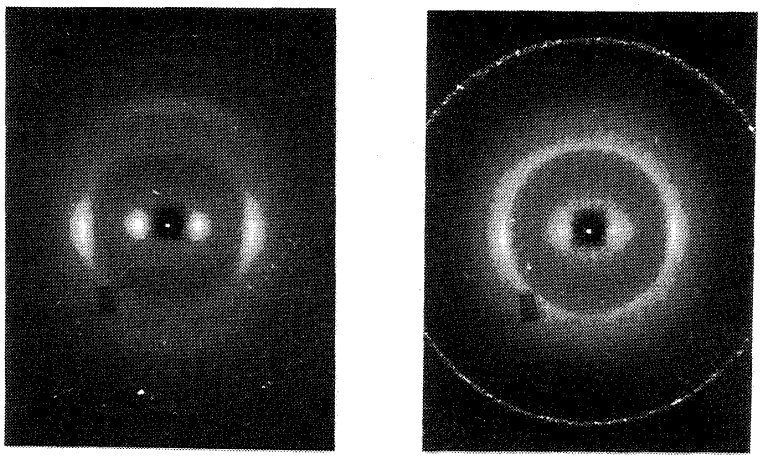

Fig 1. X-Ray fiber patterns of ethylene glycol chitin (PEGT) (left) and ethylene glycol chitosan (PEGS) (right). Fiber axes are vertical and were tilted on to the meridian. In addition to the diffraction arcs, many small white stains are observed. They appeared on the X-ray films during the long irradiation at the high (100%) relative humidity which were required to obtain these fiber patterns since both PEGT and PEGS samples were of low crystallinity and hydrated crystals. The dotted circle appeared on the right photo is the diffraction of NaF powder, which is a standard for measuring the distance between the sample and X-ray film.

The reflection on the 2nd layer line became stronger and that on the 4th layer appeared by tilting the PEGT sample, being regarded as (002) and (004) reflections, respectively. This leads the fiber axis length of the PEGT molecule in the crystal to be 1.03nm which is similar with those of common chitin, such as α-chitin (1.032nm)[9], suggesting that the backbone chitin chain of the PEGT takes an extended two-fold helical conformation.

Figure 1 right shows the fiber pattern of the ethylene glycol chitosan (PEGS). Two strong equatorial reflections have *d*-spacings of 1.51 and 0.454nm, respectively. On the 2nd layer line, two reflections were observed: one (d=0.453nm) having medium intensity and the other (0.516nm) of very weak. The latter spot appeared by titling the PEGS sample and was regarded to be a meridional (002) reflection. Thus, the fiber axis length of the PEGS chain is 1.03nm which is similar with those of common chitosan, such as annealed polymorph (1.039nm)[10], suggesting that the backbone chitosan chain of the PEGS takes an extended two-fold helix.

From the present fiber patterns of the ethylene glycol derivatives, conformations of respective backbone chain for PEGT (chitin) and PEGS (chitosan) were suggested. But those of side chains (substituents) of these derivatives could not be elucidated since each fiber pattern was of low quality to determine all the unit cell parameters except the fiber axis length. Conformational analysis by using computer modeling technique was thus performed in order to propose possible conformations of both PEGT and PEGS molecules. Chemical structure of the monomer residue of PEGT is *N*-acetyl glucosamine 2-hydroxy ethyl ether where the hydroxy ethyl ether was bonded to 0-6 of the *N*-acetyl glucosamine, and that of PEGS is glucosamine di[2-hydroxy ethyl ether] where two substituents were attached to 0-3 and 0-6 of the glucosamine, respectively. The geometries of the respective monomer residues of PEGT and PEGS were generated by the structure optimizations using MM2(77) force field[7]. Rotational positions of the 0-6 atoms were at *gt*[11] for the backbone chain of PEGT model, and at *gg* for that of PEGS model. All the carbon and oxygen atoms belonging to the side groups, 2-hydroxy ethyl ether, preferred to have near *trans* conformation. Conformational analysis of each polysaccharide derivative, PEGT or PEGS, was carried out by using the computer program PS79[8] that had been developed for the structure refinement of polymer under the constraints of helix symmetry, i.e., the two-fold helices with the fiber repeat of 1.03nm obtained from the present X-ray fiber patterns. Possible conformations of the PEGT and PEGS molecular chains with the two-fold backbone symmetry are in Fig. 2. The most distinct difference in conformational feature between PEGT and PEGS is 0-6 rotations, the former has near *gt* and the latter, *gg*. It was found that, in the PEGS models, the 0-6 side group with *gt* position caused serious steric clashes with the 0-3 side group of the next residue, and that similar steric hindrance was observed in the 0-6 *tg* side group of the PEGT with N-acetyl group, as well. On the other hand, the 0-6 *gg* model and *tg* model for PEGT and PEGS, respectively, gave a little higher values of the steric energy than the models shown in Fig. 2. The present results may be useful to analyze the complex formation of the streptococcal α-glucan with PEGT or PEGS although further study is required.

Acknowledgments

We thank Mr. Ken-ichirou Shibasaki of Lion Corporation, Tokyo, Japan for supplying the ethylene glycol derivatives of chitin and chitosan.

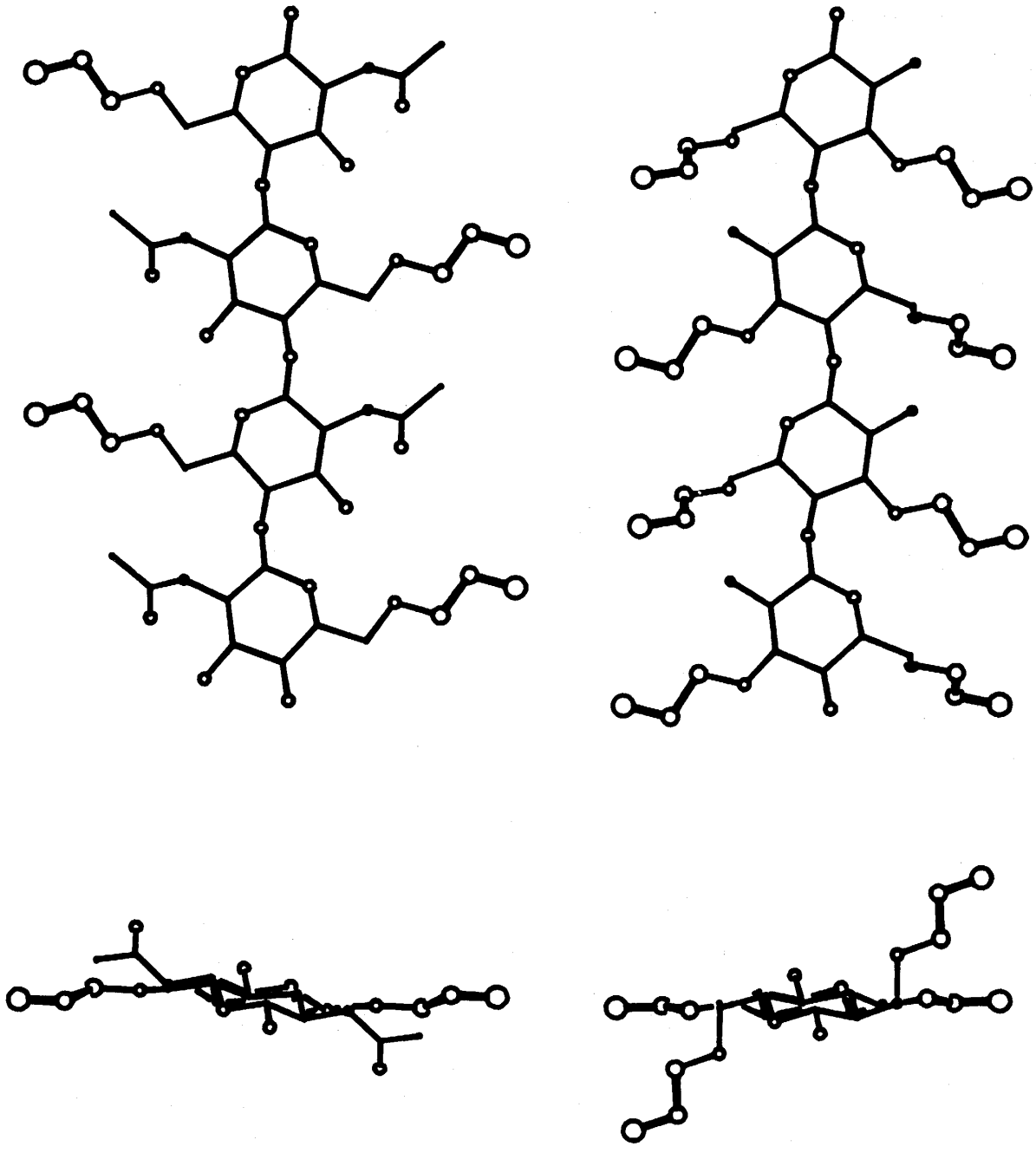

Fig 2. Possible conformations of the PEGT (left) and PEGS (right) molecular chains with the two-fold backbone symmetry, each projected perpendicular (upper) and parallel (bottom) to chain axes. The largest and middle open circles indicate oxygen and carbon atoms of the side groups, respecticely, and the smallest open and solid circles are oxygen and nitrogen atoms of the backbone chains, respectively. Hydrogen atoms are omitted for clarity.

References

1) KOMIYAMA, N., SANO, H., ITOI, H., MATSUKUBO, T., and TAKAETSU, Y. (1985). *Abstracts of Papers, 2nd Chitin Chitosan Symposium, Tottori*, March, P.9-12. (in Japanese)
2) SANO, H., MATSUKUBO, T., SHIBASAKI, K., ITOI, H., and TAKAETSU, Y. (1991). Inhibition of adsorption of oral streptococci to saliva treated hydroxyapatite by chitin derivatives. *Bull. Tokyo Dent. Coll.*, **32**, 9-17.
3) EBISU, S., MISAKI, A., KATO, K., and KOTANI, S. (1974). The structure of water-insoluble glucans of cariogenic *Streptococcus mutans*, formed in the absence and presence of dextranase. *Carbohydr. Res.*, **38**, 374-381.
4) OGAWA, K., YUI, T., OKAMURA, K., and MISAKI, A. (1994). Crystalline features of streptococcal (1→3)-α-D-glucans of human saliva. *Biosci. Biotech. Biochem. Tokyo*, **58**, 1326-1327.
5) SHIBASAKI, K., personal communication.
6) OGAWA, K. (1991). Structure and function of chitosan I. Effect of heating an aqueous suspension of chitosan on the crystallinity and polymorphs. *Agric. Biol. Chem.*, **55**, 2375-2378.
7) ALLINGER, N.L. (1977). Conformational analysis. 130. MM2. A hydrocarbon force field utilizing V_1 and V_2 torsinal terms. *J. Am. Chem. Soc.*, **99**, 8127-8134.
8) ZUGENMAIER, P. and SARKO, A. (1980). *Fiber Diffraction Methods., ACS Symposium Ser.*, **141**, American Chemical Society, Washington D.C., P.225-237.
9) MINKE, R. BLACKWELL, J. (1978). The structure of α-chitin. *J. Mol. Biol.*, **120**, 167-181.
10) OGAWA, K., HIRANO, S., MIYANISHI, T., YUI, T., and WATANABE, T. (1984). A new polymorph of chitosan. *Macromolecules*, **17**, 973-975.
11) MARCHESSAULT, R.H. and PEREZ, S. (1979). Conformatioons of the hydroxymethyl group in crystalline aldohexopyranoses. *Biopolymers*, **18**, 2369-2374.