Effect of Urea on the Conformation and Chain Flexibility of Chitosan Molecules with Various Degree of Deacetylation

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ABSTRACT

Chitosans with various degrees of deacetylation (DD) were prepared from red shrimp (*Solenocera prominentis*). DD of chitosan was determined by infrared spectrum. The effect of urea on the conformation and chain flexibility of chitosan molecules with various DD was studied. The results show: 1) The intrinsic viscosities of chitosan solutions containing 4 M urea were higher than those without urea addition. It indicated the chitosan molecules in solutions containing urea have higher hydrodynamic volume than that without urea. It may due to urea increasing the intramolecular hydrogen bond formation. 2) The persistence lengths of chitosan molecules were longer in solutions containing 4 M urea than that without urea addition. The results indicated the molecules were more extend in solutions containing urea than that without urea. 3) Addition of 4 M urea to chitosan solutions rendered the Smidsrod chain stiffness parameters lower than that without urea addition. It indicated that the possible attribute of degree of polymerization on the persistence length may be rule out.

INTRODUCTION

Lin (1974) revealed the effect of urea on the rheological properties of chitosan solution. Rheological properties of solutions are affected by hydrodynamic volume of solute which are in term affected by conformation, chain flexibility, molecular weight of the solute. Although, studies on the effect of solvent properties (pH, ionic strength, counter ion), of shear rate, and temperature on the rheological properties of chitosan are numerous. However, studies on the effect of urea on the rheological properties of chitosan are relatively rare. Report on the effect of urea on conformation and chain flexibility of chitosan molecule are even rare.

Persistence length (Rinaudo and Domard, 1989; Rinaudo et al., 1993; Kienzle-Sterzer et al., 1984), Smidsrod's stiffness parameter (Rinaudo and Domard, 1989; Rinaudo et al., 1993; Terbojevich et al., 1986), characteristic ratio (Kienzle-Sterzer et al., 1984; Anthonsen et al., 1993), Kuhn statistical segment length (Kienzle-sterzer et al., 1986; Berkovich et al., 1980;

Lyubina et al., 1983; Pogodina et al., 1986) have been used to characterize the stiffness of molecule. The Mark-Houwink exponent parameter, " a ", has been used as a molecular conformation parameter. "a" value higher than 0.8, the molecule is a rod shape, "a" value in 0.5-0.8, the molecule is in random coil, " a " value below 0.5, the molecule is very compact globular. "a" values of chitosans in different solutions range between 0.17- 0.8. It indicates the conformation of chitosans range from random coil, quasi globular and rod shape and depend on the solution conditions (Kienzle-Sterzer et al., 1986; Berkovich et al., 1980; Errington et al., 1993). Kienzle-Sterzer et al. (1986) reported the conformation of chitosan is an extended coil with Kuhn statistical segment of 12.16 nm in the presence of urea. Whereas, it is in a compact structure with a Kuhn statistical segment of 7.28 nm without urea. Mark-Houwink exponent "a" value is about 0.147 in 1% acetic/ 2.8 % NaCL without urea, change to about 0.71 when urea is present. (Kienzle-Sterzer et al., 1986). Sklyar et al. (1981) reported the addition of 4 M to 8 M urea prevents the reduction of

solution viscosity on standing. The reduction was more pronounced in solutions that are lower concentration of acid in the solvent or higher concentration of the solute in solution. The reduction in solution viscosity may due to intramolecular hydrogen bonds. Frangou et al. (1982) reported the effect of urea on the stabilization of the ordered conformation of xanthan perhaps by reduction in water activity of solution.

The objective of the studies are to explore the effect of urea on the conformation and chain stiffness of chitosans with various degree of deacetylation.

MATERIALS AND METHODS

1) Preparation of chitin

The chitin was prepared by the modified method of Stanley et al. (1976) and Lai (1979). Ground shell and head of red shrimp (*Solenocera prominentis*) were soaked in 0.5N NaOH solution at room temperature overnight to remove the surface meat. Washed with water, dried, comminuted the dried product to pass through the 60 mesh sieve. Soaked the powder in 2N HCl to remove the minerals, at the same time CO_2 was removed by suction till no further CO_2 was detected. Washed then soaked in 2N NaOH at 80°C for 1 h to hydrolyze the protein. The de-proteined powder was washed then soaked in 1% K_2MnO_4 at room temperature for 1 h to remove astaxanthin. Washed then soaked in 1% oxalic acid at room temperature to remove excess of K_2MnO_4 then washed, dried to get chitin.

2) preparation of chitosan of various DD

Soaked the chitin in 11%, 40% and 50% NaOH (v/w) at 45°C, 95°C, 110°C, and 140°C for 0.5 to 10 h. The alkali treated solutions were washed with water and dried to get chitosans of various DD (Chen et al., 1994a, 1994b).

3) The determination of the DD

Infrared spectrometry method reported by Domszy and Roberts (1985); Moore and Roberts (1980); Sannan et al. (1976) was used to determine the degree of N-acetylation of the chitosan. Powder of chitosan was mixed with KBr (1:100) and pressed into pellet. The absorbencies of amide 1 (1655 cm^{-1}) and of hydroxyl band (3450 cm^{-1}) were measured by Hitachi 260-30 infrared spectrophotometer. The band of hydroxyl group at 3450 cm^{-1} was used as an internal standard to correct for film thickness or for difference in chitosan concentration in making KBr disc. The percentage of the amine group's acetylation in a sample was given by (A1655/A3450) x 115. Here A1655, A3450 are absorbencies at 1650 cm^{-1} and 3450 cm^{-1} respectively.

4) Intrinsic viscosity determination

Cannon-Fenske capillary viscometer was used to measure the pass time of solution flow through the capillary. The solutions were prepared by different pH (pH 3 to pH 5), different ionic strength (0.M to 0.6 M), and different DD chitosans (80, 90, and 100 %). The pHs of chitosan solutions were controlled by dissolving the chitosans of various DD in pH 3 acetic acid then adjusted the solutions' pH to pH 4 or 5 with NaOH. The ionic strengths were adjusted by adding NaCl to the designated molarity. The capillary viscometer was filled with 5 ml of sample and equilibrated in water bath (Tampson, TMV40, Holland) at preseted temperature ±0.1 °C for 15 min. The sample was passed through the capillary once before running time was measured. The running time was used to calculate the relative viscosity, then reduced viscosity. Plot the reduced viscosity against the concentration. The intercept is the intrinsic viscosity (Chen et al., 1994a; 1994b).

5) The persistence length of chitosan molecule

The persistence length is the projection of the end to end distance on the direction of the first bond. The persistence length of chitosan in dilute solution was estimated according to the method of Modified Yamakawa and Fujii (1974) by using the following equation.

$$[\eta] = \phi \, (2 \, P_L / M_L)^{3/2} \, (M_w)^{1/2}$$

Here, $[\eta]$ is the intrinsic viscosity; ϕ is the Flory constant and equal to 2.3×10^{23} when $[\eta]$ is in cm^3/g; P_L is the overall persistence length; M_L is the mass per unit length and relates to molecular weight and contour length of solute as $M_L = M_w / L$. Mw and L are the molecular weight and the contour length of the solute respectively. L equals to (M_w/m_o)l. Here, mo is molecular weight of monomer ; l is the length of monomer, it is 5.33 Å for glucosamine.

6) Smidsrod's stiffness parameter

The slope S of the linear dependent between intrinsic viscosity, $[\eta]u$ and the molar salt concentration, $(C_s)^{-1/2}$ is related to the stiffness of the molecule (Smidsrod and Huag, 1971). They have obtained the following empirical relationship

$$S = B \, ([\eta]_{0.1M})^v$$

Here, B is an empirical stiffness parameter, v is an exponent between 1.2 and 1.4. Then, the logarithm slope S are plotted against the logarithm intrinsic viscosity in

0.1 M salt concentration, $[h]_{0.1}M$. The intercept is the B value

RESULTS

1) Effect of ionic strength on the intrinsic viscosity of different pH chitosan solutions

In general, intrinsic viscosities decreased with increasing ionic strength between 0 M to 0.05 M NaCl then intrinsic viscosities increased as ionic strength continuous increasing to 0,6 M for three chitosans and pHs studied. The ionic strength dependency of intrinsic viscosity was due to the polyelectrolyte effect. The polyelectrolyte effect of pH 3 solutions were more pronounced than that of pH 4 or pH 5 solutions. In pH 3, 80 % DD chitosan solutions, the intrinsic viscosity decreased sharply from 15.4 dl/g to 5.6 dl/g as NaCl concentration increase to 0.05 M from 0 M, then increased to 8.7 dl/g as NaCl further increase to 0.6 M. Different rates of reduction in intrinsic viscosities as NaCl concentration increase to 0.05 M from 0 M indicated the attributes of different DD (between 80 -100 %). The effects of DD of chitosans also can be observed on different intrinsic viscosities measured from three chitosan solutions at the same ionic strength and pH. The 80 % DD ones have the largest intrinsic viscosities, 90 % DD ones were the secondth and 100 % ones were the smallest. The smallest value of 2.18 dl/g was observed from 100 % DD chitosan in pH 5, 0.05 M NaCl solution. The largest value of 15.4 dl/g was observed from 80 % DD chitosan in pH 3, 0 M NaCl solution. The intrinsic viscosity reduction as NaCl increase to 0.05 M from 0 M were 15.4 to 5.6 dl/g; 13.4 to 4.5 dl/g and 9.0 to 3.5 dl/g for 80, 90, 100 % DD chitosans respectively.

2) The effect of pH, ionic strength and 4 M urea on the intrinsic viscosity of chitosan solutions

Fig. 2 shown The trends of the changes (due to 1. polyelectrolyte effect, 2. pH dependent, 3. DD of chitosan molecules) were overall similar to that shows in Fig. 1. but difference in absolute values. However, as NaCl concentration increasing to 0.6 M, intrinsic viscosities may be equal to or larger than that of 0 M NaCl ones. The smallest intrinsic viscosity of 11.9 dl/g was observed at solution of 100 % DD chitosan in pH 5, 0.05 M NaCl solution. Whereas, the largest intrinsic viscosity of 21.1 dl/g was observed at 80 % DD pH 3, 0 M NaCl solution. Reduction in intrinsic viscosities as NaCl increasing to 0.05 M from 0 M were 21.1 to 17.2 dl/g, 20.0 to 17.8 dl/g, and 17.1 to 17.08 dl/g for 80, 90, and 100 % DD chitosans respectively.
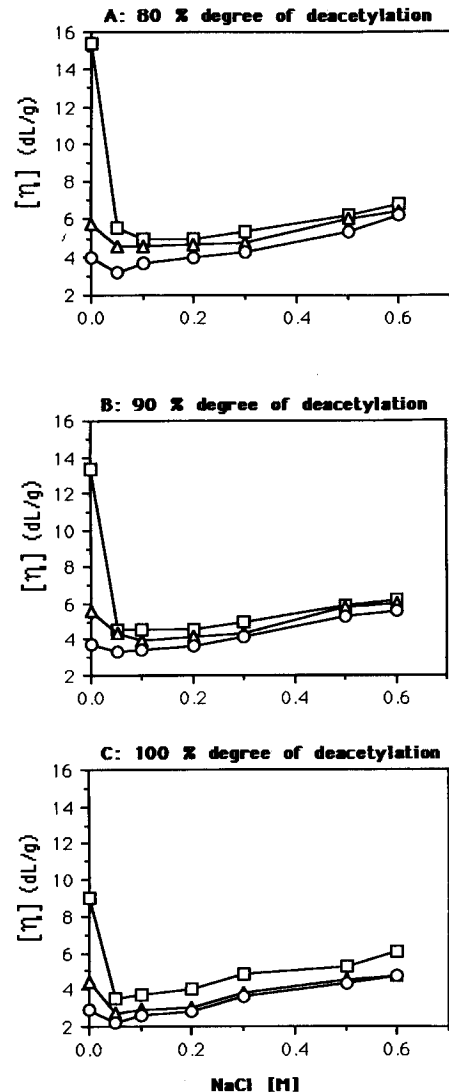
3) Effect of pH, ionic strength on the persistence length

Fig. 1. Effect of degree of deacetylation, pH, and ionic strength on intrinsic viscosity (dl/g) of chitosan at 25.0±0.1°C.
—□— pH=3   —△— pH=4   —○— pH=5

of chitosan molecules with different DD

Persistence lengths varied also with pH, ionic strength, DD of chitosans (Table 1) that were similar to the results shown in Fig. 1 . Persistence lengths decreased rapidly as ionic strength increasing from 0 M to 0.05 M. The persistence lengths further increased steadily with increasing in ionic strength from 0.05 M to 0.6 M. The smallest persistence lengths were observed at solutions of 0.05-0.1 M NaCl in three pHs and chitosans studied. Persistence lengths changed also with DD of chitosan molecules between pH 3 to pH 5. The 80 % DD chitosans have longer persistence lengths than those of 90 % DD ones in term were longer than 100 % DD ones at the same pH and ionic strength. The persistence length also pH dependent and the longest ones were found in pH 3 solutions. The decrease were proportional with increasing

in solution pH.

4) Effect of pH, ionic strength, 4 M urea on persistence length of chitosan molecules with different DD

Data in Table 1 shown the trends of the changes were similar to that shown in Fig. 2. Persistence lengths decreased with increasing ionic strength from 0 M to 0. 05 M then increased as ionic strength increasing further to 0.6 M NaCl. The changes were more pronounced for chitosans of 80 % DD ones than that of 90 % ones and also than that of 100 % DD ones. The effect of media pH were more pronounced in pH 3 solutions than in pH 5 solutions. Persistence lengths of chitosan molecules in solutions containing 4 M urea were longer than that without 4 M urea. The persistence lengths at 0.6 M ones were longer than that at 0 M NaCl ones.

5) The effect of pH, urea on Smidsrod's stiffness parameter

Table 2 shown B values increased with increasing solution pH and/or with DD of chitosan molecules. B value of 80 % DD chitosan in pH 3 solution was the smallest, 0.103 whereas, B value of 100 % DD chitosan in pH 5 solution was the largest, 0.291 in solutions without urea. The trends were similar to the solutions containing 4 M urea. However, B value were smaller compared to those without urea addition. B value was 0.021 for 80 % DD chitosan in pH 3 solution and was 0.040 for 100 % DD chitosan in pH 5 solution. The effect of pH on B value become less significant in solutions containing 4 M urea.

DISCUSSION

1) Effect of urea on intrinsic viscosity of chitosan

Fig. 1. and 2. shows intrinsic viscosities of chitosans (80-100 % DD) ranged from 15.4 dl/g to 2.2 dl/g in different pH (pH 3-5), different ionic strength (0 M to 0.6 M NaCl) solutions. The variation of intrinsic viscosity may due to different steric effect (caused by different DD), number of protonated amino group (by different solution pH), and polyelectrolyte effect (by different solution ionic strength)Intrinsic viswsity ranged between 15.4 to 2.2 de/g and 21.1 to 11.8 dl/f for solutions with on without urea respectvely. Intivision viswsity ranged between 21-1.4 dl/g urea respected in the light ature Anthonsen *et al.*, 1993 gamzazade *et al.*, 1981 for Chitosan solutions without urea addition. The effect of DD and Ionic strength on untunsic viscosity were indicated by the reduction of intrinsic viscosity from 15.4 to 5.57 dl/g, 13.4 to 4.52 dl/g, and 9.0 to 3.5 dl/g for 80, 90, and 100 % DD chitosan respectively as ionic strength increasing to 0.05M from 0 M NaCl.
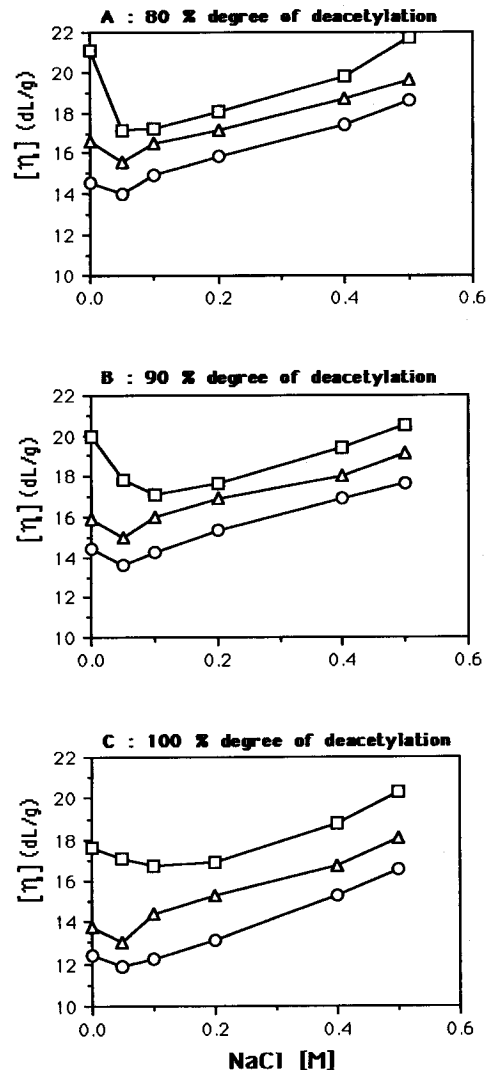

Fig. 2. Effect of 4 M urea, degree of deacetylation, pH, and ionic strength on intrinsic viscosity (dl/g) of chitosan at 25.0±0.1 °C.
—□— pH=3   —△— pH=4   —○— pH=5

Similar results were reported by Wang et al. (1991), Rinaudo et al. (1993).

Urea addition rendered the chitosans solutions increasing in intrinsic viscosity. Gamzazade et al. (1981) reported similar results and revealed the intrinsic viscosities were around 21.0 and 25 dl/g in 2 % acetic acid in the absence and presence of urea respectively. The increasing in intrinsic viscosity due to urea addition were 5.7 dl/g at 0 M NaCl (from 15.4 to 21.1 dl/g) versus 9.7 dl/g at 0.05 M NaCl (from 2.2 to 11.9 dl/g) (Figure 1 and 2). The increases at 0 M NaCl was not as significant as the increase at 0.05 M NaCl. It indicated urea addition in-fluence less on the third electroviscous effect than on the first electroviscous effect. The increase in intrinsic viscosity may be also due to conformational change caused by urea. Since Kienzle-Sterzer *et al.* (1986) reported Mark-Houwink exponent parameter "a" of 0.14 and 0.71 in 1.0 % acetic/2 0 % NaCl chitosan solution

Table 1. Effect of degree of deacetylation, pH, 4M urea, and ionic strength on persistence length (A) of chitosans at 25.0 + 0.1 C.

Degree of deacetylation	[NaCl]	pH			(4 M urea)		
(%)	(M)	3	4	5	3	4	5
80	0.00	177.95	93.75	72.70	219.42	186.86	179.42
	0.05	90.37	79.53	61.58	191.40	179.77	167.28
	0.10	83.91	78.65	68.70	192.09	186.33	174.59
	0.20	83.65	79.93	72.18	198.09	190.88	181.66
	0.30	88.00	81.37	75.50	210.19	202.45	193.13
	0.50	97.10	95.60	87.95	224.10	208.87	201.67
	0.60	103.77	99.47	97.69			
90	0.00	168.10	93.94	72.25	219.78	188.88	176.75
	0.05	81.66	79.98	66.23	203.59	181.52	170.28
	0.10	81.63	75.08	67.94	198.41	189.10	175.77
	0.20	82.25	76.71	70.41	202.64	196.30	184.54
	0.30	86.50	79.54	76.71	215.52	205.03	196.90
	0.50	97.60	95.88	90.04	223.66	213.56	202.60
	0.60	101.05	97.97	93.75			
100	0.00	137.41	85.37	65.07	215.38	182.32	170.55
	0.05	73.77	62.09	53.46	210.86	175.84	165.32
	0.10	75.81	64.95	60.93	207.56	187.89	169.10
	0.20	81.04	66.07	64.04	209.36	196.08	177.22
	0.30	90.67	77.77	74.66	224.54	207.60	195.85
	0.50	95.47	87.52	84.66	236.50	218.73	206.29
	0.60	105.96	89.98	89.65			

[NaCl] : Concentration of NaCl.

with or without urea addition respectively.

2) Effect of urea on persistence length of chitosan

Table 1 shows persistence length of chitosans (80-100 % DD) ranged from 178 Å to 53.6 Å in different pH (pH 3-5), different ionic strength (0 M to 0.6 M NaCl) solutions . The values shown in Table 1 are within the ranges reported in the literatures. Rinaudo and Domard (1989) reported in the absence of electrostatic contribution, persistence length equal to 300 Å . Rinaudo et al. (1993) reported intrinsic persistence length of 50 Å and to be independent of DD (90-79 %). Kienzle-Sterzer et al.(1984) reported total persistence length ranged from 52.25 to 79.55 Å between ionic strength of 0.05 M and 0.5 M NaCl, degree of ionization of 0.6 to 0.9. At the same conditions, electrostatic persistence length were ranged from 0.50 to 23.80 Å. Addition of urea did not influence the trends of the changes but affected the absolute values. However, upon the urea additions, the persistence length increasing from 178.0 to 219.4 Å and from 53.6 to 165.3 Å at 80 % DD chitosan in pH 3, 0.M and 100 % DD chitosan in pH 5, 0.05 M respectively. The effect of urea addition also nullifyed the effects of ionic strength and pH because the persistence length of chitosan in 0.6 M NaCl were equal to or higher than those at 0 M NaCl and less the difference cause by the effect of DD of chitosan and solution pH.

3) Effect of urea on Smidsrod's stiffness parameter

Although reports on the Smidsrod's stiffness parameter of chitosans with various DD and solution conditions are relatively rare. Terbojevich et al. (1986) reported B value ranging from 0.043 to 0.091 for chitosans (DD of chitosan between 47.8 to 88.0 %). Rinnaudo and Domard (1986) reported B value of 5.7×10^{-3} for chitosans (DD between 82.5 to 99 %). Later, Rinaudo et al. (1993) reported B value was estimated to be 0.065 ± 0.02 and was independent to the DD of chitosans and solvent conditions. Effect of urea on Smidsrod's stiffness parameter is even rarely explored. Results in Table 2 shown the effect of solution pH and DD of chitosan on B value in solution without urea were more pronounced than those solution containing 4 M urea. It indicated the effects of urea on inter- and/ or intra- hydrogen bond formation which rendered the molecule became rigid (molecule in quasi-globular structure) seems having significant effect on the Smidsrod's stiffness parameter. Although there is no data available concerning the effect of urea on the Smidsrod's stiffness parameter. However, effect of urea on Kuhn segment of chitosans has been reported (Kienzle-Sterzer et al., 1986) . Kuhn segment increased from 7.28 nm to 12.16 nm upon urea addition. Frangou et al. (1982) reported stabilizes the structure of xanthan by urea evidence by in aensing is order-disorder transition temperature pehaps by reduction in water activity.

Table 2. The chain stiffness parameter (B) of chitosan of 80, 90, and 100 % deacetylation in different solution pH with or without 4 M urea

Degree of deacetylation	pH			pH (4 M urea)		
(%)	3	4	5	3	4	5
80	0.103	0.127	0.183	0.021	0.023	0.028
90	0.118	0.166	0.211	0.023	0.024	0.029
100	0.154	0.206	0.291	0.025	0.032	0.040

[NaCl] : Concentration of NaCl.

Results of Kienzle-Sterzer *et al.* (1986) and Frangou *et al.* (1982) are accord with our finding.

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