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Polymer 45 (2004) 837–841
polymer
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Synthesis of a novel polymeric surfactant by reductive *N*-alkylation of chitosan with 3-*O*-dodecyl-D-glucose

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Received 30 September 2003; received in revised form 21 November 2003; accepted 25 November 2003

Abstract

A novel chitosan-based polymeric surfactant, DG-chitosan, was prepared via reductive *N*-alkylation of chitosan with 3-*O*-dodecyl-D-glucose in acetate buffer (pH 4.3, 0.1 M)-methanol in the presence of sodium cyanoborohydride ($NaBH_3CN$). DG-chitosan was swelling in water, partly dissolvable in pyridine and DMF, and completely soluble in 0.1% aqueous acetic acid. ^1H and ^{13}C NMR spectroscopic analyses in 2% acetic acid-d_4-methanol-d_4 together with elemental analysis showed the degree of substitution was 27%. Formation of polymeric micelles was observed by use of pyrene as a fluorescent probe, and the critical aggregation concentration (CAC) of DG-chitosan was marked equal to 28.1 mg/L.

Keywords: Chitosan; *N*-alkylation; Polymeric surfactant

1. Introduction

Self-assembling polymers have been received considerable attention of synthetic and physical chemists who work on nano-scale structures, latex stabilizers, emulsifiers, biomedical and pharmaceutical applications [1–3]. Recently, polymer micelle is regarded as one of the most promising candidates for carrier for delivery bioactive materials such as water-insoluble drugs [4–9], hormones [10,11] and plasmid DNA [12].

Chitosan, 2-amino-2-deoxy-β-(1,4)-glucan, is a natural cationic polymer prepared by *N*-deacetylation of chitin, the second most abundant biomass. Because chitosan is biodegradable and less toxic, it has been used as a starting material for various biomedical substances [13–15]. Chitosan based polymeric surfactants have been also synthesized by several groups [16–25], and they showed such advantages as structural stability, non-irritation to biomembrane of living organisms [26], and biodegradability [16,23]. However, their low degree of hydrophobic substitution may result in weakening stability of the micelle. The idea that increasing the level of hydrophobic attachment led the high stability of the polymer micelles was proposed by Li and Kwon [27] and Yokoyama et al. [28]. In addition, the hydrophobic substitution to polymeric micelles with high degree also expected a lower critical aggregation concentration (CAC). Therefore, we undertook to investigate an efficient methodology for the preparation of a chitosan based amphiphilic compound having more densely packed hydrophobic substituents.

In this paper, we would like to report the synthesis of a novel chitosan-based polymeric surfactant (DG-chitosan) that has a tetrahydroxy alkyl linker and a dodecyl hydrophobic residue. The hemiacetal group in a known amphiphilic compound, 3-*O*-dodecyl-D-glucose (DG) [29], would easily react with the amino group of chitosan through formation of Schiff's base and subsequent reduction with high degree of substitution. Introduction of both hydrophobic and hydrophilic functions into chitosan was expected to explore a novel feature for biopolymer chemistry.

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0032-3861/$ - see front matter © 2003 Published by Elsevier Ltd.
doi:10.1016/j.polymer.2003.11.034

2. Experimental

2.1. Materials

Partially acetylated chitosan (Aldrich) with medium molecular weight and degree of deacetylation (DDA) of 75–85% was treated with 50% (wt/v) aqueous sodium hydroxide for 2 h at 120 °C in an autoclave three times [30], giving 98% deacetylated chitosan. Its average molecular weight estimated by viscometry using the Mark–Houwink equation [31,32] was equal to 600 kD. 1-Bromododecane (Tokyo Kasei Kogyo), sodium cyanoborohydride (Aldrich), and other reagents (Wako Pure Chemical Industries) were used without further purification.

2.2. Instruments

^{1}H and ^{13}C NMR spectra were recorded with a Bruker ASX-300 spectrometer for solutions in 2% acetic-acid-d_4-methanol-d_4 and 2% acetic-acid-d_4-deuterium oxide at 300.13 and 75.48 MHz, respectively. Fluorescence spectra were recorded on a Hitachi F-4500 spectrometer using a xenon lamp. The slit openings for excitation and emission were set at 10 and 2.5 nm, respectively. Ultrafiltration was performed at 0.3 MPa using a membrane (Advantec UK-10) with molecular weight cutoff of 10,000.

2.3. Preparation of 3-*O*-dodecyl-D-glucose-linked chitosan (*3*: DG-chitosan)

2.3.1. Preparation of 3-*O*-dodecyl-D-glucose (1: DG)

To a solution of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose [33] (1.3 g, 5 mmol) in 4:1, v/v THF/DMF (20 mL / 5 mL) were successively added 60% sodium hydride oil dispersion (0.48 g, 12 mmol) and 1-bromododecane (1.2 mL, 5 mmol) at room temperature. The mixture was stirred overnight, quenched by successive addition of MeOH then aqueous NH_4Cl, and extracted by ethyl acetate. The extract was washed with aqueous $NaHCO_3$ and brine, dried with $MgSO_4$ and evaporated to dry oil-like solid. Without further purification, the product was subjected to hydrolysis with 5:1, v/v acetic acid/H_2O at room temperature for overnight. After evaporation, the residue was purified by column chromatography on silica gel using $CHCl_3$–MeOH, 30:1–10:1 as the eluent to give DG **1** (0.83 g, 48% yield) as white crystal [29].

2.3.2. Reductive N-alkylation of chitosan with *1*

Chitosan (92.5 mg, 0.57 mmol of D-glucosamine units) was dissolved in 0.1 M acetate buffer (pH 4.3, 15 ml) and then a solution of **1** (200 mg, 0.57 mmol) in MeOH (30 ml) was added. The mixture was stirred at room temperature for 1 h; sodium cyanoborohydride (108.2 mg, 1.71 mmol) was added to the resulting solution. The solution was stirred at room temperature overnight, subjected to ultrafiltration, washed with water and MeOH, and lyophilized to give DG-chitosan **3** (126.3 mg) with the degree of substitution (DS) of 27%; ^{1}H NMR (CD_3CO_2D–CD_3OD): $\delta_H = 4.77$ (br. s, 1H, H1), 3.58–4.05 (m, 5H, H3, H4, H5, H6a, H6b), 2.93 (br. s, 1H, H2), 1.19–1.53 (m, 5.4H, $(CH_2)_{10}$), 0.80 (t, 0.81H, J 6.24 Hz, CH_3); ^{13}C NMR (CD_3CO_2D–CD_3OD): $\delta_C = 98.54, 78.20, 77.50, 75.55, 73.07, 71.39, 70.86, 69.82, 63.89, 32.05, 29.77, 29.45, 26.17, 22.73, 19.93, 19.67, 19.41, 19.15, 18.89$ and 13.51; Anal. calcd (%) for $(C_6H_{11}NO_4)_{0.73}(C_{24}H_{47}NO_9)_{0.27}CH_3CO_2H{\cdot}H_2O$: C, 46.95; H, 8.19; N, 4.26. Found (%): C, 47.18; H, 7.88; N, 4.27.

2.3.3. Solubility test of DG-chitosan

DG-chitosan **3** (1 mg) was placed in a test tube with each of solvent (1 ml). After mixing with a vortex mixer and then with an ultrasonicator, the mixture was stored at room temperature for 7 days, and visually observed.

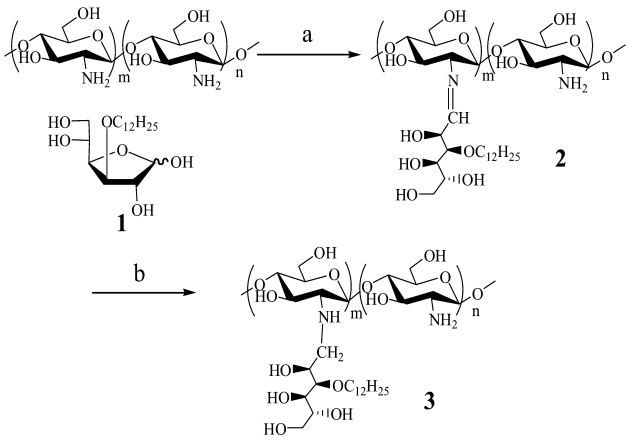
2.4. Measurement of CAC

A mixture of DG-chitosan **3** (20 mg) in 0.1% (v/v) aqueous acetic acid (20 mL) was stirred at room temperature overnight, ultra-sonicated 3 times using probe-type TAITEC ultrasonic homogenizer (VP-60 s) for 10 min, and filtered through a 0.45 μm membrane. The DG-chitosan solutions were diluted and varied in concentrations from 0.001 to 1000 mg/L. For fluoresce measurement, a small aliquot (50 μL) of a methanolic pyrene solution (25 mg/L) was added to each of the solution of **3** (3 mL) in a test tube, mixed and transferred to a quartz fluorescence cell. The fluorescence spectra were recorded by excitation at 334 nm [34].

3. Results and discussion

3.1. Synthesis of DG-chitosan polymeric surfactant (*3*)

For the coupling 3-*O*-dodecyl-D-glucose (**1**) and chitosan, we examined reductive *N*-alkylation, because this reaction proceeds chemoselectively at the amino group of chitosan. Moreover, the reaction of chitosan with aldehydo sugars is proceed smoothly and yielding the products with mostly high degrees of substitution [35,36]. The reaction was employed in the medium under weakly acidic conditions where chitosan was easily soluble and formation of Schiff's base was accelerated with a controllable to desired DS by varying in the ratio of starting materials [37]. As shown in Scheme 1, chitosan and **1** were treated at pH 4.3 acetate buffer to form a Schiff's base intermediate **2**. The resulting mixture was directly reduced with sodium cyanoborohydride to the corresponding secondary amine. The product, DG-chitosan **3**, was purified by ultrafiltration through a membrane with molecular weight cutoff of 10,000 then washed with water and MeOH, and lyophilized. Degree of substitution of DG group determined by elemental analysis was 27%. The product with DS 9.8% was also



Scheme 1. (a) Chitosan (0.57 mmol, 92.5 mg) in pH 4.3 0.1 M acetic acid, then DG (0.57 mmol, 200 mg) in MeOH, total proportion of MeOH: aqueous (2:1, v/v), RT, 1 h; (b) $NaBH_3CN$, RT, overnight yield DG-chitosan (DS 27%) 126.3 mg.

synthesized by varying the mole ratio of the starting materials (Table 1) in the similar condition as the preparation of DS 27%.

3.2. Solubility of 3

Before starting spectroscopic characterization of DG-chitosan **3**, we carried out preliminary examination of its solubility. In contrast to original chitosan, which is insoluble in organic solvents, **3** with higher DS (DS 27%) showed to be partly soluble in pyridine and DMF (*N*,*N*-dimethylformamide), and slightly soluble (swelled) in DMSO (dimethylsulfoxide) and NMP (*N*-methylpyrrolidone). DG-chitosan **3** (DS 9.8%) displayed similar solubility in organic solvents, however, slight increment of the solubility in DMSO was observed.

Since Goto et al. [38] reported that the introduction of saccharide residues in the side chain of polymer increased the water solubility; the glucose residue in **3** was expected to increase its water solubility. The lower DS (9.8%) derivative of **3** was dissolved in the water to give a transparent solution. However, **3** with DS 27% was swelled in water under neutral conditions. These results might suggest that the hydrophilic–hydrophobic balance of entire molecule was essential for theirs water solubility. The increased substitution with hydrophobic dodecyl group in **3** was supposed to suppress the hydrophilicity of the glucose residue. After various examinations to dissolve, we found that the addition of a small amount of acid improved the solubility in aqueous media. A transparent solution of **3** (DS 27%) was obtained in 0.1% (v/v) aqueous acetic acid and the solubility of it was limited to 5×10^3 mg/L. At higher concentration than this, viscous gel-like material was obtained.

Table 1
Synthesis and properties of **3**

Starting materials chitosan:DG[a]	$[\alpha]_D$[b]	EA		DS[c] (%)	CAC[d] (mg/L)
		C/N	H/N		
1:1	−9.11	11.13	1.86	27	28.1
3:1	−8.47	6.66	1.14	9.8	1200

[a] Molar ratio of glucosamine residues and DG.
[b] Measured at 25 °C with $c = 0.5$ in 0.1% aqueous acetic acid.
[c] Determined by elementary analysis (EA).
[d] 0.1% aqueous acetic acid was used for the solvent.

3.3. ^1H and ^{13}C NMR analysis of 3

The structure elucidation of product **3** was performed by ^1H and ^{13}C NMR spectroscopy. In the ^{13}C NMR spectrum of **3** in acetic acid-d_4-methanol-d_4 (Fig. 1), a signal of anomeric carbon D-glucosamine residue was observed at 98.54 and sugar carbons of both glucose and glucosamine residues were observed at $\delta_C = 63.89–78.20$ ppm. The signals due to methyl and methylene carbons of dodecyl group were revealed at upper magnetic field (13.51–32.05 ppm). On the other hand, the ^1H NMR spectrum of **3** (Fig. 2(A)) in the same solvent showed a signal of anomeric proton of D-glucosamine residues in chitosan skeleton at $\delta_H = 4.77$ ppm and other sugar protons were located at $\delta_H = 2.93–4.04$ ppm. A signal assignable to the terminal methyl group of dodecyl group significantly observed at $\delta_H = 0.77$ ppm as a triplet. Furthermore, it was noteworthy that solvent effect was observed in ^1H NMR spectra shown in Fig. 2(B). In contrast to the above mentioned NMR Spectrum of methanolic medium, that in D_2O showed the broader signal of each proton peak, suggesting that aggregation of DG-chitosan occurred in the aqueous media.

3.4. CAC measurement of the self-aggregates of 3

CAC of **3** was determined from the change of the quotient of vibrational band intensities in fluorescence emission spectrum of pyrene in a conventional way [39]. When the fluorescent probe was incorporated in polymeric micelles, the ratio of fluorescence emission intensities (I_1/I_3) corresponding to the first and third vibrational peaks at 373 and 384 nm became smaller than that in aqueous media.

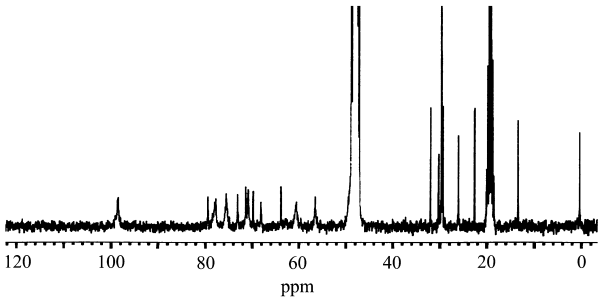


Fig. 1. ^{13}C NMR spectrum of **3** (DS 27%) dissolved in MeOH/2% acetic acid (v/v).

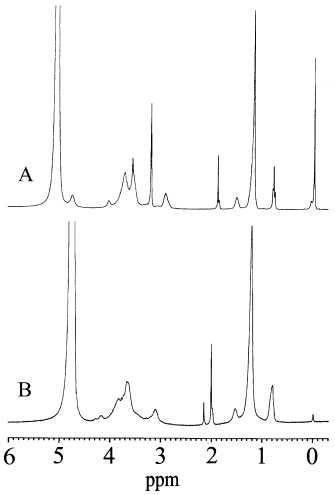


Fig. 2. Comparative ^{1}H NMR spectra of **3** (DS 27%) dissolved in difference solvents. (A) 2% acetic acid-d_4 in CD_3OD (v/v) and (B) 2% acetic-d_4 in D_2O shown broad spectrum.

Thus fluorescent spectra of pyrene in the presence of various concentration of DG-chitosan **3** were recorded and the results are shown in Fig. 3. The semi-logarithm plot of I_1/I_3 versus concentration of **3** was shown in Fig. 4. The CAC value of **3** was determined from the threshold concentration, where the intensity ratio of I_1/I_3 begins to decrease markedly [19,20].

Poor ability to form micelle aggregate was observed with the low substituted DG-chitosan (DS 9.8%). CAC values determined in neutral and acidic (0.1% aqueous acetic acid) aqueous media were 100 and 1.2×10^3 mg/L, respectively. In contrast to this, the CAC value of DS 27% was equal to 28.1 mg/L in 0.1% v/v aqueous acetic acid (Table 1). Higher degree of hydrophobic substitutions in the macromolecule of chitosan may facilitate its self-aggregation. The CAC value of **3** was much lower than that of a typical monomer surfactant SDS (sodium dodecyl sulfate) that had the same hydrophobic residues; 2.1×10^3 mg/L [34] and almost equivalent to those CAC of dodecyl-disaccharides based

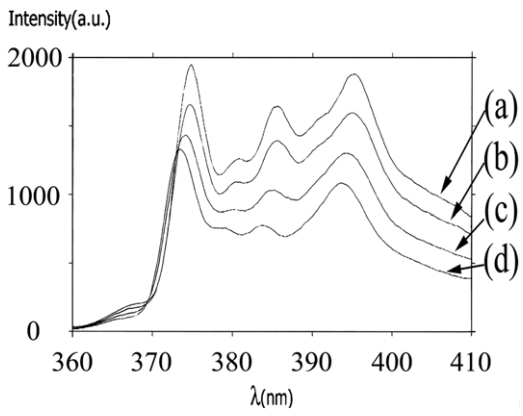


Fig. 3. Excitation spectra of pyrene (2×10^{-6} mol L^{-1}) as function of DG-chitosan **3** (DS 27%) concentrations (a) 1000, (b) 500, (c) 100 and (d) 1 mg/L in 0.1% (v/v) acetic aqueous solution.

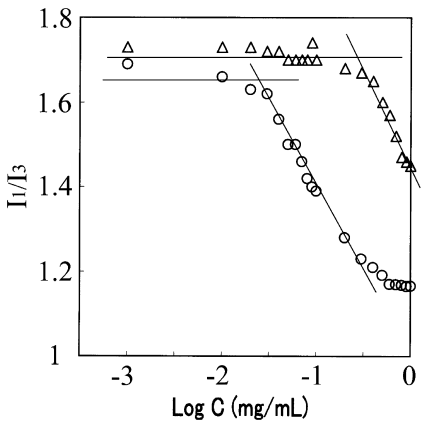


Fig. 4. Plot of the quotient of vibrational band intensities (I_3/I_1) from excitation of pyrene as a function of log C (C = [DG-chitosan] in 0.1% (v/v) acetic aqueous solution; △, DS 9.8% and ○, DS 27%.

surfactants; 86.7 and 31.6 mg/L [40,41]. In addition, the increasing of DS (9.8–27%) resulted in the significant decreasing of CAC (1.2×10^3–28.1 mg/L). The results are consistent with an earlier study by Lee and coworkers that the increasing of DS was slightly reduced the CAC of chitosan derivatives containing deoxycholic acid (DS 2.8–5.1%) [19,20].

4. Conclusion

Motivated such versatile usages of chitosan based surfactants as delivery for DNA, carrier for drugs and extractor solution for dissolve hydrophobic compounds, we have synthesized a new type of polymer surfactant (**3**: DG-chitosan). The key reaction for the preparation was reductive *N*-alkylation of chitosan with 3-*O*-dodecyl-D-dlucose (DG). CAC measurement by use of two different DS derivatives of **3** suggested that stability of the polymer micelle was highly depended on the density of hydrophobic substituents. Further investigations on physicochemical characteristics of the DG-chitosan micelle are on processing in order to show its applicability towards the control and release of bioactive materials.

Acknowledgements

We thank Ms A. Maeda of Center of Instrumental Analysis in Hokkaido University for the elemental analysis.

References

[1] Liu S, Armes SP. Curr Opin Colloid Interface Sci 2001;6:249.
[2] Webber SE. J Phys Chem B 1998;102:2618.
[3] Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Chem Rev 1999;99:3181.

[4] Kabanov AV, Nazarova IR, Astafieva IV B, Batrakova EV, Akakhov VY, Yaroslavov AA, Kabanov A. Macromolecules 1995;28:2303.
[5] Nagasaki Y, Okada T, Scholz C, Iijima M, Kato M, Kataoka K. Macromolecules 1998;31:1473.
[6] Gohy JF, Antoun S, Jerome R. Polymer 2001;42:8637.
[7] Shin IG, Kin SY, Lee YM, Cho CS, Sung YK. J Control Release 1998; 51:1.
[8] Yokoyama M, Satoh A, Sakurai Y, Okano T, Matsumura Y, Kakizoe T, Kataoka K. J Control Release 1998;55:219.
[9] Jones MC, Leroux JC. Eur J Pharm Biopharm 1999;48:101.
[10] Kawashima Y, Yamamoto H, Takeuchi H, Fujioka S, Hino T. J Control Release 1999;62:279.
[11] Brodbeck KJ, Pushpala S, McHugh AJ. Pharm Res 1999;16:1825.
[12] De Smedt SC, Demeester J. Hennink. Pharm Res 2000;17:113.
[13] De Angelis AA, Capitani D, Crescenzi V. Macromolecules 1998;31: 1595.
[14] Denkbas EB, Odabasi M. J Appl Polym Sci 2000;76(11):1637.
[15] Khor E, Lim LY. Biomaterials 2003;24(13):2339.
[16] Yoshioka H, Nonaka K, Fukuda K, Kazama S. Biosci Biotechnol Biochem 1995;59(10):1901.
[17] Nishimura S, Miura Y, Ren L, Sata M, Yamagishi A, Nishi N, Tokura S, Kurita K, Ishii S. Chem Lett 1993;1623.
[18] Miwa A, Ishibe A, Nakano M, Yamahira T, Itai S, Jinno S, Kawahara H. Pharm Res 1998;15(12):1844.
[19] Lee KY, Jo WH, Kwon IC, Kim YH, Jeong SY. Macromolecules 1998;31:378.
[20] Lee KY, Jo WH, Kwon IC, Kim YH, Jeong SY. Langmuir 1998;14: 2329.
[21] Lee KY, Kwon IC, Kim YH, Jeong SY. J Control Release 1998;51: 213.
[22] Kim YH, Gihm SH, Park CR. Bioconjug Chem 2001;12(6):932.
[23] Nonaka K, Seino H, Hamada K, Yoshioka H, Yoshioka H. J Oleo Sci 2001;50(10):773.
[24] Ramos VM, Rodriguez NM, Rodriguez MS, Heras A, Agullo E. Carborhydr Polym 2003;51:425.
[25] Chen XG, Lee CM, Park HJ. J Agric Food Chem 2003;51(10):3135.
[26] Nonaka K, Kazama S, Goto A, Fukuda H, Yoshioka H, Yoshioka H. J Colloid Interface Sci 2002;246:288.
[27] Li Y, Kwon GS. Pharm Res 2000;17(5):607.
[28] Yokoyama M, Sugiyama T, Okano T, Sakurai Y, Naito M, Kataoka K. Pharm Res 1993;10(6):895.
[29] Ikekawa T, Irinoda K, Saze K, Katori T, Matsuda H, Ohkawa M, Kosik M. Chem Pharm Bull 1987;35(7):2894.
[30] Hackman RH. Aust J Biol Sci 1954;7:168.
[31] Liu XD, Tokura S, Nishi N, Sakairi N. Polymer 2003;44:1021.
[32] Roberts GAF, Domszy JG. Int J Bio Macromol 1982;4:374.
[33] Schmidt OT, editor. Methods in carbohydrate chemistry, vol. 2. New York: Academic Press; 1962. p. 318.
[34] Dominguez A, Fernandez A, Gonzalez N, Iglesias E, Montenegro L. J Chem Educ 1997;74(10):1227.
[35] Hall LD, Yalpani M. J Chem Soc, Chem Commun 1980;1153.
[36] Yalpani M, Hall LD. Macromolecules 1984;17:272.
[37] Tojima T, Katsura H, Han SM, Tanida F, Nishi N, Tokura S, Sakairi N. J Polym Sci, Polym Chem 1998;36:1965.
[38] Goto M, Kobayashi K, Hachikawa A, Saito K, Cho CS, Akaike T. Macromol Chem Phys 2001;202:1161.
[39] Bohne C, Redmond RW, Scaiano JC, editors. Photochemistry in organized and constrained media Ramamurthy V. New York: VCH Publishers; 1991. p. 100.
[40] Vanaken T, Foxal-Vanaken S, Castleman S, Ferguson-Miller S, editors. Methods in enzymol, vol. 125. New York: Academic Press; 1986. p. 27.
[41] Izawa S, Saki-Tomita Y, Kinomura K, Kitazawa S, Tsuda M, Tsuchiya T. J Biochem 1993;113:573.