Observation of a Partially Opened Triple-helix Conformation in 1→3-β-Glucan by Fluorescence Resonance Energy Transfer Spectroscopy*

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This study used fluorescence resonance energy transfer (FRET) spectroscopy as an indirect method to investigate the effect of NaOH treatment on the conformation of a triple-helix (1→3)-β-D-glucan and then evaluated the effect of conformation on biological activity. Previous studies have suggested that treatment of the triple-helix glucans with NaOH produces single-helix conformers. FRET spectra of the triple-helix glucan, laminarin, doubly labeled with 1-amino-1-naphthoyl and fluorescein-5-isothiocyanate as acceptor probe attached at the reducing end, showed that a partially opened triple-helix conformer was formed on treatment with NaOH. Increasing degrees of strand opening was associated with increasing concentrations of NaOH. Based on these observations we propose that a partially opened triple-helix rather than a single helix, is formed by treating the triple-helix glucans with NaOH. After neutralizing the NaOH, changes in FRET indicated that the partially opened conformer gradually reverts to the triple-helix over 8 days. Laminarin was stabilized at different degrees of partial opening and its biological activity examined using the Limulus amebocyte lysate assay and nitric oxide production by alveolar macrophage. Both Limulus amebocyte lysate activity and nitric oxide production were related to the degree of opening of the triple-helix. Partially open conformers were more biologically active than the intact triple-helix.

(1→3)-β-D-Glucans have been shown to systemically enhance the immune system, resulting in antitumor, antibacterial, and wound healing activities (1). Differences in these activities are thought to be related to three factors: molecular conformation, the degree of branching, and the molecular weight (Mf) (2). Studies have shown that high Mf (100,000–200,000 g/mol) glucans with a degree of branching of 0.20–0.33 are most active (2), however, data describing the effects of molecular conformation on biological activity are less clear. This is in part due to the lack of adequate methodology to characterize the tertiary structure of glucans.

Most spectroscopic techniques only provide data about the secondary structure of glucans. Methods such as solid state 13C NMR spectroscopy (3) or multi-angle laser-light scattering (4) have had some success in relating the tertiary structure of glucans to their conformation based biological activity, however, their use is limited by availability, cost, and efficacy for evaluating glucan structure in solution. A direct observation technique, such as x-ray crystallography would assist in understanding glucan structure and its biological relevance; however, it is difficult to crystallize glucans and only a limited number of these studies have been done (5–7).

Fluorescence resonance energy transfer (FRET)1 is a phenomenon of non-radioactive energy transfer over relatively long distances that has been used to characterize the spatial relationship of donor- and acceptor-labeled molecules in biological systems (8). If a system contains two fluorophores and the donor has an emission spectrum which overlaps with the absorption spectrum of the acceptor, then the excitation energy of the donor can be transferred to the acceptor over a range of 10–80 Å (9). The efficiency (E) of this transfer is given by the following equation,

\[
E = \frac{R_0^6}{(R_0^6 + R^6)} \quad \text{(Eq. 1)}
\]

where R is the distance between centers of donor and acceptor chromophores and R0 is the Förster critical distance at which 50% of the excitation energy is transferred to the acceptor (50% transfer efficiency). \(R_0^6\) is proportional to the overlap integral of the donor emission and the acceptor absorption spectra.

Three conformers of soluble glucans have been reported, including a single-helix, triple-helix, and random coil. Among these, the single-helix and triple-helix have been considered the biologically active forms, however, it is unclear which is the most active form. Studies evaluating the molecular weight dependence of antitumor activity have suggested that the triple-helix form is the most potent conformer of schizophyllan (SPG) (10, 11). However, other studies, using solid state 13C NMR spectroscopy, have suggested that for antitumor activity (3, 12, 13) and the production of tumor necrosis factor, nitric oxide, and hydrogen peroxide by macrophage (14, 15), the single-helix is the potent conformer (15).

The conversion between triple-helix and single-helix conformers can be mediated by different chemical or physical treatments (16). Treatment of the triple-helix SPG with NaOH has been used to prepare single helix-rich forms (14, 17). Aketagawa et al. (18) suggested that treatment of SPG with NaOH causes the triple-helix to form single chains which rap-

1 The abbreviations used are: FRET, fluorescence resonance energy transfer; AP, 1-amino-1-naphthoyl; SPG, schizophyllan; FITC, fluorescein-5-isothiocyanate; LAL, Limulus amebocyte lysate.
experiments were performed after treatment with NaOH and the corresponding biological activities correlated with the degree of strand opening.

EXPERIMENTAL PROCEDURES

Reagents and Chemicals—Laminarin was obtained from Sigma, aniline blue from Polysciences, Inc. (Warrington, PA), 1-aminopyrene (AP) and sodium cyanoborohydride from Aldrich (Milwaukee, WI), and fluorescein 5-isothiocyanate (FITC) from Molecular Probes (Eugene, OR). Actigum was a gift from Dr. David L. Williams at East Tennessee State University. Actigum and laminarin are composed of β-(1→3)-α-linked glucopyranose backbone with a degree of side chain branching (β-(1→6) of 0.33 and 0.05, respectively. Actigum has a M_r of 3.51×10^6 g/mol, a polydispersity of –1.19 and root mean square radius of 36.3 nm and laminarin has a M_r of 7.7×10^5 g/mol, polydispersity of 1.17, and intrinsic viscosity of 0.07 dl/g. The M_r and polydispersity were determined by size exclusion chromatography with in-line argon-ion multiangle laser light scattering photometry and differential viscometry detectors (20) and provided by Dr. David Williams. The glucan-sensitive LAL reagent Pyrotell-T was purchased from Associates of Cape Cod (Woods Hole, MA).

Preparation of Laminarin-AP Derivative—The AP probe, designed to specifically label the reducing end of glucans, was prepared according to a modification of the procedure of Evangelista et al. (21) Specifically, 100 mg of laminarin (0.005 mmol) was mixed with 2.6 mg (0.011 mmol) of AP (in 0.5 ml MeSO_4, 2 ml of 15% acetic acid, 17.6 mg (0.28 mmol) of sodium cyanoborohydride, and 42 mg (0.31 mmol) of ammonium sulfate (NH_4)_2SO_4. The 5-ml glass reaction vial was heated in a water bath at 75 °C for 1 h, then dialyzed with double distilled water for 2 days. The final solution was divided into equal portions with one portion reserved as the donor-labeled sample and the other for labeling with FITC (laminarin-AP-FITC).

Preparation of Laminarin-FITC Derivative—Since FITC reacts with primary and secondary amines the laminarin-FITC (acceptor only) derivative was prepared by reacting FITC with laminarin-NH_2. A stock solution of FITC was prepared in dimethylformamide at a concentration of 10 mg/ml and then stored at –20 °C. Laminarin-NH_2 was prepared by the method of Liu et al. (22). Laminarin (50 mg) was dissolved in water and an excess of 2.0 m (NH_4)_2SO_4 and 0.4 m NaBH_3CN added and the solution heated at 100 °C for 120 min. After heating, the solution was cooled in an ice bath and then dialyzed against double distilled water for 2 days. The laminarin-NH_2 was then derivatized with FITC by the following procedure. One ml of pH 10 borax/ potassium hydroxide buffer and an excess of FITC (250 μl, 0.006 mmol) were added to the laminarin-NH_2 (0.76 ml) solution at room temperature. The final pH was adjusted to 10 with 0.1 m NaOH, reacted for 1 day, and dialyzed against double distilled water for 2 days to eliminate free FITC.

Preparation of Laminarin-AP-FITC (Donor and Acceptor) Derivatives—Two ml of pH 10 borax/potassium hydroxide buffer and an excess of FITC (250 μl, 0.006 mmol) were added to laminarin-AP solution (1.85 ml) at room temperature. The final pH was adjusted to 10 by adding 0.1 m NaOH, reacted for 1 day, and dialyzed against double distilled water for 2 days. Since the amount of laminarin was the same (50 mg) in the labeled derivatives (donor labeled, acceptor labeled, and doubly labeled), the labeled derivatives were adjusted to the same volume to ensure that all three solutions contain the same concentration of laminarin.

The labeling ratio of the FITC was estimated from UV absorbance by using the molar extinction coefficient for FITC, ε = 73,000 @ 494 nm (23). The labeling ratio is the proportion of fluorescein probes introduced into the glucan molecule on a molar basis and is used to identify the optimum signal response from FRET. The labeling ratio of FITC in laminarin was estimated to be about 1%. The labeling ratio for AP was calculated to be about 10%, by using the molar extinction coefficient of ε = 2618 @ 370 nm. However, actual value for AP labeling maybe smaller than 10%, because FITC could replace some of the AP during the derivatization process.

Steady-state Fluorescence Measurements and UV Measurements—Steady-state fluorescence measurements were carried out at 20 ± 1 °C on an ISS PC1 spectrofluorometer. The excitation wavelength was set at 370 nm, and the emission wavelength was scanned from 400 to 600 nm. FRET was determined by monitoring the change in the emission spectrum of both the donor and acceptor probes. UV measurements were carried out on a Beckman DU-40 spectrophotometer.

Sample Preparation for LAL Analysis—Glucan samples were prepared for LAL analysis by dissolving 400 μl (1 mg) of laminarin-AP-FITC (~8 mg/ml). The sample was neutralized with 1 N HCl followed by the addition of 10 ml of pH 7 buffer. The sample was divided into 10 equal aliquots and 6 μl of aniline blue (4.221 mg/ml, with stirring for 1 h) added to each aliquot at different times (0–100 h) to stabilize the conformational change of the triple-helix during renaturation (24).

The LAL activity of glucan-aniline blue solutions was evaluated using a kinetic turbidimetric procedure (24) based on work by Roslandsky and co-workers (25). 80 μl of each glucan preparation and 20 μl of pyrrotil T (Associates of Cape Cod) were added to each well of a microtiter plate. Plates were incubated at 37 °C in a microplate reader (Kinetic-QCL, Whittaker Bioproducts), and the absorbance measured every 30 s at 450 nm for 40 min. The onset time, defined as a change of optical density (ΔOD) of 0.02, was determined for each sample. Differences in onset time were used to evaluate differences in LAL activation.

Sample Preparation for Nitric Oxide Oxides—NaOH-treated and untreated laminarin were used to evaluate the in vitro release of nitric oxide by alveolar macrophage. NaOH-treated laminarin was used to evaluate the effect of conformation on nitric oxide release and untreated laminarin served as the control. NaOH-treated laminarin was prepared by dissolving 200 mg in 0.25 N NaOH. This preparation was neutralized with 3 N HCl, dialyzed 2 days against distilled water, and diluted with cell culture medium to a final laminarin concentration of 8.5 mg/ml. Aliquots of the preparation were evaluated for nitric oxide production daily for 5 days after dialysis. Untreated laminarin, prepared by solubilizing in phosphate-buffered saline and dialyzing to a...
with 100 m of Griess reagent (0.5% sulfanilamide, 0.05% naphthylethylendiamine dihydrochloride, 2.5% H3PO4) and measured spectrophotometrically at 546 nm. Nitrite concentrations were determined using a standard LAL curve to be 8.5 mg/ml. This preparation was treated with 20 m of laminarin-AP-FITC (~8 mg/ml) denatured with 0.27, 0.5, or 1 m NaOH, and neutralized with 1 m HCl. The final volume of each solution was adjusted to 820 l with pH 7 buffer.

For nitric oxide analysis, a rat alveolar macrophage cell line, NR8383 from American Type Culture Collection (ATCC, Manassas, VA), were cultured in RPMI 1640 media (BioWhittaker, Walkersville, MD) containing 15% heat-inactivated fetal bovine serum, 2 mM l-glutamine, 100 units/ml penicillin, and 100 mg/ml streptomycin and incubated in 150-cm² tissue culture flasks at 37 °C in a humidified atmosphere of 5% CO2. Cultured macrophage were seeded in 96-well tissue culture plates at a density of 5 × 10⁴ cells/well and incubated for 1 day at 37 °C, 5% CO2. The culture medium was then replaced with phenol red-free RPMI 1640 medium containing either NaOH-treated or polymyxin-treated laminarin (8.5 mg/ml) with 0.5% fetal bovine serum and incubated for 1 day at 37 °C in 5% CO2. After incubation the cell supernatant was collected and evaluated for the production of nitric oxide. Nitric oxide was determined as nitrite (NO₂⁻) with Griess reagent as described by Green et al. (26). Briefly, 100 μl of cell culture supernatants were mixed with 100 μl of Griess reagent (0.5% sulfanilamide, 0.065% naphthylethylendiamine dihydrochloride, 2.5% H3PO4) and measured spectrophotometrically at 546 nm. Nitrite concentrations were determined using a standard curve prepared from sodium nitrite with a linear range from 0.8 to 103 μM. Three replicates were done at each time point in the experiment.

RESULTS

This study used FRET to investigate changes in tertiary structure of laminarin induced by NaOH and correlated differences in conformation with differences in activation of the LAL assay and nitric oxide production by alveolar macrophage. The UV and fluorescence spectra of singly labeled laminarin-AP (donor) and singly labeled laminarin-AP-FITC (acceptor) derivatives are shown in Fig. 2. Laminarin-AP has a maximum UV absorbance at 370 nm and a fluorescence emission maximum at 450 nm. Laminarin-FITC has a UV absorbance maximum at 460 nm, which overlaps with AP emission, and a fluorescence emission maximum at 520 nm. On excitation at 370 nm the normal emission peak for this system is at 450 nm, however, if FRET occurs, a second emission peak will occur at 520 nm which corresponds to energy transfer from AP to FITC. The spectral overlap between donor emission and acceptor absorption results in an R0 value of 23.7 Å, providing a maximum measurable distance of 42.6 Å (a range of 1.8 R0) for FRET in this system.

Fig. 3 compares the fluorescence spectrum of laminarin-AP, laminarin-FITC, and laminarin-AP-FITC. Spectra were measured on 20 μl of laminarin-AP-FITC (~8 mg/ml) denatured with 0.27, 0.5, or 1 m NaOH, and neutralized with 1 m HCl. The final concentration of NaOH greater than 0.24M were required to convert triple-helix glucans to random coils (19, 27), which subsequently would form single helices after removing NaOH (16). FRET would not occur if there was actual strand separation (random coil conformers) due to the relatively large distance between the donor and the acceptor probes. The AP intensity of laminarin-FITC would be similar to that of laminarin-AP (Figs. 3 and 4). However, on treatment with NaOH (0.24 M) the peak increased to only two-thirds of the height of laminarin-AP (Figs. 3 and 4). The effects of other concentrations of NaOH are also shown in
Fig. 4. The highest concentrations of NaOH gave the most intense donor emission and the lowest acceptor emission. This suggests that increasing concentrations of NaOH cause increasing degrees of strand opening. To rule out the possibility that the FRET resulted from separate glucan molecules, we measured the fluorescence spectra of a mixture of singly labeled laminarin-AP and laminarin-FITC treated with NaOH and observed no significant FRET (Fig. 5). Because the intermolecular distance is large, FRET most likely occurs only from donor and acceptor probes of the same glucan molecule. Therefore, the sums of spectrums of laminarin-AP plus laminarin-FITC in a solution represents background spectra without FRET.

Fig. 6 shows the fluorescence emission spectrum over time of laminarin-AP-FITC treated with NaOH. At 0 h, the fluorescence intensity was highest at 450 nm and lowest at 520 nm, indicating that the donor and acceptor probes were far apart. As time increased, the 450 nm peak decreased and the 520 nm peak increased indicating that donor and acceptor were approaching each other. Complete conversion took about 8 days, at which time most of the fluorescence emission came from the acceptor (FITC). The time-dependent FRET phenomenon was observed at all concentrations of NaOH evaluated (from 0.0115 to 0.833 M, Figs. 4 and 6).

Fig. 7 shows the spectrum of equal concentrations of laminarin-AP-FITC, stabilized with aniline blue at different times after neutralization to stabilized the laminarin-AP-FITC conformation. 0.5 M NaOH was used as the denaturing solution, neutralized with HCl, diluted in pH 7 buffer, measured after 20 days.

Fig. 5. Comparison of the fluorescence spectra of a mixture of equal concentrations of laminarin-AP and laminarin-FITC with the doubly labeled laminarin-AP-FITC. The spectra were normalized at 450 nm for comparison.

Fig. 6. The conformational change of neutralized laminarin-AP-FITC over time as detected by FRET. Forty μl of laminarin-AP-FITC (~8 mg/ml) was denatured with 0.016 M NaOH, neutralized with HCl, and pH 7 buffer added to a final volume of 840 μl.

FIG. 7. The effect of adding aniline blue at different times after neutralization to stabilized the laminarin-AP-FITC conformation. 0.5 M NaOH was used as the denaturing solution, neutralized with HCl, diluted in pH 7 buffer, measured after 20 days.
helices are more effective in stimulating the production of nitric oxide in macrophage than the triple-helix conformer (14, 15).

DISCUSSION

This study used laminarin, a triple-helix 1→3-β-glucan, to study the effects of conformation on biological activity. The assumption that laminarin is a triple-helix is based on high-resolution solid-state 13C NMR and the molecules characteristic x-ray powder diffraction patterns reported by Saito et al. (28–30). However, because of laminarin’s low Mr, there is some disagreement regarding its characterization as a triple-helix. To verify the applicability of the FRET technique for other triple-helix 1→3-β-glucans we evaluated the effects of NaOH treatment on actigum, a higher Mr, triple-helix 1→3-β-glucan, and observed results similar to those for laminarin.

The use of FRET provides evidence that a partially opened triple-helix is formed after NaOH treatment rather than single helices as suggested by Aketagawa et al. (18). Our conclusion is based on the following evidence. First, in order for FRET to occur, the donor and acceptor must be in close proximity. If NaOH treatment produces single helices, then FRET should be observed after denaturing and re-annealing a mixture of singly labeled AP and FITC glucans. However, no significant FRET was observed in this system (Fig. 5), indicating that dissociation and re-association was not occurring after the glucans were denatured. Second, preparations of laminarin-AP-FITC, treated with NaOH at concentrations ≥0.24 M had different emission intensities at 450 nm (Fig. 4). If complete dissociation occurs after NaOH treatment, then we would expect no difference in intensity at 450 nm between the NaOH-treated preparations.

We propose that the mechanism of conformational change by NaOH treatment is via a partially opened triple-helix as shown in Fig. 1. Treatment with NaOH can break H-bonds resulting in a partially opened triple-helix structure. The degree of strand opening depends on the concentration of NaOH used. This structure better explains the observation that the Mr of native and NaOH-treated SPG is the same (13, 19). Identification of a partially opened triple-helix is difficult because most spectroscopic methods cannot distinguish between a closed and a partially opened triple-helix. By using FRET, we can monitor the degree of strand opening caused by different concentrations of NaOH and the reversion to the triple-helix conformation. Although this structure has not been previously proposed as the intermediate for NaOH treatment, electron micrographs in a recent study of SPG have shown partial strand opening with three distinct single strands (31).

We have identified two possible limitations of FRET as used in this study. First, treatment of triple-helix (1→3)-β-glucans with NaOH may also cause untwisting at the middle of the helix. Since the reducing end of the molecule is labeled with the donor-acceptor probes this technology cannot detect a conformational change in the middle of the molecule. Second, labeling the reducing end of the molecule alters the native conformation of the close triple-helix by causing some degree of partial opening at the site of the label. However, within the context of these limitations we did observe the dependence strand opening on NaOH concentration and the dynamic change in conformation during renaturation (Figs. 4 and 6).

Aketagawa et al. (13) reported that for glucans with different conformation but the same degree of polymerization, the single helix is 100 to 1000 times more potent than the triple-helix in activation of limulus coagulation factor G. We speculated that LAL activity and nitric oxide production would be dependent on the degree of partial opening of the triple-helix after NaOH treatment. We investigated this by stabilizing conformers at different degrees of strand opening with aniline blue and analyzed their relationship to LAL activation. These studies confirmed, for both a low Mr (laminarin) and high Mr glucan (actigum), that conformations with a higher degree of partial opening (single helix structure) were more effective in activating the LAL assay. Furthermore, there was a gradient of activity between conformers with a greater degree of opening and the triple-helix forms. These observations were also confirmed in studies evaluating the release of nitric oxide from alveolar macrophage.

In addition to conformation, morphology could also change during the renaturing process. Three different morphologies have been reported for renatured glucans including, circular, linear, and aggregates species (31–36). The formation of different morphological species is thought to depend on the characteristics of glucan and the conditions of denaturation. For example, Stokke et al. (31) showed that preparations of high Mr (> 7.5 × 10⁵ Da) renatured SPG had a circular morphology while those with a Mr of 1.34 × 10⁵ Da had a reconstituted linear triple-helix morphology. Stokke suggested that the tend-
oeny to form macrocyclic structures in competition with intermolecular aggregates is determined by three factors: 1) chain stiffness relative to overall length; 2) parallel or anti-parallel alignment of interacting chain segment; and 3) polymer concentration (33). There is no interconversion between different morphology except at closed to dissociating conditions (33).

Differences in biological activity based on morphology are likely to be important in understanding the structure-activity relationship of glucans. In a recent study, Kitamura et al. (34) observed that circular and linear species of renatured glucans have higher anti-tumor activity compared with the aggregate species but that aggregate species had higher G activity than circular or linear species. However, it is unlikely that FRET can be used to evaluate the effects of morphology on biological activity because the technique, as used in these studies, is limited to a maximum measurable probe distance of 42.6 Å. Since we did not observe FRET in a mixture of two singly labeled laminarin preparations, it is unlikely we would observe FRET in a labeled preparation of aggregate morphology because the distance between probes would be too large.

In summary, we applied a FRET technique to evaluate the effect of NaOH treatment on glucan conformation by adding donor and acceptor fluorophore groups to the reducing end of donor and acceptor fluorophore groups to the reducing end of triple-helix glucan. Using this technique we demonstrated that the NaOH-induced conformational change is between the closed-triple helix and the partially opened triple helix. These tertiary structural changes in triple-helix glucan occurred in all concentrations of NaOH tested (0.0115 to 0.83 M) and the degree of strand opening was dependent on the strength of the denaturing agent. We also observed a strong correlation between conformation and biological activity as measured by the tertiary structural changes in triple-helix glucan occurred in all concentrations of NaOH tested (0.0115 to 0.83 M) and the degree of strand opening was dependent on the strength of the denaturing agent. We also observed a strong correlation between conformation and biological activity as measured by the tertiary structural changes in triple-helix glucan occurred in all concentrations of NaOH tested (0.0115 to 0.83 M) and the degree of strand opening was dependent on the strength of the denaturing agent.

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REFERENCES
1. Stone, B. A., and Clarke, A. E. (1992) Chemistry and Biology of 1–3-β-Glucans, La Trobe University Press, Victoria, Australia
2. Bohn, J. A., and BeMiller, J. N. (1995) Carbohydr. Polym. 28, 3–14
3. Yoshioka, Y., Uehara, N., and Saito, H. (1992) Chem. Pharm. Bull. 40, 1221–1226
4. Kulicie, W.-M., Lettau, A. I., and Thielking, H. (1997) Carbohydr. Res. 297, 135–143
5. Marchessault, R. H., and Sarko, A. (1967) Adv. Carbohydr. Chem. Biochem. 22, 421–482
6. Atkins, E. D. T., Parker, K. D., and Preston, R. D. (1969) Proc. R. Soc. Lond B Biol. Sci. 173, 209–221
7. Jelsma, J., and Kreger, D. R. (1975) Carbohydr. Res. 43, 200–203
8. Szollosi, J., Damjanovich, S., and Matys, L. (1998) Cytometry 34, 159–179
9. Cheung, H. C. (1991) in Topics in Fluorescence Spectroscopy (Lakowicz, J. R., ed) Vol. 2, pp. 127–176, Plenum, New York
10. Kojima, T., Tabata, K., Ishii, W., and Yanaki, T. (1986) Agric. Biol. Chem. 50, 231–232
11. Yanaki, T., Ito, W., Tabata, K., Kojima, T., Norisuye, T., Takano, N., and Fujita, H. (1983) Biophys. Chem. 17, 337–342
12. Saito, H., Yoshioka, Y., Uehara, N., Aketagawa, J., Tanaka, S., and Shibata, T. (1991) Carbohydr. Res. 217, 181–190
13. Aketagawa, J., Tanaka, S., Tabata, Y., Shibata, Y., and Saito, H. (1993) J. Biochem. (Tokyo) 113, 683–686
14. Ohno, N., Miura, N. N., Chiba, N., Adachi, Y., and Yadamoe, T. (1995) Biol. Pharm. Bull. 18, 1242–1247
15. Ohno, N., Hashimoto, T., Adachi, Y., and Yadamoe, T. (1996) Immunol. Lett. 52, 1–7
16. Yadamoe, T., and Ohno, N. (1996) Recent Res. Dev. Chem. & Pharm. Sci. 1, 23–33
17. Miura, N. N., Ohno, N., Adachi, Y., Aketagawa, J., Tabata, H., Tanaka, S., and Yadamoe, T. (1995) Biol. Pharm. Bull. 18, 185–189
18. Aketagawa, J., Tabata, H., and Saito, T. (1994) in Third Glucan Inhalation Toxicity Workshop; Committee on Organic Dusts, ICOH (Nylander, R., and Goto, H., eds) pp. 4–16, ICOH, Tokyo
19. Kitamura, S., Hirano, T., Takeo, K., Fukuda, H., Takahashi, K., Falch, B. H., and Stokke, B. T. (1996) Biopolymers 39, 407–416
20. Muller, A., Pretus, H. A., McNamee, R. B., Jones, K. L., Brown, I. W., and Williams, D. L. (1995) J. Chromatogr. B 666, 283–290
21. Evangelista, R., Liu, M.-S., and Chen, F.-T. A. (1995) Anal. Chem. 67, 2239–2245
22. Liu, J., Shirato, O., Wiesler, D., and Novotny, M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2302–2306
23. Haugland, R. P. (1996) Handbook of Fluorescent Probes and Research Chemicals, 6th Ed., Molecular Probes, Inc., Eugene, OR
24. Young, S.-H., and Jacobs, R. R. (1998) Anal. Biochem. 270, 230–236
25. Remillard, J. F., Roslansky, P. F., and Novitsky, T. J. (1993) Anal. Biochem. 223, 223–2245
26. Ohno, N., Hashimoto, T., Adachi, Y., and Yadamoe, T. (1996) Proc. R. Soc. Lond B Biol. Sci. 260, 425–4266
27. Saito, H., Tabata, K., Yoki, M., and Erata, T. (1987) Bull. Chem. Soc. Jpn. 60, 113–118
28. Saito, H., Tabata, K., Yoki, M., and Erata, T. (1987) Bull. Chem. Soc. Jpn. 60, 119–121
29. Saito, H., and Yoki, M. (1989) Bull. Chem. Soc. Jpn. 62, 392–398
30. Stokke, B. T., Elgsaeter, A., Kitamura, S., and Cheung, H. C. (1991) Macromolecules 24, 6349–6351
31. Kitamura, S., Tabata, Y., Uehara, N., and Saito, H. (1992) Carbohydr. Res. 23, 395–399
32. Kitamura, S., Hori, T., Kurita, K., Takeo, K., Ishii, W., Tabata, K., Elgsaeter, A., and Stokke, B. T. (1994) Carbohydr. Res. 236, 111–121
33. Stokke, B. T., Elgsaeter, A., and Kitamura, S. (1995) Int. J. Biol. Macromol. 15, 65–68
34. Kitamura, S., Hirano, T., Hori, T., Stokke, B. T., Ishii, W., Tabata, K., and Takeo, K. (1992) Nippon Nogeishakagu Kaishi 66, 305
35. McIntire, T. M., Penner, R. M., and Brant, D. A. (1995) Macromolecules 28, 6575–6577
36. McIntire, T. M., and Brant, D. A. (1998) J. Am. Chem. Soc. 120, 6909–6919
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