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Interactions between Oat β-Glucan and Calcofluor Characterized by Spectroscopic Method

JIA WU, XIAO DENG, BINQIANG TIAN, LAN WANG, AND BIJUN XIE*

College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China, and Wuhan Scientific Research and Design Institute, State Administration for Grain Reservation, Wuhan 430079, China

This paper describes the binding of Calcofluor, a fluorescent probe, to oat β-glucan in buffer solutions. The binding equilibrium constant (*K*), the total number of binding sites per β-glucan molecule (*N*), and the average binding number of Calcofluor per β-glucan molecule (*n*) were determined by UV spectroscopic method. The results indicate that the association of Calcofluor and β-glucan is driven by both enthalpy and entropy and that the process involves hydrogen bonding, van der Waals forces, and hydrophobic interaction. Higher buffer concentration and NaCl facilitate the binding of Calcofluor to β-glucan. The adsorption isotherm fits a Langmuir model quite well.

KEYWORDS: Oat β-glucan; Calcofluor; intermolecular forces; UV spectroscopy; Langmuir adsorption

INTRODUCTION

Mixed-linkage (1→3),(1→4)-β-D-glucan, or β-glucan, is a linear polysaccharide composed of consecutively (1→4)-linked β-D-glucopyranosyl residues in blocks separated by single (1→3)-linkages (*1–3*). β-Glucan mainly occurs in oat and barley in relatively high amounts. In recent years oat β-glucan has begun to receive greater attention due to its health benefits. As a water-soluble dietary fiber, oat β-glucan displays physiological effects on lowering cholesterol and decreasing postprandial glucose levels in serum (*4–7*). Specific benefit of soluble oat β-glucan on lung tumor metastases and macrophage antitumor cytotoxicity has also been reported (*8*). It is generally considered that the positive effects of oat β-glucan on plasma glucose and insulin are mainly attributable to viscosity (*7, 9*). Because the viscosity is related to the concentration and molecular weight, determination of β-glucan content in cereal food is of great importance to predict their physiological effects.

The techniques most commonly employed in the determination of β-glucan are those based on a purified β-glucanase from *Bacillus subtilis* (*10*) and fluorometric Calcofluor−FIA methodology (*11–13*), based on the specific binding of the fluorochrome Calcofluor to β-glucan (*14*) followed by the fluorometric detection of the complex formed. The Calcofluor−FIA method is rapid, easy, and accurate, but the mechanism by which the Calcofluor/β-glucan complex develops is unknown.

The purpose of this study is to explore the interactions between Calcofluor and oat β-glucan molecules. Previous papers have suggested that the binding between Calcofluor and β-glucan is dependent on the ionic strength of the solution and molecular weight of β-glucan (*15*). Temperature, ethanol, and maltose were mentioned to be factors that interfered with the combination of Calcofluor with β-glucan (*12*).

Calcofluor (**Figure 1**) is a fluorescent probe capable of binding with β-(1→4)- and β-(1→3)-linked polysaccharides. It has been used commercially to whiten cellulosic textiles and paper. Biologists use it to localize cellulose and chitin (*16*). Precipitation of the Calcofluor−glucan complex and changes in the UV absorption and fluorescence spectra of Calcofluor have been studied. Changes in the absorption spectra, which were detectable with as little as 0.5 μg mL^{-1} of glucan, could be used to determine the effect of β-glucan concentration on free and bound Calcofluor (*17*).

The interactions between proteins and small molecules have been extensively investigated; however, there is little research on the association of polysaccharide with small molecules (*18–21*), probably due to the complexity of polysaccharide. We present here a simple spectroscopic method to study the interactions between the polysaccharide and the dye molecule. It will be helpful for understanding the interactions between polysaccharide and other molecules in aqueous solution.

EXPERIMENTAL PROCEDURES

Oat β-Glucan Preparation. Oat β-glucan was extracted from hulless oat (Jinyan 8, obtained from Shanxi Plateau Plant Institute) whole flour according to the protocol proposed by Lazaridou et al. (*22*). The procedure of extraction and purification involves a dual-enzyme digestion with a thermostable α-amylase (Termamyl 120L, batch AXS 30008, Novozymes, Denmark) and pancreatin (batch 045K0673, Sigma, St. Louis, MO). The β-glucan content of the preparation was determined to be 94.2% with the method of McCleary and Glennie-Holmes using the Megazyme β-glucan assay kit (*10*). The molecular weight of the β-glucan was determined by GPC-LLS (DLS-700, Otsuka Electronics, Japan) employing the same equipment and operating under the same conditions as we used previously (*23*). The value for the weight-average molecular weight was 1.5×10^5, with a polydispersity index of 1.3.

* Corresponding author (telephone 86-027-87283201; fax 86-027-87282966; e-mail bijunxie@sina.com).

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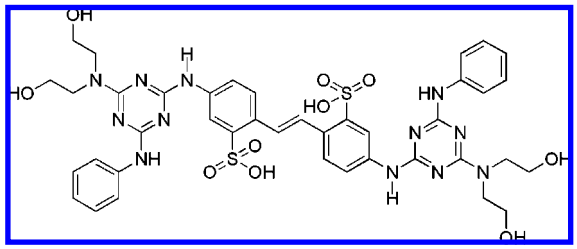

Figure 1. Schematic structure of Calcofluor molecule.

UV Absorption Spectroscopy. The purity of Calcofluor (batch 020K1578, Sigma) was determined to be 95.8% by elemental analysis based on the molecular formula as suggested by the provider. Calcofluor solutions of 2.18×10^{-4} M were prepared in Na_2CO_3/$NaHCO_3$ buffers (3.125, 6.25, 12.5, 25, 50, and 100 mM, pH 10) to produce six different ionic strengths. Calcofluor solutions were stored in dark containers to preserve them from light. Ten milligrams of β-glucan was heated at 70 °C with magnetic stirring in the same buffer for about 1 h until complete solubilization. The solution was allowed to cool to room temperature and adjusted to 100 mL with buffer solution. The resulting β-glucan solution was 6.67×10^{-7} M (on the basis of the weight-average molecular weight). Then 0.2 mL of Calcofluor solutions was mixed with various volumes of β-glucan solutions. The final concentration of Calcofluor in the reaction system was kept constant at 1.09×10^{-5} M. After the two solutions were blended on a vortex or by shaking to make an adequate combination, UV absorption spectra or intensities were collected within 5 min. A UV−vis spectrophotometer from Beckman Coulter (DU 800) with a circulating water temperature control unit was utilized. The changes of absorbance were determined in different solutions using 1 cm path length quartz cuvettes. The thermodynamic parameters were determined at 20, 25, 28, and 35 °C. Each determination was repeated three times.

Solutions of 0.8 M NaCl and 8 M urea were prepared in Na_2CO_3/$NaHCO_3$ buffers (100 mM, pH 10). The NaCl solution was added to β-glucan solutions followed by blending with Calcofluor to make the final concentrations of 25, 50, 100, and 200 mM. The urea solution was added the same way as NaCl solution, and the final concentrations were 0.25, 0.5, 1, and 2 M. The UV absorption intensities of the mixed solutions were determined using the same way and spectrometer.

Quantitative UV Spectroscopic Analysis on Binding of Calcofluor to *β*-Glucan. On the basis of the simplest mechanism able to describe the reversible interaction between Calcofluor and β-glucan (*24*)

$$C_F + \text{glucan} \overset{K}{\rightleftharpoons} C_B \tag{1}$$

where C_F represents the molar concentration of free Calcofluor and C_B refers to the molar concentration of Calcofluor that bound on β-glucan. The absorbance of the Calcofluor/β-glucan mixture, A, is attributed to the free Calcofluor and Calcofluor/β-glucan complex. It can be analyzed according to Beer's law as

$$A = \epsilon_F C_F + \epsilon_B C_B = \epsilon_F C_T + (\epsilon_B - \epsilon_F) C_B \tag{2}$$

where ϵ_F is the molar absorptivity of free Calcofluor and ϵ_B is that of the bound Calcofluor. C_T is the total molar concentration of Calcofluor in solution. Rearranging eq 2 yields

$$C_B = (A - \epsilon_F C_T)/(\epsilon_B - \epsilon_F) \tag{3}$$

where A is the absorbance of the Calcofluor/β-glucan mixture and $\epsilon_F C_T$ is the absorbance of the Calcofluor solution without β-glucan. Both A and $\epsilon_F C_T$ can be measured. Define

$$\Delta A = A - \epsilon_F C_T \tag{4}$$

and

$$\Delta\epsilon = \epsilon_B - \epsilon_F \tag{5}$$

then eq 3 may be simplied as

$$C_B = \Delta A/\Delta\epsilon \tag{6}$$

If ϵ_B and ϵ_F are determined from the absorbance of Calcofluor solutions with an excess of β-glucan and without β-glucan, then $\Delta\epsilon$ can be obtianed. The average binding number of Calcofluor per glucan molecule, n, can be expressed as

$$n = \frac{C_B}{C_G} \tag{7}$$

Because C_B can be calculated from eq 6 and C_G is the molar concentration of β-glucan, we can obtain the value of n from eq 7 . For convenience in calculation, a "glucan molecule" is assumed to be the molecule with the molecular weight 1.5×10^5, which is the weight-average molecular weight determined by GPC-LLS as described above. The binding equilibrium constant of the binding reaction in eq 1 should be

$$K = C_B/[\text{glucan}]C_F \tag{8}$$

where [glucan] represents the concentration of unoccupied binding sites on glucan

$$[\text{glucan}] = NC_G - C_B = (N - n)C_G \tag{9}$$

where N is the total number of binding sites per β-glucan molecule and n is the average binding number of Calcofluor per glucan molecule. Substituting eq 9 into eq 8 gives

$$n = \frac{KNC_F}{1 + KC_F} \tag{10}$$

Substituting eq 6 into eq 7 yields

$$n = \frac{\Delta A}{\Delta\epsilon C_G} \tag{11}$$

From eqs 10 and 11, we get a new equation,

$$\Delta A = \frac{\Delta\epsilon C_G KNC_F}{1 + KC_F} \tag{12}$$

It is clear that

$$C_F = C_T - C_B = C_T - \Delta A/\Delta\epsilon \tag{13}$$

From the above equations, Jiao et al. (*24*) deduced the equation

$$\Delta A = \Delta\epsilon(1 + KC_T)/K - \Delta\epsilon N(C_T\Delta\epsilon/\Delta A - 1)C_G \tag{14}$$

Because $\Delta\epsilon N$ is a constant, and $\Delta\epsilon\ (1 + KC_T)/K$ has a fixed value at a given Calcofluor concentration C_T, $\Delta A \sim (C_T\Delta\epsilon/\Delta A - 1)C_G$ is a linear equation. From the slope, $\Delta\epsilon N$, and intercept, $\Delta\epsilon(1 + KC_T)/K$, of this equation, the value of K and N can be obtained.

Zeta Potential Determination. The zeta potential was measured using a zeta meter (Zetasizer Nano ZS90, Malvern) at 20 or 25 °C with a laser wavelength of 633 nm and a scattering angle of 90°. To evaluate the effect of pH 10 Na_2CO_3/$NaHCO_3$ buffer concentration on the zeta potential of β-glucan, oat β-glucan was dissolved in 3.125, 6.25, 12.5, 25, 50, and 100 mM buffer to a final concentration of 3.33×10^{-7} M. To evaluate the effect of NaCl, 0.8 M NaCl solutions (prepared in 100 mM Na_2CO_3/$NaHCO_3$ buffer) were added to β-glucan solutions (prepared in 100 mM Na_2CO_3/$NaHCO_3$ buffer) to make the final NaCl concentrations of 25, 50, 100, and 200 mM; the glucan concentration was kept at 3.33×10^{-7} M. Each determination was repeated three times.

RESULTS AND DISCUSSION

UV Absorption Spectra of Calcofluor/*β*-Glucan Complex. Calcofluor/β-glucan complex was detected by changes in the absorption spectra of Calcofluor below the concentration at which precipitation occurred. The effect of oat β-glucan, which alone showed negligible absorption above 250 nm, on the absorption spectra of 1.09×10^{-5} M Calcofluor in Na_2CO_3/

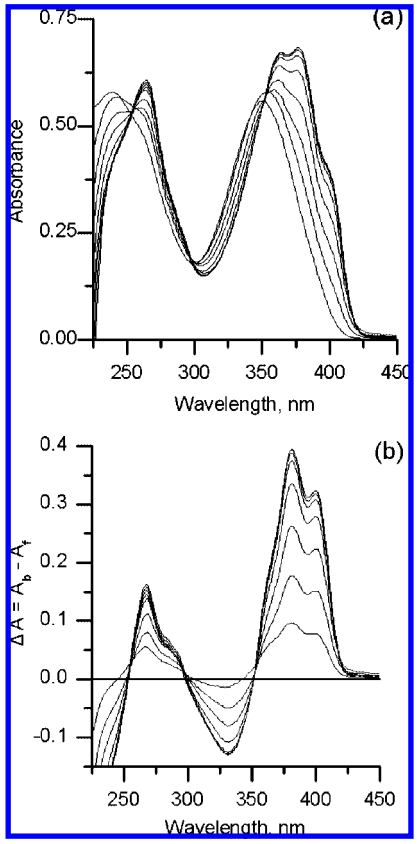

Figure 2. (**a**) UV absorption spectra of 1.09×10^{-5} M Calcofluor in pH 10 $Na_2CO_3/NaHCO_3$ buffer (0.1 M) in the presence of various concentrations of oat β-glucan. In order of increasing peak absorbance at 380 nm, β-glucan concentrations are 0, 0.33, 0.67, 1, 1.33, 1.67, 2, 2.67, and 3.33×10^{-7} M. (**b**) UV difference spectra for Calcofluor/β-glucan interaction, obtained from the spectra in **a** by subtracting the spectra of the free Calcofluor (A_f) from the spectra of Calcofluor/β-glucan mixtures (A_b).

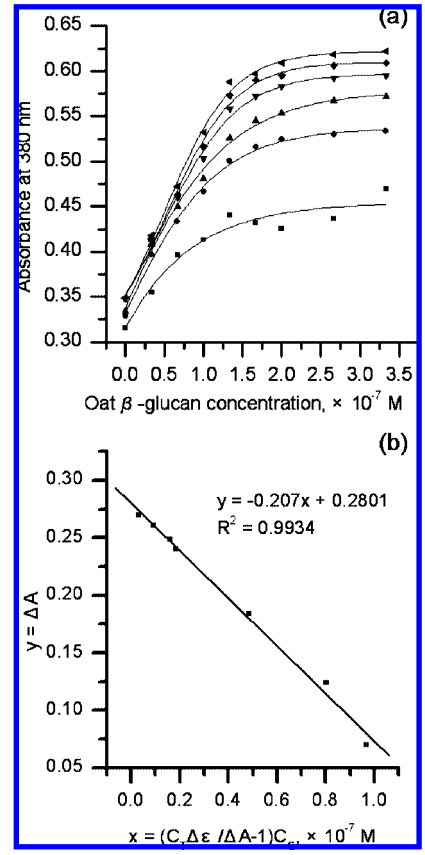

Figure 3. (**a**) Absorbance at 380 nm for 1.09×10^{-5} M Calcofluor mixed with various concentrations of oat β-glucan. From the lowest curve to the highest curve, the corresponding $Na_2CO_3/NaHCO_3$ buffer concentrations are 3.125, 6.25, 12.5, 25, 50, and 100 mM. (**b**) Linear relationship between ΔA and $(C_T\Delta\epsilon/\Delta A - 1)C_G$, obtained from 1.09×10^{-5} M Calcofluor binding to β-glucan in 100 mM buffer. The data in **a** and **b** were obtianed at 20 °C.

$NaHCO_3$ buffer (0.1 M, pH 10) is shown in **Figure 2a**. There was a red shift in the wavelength of maximum absorption from about 350 to 380 nm when oat β-glucan was added into the Calcofluor solution. The absorption intensity at 380 nm increased with the rise of oat β-glucan concentration from 0 to 3.33×10^{-7} M; meanwhile, the absorption intensity at 310 nm decreased. The isosbestic points at 353 and 298 nm strongly suggested that the spectra in the presence of oat β-glucan resulted from two forms of Calcofluor molecules, namely, bound and unbound. The difference spectra (**Figure 2b**) between bound and free Calcofluor showed a maximum at 380 nm; the wavelength was chosen for investigating the association of β-glucan with Calcofluor. The results were in accordance with previous results (*17, 25*).

Determination of *K*, *N*, and *n*. The absorption curves of Calcofluor/β-glucan complex in $Na_2CO_3/NaHCO_3$ buffer of various concentrations at 20 °C are shown in **Figure 3a**. The results revealed that the absorption intensity at 380 nm of Calcofluor/β-glucan complex was intensely dependent on buffer concentration. As shown in **Figure 3a**, absorbency of free Calcofluor increased slightly with the rise of buffer concentration, possibly attributed to the changes in the state of dissolution for Calcofluor. Maybe higher buffer concentration could break the aggregation of Calcofluor and disperse Calcofluor to molecular solution state. It seemed from our experience that the UV absorption of Calcofluor showed a better reproducibility in higher ionic strength solution, so the molar absorption coefficient of free Calcofluor was calculated from 100 mM buffer. From the data in **Figure 3a**, the linear relationship between ΔA and $(C_T\Delta\epsilon/\Delta A - 1)C_G$ was obtained, and a typical regression equation of 1.09×10^{-5} M Calcofluor binding to β-glucan in 100 mM buffer is shown in **Figure 3b**. The binding equilibrium constant, *K*, and the total number of binding sites per glucan molecule, *N*, were calculated from the intercept and slope of the linear equation. The values of *K* and *N* are 3.53×10^6 and 82.7, respectively. The average binding number of calcofluor per glucan molecule, *n*, was determined using eq 11 to be 71.9 for 1.33×10^{-7} M glucan binding with 1.09×10^{-5} M Calcofluor in 100 mM buffer. According to the assumption that the molecule weight of a "β-glucan molecule" is 1.5×10^5, we calculated that on average there is a binding site on β-glucan every 11.2 anhydroglucose units in 100 mM buffer at 20 °C.

It is important that Calcofluor solutions should be prepared in a dark environment; otherwise, the Calcofluor could be isomerized from the trans to the cis form, and the cis form Calcofluor molecules have no ability to bind on glucan (*26*). Therefore, the Calcofluor solutions were prepared in an amber bottle wrapped with aluminum foil. Calcofluor and glucan were mixed in a dark room for binding experiments, and then the mixture was poured into a cuvette and protected form light until equilibrium; finally, a UV–vis light beam was transmitted through the cuvette, and the absorbance was recorded. Exposure of the mixture to light during determination on a spectrometer actually had little effect on the absorption spectra, because the

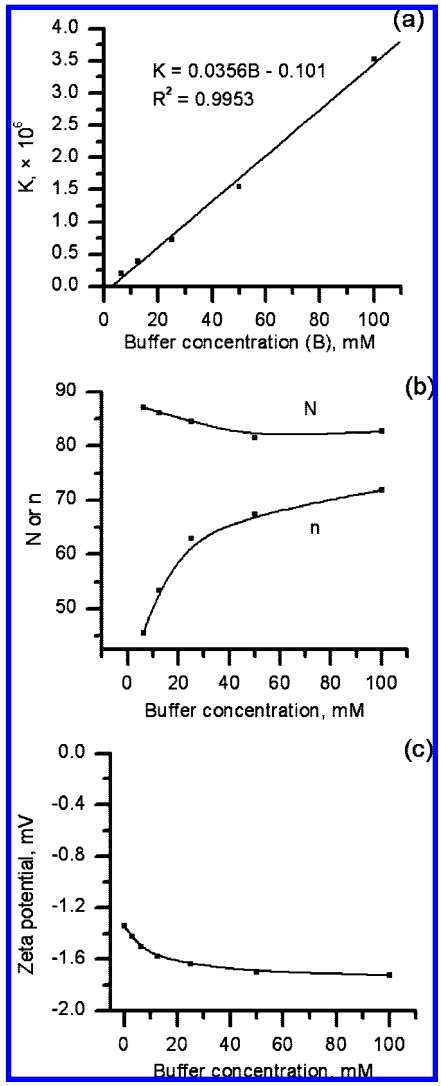

Figure 4. (**a**) Linear relationship between the binding equilibrium constant (*K*) and the concentration (*B*) of pH 10 Na_2CO_3/$NaHCO_3$ buffer. (**b**) Relationship between the total number of binding sites per glucan molecule (*N*) or the average binding number of Calcofluor per glucan molecule (*n*) and the concentration (*B*) of the same buffer. (**c**) Zeta potential of 3.33 $\times$ 10^{-7} M glucan in various concentrations of buffer solution. The data in **a**−**c** were obtianed at 20 °C.

spectrum of the same sample scanned for a consecutive three times showed little difference. This indicated the measurement itself did not affect the binding.

Factors That Influence the Adsorption of Calcofluor to *β*-Glucan. *Influence of Buffer Concentration.* It is easy to calculate the values of *K*, *N*, and *n* in various concentrations of the buffer using the data in **Figure 3a**. The binding equilibrium constant, *K*, of the reversible reaction has a positive linear correlation with the concentration of pH 10 Na_2CO_3/$NaHCO_3$ buffer, and the trend is shown in **Figure 4a**. The increase of *K* attributed to the hydration of the ions in the buffer, which disturbed the interactions between water and Calcofluor or β-glucan, thus facilitated the approach and affinity of Calcofluor and β-glucan molecules. Because the bound Calcofluor molecules increased with the rise of the buffer concentration, it was reasonable that the *K* value would increase. The total number of binding sites per glucan molecule (*N*) and the average binding number of Calcofluor per glucan molecule (*n*) showed opposite trends with increasing buffer concentration, which is shown in **Figure 4b**. The rising value of *n* meant there were more Calcofluor molecules binding on glucan, which was in accordance with the change in *K*, but the decline of the *N* value was unexpected. The changes in zeta potential of glucan at various buffer concentrations may shed some light on this abnormal downtrend. As shown in **Figure 4c**, the zeta potential of glucan was negative in the buffer, and the higher buffer concentration led to the decline of the zeta potential. In other words, the negative charge density at the surface of glucan increased with the elevated buffer concentration. These negative charges on the surface of glucan occupied the binding sites for Calcofluor and caused the decrease of *N*. Hence, increasing the buffer concentration had two aspects of impact on the binding of Calcofluor to glucan, including enhanced binding ability and decreased potential binding sites.

Because Calcofluor cannot be dispersed into a clear solution in pure water, pH 10 carbonate buffer was used to solubilize Calcofluor. The resulting relative standard deviations (RSD) of the three repeats of each determination were between 5 and 15% in carbonate buffer below 20 mM, whereas the RSD of the repeated determination was below 5% when the buffer concentration was above 20 mM, which indicated the combination between Calcofluor and β-glucan is more reproducible in relatively higher buffer concentration. Therefore, 100 mM carbonate buffer was used for the latter experiments to get more reliable results. We speculate that carbonate buffer above 20 mM could completely disperse Calcofluor into free molecules and that Calcofluor in carbonate buffer below 20 mM formed aggregates and also that the concentration of free Calcofluor could not be kept stable in the three repeats of measurement.

Influence of NaCl. NaCl was usually used to enhance the combination of Calcofluor with β-glucan (*13, 27*); hence, it is valuable to investigate the mechanism by which NaCl affects the binding of the two kinds of molecules. To obtain the best dissolution state of Calcofluor, 100 mM Na_2CO_3/$NaHCO_3$ (pH 10) was used as buffer solution. Because high concentration NaCl may decrease the solubility of Calcofluor and cause aggregates, the absorption intensity at 380 nm was determined at 25 °C (5 °C higher than previous experiment) to eliminate this possibility, although the NaCl concentration in the solution was relatively low and did not cause perceivable aggregation. It is evident from the *K* values shown in **Figure 5a** that the value of the binding equilibrium constant was in direct proportion to the NaCl concentration, which is similar to the effect of Na_2CO_3/$NaHCO_3$ on *K* (**Figure 4a**). The buffer with additional NaCl caused higher *N* and *n*, which is displayed in **Figure 5b**. The *N* value, which reflects the total number of binding sites per β-glucan molecule, remained at around 67 when the NaCl concentration was below 25 mM, and then the value of *N* increased sharply with additional NaCl until finally reaching 76 in 200 mM NaCl solution. The value of *n* has the ascending trend in accordance with that of *N*. The zeta potential of β-glucan in NaCl solution is shown in **Figure 5c**, and it is clear that the higher NaCl concentration elevated the zeta potential of glucan, which means the negative charge density on the surface of the glucan reduced and it is in favor of the binding of negatively charged Calcofluor on glucan. The higher salt concentration also increased the dielectric constant of the solution and enhanced the binding of Calcofluor on glucan molecules. We note that the *K* value at 100 mM buffer is 3.5 $\times$ 10^6 in **Figure 4a**, whereas in **Figure 5a** the *K* value for no NaCl additions, which means 100 mM buffer only, is only about 2.8 $\times$ 10^6. The discrepancy derives from different experimental temperature. Data for **Figure 4** were obtained from an experiment at 20 °C, whereas **Figure 5** used the data under 25 °C. The higher

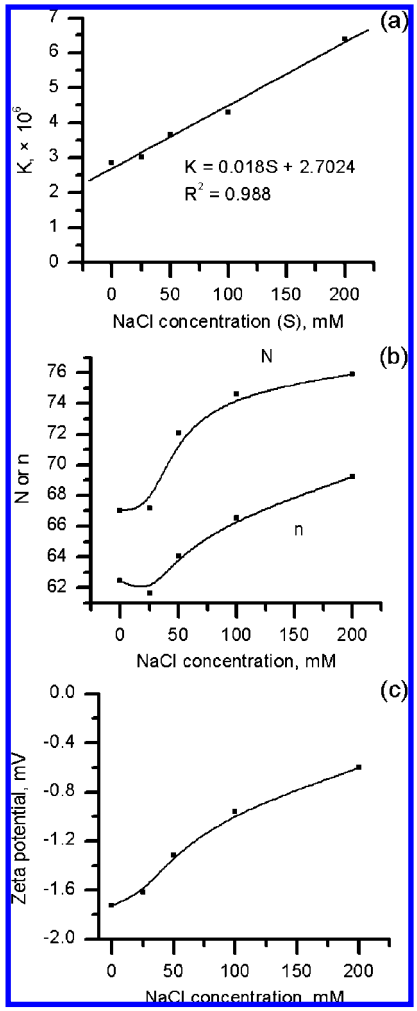

Figure 5. (**a**) Linear relationship between the binding equilibrium constant (*K*) and the NaCl concentration (*S*). (**b**) Relationship between the total number of binding sites per glucan molecule (*N*) or the average binding number of Calcofluor per glucan molecule (*n*) and the NaCl concentration. (**c**) Zeta potential of 3.33 × 10^{-7} M glucan in various concentrations of NaCl solution. The data in **a**−**c** were obtained in 100 mM, pH 10, Na_2CO_3/$NaHCO_3$ buffer at 25 °C.

temperature was not favorable for the binding and caused the decrease of *K* and *N*. The impact of temperature on the binding process will be discussed later.

Influence of Urea. To examine the possible role of hydrogen bonding, the binding test was carried out in the presence of urea. Urea is a strong hydrogen bonding acceptor, and it can affect the hydrogen bonding between the polymer and the small molecules in solution by preferential formation of hydrogen bonds between themselves and the polysaccharides or water (*33*). Absorption intensities at 380 nm of Calcofluor/β-glucan complex in the presence and absence of urea are shown in **Figure 6**. It is evident that urea prevented the binding of Calcofluor to β-glucan, which led to the decline of absorbance. Two molar urea seems to nearly completely prevent Calcofluor from binding to β-glucan. Therefore, it is proposed that hydrogen bonding plays an important role in the combination of Calcofluor and β-glucan.

The hydroxy groups in β-glucan or Calcofluor and imine groups in Calcofluor are functional groups responsible for hydrogen bonding between β-glucan and Calcofluor. After the formation of hydrogen bonding, the distance between aromatic rings in Calcofluor and sugar rings in β-glucan becomes very

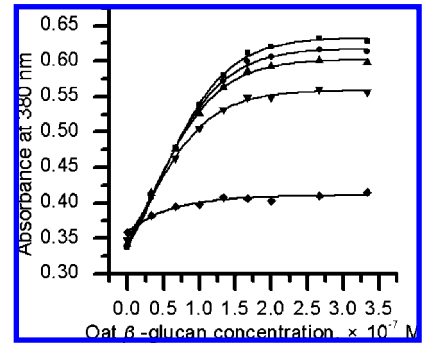

Figure 6. Absorbance at 380 nm for 1.09 × 10^{-5} M Calcofluor mixed with various concentrations of oat β-glucan in the presence of urea. From the highest curve to the lowest curve, the corresponding urea concentrations are 0, 0.25, 0.5, 1, and 2 M.

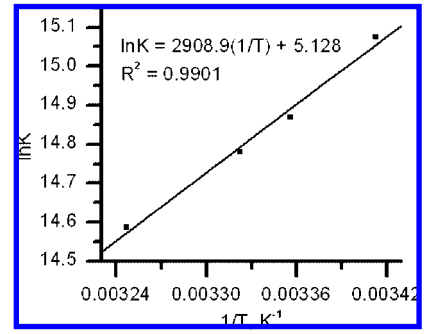

Figure 7. Gibbs–Helmholtz plot for the binding of Calcofluor to β-glucan.

low and permits the generation of van der Waals interactions. This attraction is favored by a large planar structure of the Calcofluor molecule (*28*). Therefore, there are at least two kinds of intermolecular forces, namely, hydrogen bonding and van der Waals forces, involved in the binding of Calcofluor with β-glucan.

Influence of Temperature. To explain the effect of temperature on the combination, the UV absorption intensities of Calcofluor with the various β-glucan concentrations were determined between 20 and 35 °C. Thermodynamic parameters were calculated on the basis of the temperature dependence of the binding equilibrium constant (*K*). The enthalpy change (Δ*H*°) was calculated from the slope, and the entropy change (Δ*S*°) was obtained from the intercept of the Gibbs–Helmholtz equation

$$\ln K = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (15)$$

where *R* is the gas constant and *T* is the absolute temperature. The free energy change (Δ*G*°) was calculated from the following relationship:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (16)$$

The thermodynamic parameters were calculated from the linear relationship between ln *K* and the reciprocal absolute temperature (**Figure 7**). The values of standard enthalpy change (Δ*H*°) and standard entropy change (Δ*S*°), which are shown in **Table 1**, indicate that the binding of Calcofluor to β-glucan is an exothermic process accompanied by a positive value of Δ*S*°. Because there are no charged groups in oat β-glucan, the electrostatic interactions between Calcofluor and β-glucan are negligible. With regard to typical hydrogen bonding and van der Waals forces, both Δ*H*° and Δ*S*° are negative. It is clear

Table 1. Thermodynamic Parameters for the Combination of Calcofluor with β-Glucan

temperature (K)	$\Delta G°$ (kJ mol^{-1})	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})
293	−36.68		
298	−36.89	−24.18	42.63
301	−37.02		
308	−37.32		

that neither of the forces can cause the positive entropy change, so there must be another interaction that causes the positive entropy change in the binding process. Positive entropy change may arise from hydrophobic interaction (*29, 30*); therefore, the binding process is likely to involve hydrophobic interaction. The enhancement effect of carbonate and NaCl on the binding of Calcofluor to oat β-glucan confirmed hydrophobic interactions between them. It is reported that glucose residues with β-linkages predominantly interact with the directly attached sugar residues and have a limited ability to be involved in water bridges (*32*). Oat β-glucan may be considered as having two hydrophobic surfaces with a hydrogen-bonding edge (*33*). The molecule retains much of the characteristics of the cellulose ribbon-like conformation including the extended, flat, hydrophobic surfaces of the glucopyranose ring. Planar Calcofluor molecules may be readily accommodated on oat β-glucan. This coplanar arrangement would also allow both hydrogen bonding and van der Waals forces to participate in binding (*31, 34*). The "hydrophobic effect" is primarily a consequence of changes in the surrounding water rather than water−solute interactions. When Calcofluor molecules bind to β-glucan, the ordered water molecules are released from the surface of the glucan and this positive entropy more than compensates for the decrease of entropy by the binding of Calcofluor on the glucan. The positive entropy change ($\Delta S°$) in **Table 1** is the result of the release of water from the hydrophobic surfaces of β-glucan in the binding process of Calcofluor to β-glucan. It is obviously shown in **Table 1** that the absolute value of free energy change ($\Delta G°$) increased with the rise of temperature, which can be ascribed to hydrophobic interaction rather than hydrogen bonding between β-glucan and Calcofluor (*35*).

Langmuir Adsorption Model. Considering the saturation adsorption phenomenon in the binding of Calcofluor to β-glucan, the Langmuir adsorption model was used to obtain a better understanding of the adsorption mechanism. According to the Langmuir adsorption model applied to the polymer system (*36*), surface coverage

$$\theta = \frac{(x/m)}{(x/m)_{max}} = \frac{bC}{1+bC} \quad (17)$$

where x is the amount of Calcofluor adsorbed, m is the mass of β-glucan, $(x/m)_{max}$ is the maximum amount of Calcofluor adsorbed per mass of β-glucan, b is the adsorption equilibrium constant, and C is the equilibrium solution concentration of Calcofluor. Equation 17 can be expressed as

$$\frac{C}{(x/m)} = \frac{C}{(x/m)_{max}} + \frac{1}{(x/m)_{max}b} \quad (18)$$

A plot of $C/(x/m)$ against C should yield a linear relationship. The values of $(x/m)_{max}$ and b can be determined from the slope and intercept of such a plot as shown in **Figure 8a**, in which experimental data of adsorption in the absence and presence of 200 mM NaCl were used. The adsorption isotherms calculated from the Langmuir model are shown in **Figure 8b**. All of the data in **Figure 8b** exhibit very good agreement with the

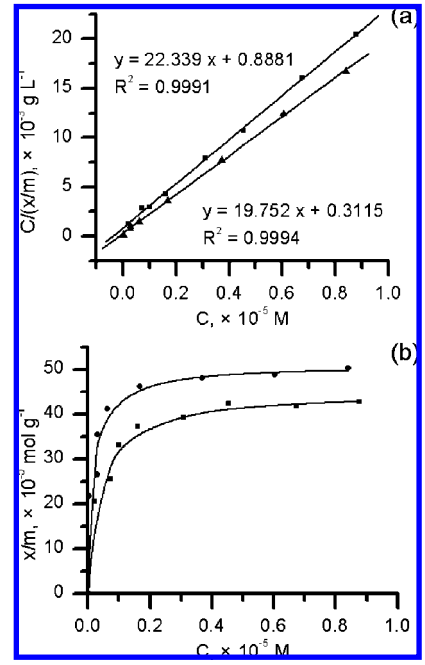

Figure 8. (**a**) Langmuir plot for the adsorption of Calcofluor on β-glucan in the absence (higher curve) and presence (lower curve) of 200 mM NaCl. (**b**) Adsorption data and fitted isotherm with Langmuir adsorption model in the absence (lower curve) and presence (higher curve) of 200 mM NaCl.

Langmuir adsorption isotherms. There are a number of surface sites on the β-glucan molecule responsible for binding with Calcofluor through hydrogen bonding, van der Waals forces, and hydrophobic interaction. When these binding sites were occupied by a single layer of Calcofluor molecules, the adsorption would reach saturation and the binding number of Calcofluor would come to a maximum. The nature of the binding of Calcofluor to β-glucan is in accordance with the basic assumptions of Langmuir adsorption, so the adsorption data fit the Langmuir model quite well.

Conclusion. In this work we have described the binding of Calcofluor to oat β-glucan in various solution environments through a UV spectroscopic method and thermodynamic analysis. UV spectroscopy proved to be a technique useful to study the binding of Calcofluor to oat β-glucan. Several factors that influence the binding were investigated. Our results showed that higher buffer concentration enhances the dissolution of Calcofluor and the combination of Calcofluor with β-glucan. NaCl can shield the negative charge on Calcofluor so as to reduce the electrostatic repulsion and improve the binding. Hydrogen bonding, van der Waals forces, and hydrophobic interaction are the major interactions between Calcofluor and β-glucan molecules. The data from the adsorption of Calcofluor to β-glucan show good agreement with the Langmuir adsorption model.

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