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Modelling of xyloglucan, pectins and pectic side chains binding onto cellulose microfibrils

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Abstract

Binding modelling of tamarind and pea xyloglucans, sugar beet and potato pectins, and pectic side chains (branched arabinan, debranched arabinan, galactan) onto microcrystalline Avicel cellulose and primary cell wall (PCW) cellulose was performed. The most commonly used binding models, namely the Langmuir, the Freundlich and the Scatchard models, were applied to the data. It appeared that the Freundlich model was more appropriate to describe the binding of all the polysaccharides used in this study. The heterogeneity index calculated from the slope of Freundlich isotherms highlights an important heterogeneity of Avicel and PCW cellulose surfaces, in agreement with the Scatchard representation.

Keywords: Adsorption isotherms; Langmuir model; Freundlich model; Heterogeneity; Mono/multilayers; Scatchard isotherm

1. Introduction

Pectins, xyloglucans and cellulose are the three main polysaccharides within the primary cell walls of higher plants. Pectins are bloc copolymers composed of two main domains, namely homogalacturonans (HG) and rhamnogalacturonans I (RG I) (O'Neill, Albersheim, & Darvill, 1990). HG is a linear chain composed of (1 → 4)-linked α-D-galacturonic acids units, whereas RG I contains the repeating disaccharide unit: (1 → 2)-α-L-rhamnopyranosyl-(1 → 4)-α-D-galacturonic acids (Renard, Crépeau, & Thibault, 1995). RG I are predominantly substituted by side chains composed mainly of arabinose and galactose residues (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Xyloglucans have a backbone composed of (1 → 4)-linked β-D-glucopyranosyl units that can be branched at *O*-6 by α-D-xylopyranosyl residues, which can be further substituted at *O*-2 by β-D-galactopyranosyl residues (Fry, 1989). Some of the galactose residues may be substituted at *O*-2 by α-D-fucopyranosyl residues. Cellulose microfibrils are formed by the association through hydrogen bonds of (1 → 4)-linked β-D-glucopyranosyl units to give highly crystalline and resistant structures called microfibrils. Cellulose microfibrils are supposed to be composed of a crystalline core and less organized (amorphous) regions at the microfibril surface (Earl & VanderHart, 1981). It is likely that crystalline and amorphous domains are linked together and arranged periodically along microfibrils (Nishiyama et al., 2003). The degree of cellulose crystallinity varies with respect to cellulose origin, from highly crystalline cellulose (bacteria or tunicin) to less crystalline one (Avicel or primary cell wall). The degree of cellulose crystallinity is proportional to the lateral size of cellulose microfibrils. It can then be thought that the total surface of low crystalline cellulose is bigger than that of highly crystalline cellulose (for the same amount of cellulose).

Both xyloglucans and neutral sugar-rich pectins are thought to be involved in the primary cell wall assembly.

Abbreviations: CWM, cell wall material; PCW cellulose, primary cell wall cellulose.

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Several authors have used *in vitro* approaches to demonstrate that xyloglucan can coat and/or tether cellulose microfibrils through non-covalent interactions (Hayashi, Marsden, & Delmar, 1987; Pauly, Albersheim, Darvill, & York, 1999; Vincken, Keizer, Beldman, & Voragen, 1995; Whitney, Brigham, Darke, Reid, & Gidley, 1995). Similar artificial composites created with pectins or isolated pectic domains have shown that pectins can bind to cellulose microfibrils too (Zykwinska, Ralet, Garnier, & Thibault, 2005; Zykwinska, Thibault, & Ralet, 2007a). In order to get more information about the properties of the cellulose surface (homo/heterogeneity), the binding affinity between xyloglucan or pectins and cellulose, and the possibility of mono/multilayer formation by xyloglucan or pectins adsorbed onto cellulose, the data can be fitted with different binding models. Two binding models are commonly used to describe binding data, namely the Langmuir and the Freundlich models. The Langmuir (Langmuir, 1918) model is based on a homogeneous surface and assumes a monolayer adsorption, whereas the Freundlich model (Freundlich, 1928) applies to adsorption processes on heterogeneous surfaces and can assume a multilayer adsorption. The Langmuir model was previously applied by Hayashi, Ogawa, and Hitsuishi (1994) to describe the binding of xyloglucan onto cellulose surface. A good fit of experimental data with this model allowed to suppose that xyloglucan binds to cellulose as a monolayer.

In the present study, the adsorption isotherms data of tamarind and pea xyloglucans, sugar beet and potato pectins, and pectic neutral sugar side chains onto Avicel and PCW cellulose obtained in our previous studies (Zykwinska et al., 2005, 2007b), were fitted to the Langmuir and the Freundlich models. The Scatchard modelling (Scatchard, 1949) was applied in order to complete the results obtained. Tamarind xyloglucan is an example of non-fucosylated storage xyloglucan, whereas pea xyloglucan is more representative of primary cell walls due to its fucosylation. Sugar beet and potato pectins can be considered as primary cell wall pectins because they are highly branched by neutral sugar side chains composed of, respectively, arabinose and galactose. Celluloses from two different origins: (i) commercial Avicel microcrystalline cellulose and (ii) PCW cellulose prepared from sugar beet were selected in order to study the influence of their structures on polysaccharide binding.

2. Materials and methods

2.1. Substrates

Non-fucosylated xyloglucan was extracted from a powder of tamarind seeds (Zykwinska et al., 2005), whereas fucosylated xyloglucan was isolated from pea pods cell wall material.

Arabinan-rich pectin (SB pectin) and galactan-rich pectin (P pectin) were extracted, respectively, from sugar beet and potato cell wall materials, as described elsewhere (Zykwinska et al., 2005).

Branched and debranched arabinans (from sugar beet) and galactan (from potato) were purchased from Megazyme (Ireland).

PCW cellulose was prepared from sugar beet cell wall material (CWM) as described by Heux, Dinand, and Vignon (1999) and Zykwinska et al. (2005). Briefly, sugar beet CWM was sequentially treated with hot dilute acid (0.1 M HCl, 85 °C, 3 × 30 min) and hot dilute alkali (0.5 M NaOH, 80 °C, 3 × 30 min) to solubilise pectins and hemicelluloses. PCW cellulose thereby obtained was suspended in distilled water, mixed in a Warring Blender and homogenized by 10 passes through an APV Gaulin homogenizer operating at 1000 bars.

Avicel microcrystalline cellulose PH-101 was purchased from Fluka (Germany).

2.2. Binding isotherms

Binding assays were performed as described elsewhere (Zykwinska et al., 2005). Briefly, solutions of the different polysaccharides were prepared at different concentrations and aliquots were mixed with a known amount of Avicel or PCW cellulose. After incubation under continuous head-over-tail mixing at 40 °C for 6 h, the blends were centrifuged, and the supernatants were tested for their total neutral sugar or galacturonic acid contents by colorimetric assays. The amount of adsorbed matter was calculated from the difference in sugar content measured for polysaccharides solutions and blends supernatants, taking into account the amount of sugars released in the blank (cellulose in Na–acetate buffer, pH 5.8). Binding assays were performed in triplicate. The average and the corresponding error of measurement were calculated for each point.

2.3. Analytical

Uronic acid (as galacturonic acid) and total neutral sugars (as arabinose or galactose for pectic neutral sugar side chains or glucose for xyloglucan) were determined colorimetrically by the automated *m*-hydroxybiphenyl and orcinol methods, respectively (Thibault, 1979; Tollier & Robin, 1979).

3. Results

3.1. Binding models

Binding modelling of tamarind and pea xyloglucans, sugar beet and potato pectins, as well as pectic side chains (branched arabinan, debranched arabinan, galactan) onto microcrystalline Avicel cellulose and PCW cellulose was performed. Tamarind and pea xyloglucans differ by their composition and molar masses. Tamarind xyloglucan is

an example of storage galactoxyloglucan of high molar mass estimated at 763 kDa (Zykwinska et al., 2005), whereas xyloglucan isolated from pea pods is fucosylated and thus more representative of hemicelluloses found in majority of primary cell walls. Its molar mass is more than 5 times lower compared to tamarind xyloglucan (143 kDa). Sugar beet pectin is particularly rich in arabinan side chains, which constitute 20% of the pectin weight, while potato pectin is highly branched by galactan side chains, representing 46% of the total pectin (Zykwinska et al., 2005). Both pectins are representative of primary cell wall pectins, in opposite to commercial pectins which are almost free of side chains due to their extraction in acidic conditions. Pectic arabinan and galactan side chains, isolated, respectively, from sugar beet and potato, are mainly composed of arabinose and galactose (Zykwinska et al., 2005). The two celluloses used in the present study were of similar composition. They are mainly composed of glucose, which represents more than 90% of the samples. In both cases, cellulose preparation did not lead to total extraction of pectic and hemicellulosic sugars, which represent less than 7% of the cellulose weight (Zykwinska et al., 2005). Despite their similar chemical composition, Avicel and PCW celluloses differ by their structures. PCW cellulose was defibrillated after extraction, which led to separation of cellulose microfibrils and formation of a stable suspension in water, whereas Avicel cellulose did not form a stable suspension, which suggests that cellulose microfibrils are more aggregated and present as bundles.

The binding data used for modelling of tamarind and pea xyloglucans, sugar beet pectin and pectic neutral sugar side chains were previously described and discussed (Zykwinska et al., 2005, 2007b). The values of maximum binding obtained for xyloglucans, pectins and pectic side chains onto cellulose (q_e) are presented in Table 1. Two different binding models were applied to the experimental data, namely the Langmuir and the Freundlich models.

The Langmuir model (Langmuir, 1918) can be expressed as follows:

$$q_e = \frac{q_m b C_e}{1 + bC_e} \tag{1}$$

where q_e is the amount adsorbed per mg of cellulose, q_m is the maximum adsorption capacity, b is the adsorption affinity and C_e is the concentration of free polymer remaining in solution at equilibrium concentration.

The Freundlich model (Freundlich, 1928) can be expressed by the following equation:

$$q_e = mC_e^{1/n} \tag{2}$$

where m is a complex function describing both the maximum capacity (q_m) and the average binding affinity (b), $1/n$ is the surface heterogeneity index, which varies from 0 for heterogeneous systems to 1 for homogeneous systems. The surface heterogeneity index depends on the surface properties, the crystallinity degree and the distribution of active adsorption sites.

Table 1
Constant parameters and correlation coefficients for the Freundlich model

	q_e (μg/mg of cellulose)	m	$1/n$	R^2
Tamarind xyloglucan/Avicel$_c$	13*	1.011	0.36	0.96
Tamarind xyloglucan/PCW$_c$	33*	7.725	0.23	0.90
Pea xyloglucan/PCW$_c$	112*	3.322	0.54	0.99
Sugar beet pectin/Avicel$_c$	4*	0.297	0.45	0.92
Sugar beet pectin/PCW$_c$	8*	0.550	0.44	0.91
Potato pectin/Avicel$_c$	2.5*	0.106	0.67	0.95
Potato pectin/PCW$_c$	9	0.175	0.43	0.97
Debranched arabinan/Avicel$_c$	11*	0.072	0.77	0.98
Debranched arabinan/PCW$_c$	15.5*	0.119	0.71	0.96
Branched arabinan/Avicel$_c$	5*	0.015	0.90	0.98
Branched arabinan/PCW$_c$	7*	0.270	0.42	0.91
Galactan/Avicel$_c$	5*	0.090	0.61	0.98
Galactan/PCW$_c$	14.5*	0.307	0.47	0.89

Freundlich constant $1/n$ and m were obtained from the linear equations depicted in Figs. 3–5. The values for R^2 are the correlation coefficients for the linear relationships.

* q_e – amount of adsorbed matter (μg) per mg of cellulose (data from Zykwinska et al., 2005, 2007b).

The experimental binding isotherms data were plotted by the linearized forms of the two models with the following equations:

$$\frac{C_e}{q_e} = \frac{1}{bq_m} + \frac{C_e}{q_m} \quad \text{Langmuir} \tag{3}$$

$$\ln(q_e) = \ln(m) + \frac{1}{n}\ln(C_e) \quad \text{Freundlich} \tag{4}$$

The experimental data for xyloglucans, pectins and pectic neutral sugar side chains used in binding assays with Avicel and PCW cellulose could not be linearized by the Langmuir model in the whole range of concentrations used in this study. Fig. 1 presents an example of a Langmuir isotherm obtained for tamarind xyloglucan adsorbed onto Avicel cellulose. It appeared that two lines are required to fit the data, the first one in the range of low concentrations from 30 μg/mL to 100 μg/mL and the second one in the concentration range from 150 μg/mL to 1000 μg/mL of xyloglucan. The maximum adsorption capacity, q_m calculated from the inverse of a slope of linear plots are 4.3 μg/mg and 17.9 μg/mg of cellulose for plots obtained in low and high range of concentrations, respectively. Very similar plots were obtained for xyloglucan bound onto PCW cellulose. An example of binding isotherm of one of the neutral sugar side chains adsorbed onto PCW cellulose surface is presented in Fig. 2. Two lines were necessary to fit the debranched arabinan data, as previously observed for xyloglucan. The maximum adsorption capacity value q_m of 5 μg/mg of cellulose was calculated for a plot obtained in low range of concentrations, while an infinite value of q_m was obtained for a plot in high range of concentrations. Two linear plots were also obtained for

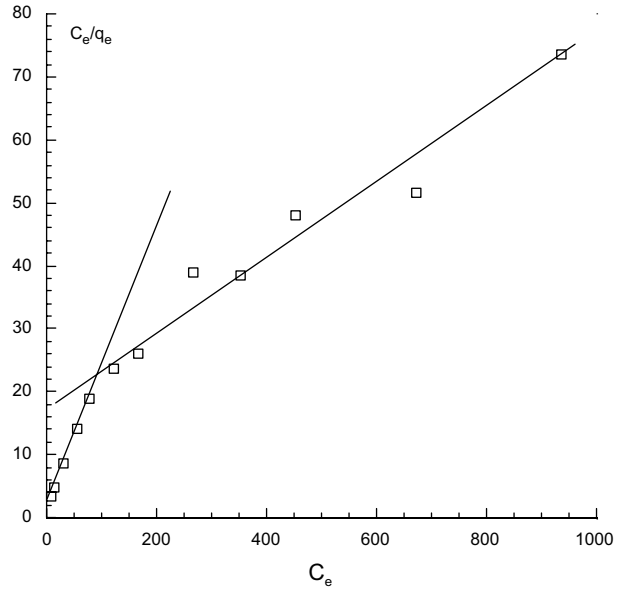

Fig. 1. Langmuir isotherm for adsorption of tamarind xyloglucan onto Avicel cellulose.

branched arabinan and galactan side chains adsorbed onto Avicel and PCW cellulose.

On the opposite, xyloglucans, pectins and neutral sugar side chains binding results fitted extremely well to the linear form of the Freundlich model in the whole range of concentrations used. The values for the slope (the heterogeneity index, $1/n$) and intercept ($\ln m$, given as m) of the linear regression lines together with the correlation coefficients (R^2) are reported in Table 1. The linearized plots of the Freundlich model obtained for xyloglucans binding in tamarind xyloglucan/Avicel and tamarind or pea xyloglucans/PCW cellulose systems are presented in Fig. 3. Heterogeneity index $1/n$ of 0.23, 0.36 and 0.54, and m parameter of 1.011, 7.725 and 3.322 were obtained, respectively, for tamarind xyloglucan/Avicel cellulose, tamarind and pea xyloglucans/PCW cellulose systems (Table 1). The greater m value obtained for tamarind xyloglucan adsorbed onto PCW cellulose is in good agreement with the binding isotherms. Indeed, the *in vitro* adsorption of tamarind xyloglucan onto PCW cellulose was about 3 times greater than the adsorption onto Avicel cellulose. It is worth mentioning that pea xyloglucan exhibited the most important binding onto cellulose microfibrils (Table 1).

Binding data obtained for sugar beet and potato pectins were also well fitted to the linear form of the Freundlich model (Fig. 4). The heterogeneity index $1/n$ for both pectins was <1 (Table 1). The m parameter values of 0.23 and 0.55 were obtained for sugar beet pectin adsorbed onto Avicel and PCW cellulose, respectively, while values of 0.106 and 0.175 were calculated for potato pectin bound onto Avicel and PCW cellulose (Table 1). The m parameter values obtained are in good agreement with the binding data, where PCW cellulose is able to adsorb more pectins than the Avicel one (Table 1).

The linear plots of debranched arabinan, branched arabinan and galactan side chains are presented in Fig. 5. The heterogeneity index $1/n$ calculated for all pectic side chains varied from 0.42 to 0.90 (Table 1). The m parameter values, greater for side chains adsorbed onto PCW cellulose than onto Avicel one, are in good concordance with the binding results. Indeed, debranched arabinan and galactan displayed more important binding to PCW cellulose than to Avicel one. On the opposite, very similar

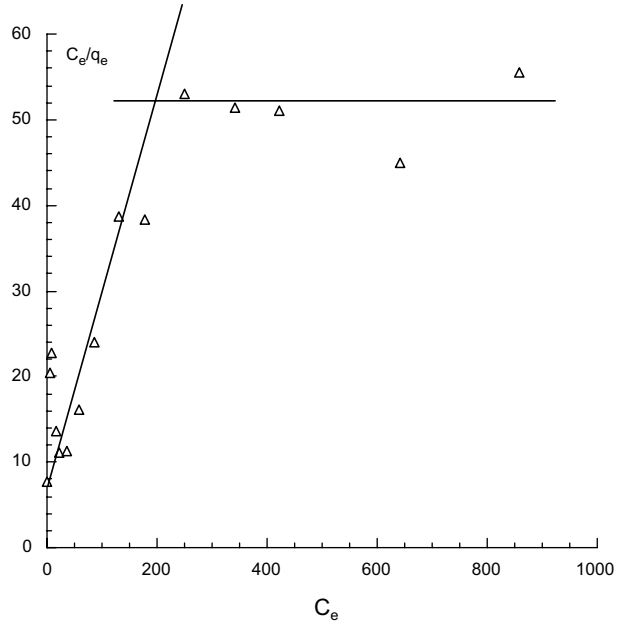

Fig. 2. Langmuir isotherm for adsorption of debranched arabinan onto PCW cellulose.

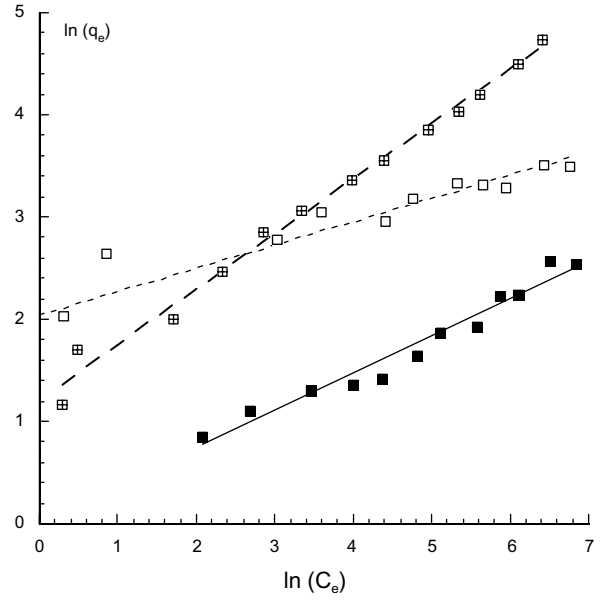

Fig. 3. Freundlich isotherm for adsorption of tamarind xyloglucan onto Avicel (■) and PCW (□) celluloses, and pea xyloglucan onto PCW cellulose (⊞).

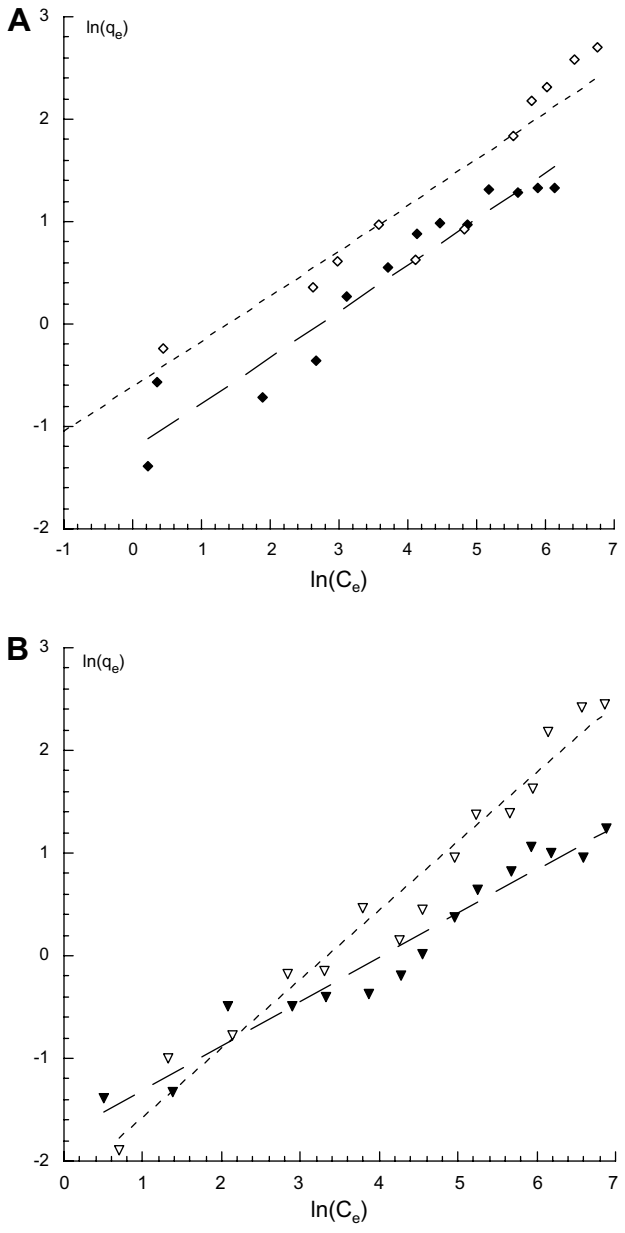

Fig. 4. Freundlich isotherms for adsorption of (A) arabinan-rich sugar beet pectin onto Avicel (♦) and PCW (◇) celluloses; (B) galactan-rich potato pectin onto Avicel (▼) and PCW (▽) celluloses.

bindings were observed for branched arabinan side chains adsorbed onto both PCW and Avicel celluloses (Table 1). It is worth mentioning that *m* parameter values obtained for pectic neutral sugar side chains are very low in comparison to the ones calculated for xyloglucan.

3.2. Scatchard plots

The Scatchard representation was applied to the binding results. Fig. 6 presents plot examples obtained for tamarind and pea xyloglucans, galactan and debranched arabinan side chains. A concave shape of the curves was observed for both xyloglucans adsorbed onto PCW cellulose (Fig. 6A), sugar beet and potato pectins (data not shown), branched arabinan (data not shown) and galactan side chains (Fig. 6B) adsorbed onto Avicel and PCW cellulose. A concave curves shape is usually attributed to binding site heterogeneity, indicating the presence of more than one class of binding sites onto solid surface and an anticooperative mechanism of adsorption of soluble polymers, suggesting that the adsorption of one molecule precludes the adsorption of a second one (Cantor & Schimmel, 1980, chap. 5). The shape of the curves observed for debranched arabinan adsorbed onto Avicel and PCW cellulose (Fig. 6C), different from those obtained for xyloglucans, pectins or branched arabinan and galactan side chains, may indicate that the presence of debranched arabinan chains bound onto cellulose surface neither prevents nor favours the adsorption of another one.

4. Discussion

In the present work, the linear form of the Langmuir and the Freundlich models were applied to the binding data obtained for tamarind and pea xyloglucans, pectins and pectic side chains adsorbed *in vitro* onto Avicel and PCW cellulose (Zykwinska et al., 2005, 2007b). Our previous results revealed that pectins rich in neutral sugar side chains are able to bind to cellulose microfibrils under *in vitro* conditions. The use of isolated pectic domains representative of the entire pectin allowed to determine that the interactions with cellulose are most likely mediated through pectic neutral sugar side chains (Zykwinska et al., 2007b). In order to get more information about xyloglucans, pectins and pectic side chains binding onto cellulose surface (e.g. mono/multilayer formation, cellulose surface homo/heterogeneity), we applied few binding models to the adsorption data.

It appeared that the Langmuir model was inadequate to fit the binding results obtained for xyloglucans, pectins and pectic side chains in the whole range of concentration used (2.5–1000 μg/mL). Hayashi et al. (1994) reported that the xyloglucan binding to cellulose could be fitted by the Langmuir model. However, these authors used a narrow range of concentrations (200–1000 μg/mL). The Langmuir model is useful when there is a strong specific interaction between the surface and the adsorbate so that a single adsorbed layer is formed and no multilayer adsorption occurs. Indeed, this could be the case of xyloglucan/cellulose associations. An outstanding binding between xyloglucan and cellulose is most likely to be due to excellent surface complementarities between cellulose and cellulose-like backbone of xyloglucan, as showed by Levy, York, Stuike-Prill, Meyer, and Staehelin (1991). This remarkable complementarity is obtained by the change of xyloglucan conformation from a "twisted" to a "flat" one, required for the interaction with cellulose. Recently, Hanus and Mazeau (2006) studied the assembly of xyloglucan and cellulose at the atomistic scale using molecular dynamics simulations. The authors used Iβ allomorph as a model

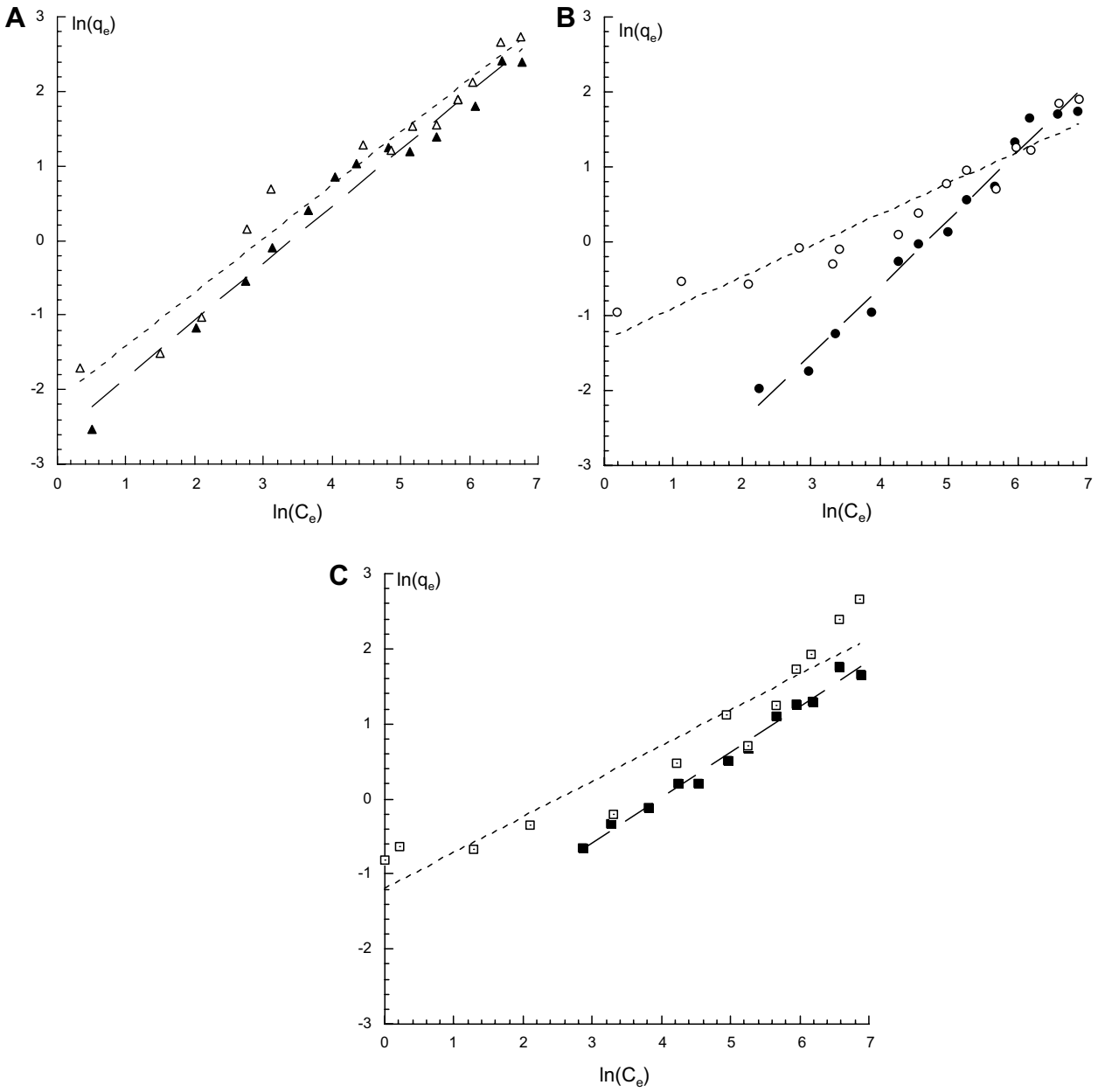

Fig. 5. Freundlich isotherms for adsorption of (A) debranched arabinan onto Avicel (▲) and PCW (△) celluloses; (B) branched arabinan onto Avicel (●) and PCW (○) celluloses; (C) galactan onto Avicel (■) and PCW (⊡) celluloses.

of cellulose microfibrils with five crystalline surfaces: (1 1 0), (1 0 0), (0 1 0), (1 1 0) and (1 −1 0). The (1 0 0) surface is flat and hydrophobic because the CH groups are exposed at the surface. The (1 1 0) and (1 −1 0) surfaces are hydrophilic, where hydroxyl groups pointed outward are involved in hydrogen bonding formation. It came out that all cellulose microfibril surfaces bind the xyloglucan molecules with comparable efficiency. Adsorption of xyloglucan onto cellulose surfaces is stabilized by both electrostatic and van der Waals contributions (Hanus & Mazeau, 2006). However, the presence of hydrophilic and hydrophobic surfaces onto cellulose microfibrils suggests two different binding sites that may (but do not have to) exhibit two different binding affinities *versus* the adsorbate molecules. Therefore, it can be thought that the Langmuir model which assumes that there is only a single class of binding sites is not well adapted to study the binding of xyloglucan or pectic side chains onto cellulose surface. Indeed, the Freundlich model was more appropriate to describe the binding data of xyloglucan and pectic neutral sugar side chains. The Freundlich model assumes an infinite number of different types of binding sites differing in their binding affinity. Indeed, an important heterogeneity of cellulose surface was suggested by the heterogeneity index $1/n$ calculated from the slope of linear Freundlich isotherms. It came out that the heterogeneity index was

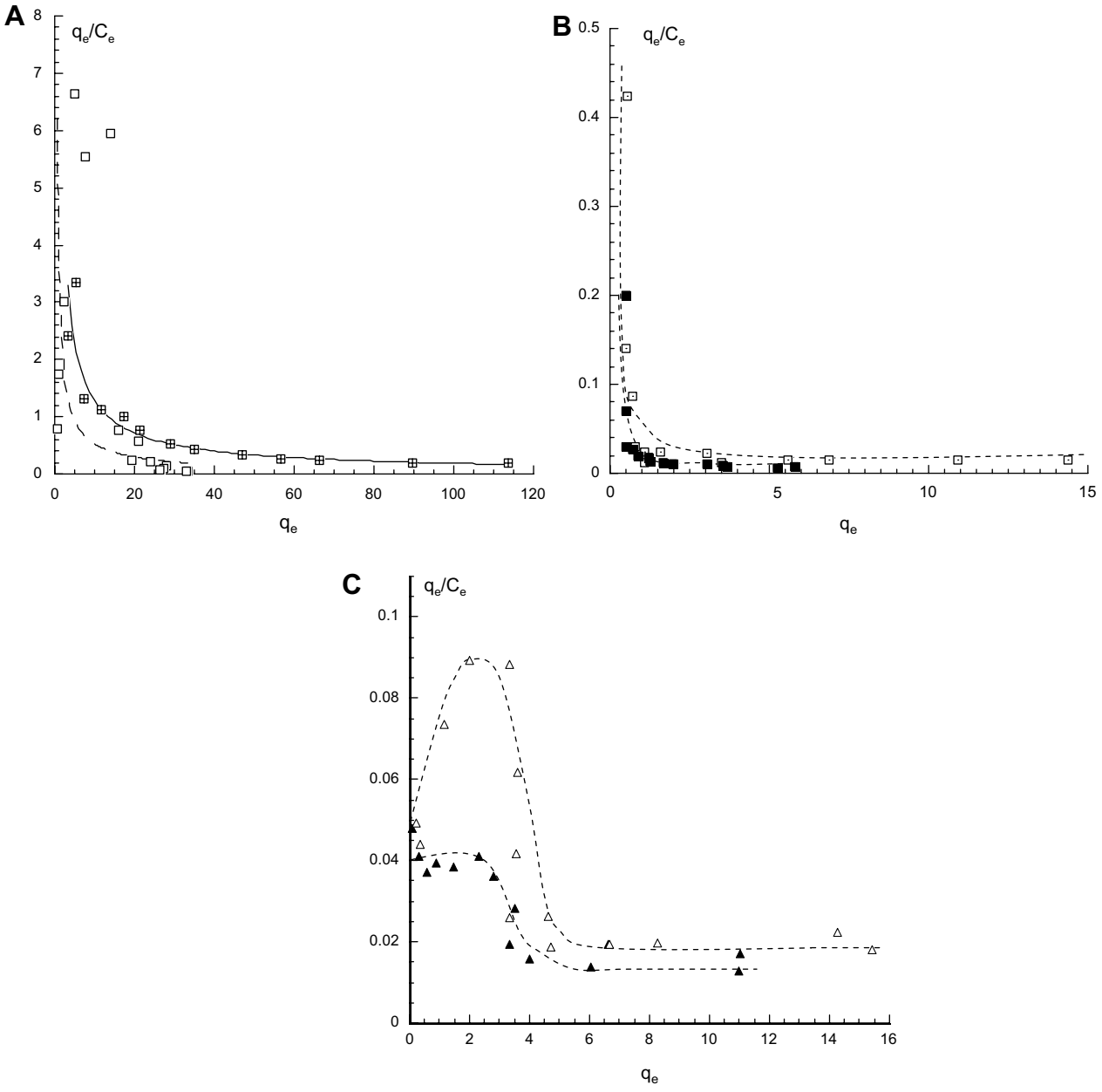

Fig. 6. Scatchard plots for adsorption of (A) tamarind xyloglucan (□) and pea xyloglucan (⊞) onto PCW cellulose; (B) galactan onto Avicel (■) and PCW (⊡) cellulose; (C) debranched arabinan onto Avicel (▲) and PCW (△) celluloses.

lower than 1 for all polysaccharides used and lower values were obtained for the polysaccharides adsorbed onto PCW cellulose than for polysaccharides adsorbed onto Avicel one. This observation suggests that PCW cellulose surface available for binding is either more heterogeneous (more binding sites available) or bigger than the Avicel one.

The possibility that xyloglucan is able to form multilayers onto cellulose surface is very low. Indeed, when taking into account the adsorption mechanism between xyloglucan and cellulose, the self-association of xyloglucan molecules is very unlikely (Levy et al., 1991). It can then be hypothesized that the Freundlich model used to describe the xyloglucan binding highlights more the important heterogeneity of the cellulose surface than the possibility of multilayer formation. On the opposite, the Freundlich model applied to debranched arabinan side chains adsorbed onto cellulose surface may suggest either the heterogeneity of cellulose surface or the formation of a multilayer system. The lack of branches onto arabinan backbone may facilitate, on the one hand, the alignment with cellulose surface, and on the other hand, with another arabinan molecule. The hypothesis of multilayer formation by debranched arabinans was also suggested by the fact that an infinite value of maximum adsorption capacity (q_m) was calculated from the Langmuir plot. Moreover, the Scatchard representation also seems to sup-

port a multilayer binding mechanism. Contrary to debranched arabinan, branched arabinan and galactan side chains are most likely not able to form multilayers. Assuming that adsorption mechanism of branched pectic side chains is similar to that of xyloglucan, the presence of short branches onto arabinan and galactan backbones pointed out are probably the limiting factor for self-association due to the steric hindrance of the substituants. Additionally, the Scatchard representation suggests an anticooperative binding mechanism for both side chains. It can also be thought that the complex pectin structure limits the possibility of a multilayer formation.

In the present work, the binding data describing the interaction between xyloglucans, pectins or pectic neutral sugar side chains and cellulose were fitted with the Langmuir, the Freundlich and the Scatchard models. Both Freundlich and Scatchard isotherms highlight the important heterogeneity of cellulose surfaces, with at least two different main binding sites. Moreover, all models applied suggest the possibility of multilayer formation by self-association of debranched arabinan chains.

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