JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 11, No. 10, October 2009, p. 1428 - 1431

Binding of cationic polyelectrolytes to pectin in solution and in multilayered structures♣

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The binding of two polycations, poly-L-lysine (PLL) and chitosan, to pectin in dilute solutions and in multilayered structures on a solid substrate was investigated. The attractive electrostatic interaction was emphasized as a main factor, affecting the interaction between the examined polyelectrolytes. pH ranges, at which binding caused formation of soluble and insoluble complexes, were qualitatively determined by turbidimetric titration. Potentiometric titration of pectin solutions with PLL or chitosan was used for measuring the concentration of the bound polycation, and for determining the binding isotherm. Surface Plasmon Resonance was used for building-up and monitoring the kinetics of the multilayer deposition. The binding parameters in solutions and multilayers were evaluated by Hill's equation and Karlsson's model respectively. In both systems, the binding of PLL to pectin was cooperative, whereas that of chitosan was anti-cooperative. The observed phenomena could be explained by the larger flexibility of the PLL chain. Because of the cooperative effect, the binding constant of the PLL/pectin interaction is higher for concentrated systems.

(Received November 5, 2008; accepted December 15, 2008)

Keywords: Binding constant, Polyelectrolyte, Multilayer, Cooperative binding, Anticooperative binding

1. Introduction

The interactions of polyelectrolytes, governed mainly by electrostatic forces, are a subject of growing interest, because they provide a possibility for the development of new materials with desirable properties [1]. Due to the large size of the macromolecules and the interactions between the binding sites, the polyelectrolyte binding may exhibit cooperativity. Positive or negative cooperativity could be distinguished, based on the effect of the already bound polyions on the binding affinity [2]. The simplest model (Hill's equation), analyzes the binding equilibrium for ligand-receptor interactions:

$$\lg(L) = -\frac{1}{\alpha_H}\lg\left(\frac{n_H}{\nu} - 1\right) - \lg K_B \qquad (1)$$

where ν is the amount of the ligand bound by the receptor; L is the concentration of the free ligand; n_H is the number of sites per segment able to bind ligands; K_B is the binding constant and α_H is the Hill constant, which is an index of the cooperativity. When $\alpha_H > 1$, the binding is cooperative and anticooperative when $\alpha_H < 1$.

Hill's equation does not reflect a physically possible reaction scheme for a receptor with more than one ligand binding site. An appropriate model, which takes into account the nonspecific binding, "overlapping binding site" effect and interactions between the ligands has been proposed by McGhee–von Hippel [3]. However it describes only interactions between adjacent bound ligands, and any interactions between bound ligands separated by one or more monomer residues are completely ignored. According to Weiss [4], the results for data evaluation with models taking into account the ligand interactions are very close to those obtained by the Hill equation.

Pectin – a plant polyanionic saccharide, is subjected to intensive investigations because of its functions in plant tissues, various of applications in the food industry, biocompatibility and biodegradability [5]. Its polyelectrolyte complexes with polycations possess their own antibacterial activity, and are promising materials for pharmaceutical and food packaging [6, 7].

The present work aims to investigate and to compare the binding behaviour of flexible (PLL) and stiff (chitosan) polycations to pectin, in solution and in multilayered structures.

♣ Paper presented at the International School on Condensed Matter Physics, Varna, Bulgaria, September 2008

2. Experimental

2.1 Materials

Enzyme deesterified pectin with a molecular weight of 120,000 was obtained from CP Kelco. Reported values for the degree of esterification and the galacturonic acid content were 36.7% and 92.4% respectively. PLL hydrobromide, molecular weight 70,000-150,000, and LMW chitosan, with degrees of deacetylation of 75–85%, were bought from Sigma and used without further purification. All other chemicals were of analytical grade.

2.2 Binding in polyelectrolyte solutions

Potentiometric titrations were done with a two-point calibration Corning pH-meter 240, equipped with a combined electrode, under N_2, at 25 °C. In "Type 1" titrations [8], a 0.1 M NaOH was added to 3 wt % pectin in 0.03 M NaCl solution with 1 wt% polycation. In "Type 3" titrations, 25 g/L polycation in 0.03 M NaCl was added gradually to 15 mL of 1.0 g/L pectin in 0.03 M NaCl, after adjusting both to the desired initial pH. The method applied for the investigation of the binding equilibrium was based on measuring the amount of dissociated H^+ ions from the differences in the pH values for "Type 3" titration. In solutions of a weak polymeric acid (like pectin) and a polymeric cation (PLL or chitosan), it is equal to the amount of bound polycation (expressed in monomer units) [9]. Binding isotherms were evaluated by Hill's equation (eq. 1). Turbidimetric "Type 1" titration was carried out with a "SPECOL 11" spectrophotometer at a wavelength of 420 nm. The spectrophotometer was calibrated to 100%T with distilled water. Titrations were carried out as described above, and the turbidity was reported as 100–%T.

2.3 Binding in polyelectrolyte multilayers

Surface Plasmon Resonance Spectroscopy (SPRS) was applied for the formation of multilayered structures and for monitoring the kinetics of polyelectrolyte deposition. A BIAcore instrument with a BIAcore sensing chip was used as described elsewhere [10]. For layer deposition, 0.08 mg/mL solutions of the polymers were prepared in a 0.05 M pH 7.0 phosphate buffer containing 0.05 M NaCl. The layers were laid down by injecting consecutively 50 μL of polyelectrolyte solutions and 75 μL of buffer. The flow rate was the same for all solutions – 5μL/min. Under these , the adsorption rate was kinetic limited [10], and the genuine value for the binding constant could be evaluated. The data analysis was done with the Karlssonmodel [11]:

$$\frac{[AB]}{[AB_{\max}]} = \frac{\exp\left(\left(f_1 + f_2\right)k_a[A]t\right) - 1}{f_1 + f_2\exp\left(\left(f_1 + f_2\right)k_a[A]t\right)} \quad (2)$$

where $[AB]$ is the concentration of the complex at time t; $[AB_{\max}]$ is the concentration of the complex at saturation; k_a is the kinetic rate constant, $[A]$ is the concentration of the injected sample; f_1 is a factor, through which the apparent kinetic rate constant will depend on the saturation level – negative values of f_1 will result in a decreasing k_a with increasing saturation levels; f_2 is a factor determining the number of binding sites that disappear for each binding event.

3. Results

3.1 Binding of PLL and chitosan to pectin in solution

Results from the "Type 3" turbidimetric titration are presented in Fig. 1.

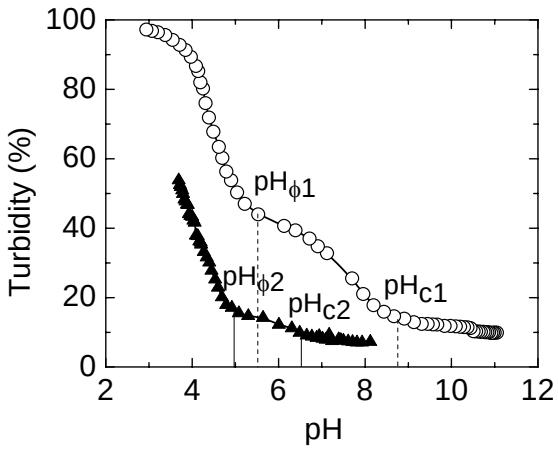

Fig. 1. Turbidimetric titration of pectin with PLL – ○ and chitosan –▲.

A region of soluble complex formation was revealed by the increase in the turbidity between the pH values of pH_c and pH_ϕ. At pH values greater than pH_c the coulombic attractive interactions were not sufficiently strong to ensure complex formation. At pH values less than pH_ϕ a substantial increase in the turbidity indicated the presence of insoluble precipitates. Further studies on the pectin complexes were done in the range pH_c–pH_ϕ.

The effect of polycation concentration on the pH values of the mixed pectin/polycation solutions is shown in Fig. 2. The diminution in pH upon the addition of he polymer must arise from the release of H^+ ions from the carboxyl groups of pectin, due to the binding of polycation.

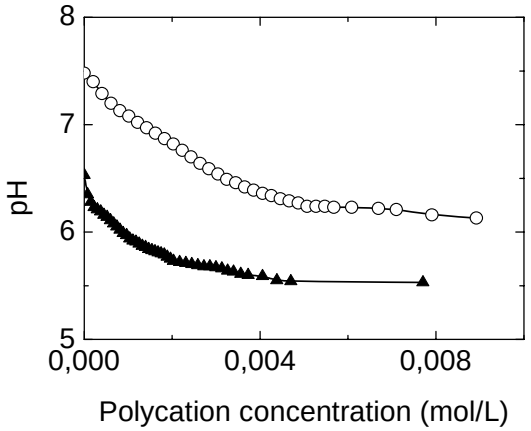

Fig. 2. Effect of polycation concentration on the pH of the mixed solutions: ○ – PLL; ▲ –chitosan.

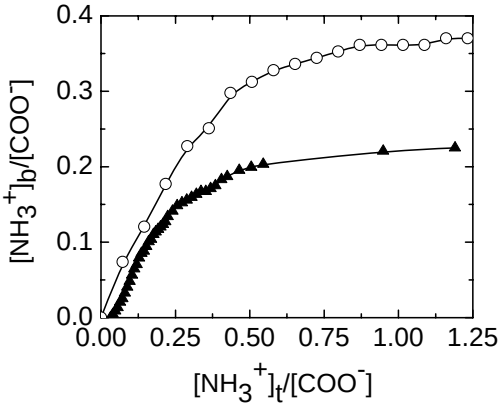

Fig. 3. Binding isotherms of PLL – ○ and chitosan –▲ to pectin.

The increase in the hydrogen ion concentration is relevant to the amount of polycation bound, and therefore the binding isotherms could be evaluated as in Fig. 3. They present the amount of bound polycation as a function of its total amount.

The values of the parameters in the Hill's equation, as obtained from the binding isotherms, are presented in Table 1.

Table 1. Hill's parameters for polycation binding in solution.

Polycation	α_H	K_B	R^2
PLL	2.66±0.2	286±12	0.99
Chitosan	0.9±0.06	196±8	0.99

3.2 Binding of PLL and chitosan to pectin in multilayered structures

Binding of the polyelectrolytes in multilayered structures was examined by a BIAcore SPR refractometer. All the data are presented in responsible Units, which are directly proportional to the surface refractive index, as in Fig. 4.

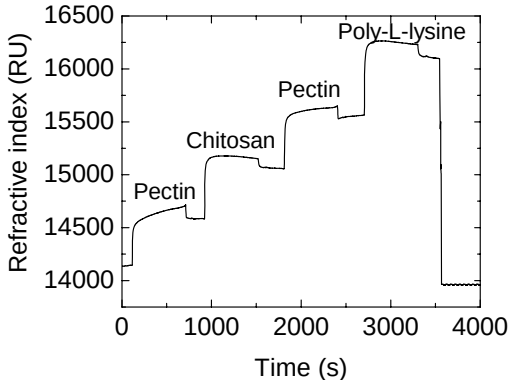

Fig. 4. Plot of resonance response versus time for the multilayers built-up.

The increase in the refractive index confirmed the deposition of the polyelectrolyte on the surface during each injection. The deposition was irreversible over the time of experiment. The decay in the responsible signal immediately after the end of the injection was due to the smaller refractive index of the buffer solution, in comparison to that of the polymer solutions.

The binding at each deposition step was analyzed based on equation (2). The values for the parameters are presented in Table 2.

Table 2. Parameters of polycation binding in multilayer structures, based on Karlsson's model.

Polycation	k_a, $Lmol^{-1}s^{-1}$	f_1	f_2	R^2
PLL	1672±23	1.12	1.02	0.98
Chitosan	274±7	-0.51	0.98	0.99

4. Discussion

The main reason for the binding of polyelectrolytes is the electrostatic attractive interaction between oppositely charged functional groups. The data, obtained from the turbidimetric titration (Fig. 1) confirmed that PECs could be formed in a pH range where both of the polyelectrolytes are charged. The increase of the charged density leads to a transition from water soluble to insoluble complexes. Since the pK_a value of PLL is higher than that for chitosan (10.72 and 6.5 respectively), PECs between pectin and PLL formed at greater pH values.

As could be seen from the binding isotherms (Fig. 3), both polycations possessed high binding affinities to pectin at low concentrations. The binding isotherm for PLL showed little tendency to reach a plateau at $[NH_3^+]_t/[COO^-]$ ratios less than 1.25. In contrast, the asymptotic behaviour of the chitosan binding isotherm determined the point at which saturation occurred and no more chitosan could be bound. The plateau level was less than 1 ($[NH_3^+]_b/[COO^-]$=0.2), and therefore some hindrance for further binding must exist.

The detailed analysis of the binding parameters (Table 1) suggested that the main difference between the binding of PLL and chitosan was the values of Hill's parameter and the factor f_1. They indicated that the binding is cooperative in the case of PLL, and anticooperative for chitosan. Similar results were achieved for the binding behaviour of PLL and chitosan to pectin in gels [12]. This observation could be related to the fact that PLL possesses a flexible chain, which makes the motion of the macromolecules and the binding easier. The value of the binding constant K_B for PLL in solution is smaller than in pectin/PLL gels [12], which is particular to the cooperative binding and is in good agreement with the results of other authors [13]. The effect of chain flexibility on the cooperativity was more pronounced for the binding of the polycations to pectin in multilayer structures. Polymer adsorption on a solid surface is frequently a non-equilibrium process [14]. Following an initial contact and attachment to the surface, there is a process of structural rearrangement and spreading of the polymer on the surface. As a result of the relatively rigid structure of chitosan, its binding to the surface will impede the spreading process. The extension of the loops and tails prevents further molecular deposition, and the binding is anticooperative. The values of K_B for both PLL and chitosan were larger than those observed for the binding of Ca^{2+} to pectin [13]. This phenomenon could be explained by the important role of the polyelectrolyte chain length in the cooperative electrostatic interaction [15]. An expression relating the equilibrium binding constant K_B between two polymers to the equilibrium constant of binding between one oligomer unit and the matrix (K_1) has the form:

$$K_B = K_1^n = \exp\left(-\frac{n\Delta G_1^0}{RT}\right) \quad (3)$$

where n is the degree of polymerization and ΔG_1^0 is the change in the free energy caused by the interaction of a monomer unit with the matrix. It is evident that the value of the binding constant increases with an increasing degree of polymerization.

5. Conclusions

Binding of PLL and chitosan to pectin was investigated in solutions and in multilayers. Due to its flexible molecule PLL binds to pectin cooperatively. The stiff chain of chitosan hampers the electrostatic interaction and does the binding anti-cooperative.

Acknowledgements

This study was supported by Projects No.IS-6-F and. MU-20 to the PU Research Activity Dept.

References

[1] V. A. Kabanov, Russia Chemical Reviews **74**(1), 3 (2005).
[2] Ch. R. Cantor, P. R. Schimmel, in Biophysical Chemistry, W. H. Freeman, London, 1980.
[3] J. D. McGhee, P. H. von Hippel, J. Mol. Biol. **86**, 469 (1974).
[4] J. N. Weiss, The FASEB Journal **11**, 835 (1997).
[5] B. R. Thakur, R. K. Singh, A. K. Handa, Crit Rev Food Sci Nutr. **37**(1), 47 (1997).
[6] L. S. Liu, M. L. Fishman, K. B. Hicks, Cellulose **14**(1), 15 (2007).
[7] P. A Williams, G. O. Phillips, Gums and Stabilisers for the Food Industry 14, The Royal Society of Chemistry, Cambridge, 2008.
[8] Y. P. Wen, P. L. Dubin, Macromolecules **30**, 7856 (1997).
[9] A. B. Zezin, V. A. Kabanov, Russian Chemical Reviews **51**(9), 1447 (1982).
[10] M. Marudova, S. Lang, G. J. Brownsey, S. G. Ring, Carbohydrate Res. **340**, 2144 (2005).
[11] R. Karlsson, H. Roos, L. Fagerstam, B. Persson, METHODS: A Companion to Methods in Enzymology **6**, 99 (1994).
[12] M. Marudova, A. J. MacDougall, S. G. Ring, Carbohydr. Res. **339**, 209 (2004).
[13] C. Garnier, A. V. Axelos, J.-F. Thibault, Carbohydr. Res. **256**, 71 (1994)
[14] J. J. Ramsden, Quart. Rev. Biophys. **27**, 41 (1994).
[15] V. A. Izumrudov, Russia Chemical Reviews, **77**(4), 401 (2008).

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