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Induced circular dichroism of the interaction between pinacyanol and algal alginates

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ABSTRACT

The interaction between pinacyanol chloride and sodium alginate or guluronate-rich alginate is found to effect profound changes in the visible absorbance and circular dichroism spectra. Two different types of aggregates are observed depending on the relative dye/alginate concentrations. With a dye/alginate ratio at 1:1, a complex is deduced based on an analysis of Job's method and conductometric titrations. Another complex forms at 1:10 dye/alginate ratio and only in the presence of alginate or guluronate-rich alginate. The two aggregates are in dynamic equilibrium according to the presence of isosbestic points in the visible spectra. The effects of pH and divalent cations on the spectra are studied. The 1:10 complex is damaged by addition of hydrochloric acid and divalent cations; however, at low concentration of these agents the spectra indicate conversion of the complex into the 1:1 aggregate. Models for the two complexes are proposed taking into account the preference of guluronate binding sites for chelating ions.

1. Introduction

Algal alginates are structural polysaccharides found in high concentration in various types of brown seaweeds, which have considerable commercial interest due to their gelling properties.[1] Algal alginates are used extensively in food products, pharmaceuticals and cosmetics and for a wide range of other industrial applications.[2] The polymers are 1,4-linked block of β-D-mannuronate (M for short) and α-L-guluronate (G).[3] Alternating (MG) sequences with composition vary according to the source and the treatment methods of algal alginates (Fig. 1).[4]

Due to the presence of carboxylate groups, which absorb at about 215 nm (corresponding to the $n \rightarrow \pi^*$ transition) circular dichroism (CD)—the differential absorbance between left and right circularly polarized light—has played a major role in elucidating the structure of alginates and their cation-mediated aggregation properties.[5,6] Interaction of alginates with dyes can shift the UV and the CD absorptions into the visible region and allows the study of biopolymers in a wavelength range which is spectroscopically more easily accessible.[7] Seely and Hart have investigated the interaction between methylene blue and several types of sodium alginate by UV/vis and CD spectroscopies at high polymer to dye ratios,[8] and Pal and Mandal have used several cationic dyes, among them pinacyanol chloride, to study the binding to potassium alginate.[9] Using this approach we have studied the interaction of pinacyanol dye with algal alginates from different sources; sodium alginate (Trade name: Manucol-LHF), mannuronate-rich alginate, and guluronaterich alginate) in different concentrations. We have found what we believe is an alginate bound dye, which holds promise as an indicator for the various processes (e.g., changing alginate concentrations or adding divalent cations) involving alginate chain conformations without the need to solve the experimental problems that are associated with making CD measurements in the far UV region.

2. Experimental

2.1. Materials

Sodium alginate (Trade name: Manucol-LHF) was purchased from Kelco, UK. According to manufacturer's specifications the alginate has an average molecular mass of 88,000 g/mol and consists of approximately 445 monomers. The relative content in mannuronate to guluronate in the sample is 63/37. Mannuronate-rich and guluronate-rich alginate were obtained from a sodium alginates sample (Trade name: Manucol-LB),[10] which has an average molecular mass of 60,000 g/mol and consists of approximately 300 monomers. The relative content in mannuronate to guluronate in the former was 83/17, in the latter it was 26/74. These ratios were estimated experimentally by a reliable non-destructive method by measuring the CD spectrum of 0.80 mg/ml alginate

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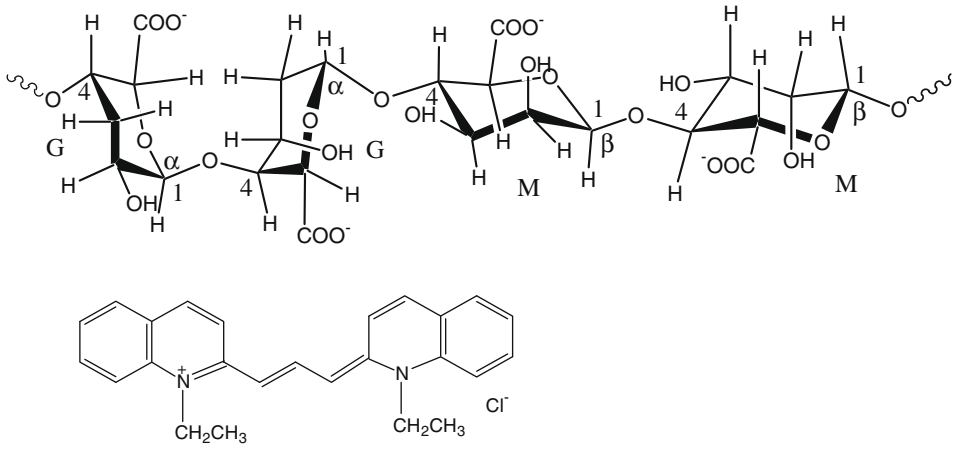

Figure 1. Glycosidic bonds between β-(1,4)-linked D-mannuronate (M) and α-(1,4)-linked L-guluronate (G) residues in the polymer chain of alginate (top), and the chemical structure of 1,1′-diethyl-2,2′-carbocyanine chloride (pinacyanol chloride) (bottom).

between 195 and 250 nm, corresponding to 4.0×10^{-3} M, if monomeric sodium mannuronate or guluronate, $C_6H_7O_6Na$ are taken as the molecular mass unit.[6] Pinacyanol chloride (1,1′-diethyl-2,2′-carbocyanine chloride) was obtained from Sigma and used without further purification. For the spectra measurements, we used spectroscopy grade ethanol from Merck, and water used was distilled three times.

2.2. Methods and Instruments used

Standard dye and alginate solutions were prepared in 25-ml volumetric flasks. Alginate-dye solutions (4.00 ml) were prepared in stoppered rolled rim glasses of 10-ml capacity. To prevent the dye from precipitating at the glass walls the alginate solutions were added first, followed by the required amount of the dye solution. The tendency of cyanine dyes to aggregate in aqueous solution is well-known. In order to extend the concentration range we added a constant low concentration of 7.5% (v/v) ethanol in all our spectroscopic investigations.

The UV/vis spectra were recorded with a Perkin–Elmer Lambda 5 spectrophotometer, and CD-spectra were measured with AVIV circular dichroism spectrometer (Model 62 ADS) in the wavelength range of 400–700 nm. A spectral bandwidth of 1.0 nm and a scan rate of 50 nm/min were used. Both instruments were connected to a personal computer for data collection in ASCII-file format. The conductivities in conductometric titration part were measured using LF 42 WTW conductometer, with a cell constant of 0.73 cm^{-1}.

3. Results

3.1. UV/vis and CD spectra

Figure 2 presents the UV/vis spectrum of a 1.50×10^{-5} M aqueous solution of pinacyanol chloride in the presence of 8 different concentrations of the sodium alginate used in this study, from 2.97 μg/ml to 74.3 μg/ml, corresponding to 1.50×10^{-5} to 3.75×10^{-4} M (molar concentrations of alginate monomers), which corresponded to molar concentration ratios of polysaccharide to dye from 1:1 to 25:1, respectively. The aggregating effect exerted by the polysaccharide is easily seen. The absorption maxima of the alginate-free dye (spectrum 1 in Fig. 2) are found at 600 and 546 nm with a shoulder at 505 nm, which were attributed to monomer, dimer, and higher aggregate, respectively.[11,12] The initial effect of alginate is to broaden the dye spectrum between 550 and 600 nm, and develop a band at 485 nm. Increasing the polysaccharide concentration shifts the intensity more and more into the 485-nm band. The highest absorbance of this band is observed at a polysaccharide to dye ratio of 10:1 (spectrum 6 in Fig. 2). Further increase of this ratio reverses this effect, and the intensity of the 485 nm band starts to decrease, while the broad absorbance between 555 and 585 nm starts to increase again, in addition, the appearance of a red-shifted shoulder around 630 nm.

The CD spectra of these solutions taken under identical conditions as shown in Figure 2 are shown in Figure 3. No circular dichroism is observed in the absence of alginate, and only the base line is recorded. At low polysaccharide/dye ratios a broad negative band develops between 500 and 680 nm and the monomer absorbance as well as the red-shifted shoulder acquires positive CD absorbance. Increasing the alginate to dye ratio leads to what appears to be a negative exciton couplet, with two oppositely signed bands and a zero-point crossing at 485 nm. This couplet reaches its maximum amplitude at a polymer to dye ratio of 10:1, which also corresponds to the maximum absorbance of the 485-nm band shown in Figure 2. Also, as observed in the UV/vis spectra, the amplitude of the couplet decreases as the polymer to dye ratio is

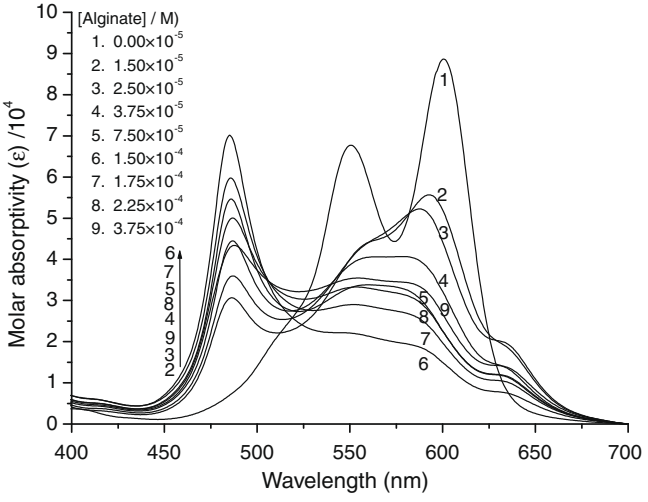

Figure 2. The visible absorption spectra of an aqueous solution of 1.50×10^{-5} M pinacyanol chloride with 7.5% v/v ethanol in the presence of eight different concentrations (from 2.97 to 74.3 μg/ml) of algal alginate at room temperature.

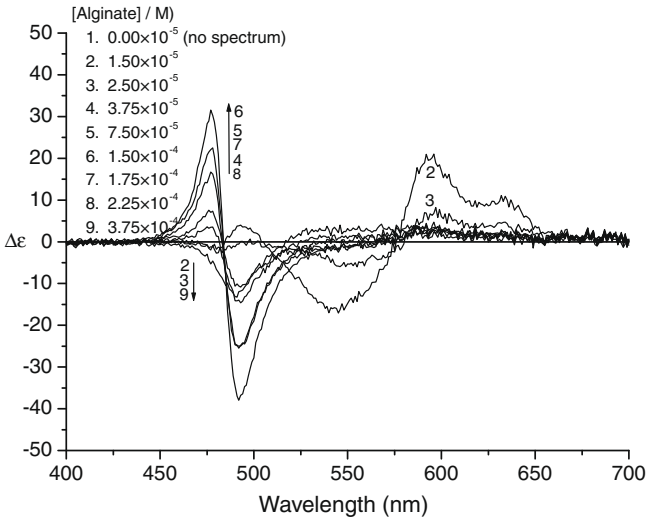

Figure 3. CD spectra of all solutions in Figure 2.

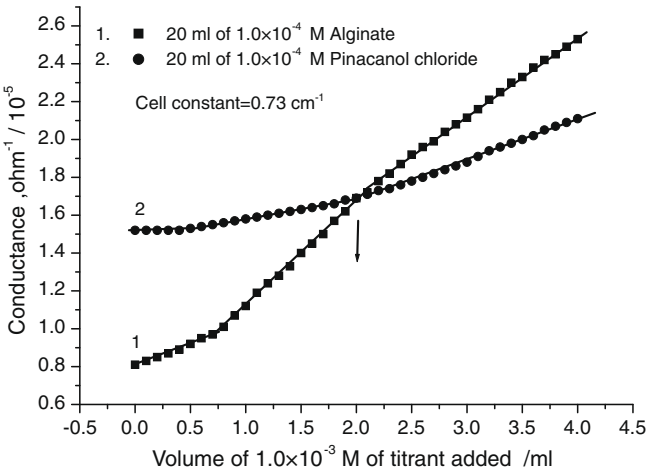

Figure 5. Conductometric titration of alginate (20.0 ml, 19.8 μg/ml or 1.0×10^{-4} M) with pinacyanol chloride (1.0×10^{-3} M), (plot 1). Conductometric titration of pinacyanol chloride (20 ml, 1.0×10^{-4} M) with alginate (198 μg/ml or 1.0×10^{-3} M) (plot 2).

increased further, until it finally collapses and a negative band remains.

3.2. Stoichiometry of the complex formation

To determine the stoichiometry of the complex formed at approximately equimolar ratio of the sodium alginate and pinacyanol dye used in this study, we employed Job's plots (or the Method of Continuous Variation).[13] For this, solutions were prepared containing the same total number of moles of the two reactants (molar concentrations of alginate monomers are used here), but differing in the molar fraction of each reactant. The reaction between pinacyanol chloride and sodium alginate belongs to the so-called double displacement reaction type: the sodium cation of the carboxylate group of alginate is replaced by the cationic dye molecule. The maximum absorbance of the complex formed is then determined from the spectrum at 485 nm for the set of solutions, and also the absorbance of the monomer of the dye at 600 nm. Figure 4 shows the plot of absorbance of the complex formed at 485 nm and the dye monomer absorbance at 600 nm against the mole fraction of added sodium alginate. For both absorbances, the two straight lines intersect at a ratio of 0.5 of polymer to polymer plus dye showing the formation of a 1:1 complex between the dye and polysaccharide.

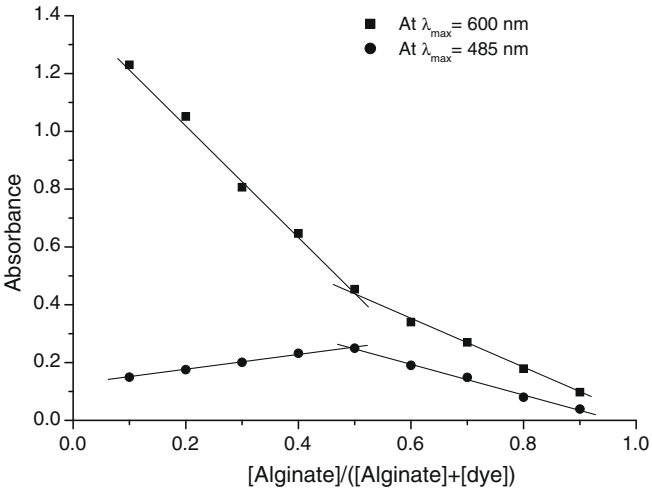

Figure 4. Job's plot (absorbance at 485 nm and at 600 nm vs alginate mole fraction) of the interaction between alginate and pinacyanol chloride. The total concentration [Alginate] + [dye] is 1.50×10^{-5} M.

The stoichiometry of the complex was also determined by following the titration reaction between the two ionic reactants in a conductometric cell, with one reactant present in a fixed amount and the other being gradually added.[14,15] The conductometric behavior (molar conductivity against concentration) of pinacyanol chloride and sodium alginate in a limited range of concentrations 0.890–19.8 μg/ml (4.50×10^{-6}–1.00×10^{-4} M) in non-alcoholic aqueous solution reflects the type of these electrolytes. As expected the two reactants represent weak electrolytes according to the nonlinear shape of the plots of molar conductivity against concentration (spectra not shown). However, both titration curves—sodium alginate titrated against pinacyanol chloride and vice versa—show distinct breaks in their linear behavior at approximately 1:1 stoichiometry (Fig. 5).

Concentrations in the plots are calculated on the basis of only monomers being present. This is a valid approximation for the polysaccharide where the carboxylate groups react independently; however, pinacyanol chloride under the experimental conditions is present not as a pure monomer, but as a mixture of monomers, dimers, and also of higher aggregates. The interaction between the two reactants seems to occur between pinacyanol monomers and alginate, since dye dimers (and higher aggregates) for steric reasons probably do not interact directly with alginate. From Figure 2, it is obvious that the dimer concentration decreases with increasing alginate concentration. Therefore, there will be a shift in the equilibrium to the side of monomer form during the titration. So, the conspicuous initial breaks in curves 1 and 2 of Figure 5 probably reflect the dimer to monomer shift of the dye in the presence of alginate.

3.3. Effect of pH and divalent cations

The UV/vis and the CD spectra shown in Figures 2 and 3 are significantly pH dependent. The pH of a pinacyanol/alginate 1:10 solution is approximately 8.3. Upon addition of HCl the intensity of the 485-nm UV/vis absorption band and the amplitude of the excitonic doublet in CD spectra decrease gradually until a pH of 6.45 is reached, after which there is no significant change in the spectra.

When divalent metal ions (as chloride salts) are allowed to diffuse into an alginate solution, chain–chain associations will be in-

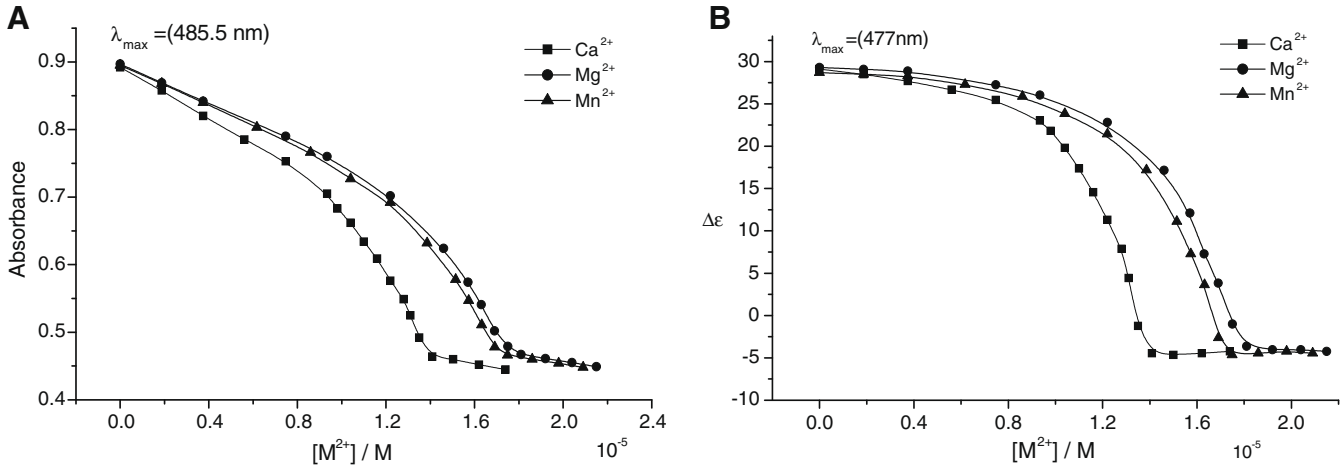

Figure 6. Visible absorbance at 485.5 nm (A) and CD absorbance at 477 nm (B) of aqueous solutions of pinacyanol chloride (1.50×10^{-5} M) and alginate (29.7 μg/ml or 1.50×10^{-4} M) titrated against Ca^{2+}, Mg^{2+}, and Mn^{2+}.

duced with the ions acting as junction zones responsible for gel formation.[15,16] In studying the effect of divalent cations on the dye-alginate complexes we found that the formation of the 1:1 complex is practically not affected by addition of a dilute solution of $CaCl_2$. In the case of the 1:10 complex things are completely different: adding Ca^{2+} ions gradually breaks down the intense 485 nm absorption peak and the associated exciton couplet.

For more quantitative data, Figure 6 shows the corresponding titration curves (series of prepared solutions with different concentrations were measured separately) of the 480-nm UV/vis maximum and the amplitude of the CD couplet as three different divalent cations, Ca^{2+}, Mg^{2+}, and Mn^{2+} (solutions of chloride salts) are added to the dye/alginate solution. Both plots show that Ca^{2+} exhibits a significantly stronger effect than the two other cations. They also show that there are no more spectral changes when the concentrations of the Ca^{2+}, Mg^{2+}, and Mn^{2+} cations have reached values of, respectively, 1.41, 1.75, and 1.81×10^{-5} M (calculated ionic strength are 4.23, 5.52, and 5.43×10^{-5}, respectively). The UV/vis spectra at these limiting cation concentrations are similar to the spectrum when the alginate concentration is 1.50×10^{-5} M (spectrum 2 in Fig. 2).

3.4. Guluronate- and mannuronate-rich alginates

Comparison of the UV/vis and CD spectra of pinacyanol chloride with alginates rich in mannuronate and rich in guluronate reveals significant differences from the alginate used in this study (Fig. 7). At an alginate to dye ratio of 10:1, the monomer absorption of the dye in the guluronate-rich alginate has almost completely disappeared in favor of the sharp 485-nm band and the broad absorbance around 550 nm. In contrast, the monomer absorbance is still strong in the mannuronate-rich alginate under these conditions. Even more striking is the complete absence of the CD exciton couplet at 485 nm in the mannuronate-rich alginate, while the CD spectra at low alginate/dye ratio are rather similar.

4. Discussion

Solutions of algal alginates and pinacyanol chloride become optically active in the spectral region of the dye. This, and the appearance of aggregate bands in the absorption spectra, is indicative of complex formation between the alginates and the dye. Complexation is mediated by electric charges, negative on the algi-

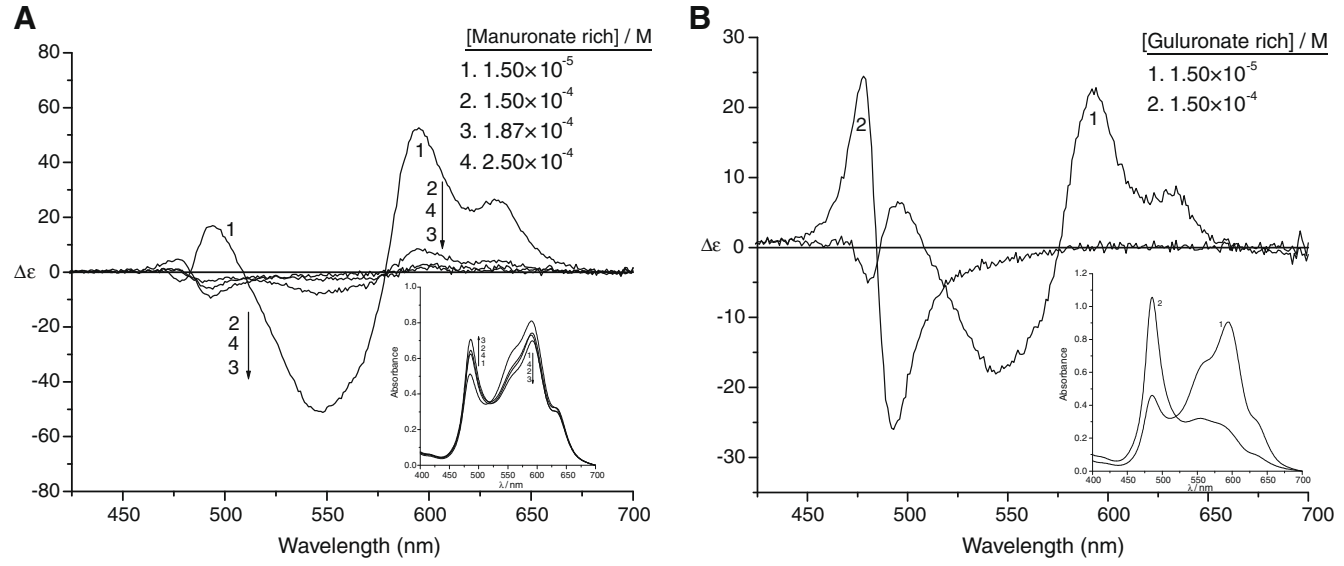

Figure 7. CD spectra (insets: corresponding to UV/vis spectra) of an aqueous solution of 1.50×10^{-5} M pinacyanol chloride with 7.5% v/v ethanol at room temperature in the presence of different concentrations of mannuronate-rich alginate (A), and guluronate-rich alginate (B).

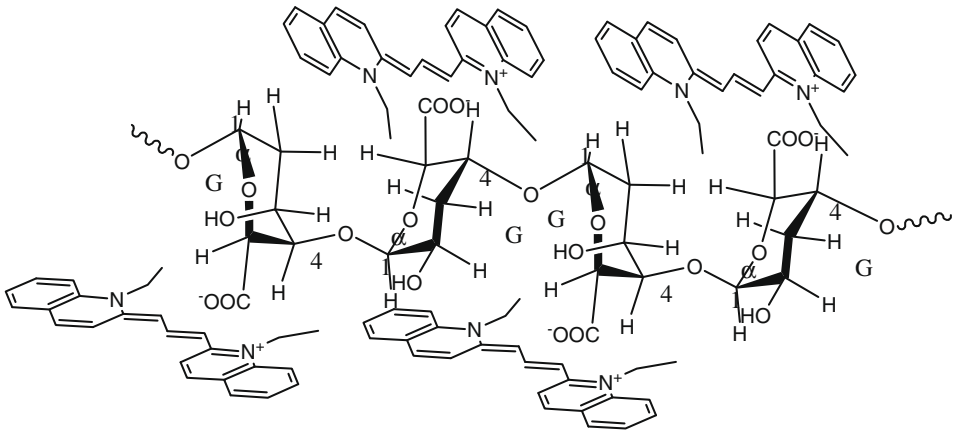

Figure 8. Hypothetical structure of the interaction between guluronate residues with pinacyanol dye cation.

nate due to the ionized carboxylate groups and positive on the cyanine dye. Coupling with the alginate makes the dye chiral and causes the absorption bands to become optically active. Coupling with the dye molecules may also induce conformational changes in the alginate.

The visible and CD absorption spectra of solutions containing the alginate and the dye in varying ratios show two kinds of spectra from which the presence of two different types of complexes can be deduced. One type is formed when the alginate and pinacyanol chloride are present in the solution in a 1:1 stoichiometry. This ratio is also found from Job's plots and conductometric titrations. Apart from the fact that the oppositely charged reactants (positive univalent pinacyanol and negative univalent alginate) are electrically neutralized at this point, the broad unstructured absorbance of this complex indicates a rather undefined structure of this complex in which the tendency of the dye to self-aggregate competes with the interaction with the alginate. This analysis is supported by the CD spectra with at least five bands ranging from 480 to 630 nm. Prominent among these bands are the monomer absorbance at 600 nm and the blue-shifted absorbance at 550 nm, which would be expected for a sandwich type dye dimer. Self-aggregation of the dye implies that not all of the carboxylic groups of the alginate are in contact with the dye cations; however, all dye species are somehow in contact with the alginate matrix; otherwise there would be no CD activity. Also, the fact that mannuronate- and guluronate-rich alginates induce the same CD pattern of bands indicates that the interaction is very local and not dependent on the secondary structure of the alginate.

The second type of complex is preferred in the presence of a large excess of alginate or of guluronate-rich alginate; the highest concentration is obtained when the molar ratio is 10:1 in aqueous solution with 7.5% ethanol added. The complex is characterized in the UV/vis by a sharp blue-shifted absorption at 485 nm and in the CD by a narrow exciton-like negative couplet. In mannuronate-rich alginate the band is much weaker, and the CD characteristic is completely missing. This complex represents a more regular or uniform kind of aggregation than the type discussed above. One reason for this may be the absence of excess dye aggregation and to the fact that only a fraction of the anionic sites of the polysaccharide are occupied by dye molecules. Thus, the very unspecific interaction between alginate and the dye in the case of 1:1 stoichiometry changes to a more regular one at a ratio of 10:1. Direct interaction through the charged sites is also suggested by the titration experiment with divalent cations which affect only the latter type of complex.

From the preferred interaction of the dye with alginate or guluronate-rich alginate at high polysaccharide/dye ratio we conclude that the dye interacts with identical sequences of guluronate residues (-G-G-G-G-) rather than the mannuronate residues (-M-M-M-M-) or the disordered conformation (-G-G-M-G-M-M-). It is tempting to interpret the CD-spectrum of this complex as originating from the pairwise interaction of dye molecules which would give rise to the observed exciton couplet structure.[17] This interaction might be provided by pairs of guluronate residues and the attached dye molecules in a specific orientation mediated by the α-(1,4)-glycosidic bond (Fig. 8).

Acknowledgments

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