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On the Optical Activity of Pseudoisocyanine Bound by Sulfated Polysaccharides in Dilute Aqueous Solution

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Summary

Visible absorption and circular dichroism spectra of the cationic dye pseudoisocyanine bound to sulfated polysaccharides in dilute aqueous solution are reported. Characteristic, strong optical activity is associated with stacks of dye molecules bound by dextran sulfate as well as by i-carrageenan in the random coil states. Conversion of i-carrageenan chains to an ordered state (by addition of NaCl) markedly changes the dye circular dichroism spectrum. The possible configurational origins of the observed effects are pointed out.

Results and Discussion

Linear, intact i-carrageenan as well as segmented i-carrageenan chains are randomly coiled in water but would assume ordered conformations in the presence of relatively low NaCl concentrations and at around room temperature (REES, WELSH 1977; KARIMIAN 1978).

In the latter case, increasing the temperature brings about a (reversible) order-disorder transition of the polysaccharides, usually monitored by studying their intrinsic, conformation dependent optical activity.

Considering that cationic dyes have been often found useful in probing the conformational state of chiral macroions in solution, we decided to explore the spectral properties of the dye pseudoisocyanine·HCl(PIC) in the presence of i-carrageenan in dilute aqueous media. Dextran sulfate a synthetic, branched ionic polysaccharide has also been used for comparative purposes. The absorption spectrum of PIC in the presence of the above polysaccharides is given in Figs. 1, 2. Interaction with the macroions elicits the development of a sharp intense band in the dye spectrum centered at around 574 nm: this so called J-band has been attributed in other, similar cases to bound dye aggregates, (PAL, MANDEL 1977).

Data of Figs. 1, 2 also show that addition of NaCl to the dye polymer solution induces a decrease in the intensity of the

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J-band. Comparison of the spectra recorded with carrageenan and with dextran sulfate reveals that with the latter the extent of dye binding and of dye stacking would be larger. This may be qualitatively explained in terms of the higher charge density of our dextran sulfate sample (D.S.=2.0) as compared to i-carrageenan (D.S.=1.7)*. Representative results of the CD spectral measurements are given in Figs. 3 and 4.

Data of Fig. 3 clearly show that PIC aggregates bound onto randomly coiled i-carrageenan segments in water (spectrum a) display strong elliplicity in the wavelength range comprised between 580 and 500 nm. Addition of NaCl (5.10^{-2}M) which induces the uptake of an ordered conformation by i-carrageenan chains (below 25°C, approximately) changes markedly the CD spectrum of bound PIC aggregates. (Fig. 3b)

This evidence taken by itself might incline to believe that the CD spectrum b of Fig. 3 typically results by PIC molecules dissimmetric stacking onto one-handed helices of i-carrageenan.

In our opinion, however, this is not the case. If one considers in fact the CD spectra reported in Fig. 3b and 4 it emerges that PIC bound by i-carrageenan (in 5×10^{-2}M NaCl, 20°C) and bound by dextrane sulfate (in H_2O and/or in 5×10^{-2}M NaCl, 20°C) do exhibit nearly exactly opposite chirality. As it appears reasonable to think that dextran sulfate should not be capable of assuming an ordered conformation, our finding cannot be explained in terms of an opposite handedness between dextran sulfate and i-carrageenan helices.

We believe, to the contrary, that asymmetric stacks of PIC molecules reflect essentially local configurational features of the binding sites on the different chains which would thus happen to be nearly specular. The "configurational" hypothesis is qualitatively in line also with the observation (see Fig. 3a) that strong extrinsic optical activity is associated with the PIC-randomly coiled i-carrageenan system too. Probably our case has points of similarity with that of PIC - random coil poly(L-glutamic acid) described by PAL and MANDEL (1977).

Our conclusion moreover is analogous to that arrived at by SCHEIBE ed al. (1968) who have adsorbed PIC to heparin and found that the dye CD spectrum changes sign by simply varying the heparin concentration or upon adding NaCl. These authors conclude that it is very unlikely that under such condi-

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* In the case of dextran sulfate the backbone repeating unit is assumed to consist of only one glucose residue each of which thus bears two-SO_3^- groups, on the average. On the contrary, for i-carrageenan the repeating unit consists of <u>two</u> sugar residues and bears 1.7- SO_3^- groups, on the average.

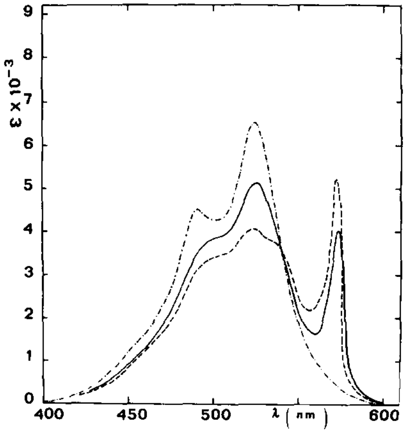

Fig. 1- Pseudoisocyanine absorption spectra in water (-.-.-.); dye concentration; D=1.1 x 10^{-5}M) and in the presence of i-carrageenan in water (- - -) and in 5 x 10^{-2} M NaCl (——); Polymer concentration: P = 1.2 x 10^{-3} equiv/l; P/D = 85; temperature: 20°C.

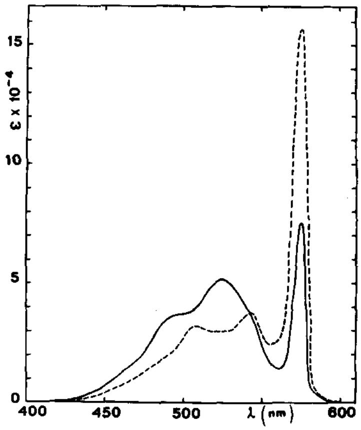

Fig.2 - Pseudoisocyanine absorption spectra in the presence of dextran sulfate in water (- - -) and in 5 x 10^{-2} M NaCl (——). Polymer concentration: P = 1.2 x 10^{-3} equiv/l; P/D = 85; temperature: 20°C.

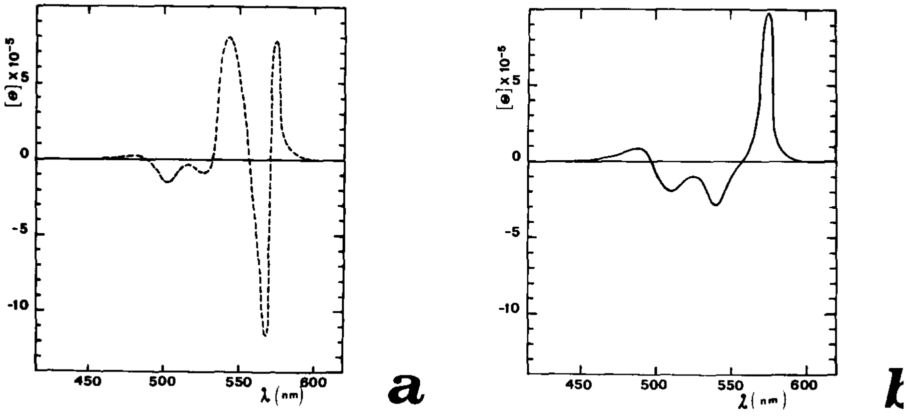

Fig. 3 - Circular dichroism spectra of pseudoisocyanine in the presence of i-carrageenan in water (Fig. 3a) and in 5 x 10^{-2} M NaCl (Fig. 3b). Polymer concentration: P = 1.5 x 10^{-3} equiv/l; P/D = 90; temperature: 20°C, θ in deg cm^2/dmol.

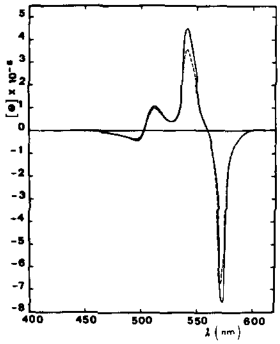

Fig. 4 - Circular dichroism spectra of pseudoisocyanine in the presence of dextran sulfate in water (- - -) and in 5 x 10^{-2} M NaCl (———), Polymer concentration: P = 1.2 x 10^{-3} equiv/l; P/D = 85; temperature: 20°C, θ in deg cm^2/ dmol.

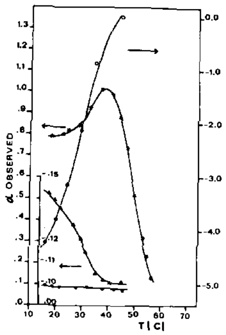

Fig. 5 - Optical activity readings (degrees) as a function of temperature (cell optical path: 10.0 cm)

● : i-carrageenan in water (λ = 300nm)

▲ : i-carrageenan in 5 x 10^{-2} M NaCl (λ = 300 nm)

★: pseudoisocyanine and i-carrageenan in 5 x 10^{-2} M NaCl (λ = 579 nm)

O: pseudoisocyanine and dextran sulfate in 5 x 10^{-2} M NaCl (λ = 579 nm)

Polymer concentration: P = 1.65 x 10^{-3} equiv/l; P/D = 110. Optical activity-temperature profiles may be reproduced by letting the solutions stand at ca. 15° for about 12h prior to each run.

tions the screw sense of heparin chains changes.

It is, in fact, much more likely that in the heparin as well as in the carrageenan case, only the dye absorption equilibrium is changed (e.g. by NaCl) leading to a variation of the geometrical details of the contact between dye aggregates and the optically active centres of the matrix, sufficient to profoundly change the CD spectrum.

In our case, however, we know that i-carrageenan passes from random coil to an ordered conformation, most probably a helical one, going from water to 5 x 10^{-2}M NaCl at 20°C: we may thus observe this passage via the CD differences due to bound PIC(Fig. 3a and b) but cannot derive any reliable information on the screw sense.

The very fact that PIC is bound to ordered chains of i-carrageenan in 5 x 10^{-2}M NaCl at 20°C while, under similar conditions, this is not the case with dextran sulfate is also made evident by the data reported in Fig. 5. We find that upon increasing the temperature, the PIC i-carrageenan-NaCl system shows a rather anomalous trend in optical activity reconcilable, at least on qualitative grounds, with a conformational transition of i-carrageenan, while in the PIC-dextran sulfate case the optical activity tends to zero in a simple, regular manner.

In both cases, increasing the temperature markedly disfavours bound dye aggregation (a distinctly exothermic process, in general) and eventually leads to monomeric dye binding with no extrinsic optical activity.

In conclusion it appears that due care must be exercised in deriving conjectures on the conformational state or helix sense of polysaccharide macroions in aqueous solutions using optical probes, (STONE 1972; EYRING et al. 1968), at least using pseudoisocyanine.

Experimental

The segmented i-carrageenan sample was a kind gift of Dr.S. REID, Unilever Research Laboratory (England). The intact i-carrageenan sample was a SIGMA-4 (USA) product, purified in the CERMAV Laboratory of the University of Grenoble (France). Dextran sulfate was a PHARMACIA (Sweden) sample, $\overline{M}$ ca 5 .10^5, which has been purified by dialysis.

Elemental analysis of the above said ionic polysaccharides (Na salts) and potentiometric titrations carried out on the same samples after conversion to the free acids by ion exchange, have yielded the following results (equivalent weights): 1) i-carrageenan segments: 270; 2) intact i-carrageenan 270;

3) dextran sulfate: 219, taking into account of course the water content of the samples.

Pseudoisocyanine·HCl (PIC), a SERVA(W. Germany) product, was used without further purification. Dye stock solutions were prepared by dissolving 10 mg of dry material in 10 ml of methanol and adding water to 250 ml.

The molar extinction coefficient of PIC at 524 nm was determined to be 65,000 M^{-1} cm^{-1} in 0.4% methanol/water solution. Dye·polymer solutions were prepared by adding to a stock polyelectrolyte solution prescribed amounts of 0.1 NaCl and of freshly prepared dye stock solution, in the order given, under constant, prolonged stirring and finally bringing to volume. The temperature of the solution was always kept at 10-15°C prior to the measurements. Water was deionized and bidistilled. The P/D ratios in the range 80-110 were so chosen as to minimize the possible influence of dye binding upon the conformational state of polyanions and because at P/D values higher than about 200 the optical and chirooptical effects result very small. Following the protocol given above, the data have been found reproducible within 5%, approximately.

Spectral measurements were carried out with a Beckman DK-2A spectrophotometer; optical activity measurements with a Cary-61 dichograph. The temperature of the cells was in all cases controlled ($\pm$ 0.1°C) by circulating water through an ultrathermostat.

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