

Interaction of biopolymers with cationic dyes : Spectral and thermodynamic studies

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The interaction between a cationic dye pinacyanol chloride and an acidic capsular polysaccharide isolated from *Klebsiella* serotype K14 has been studied by spectral measurements. Thermodynamic parameters like entropy change, enthalpy change and free energy change have also been evaluated for the interaction. The acidic capsular polysaccharide, having two potential anionic sites, glucuronic acid and pyruvic acid, interacts strongly with dye cations by inducing metachromasy and a blue shift of about 110 nm is observed in the visible absorption spectrum of the dye. At [polymer]/[dye] $\approx$ 30, a distinct μ - band at 490 nm appears as a single banded spectrum with complete removal of α - and β - bands. Studies on reversal of metachromasy by addition of different co-solvents indicate progressive destruction of metachromatic compounds. It is also observed that the added substances are used as hydrophobic bond breakers and thus a reversal of metachromasy occurs. Thermodynamic parameters evaluated from spectral data have been used to elucidate the nature of interaction of the polymers with dye cations.

Metachromasy is a well-known phenomenon in the case of dye- polymer interactions and it is generally found in the case of aggregation of cationic dyes on anionic polymers¹⁻³. However, metachromasy has also been observed in some cationic polyelectrolyte and anionic dye^{4,5} systems. Interaction of cationic dyes with different hetero polyanions has been studied by different authors⁶⁻⁹.

Formation of metachromatic compound is characterized by the shift of the absorption maximum to shorter wavelengths. Generally, a polyelectrolyte with relatively high charge density is found to be more efficient in inducing metachromasy. It has also been observed that the metachromasy depends on the conformation of the polyanions as well as the dye ions in solution.

Klebsiella K14 is one of the 82 strains of *Klebsiella* K serotype which produce acidic capsular polysaccharide having definite repeating units. The primary structure of this polysaccharide consists of hexasaccharide repeating units¹⁰ having one residue each of glucuronic acid, galactose, glucose and mannose in the main chain. Mannose in the chain is the branching point to which one unit of rhamnose and one unit of glucose are linked. One unit of pyruvic acid is linked to 4 and 6 positions of the branched glucose.

In the present studies, we report the spectral results of the interaction between the cationic dye pinacyanol chloride and the anionic polysaccharide isolated from *Klebsiella* K14. The thermodynamic parameters of the interaction have also been evaluated.

Materials and Methods

The serological test strain for *Klebsiella* K14 was kindly supplied by Dr. S. Schlecht of the Max-Planck Institute for Immunobiology, Freiburg, Germany. The strain was checked for agglutination in Difco type-specific antisera. The bacterial cells were grown on nutrient agar medium, harvested in large quantity on big agar plates, killed, dried, and the capsular polysaccharide was isolated and purified by the phenol-water- cetavlon extraction method¹¹. The dye pinacyanol chloride (PCYN) was purchased from Sigma Chemical Co. USA and used directly without further purification.

A stock solution ($10^{-4}M$) of the dye was prepared in doubly- distilled water containing 4 % methanol and kept in the dark at 4°C protected from light by wrapping the container with black paper. The stock solution was diluted to appropriate concentrations with distilled water as required.

Absorbances of the pure dye solution and the mixtures containing different polymer/dye (P/D) mo-

lar ratios were measured at 400-700 nm with the UV-visible spectrophotometer "Spectronic 21D (Milton Roy)". Concentrations of the pure dye solutions and the aqueous polymer solutions were used within the range of 10^{-4} - 10^{-5} M. Here one mole of polymer refers to the mass of one repeating unit of the polymer.

The reversal of metachromasy¹² was investigated by measuring absorbance of the metachromatic solution ($[P]/[D] = 5.0$) at the metachromatic band (μ -band) of the spectra and also at the monomeric band (α -band, 600 nm) of the dye solution upon addition of different co-solvents like 1-propanol, ethanol, methanol and urea in increasing amounts (alcohols, 0-70% by vol; urea, 0-10M). The reversal of metachromasy was also confirmed by measuring absorbances of the pure dye solution and metachromatic solution ($[P]/[D]=5$) at 450-650 nm with and without addition of co-solvents. The effect of NaCl and EDTA on the polyelectrolyte-induced metachromasy in pinacyanol chloride was also studied similarly.

In order to determine the thermodynamic parameters¹³ absorbances of the dye solution (A_0) and dye-polymer mixture (A) at the metachromatic band (μ , 490 nm) were measured at different temperatures in the range 30° to $50^\circ \pm 0.1^\circ$ for different sets of solutions containing varying amounts of polymer (C_S) in a fixed volume of dye solution (C_D). The temperature was maintained by a thermostatic bath. The interaction constant, K_c , of the dye-polymer system, $D + S = DS$, was calculated at each temperature using the equation of Rose and Drago¹⁴.

$$\frac{C_D C_S}{(A - A_0)} = \frac{L}{K_c L (E_{DS} - E_D)} + \frac{C_S}{(E_{DS} - E_D)L}$$

where, C_D is initial molar concentration of dye; C_S , initial molar concentration of polymer; A_0 , absorbance of the dye solution; A , absorbance of the dye-polymer mixture; E_{DS} , molar absorption coefficient of the dye-polymer complex; E_D , molar absorption coefficient of the dye; and L is the length of the light path.

The value of K_c was calculated at each temperature from the slope and intercept of the linear plot of $C_D C_S / (A - A_0)$ versus C_S . By plotting $\log K_c$ against $1/T$, change in enthalpy of complex formation (ΔH) was calculated from the slope of the straight line obtained according to van't Hoff equation. Free en-

ergy change (ΔG) was then obtained from the expression $\Delta G = -RT \ln K_c$ at all the temperatures. Finally, entropy change (ΔS) was obtained from the linear plot of ΔG against T according to the thermodynamic expression, $\Delta G = \Delta H - T\Delta S$.

Results and Discussion

The absorption spectra of the aqueous solution of cationic dye pinacyanol chloride (1.00×10^{-5} M) along with the spectra obtained upon addition of *Klebsiella* K14 polysaccharide at different polymer/dye molar ratios ($[P]/[D]=0$ to 30) are shown in Fig.1. The pure dye solution shows two peaks at 600 and 545 nm corresponding to monomeric (α -band) and dimeric (β -band) bands respectively. A distinct metachromatic band (μ) with the peak at 490 nm appears at $[P]/[D]=0.5$. The peak intensity of the μ -band increases with subsequent reduction in the intensities of α and β -bands with the increasing $[P]/[D]$ values. It is observed that at $[P]/[D] \approx 30$, there is complete removal of the α and β -bands and a single-banded spectrum appears with the peak around 490 nm. However at moderate $[P]/[D]$ ratios, multiple-banded (α, β, μ -bands) spectral behaviour is observed. Thus, the shape of the metachromatic spectrum is sensitive to $[P]/[D]$ values and a metachro-

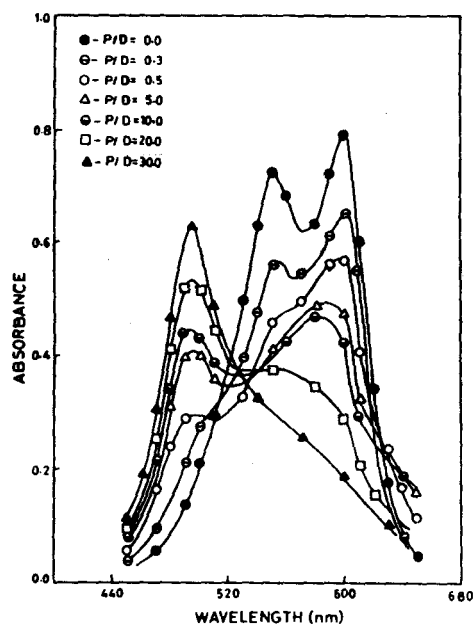


Fig.1 — Absorption spectra of metachromatic solutions; *Klebsiella* K14 polymer/pinacyanol chloride dye at different molar ratios (dye conc., 1.00×10^{-5} M).

Table 1 — Results of the reversal of metachromasy in *Klebsiella* K14 polymer/pinacyanol chloride at [P]/[D] = 5 by 35 % ethanol

Absorbance of dye solution and metachromatic solution with and without addition of 35% ethanol

Wavelength (λ)	Pure dye ^a (P/D=0)	Dye+ethanol (P/D=0)	Dye+polymer ^b (P/D=5)	Dye+polymer +ethanol
450	0.030	0.020	0.070	0.015
460	0.045	0.030	0.105	0.020
470	0.060	0.035	0.185	0.030
480	0.090	0.045	0.295	0.055
490	0.140	0.060	0.360	0.085
500	0.210	0.100	0.355	0.090
510	0.290	0.145	0.330	0.140
520	0.385	0.200	0.325	0.190
530	0.490	0.275	0.330	0.250
540	0.620	0.360	0.360	0.360
550	0.710	0.510	0.390	0.490
560	0.700	0.690	0.420	0.670
570	0.640	0.725	0.450	0.695
580	0.650	0.780	0.480	0.760
590	0.740	0.940	0.500	0.915
600	0.800	1.050	0.475	1.050
610	0.580	0.790	0.350	0.770
620	0.350	0.550	0.285	0.540
630	0.185	0.265	0.250	0.260
640	0.095	0.120	0.205	0.110
650	0.055	0.060	0.145	0.055

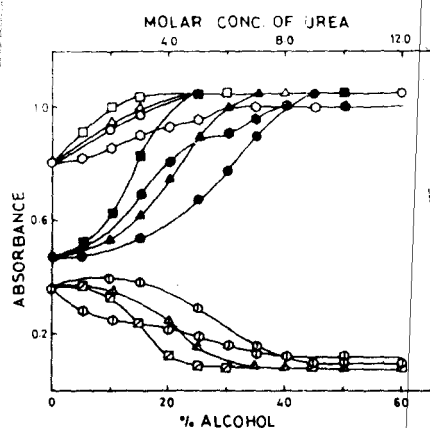
^aDye concentration = $1.02 \times 10^{-5} M$ ^bPolymer concentration = $5.10 \times 10^{-5} M$ 

Fig.2 — Reversal of metachromasy in *Klebsiella* K14 polymer/pinacyanol chloride dye ([P]/[D] = 5) by the addition of alcohols and urea [Upper curves at 600 nm and lower curves at 490 nm; O - dye + methanol, Δ - dye + ethanol, $\square$ - dye + 1-propanol, $\circ$ - dye + urea; $\oplus$, $\bullet$ - dye-polymer + methanol, $\blacktriangle$, $\blacktriangle$ - dye-polymer + ethanol, $\boxplus$, $\blacksquare$ - dye-polymer + 1-propanol, $\oplus$, $\bullet$ - dye-polymer + urea].

matic blue shift of about 110 nm demonstrated induction of strong metachromasy in the dye.

The spectral behaviour reveals that the conformation of the polyanionic chromotrope influences the shape of the metachromatic spectra, produced by interaction with the metachromatic dye cations. It has been reported^{15,16} that polyglutamate in acidic pH range produces a sharp single-banded metachromatic spectrum in pinacyanol chloride dye due to α -helical form of the polypeptide, but it produces a broad multiple-banded spectrum in the dye as a result of random coil conformation of the polyglutamate at neutral pH. Thus, the appearance of multiple-banded broad spectra indicates that the polymer K14 might have random coil structure in the solution. At higher concentration, the conformations of the polyanions, possibly change from random coil to helical form exhibiting single-banded spectra. The absorption spectra at the intermediate [P]/[D] values demon-

Table 2 — Thermodynamic parameters for interaction between pinacyanol chloride dye and *Klebsiella* K14 polymer

Temp.(K)	K_c (lit mol ⁻¹)	ΔG (kcal mol ⁻¹)	ΔH (kcal mol ⁻¹)	ΔS (cal mol ⁻¹ deg ⁻¹)
304	14598	-5.81		
307	12470	-5.79		
313	9965	-5.76	-7.37	-5.10
323	7031	-5.72		

strated gradual transition in conformation of the polymer in solution.

The results of the reversal of metachromasy in *Klebsiella* K14 polymer/pinacyanol chloride at [P]/[D] = 5.0 by the co-solvents methanol, ethanol, 1-propanol and urea are shown in Fig.2. Absorbances at 600 nm (α -band) and 490 nm (μ -band) were measured after addition of increasing amounts of the co-solvents to the dye solution and also to the dye-polymer mixtures. Absorbance of the metachromatic solution at 600 nm increased with the increase in concentration of the co-solvent and finally reached the constant value corresponding to that of the pure dye solution, indicating complete destruction of metachromasy. However, absorbance at 490 nm decreased simultaneously and reached a constant minimum value. Figure 2 indicates that 45% methanol, 35% ethanol, 25% 1-propanol or 8M urea could cause complete reversal of metachromasy. The spectral data on the reversal of metachromasy in K14 polymer/PCYN at [P]/[D] = 5 by 35% ethanol are given in Table 1 as representative values.

The gradual destruction of metachromatic compound by alcohols and urea is attributed to the involvement of hydrophobic bonds in the induction process of metachromasy. The extent of destruction of metachromatic compounds by alcohols and urea was followed by measuring the increase in absorbance of solutions of dye and chromotrope at the α -band or by the decrease in absorbance at the μ -band as a function of concentration of alcohols and urea. Destruction of metachromasy is, perhaps, mainly due to breaking of hydrophobic bonds by alcohols and urea¹⁷. The efficiency of alcohols in disrupting metachromasy was found to be in the order: methanol < ethanol < 1-propanol, indicating that reversal became quicker with increasing hydrophobic character of alcohols. This agrees with the results of other polyelectrolyte systems¹⁸. Thus, the metachromasy can be destroyed by different means and the concept of reversal of metachromasy can be used to determine

the stability of the metachromatic compound and the nature of binding.

It was also observed that metachromasy could be reversed by NaCl and EDTA. This could be ascribed to the competitive binding of the effective cations to the polyanionic sites, causing disruption of the dye-polymer compound.

It is very significant to note that no metachromatic band is obtained when methylated or carboxyl-reduced polymers of *Klebsiella* K14 are interacted with the cationic dye pinacyanol chloride. Polyelectrolytic property of the polysaccharide was destroyed after methylation or carboxyl-reduction when it was converted into neutral polysaccharide. The main driving force between the dye-polymer interaction, which is electrostatic in nature¹⁹, is lost and the polymers then fail to induce metachromasy in the cationic dye.

By measuring spectral changes of the dye-polymer complexes at different temperatures, thermodynamic parameters such as K_c , ΔH , ΔG and ΔS of the interaction were determined. The results are given in Table 2.

Table 2 shows that the value of K_c decreases with increase in temperature which suggests that the interaction is exothermic in nature. It also indicates that the electrostatic attraction might be an important factor in securing close association between the dye cations and polyanions, causing aggregation of the dye molecules. The negative value of ΔH (-7.37 kcal mol⁻¹) also confirms the exothermic nature of the interaction and such a value is quite reasonable for this type of interactions. The negative value of ΔG (-5.79 kcal mol⁻¹) at 307 K is also within the range of a reversible biological process and such a small value of free energy change suggests a non-chemical type of interaction. The negative value of ΔS (-5.10 cal mol⁻¹ deg⁻¹) indicates more ordered state of the ions due to aggregation of dye molecules. Thus, the thermodynamic parameters suggest that there is interaction between the anionic sites of the polyanions and the dye counter ions, resulting in induction of

metachromasy. The data also give a clear idea about the favourable conditions for the dye-polymer interaction.

The results discussed above establish the chromotropic character of the capsular polysaccharide in inducing metachromasy in cationic dye pinacyanol chloride. The anionic sites in the hexasaccharide repeating units would remain far apart if the polysaccharide had been a rigid straight chain polymer rendering it a weak chromotrope. But the blue shift value of about 110 nm supports the strong chromotropic character, which is perhaps due to the random coil conformation of the polysaccharide in solution that brings anionic sites relatively closer to each other. Moreover the shape of the spectra depends on the type of aggregation²⁰ of the dye molecules on the polymer in affecting metachromasy. Overcrowding of the dye molecules usually produces broad and multiple-banded spectra. However, appearance of sharp-banded spectra at higher [P]/[D] values suggests that there is no overcrowding in aggregation and anionic sites in the polysaccharide are not closely packed, i.e., at higher [P]/[D] values the random coil conformation changes to a helical one.

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