Electric dichroism studies of the bonding of Co(III) chelate with synthetic polyelectrolytes

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The interactions of bis-2-(2-pyridylazo)-1-naphthol Co(III), [Co(III) $(\alpha\mathrm{PAN})_2^+$], with five kinds of synthetic polyelectrolytes have been studied by spectrophotometric and transient electric dichroism measurements. The polyelectrolytes were: poly(styrene sulphonic acid) (PSS); poly(acrylic acid) (PAA); poly(L-glutamic acid) (PLG); poly(N^e,N^e-dicarboxylmethyl-L-lysine (PDCML); and poly(L-lysine) (PLL). The equilibrium constant of the reaction:

$$\mathrm{Co(III)}\ (\alpha\mathrm{PAN})_2^+ + \mathrm{P} \xrightarrow{K_1} \mathrm{Co(III)}\ (\alpha\mathrm{PAN})_2^+ \cdot \mathrm{P}$$

with P = polyelectrolyte residue was determined spectrophotometrically: K_1 is $>10^7\ \mathrm{M}^{-1}$ (PSS); $(1.4\pm0.2)\times10^4\ \mathrm{M}^{-1}$ (PAA); $(4.0\pm0.4)\times10^3\ \mathrm{M}^{-1}$ (PLG); $(1.4\pm0.2)\times10^6\ \mathrm{M}^{-1}$ (PDCML); and $<10^2\ \mathrm{M}^{-1}$ (PLL) at pH 6–8. From transient electric dichroism, the angle (ψ) between the αPAN plane and the polymer axis was determined to be 65° (PSS); 52° (PAA); 55° (PLG); and 52° (PDCML). The large K_1 and ψ values for PSS are ascribed to the hydrophobic interaction between the aromatic αPAN ring and the styrene sulphonate residues of PSS. Using stopped-flow electric dichroism measurements, rapid transfer of a bound Co(III) chelate from the PDCML to PSS chains was shown to occur.

Keywords Electric dichroism; cobalt(III) chelate–polyelectrolyte complex; synthetic polyelectrolyte; stopped-flow electric dichroism

INTRODUCTION

The bonding of a metal ion to a synthetic polyelectrolyte has been widely researched[1] and is important in understanding the role metal ions play in macromolecules with catalytic properties such as enzymes[1]. It is thus desirable to establish the relation between the structure of a metal–polyelectrolyte complex and the nature of the metal–polymer interactions[2].

A number of spectroscopic methods have been used to find the structure of the metal–polymer complex in solution: electronic spectra, optical rotatory dispersion, o.r.d., circular dichroism, c.d., and magnetic resonance measurements. The results have been (so far) qualitative especially in the case of diamagnetic ions or coiled polymers[3].

One of us (A.Y.) has measured the transient electric dichroism of the ternary system Cu(II)–bovine serum albumin (BSA)–terdentate chelating ligand[4]. The orientation of the molecular plane of the chelating ligand was determined with respect to the long axis of the BSA molecule. The results are a clue to the binding states of Cu(II) chelate.

The same method can be applied to coiled polymers, if the bound metal moves together with the orienting polymer segment in an electric field[5]. The resultant optical anisotropy may reveal the conformation of a metal–polymer complex in the vicinity of the bound metal ion.

Here, we deal with the bonding of a Co(III)–chelate, bis-2-(2-pyridylazo)-1-naphthol Co(III) (denoted Co(III) $(\alpha\mathrm{PAN})_2^+$, *Figure 1*) with five kinds of synthetic polyelectrolytes. The polyelectrolytes used are poly(styrene sulphonic acid) (PSS), poly(acrylic acid) (PAA), poly(L-glutamic acid) (PLG), poly(N^ε,N^ε-dicarboxylmethyl-L-lysine) (PDCML) and poly(L-lysine) (PLL). The first four compounds were chosen because they were expected to bind Co(III) $(\alpha\mathrm{PAN})_2^+$ with their sulphonate carboxylate groups. The interaction with PLL has also been studied to investigate the effect of a positive charge in the ε-amino group on the binding of the metal chelate.

The binding equilibria and structures of Co(III) $(\alpha\mathrm{PAN})_2^+$–polyelectrolyte complexes have been studied using electronic spectra and transient electric dichroism measurements. Among the results, the importance of hydrophobic interactions on the structures is evident. In addition, using stopped-flow transient electric dichroism, the rapid transfer of a bound Co(III) chelate from one polyelectrolyte chain to another is confirmed to occur.

EXPERIMENTAL

Co^{2+} ion was added as the perchlorate salt, $\mathrm{Co(ClO_4)_2\cdot6H_2O}$. 2-(2-Pyridylazo)-1-naphthol-(αPAN·H) was synthesized according to Anderson and Nickless' procedure[6]. PSS (molecular weight 3.5×10^5) was donated by Dr Shigeharu Harada of Shizuoka Women's College, Japan. The solution was neutralized by KOH. PAA (Wako, $MW = 6.6\times10^5$), PLG (Protein Research Foundation, $MW = 7\times10^4$) and PLL (Sigma Chem.

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Company, Type V, $MW = 3 \times 10^4$) were used as purchased. Preparation of PDCML ($MW = 3.3 \times 10^5$) was as described in the previous paper[7]. The concentration of polyelectrolyte was calculated from its dissolved weight.

Electronic spectra were recorded on a Hitachi EPS-3T spectrophotometer at 20°C. pH was measured with a Radiometer PHM-4d.

Transient electric dichroism was obtained using the high electric field generated by a Joule heating temperature-jump apparatus (Union Giken T-jump apparatus)[8]. Electric energy was stored in a coaxial cable 157 m long. A linear polarizer was placed in between the T-jump cell and the monochromator[9].

Stopped-flow electric dichroism measurements were performed by mounting a mixing compartment (Union Giken MX-7 apparatus) onto the T-jump cell. The dead-time of the mixing apparatus was about 0.2 s.

RESULTS

Electronic spectra of Co(III) chelate and polyelectrolyte solution

$Co(III)(\alpha PAN)_2^+$ solution was prepared by quantitative oxidation of a 1:2 Co^{2+} and αPAN·H solution with $K_3Fe(CN)_6$. The electronic spectra of the free αPAN molecule (αPAN·H), $Co(II)(\alpha PAN)_2$ and $Co(III)(\alpha PAN)_2^+$ are shown in *Figure 2*.

The interaction of $Co(III)(\alpha PAN)_2^+$ with a polyelectrolyte was studied spectrophotometrically. When PDCML is added to a $Co(III)(\alpha PAN)_2^+$ solution, the absorbance at 600 nm (λ_{max} of free $Co(III)(\alpha PAN_2^+)$) decreases with the isosbestic point at 655 nm (curves C–E in *Figure 2*). This change is ascribed to the binding of the Co(III) chelate with PDCML as below.

$$Co(III)(\alpha PAN)_2^+ + PDCML \rightleftarrows Co(III)(\alpha PAN)_2^+ \cdot PDCML \qquad (1)$$

Since the absorbance at 480 nm (λ_{max} of free αPAN·H) stays constant during this reaction, no free αPAN·H is generated on addition of the polyelectrolyte.

Assuming that one Co(III) chelate binds with one residue in PDCML, the binding equilibrium constant for reaction (1), K_1, is calculated from the expression:

$$K_1 = \frac{\Delta A_{600}/\Delta\varepsilon_{600}}{([M]_0 - \Delta A_{600}/\Delta\varepsilon_{600})([P] - \Delta A_{600}/\Delta\varepsilon_{600})} \qquad (2)$$

where ΔA_{600} and $\Delta\varepsilon_{600}$ denote the decrease of absorbance at 600 nm, and the difference in molar extinction coefficients between the free and bound Co(III) chelates respectively. $[M]_0$ and $[P]$ are the concentrations of the Co(III) chelate and polyelectrolyte added, respectively. [P] is measured as the number of moles of residue/l.

ΔA_{600} is plotted against [P] at constant $[M]_0$ in *Figure 3*. K_1 is determined from the curve to be $(1.4 \pm 0.4) \times 10^6$ M^{-1} for PDCML. For PAA and PLG, K_1 is obtained in a similar way. For PLL, no absorbance change is observed up to $[PLL] = 3 \times 10^{-3}$ M, implying that $Co(III)(\alpha PAN)_2^+$ does not bind with PLL. For PSS, the absorbance at 600 nm, A_{600}, decreases linearly until the ratio of [P] to $[M]_0$ becomes unity. With a further increase in PSS, however, A_{600} increases to a saturation value above $[P]/[M]_0 = 6$ (*Figure 3*). From the results, K_1 for PSS is estimated to be larger than 10^7 M^{-1}. The two-step behaviour for PSS suggests that the bound Co(III)-species interact with each other when they occupy neighbouring sites on a

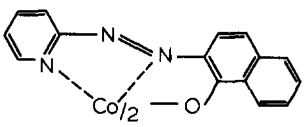

Figure 1 $Co(III)(\alpha PAN)_2^+$

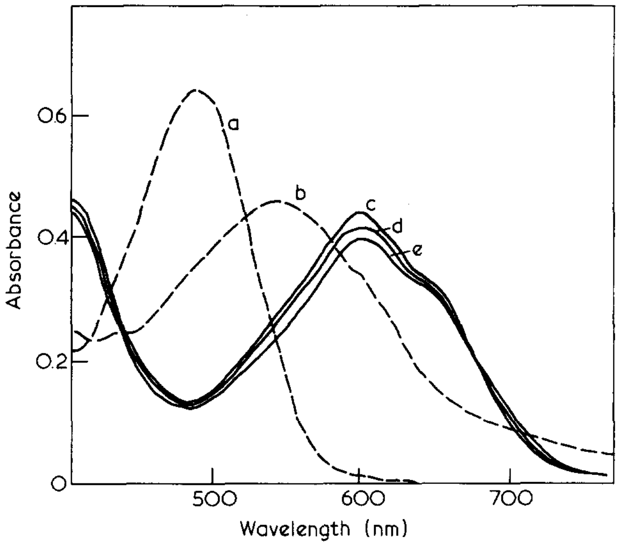

Figure 2 Electronic spectra of a $Co(III)(\alpha PAN)_2^+$ with PDCML solution: (a) free αPAN.H (3.3×10^{-5} M); (b) free $Co(II)(\alpha PAN)_2$ (1.6×10^{-5} M); (c) free $Co(III)(\alpha PAN)_2^+$ (1.6×10^{-5} M); (d) PDCML (8.0×10^{-6} M) added to (c); (e) PDCML (1.6×10^{-5} M) added to (c). pH, 7.80 ± 0.05

Table 1 Spectrophotometric and electric dichroism results for $Co(III)(\alpha PAN)_2^+$ and polyelectrolyte systems

Polyelectrolyte[a]	PSS	PAA	PLG	PDCML	PLL
K_1 (M^{-1})[b]	$>10^7$	$(1.4 \pm 0.2) \times 10^4$	$(4 \pm 0.4) \times 10^3$	$(1.4 \pm 0.4) \times 10^6$	$<10^2$
$\rho^{\infty}([P] = 0)$[c]	−0.66	+0.22	0.02	0.14	–
ψ (degrees)[d]	65	52	55	52	–
e/l (electron/Å)[e]	0.4	0.4	0.3	0.3	0.3

[a] PSS (poly(styrene sulphonic)acid), PAA(Poly(acrylic acid)), PLG(poly(L-glutamic acid)), PDCML(poly(N^{ϵ},N^{ϵ}-dicarboxylmethyl-L-lysine)), and PLL(poly-L-lysine)

[b] Equilibrium constant of reaction (1)

[c] Reduced linear dichroism extrapolated to infinite field strength and infinite dilution (from *Figure 6*)

[d] Angle between the transition moment of the α-PAN molecule and the oriented axis of a polymer segment. ψ for PDCML is estimated assuming Δ = 60° (see Discussion)

[e] Charge density of a polyelectrolyte chain expressed as the number of electron charges per Å

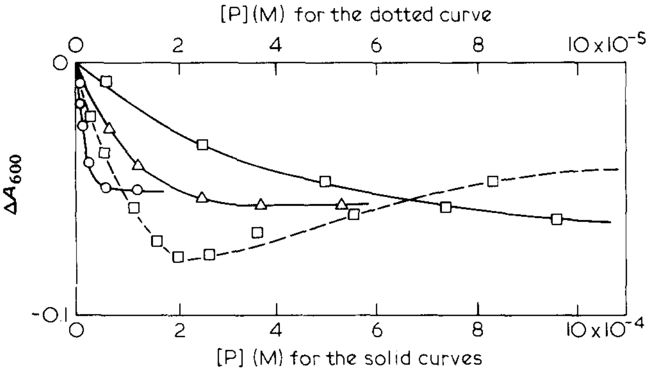

Figure 3 Dependence of the change in absorbance at A_{600}, ΔA, of a Co(III)(αPAN)$_2^+$ solution on the amount of polyelectrolyte added, [P]. [Co(III)(αPAN)$_2^+$]$_0$ = 1.9 × 10^{-5} M. pH: 6.50 (- - -□- - -, PSS); 7.80 (—○—, PDCML); 6.65 (—△—, PAA); and 7.00 (—□—, PLG)

polyelectrolyte chain. Thus, the gradual increase of A_{600} as $[P]/[M]_0$ goes from 1 to 6 may correspond to the dilution of the bound species among empty sites. Spectral results are summarized in *Table 1*.

Electrid field effect on bound Co(III) chelate solution

The effect of an electric field on the Co(III) chelate and polyelectrolyte solution has been studied under conditions in which all the metal chelates exist as isolated bound species (or $[P]/[M]_0 \gg 1$).

Figure 4a shows an example of the signals when electric discharge experiments are performed on a solution of a Co(III) chelate bound with PDCML. The monitoring light is polarized parallel to the direction of the electric field (denoted by $\theta = 0°$). The initial rapid decrease in transmittance occurs within 10 μs and recovers to the original level after about 300 μs. When the light is polarized vertically with respect to the electric field ($\theta = 90°$), similar spike-shaped signals are observed but in the opposite direction (*Figure 4b*). *Figure 4c* shows the time-course of electric field under the same conditions.

When the build-up of orientation dichroism is monitored by linearly-polarized light, relative absorbances change with the angle (θ) between the electric field and the direction of polarization of the light[9]:

$$\frac{\Delta A}{A}=\frac{\rho}{6}(1+3\cos 2\theta), \qquad (3)$$

where ΔA is the change in absorbance, A the absorbance in the absence of electric field and ρ is defined as $\rho = (\varepsilon_{\parallel} - \varepsilon_{\perp})/\varepsilon$ with ε the isotropic molar extinction coefficient, $\varepsilon_{\parallel}$ the molar extinction coefficient for the incident light polarized parallel to the electric field, and $\varepsilon_{\perp}$ the molar extinction coefficient vertical to electric field. Since the experimental θ dependence of the maximum amplitude of the signal, ΔA_m in *Figures 4a* and *4b*, agrees well with the theoretical prediction (3) (*Figure 5*), the observed transient change is due to the orientation of the bound Co(III) chelate under electric field. The absence of changes in transmittance at $\theta = 55°$, where $\Delta A/A$ is zero according to equation (3), excludes the possibility that the concomitant rise in temperature causes any isotropic transmittance change. Also, no change is induced by electric discharge, when KCl is added instead of PDCML.

In an electric dichroism apparatus, the electric field is supplied as a square pulse[10]. The decay of the induced dichroism represents the disorientational process of the chromophore[11]. This apparatus, however, does not provide a square-pulsed field but a spike-shaped pulse as seen in *Figure 4c*. Since the half-life of the signal decay, $t_{1/2}$(signal) = 120 μs, is close to that of the field decay, $t_{1/2}$(field) = 100 μs, we conclude that the observed signal

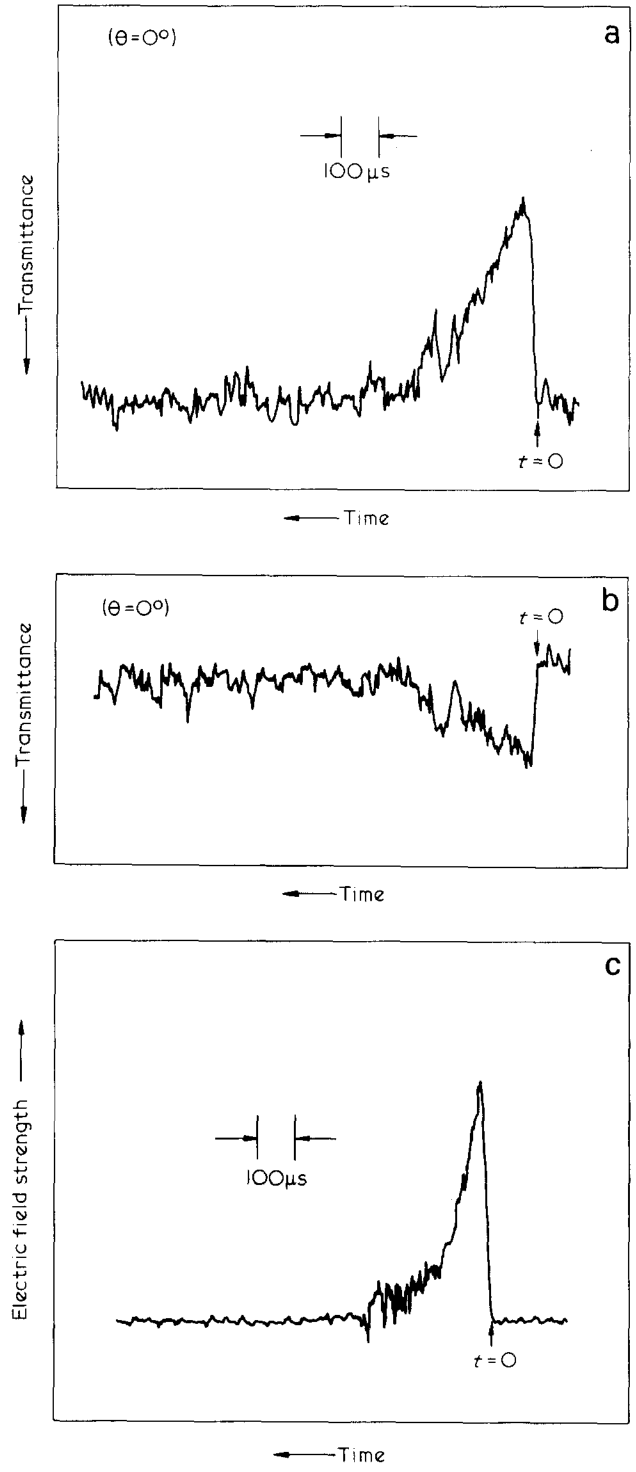

Figure 4 Transient signals observed during electric discharge for Co(III)(αPAN)$_2^+$ and PDCML solution. [Co(III)(αPAN)$_2^+$] = 1.8 × 10^{-5} M; [PDCML] = 1.5 × 10^{-3} M. Wavelength, 600 nm. pH = 7.80 ± 0.20. Horizontal scale 100 μs/div. Monitoring light is polarized parallel to electric field in (a), and vertical to electric field in (b), (c) denotes the time course of the imposed electric field

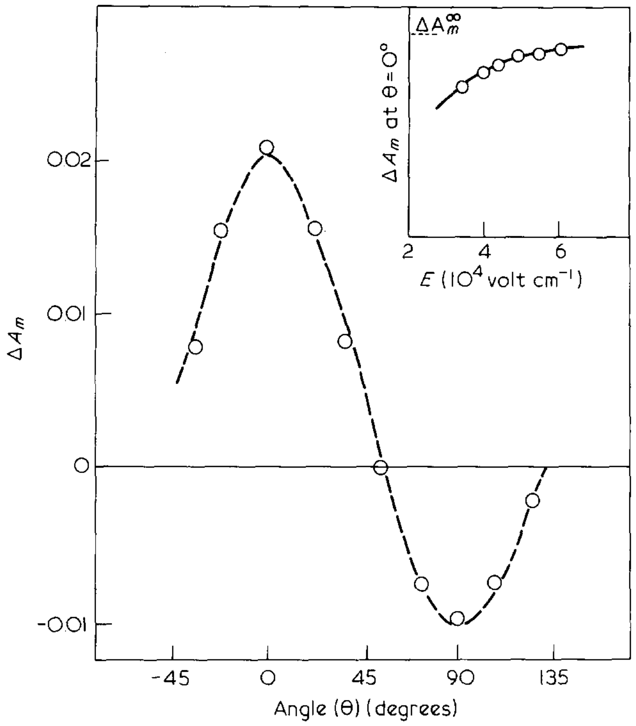

Figure 5 Dependence of maximum amplitude of the transient signal on θ. Inset shows the dependence of the maximum amplitude at $\theta = 0°$ on electric field strength

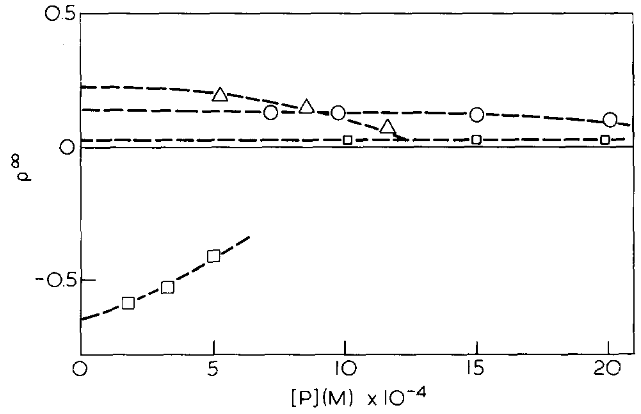

Figure 6 Dependence of reduced linear dichroism at infinite field strength, ρ^∞, on the polyelectrolyte concentration. Large □ (PSS); ○(PDCML); △ (PAA); and small □ (PLG). [Co(III)(αPAN)$_2^+$] = 1 ~5 x 10^{-5} M. Added Co(III) chelates are bound to the polyelectrolytes

decay does not represent a disorientational process, but a decrease in the stationary value of the dichroism, corresponding to the decrease of electric field strength.

This conclusion is supported by the following facts. Both $t_{1/2}$(signal) and $t_{1/2}$(field) reduce to about one-half of their original values, when the length of a coaxial cable is shortened from 157 m to 70 m[12]. When $t_{1/2}$(field) is lowered to about 3 μs adding KCl to the solution, $t_{1/2}$(signal) is also lowered to about 5 μs. These results imply that both the rates of the orientational and disorientational processes of the chromophore under investigation are much faster than the rise and decay rates of the electric field, so that we observe only a stationary value of the dichroism over the whole time range.

The maximum amplitude of the signal in *Figure 4a*, ΔA_m, increases slightly with an increase in electric field strength (E) as seen in the inset in *Figure 5a*. ΔA_m can be extrapolated to infinite field strength, to give ΔA_m^∞. The corresponding extrapolated reduced linear dichroism is given by $\rho^\infty = 1.5\,\Delta A_m/A$ by setting $\theta = 0°$ in equation (3). ρ^∞ depends on the concentration of PDCML at a constant ratio of [PDCML] to $[M]_0$ as shown in *Figure 6*. ρ^∞ is extrapolated to infinite dilution by the dotted line in *Figure 6*. For other kinds of polyelectrolyte (except PLL), the extrapolated ρ^∞ values are obtained in a similar manner. The results are summarized in *Table 1*. A Co(III)(αPAN)$_2^+$ and PLL solution does not show any transient transmittance change with the electric field. This is reasonable, since the Co(III) chelate does not bind with PLL and the free metal chelate alone does not orient under the present electric field strength.

The interaction of Co(III)(αPAN)$_2^+$ bound on a PDCML chain with a PSS molecule was investigated by a stopped-flow transient electric dichroism method. Electric dichroism was measured immediately after mixing a solution of Co(III)(αPAN)$_2^+$ and PDCML with a PSS solution. As seen from *Figures 7a* and *7b*, 0.2 s after mixing the electric dichroism exhibits a negative ρ value. The dichroism is due to the Co(III) chelate bound with PSS. The results imply that a Co(III) chelate, initially bound with PDCML, transfers to a PSS chain within the time of mixing (~0.2 s). The reaction is written as:

$$\mathrm{Co(III)(\alpha PAN)_2^+{\cdot}PDCML + PSS \rightleftarrows DPCML + Co(III)(\alpha PAN)_2^+{\cdot}PSS} \quad (4)$$

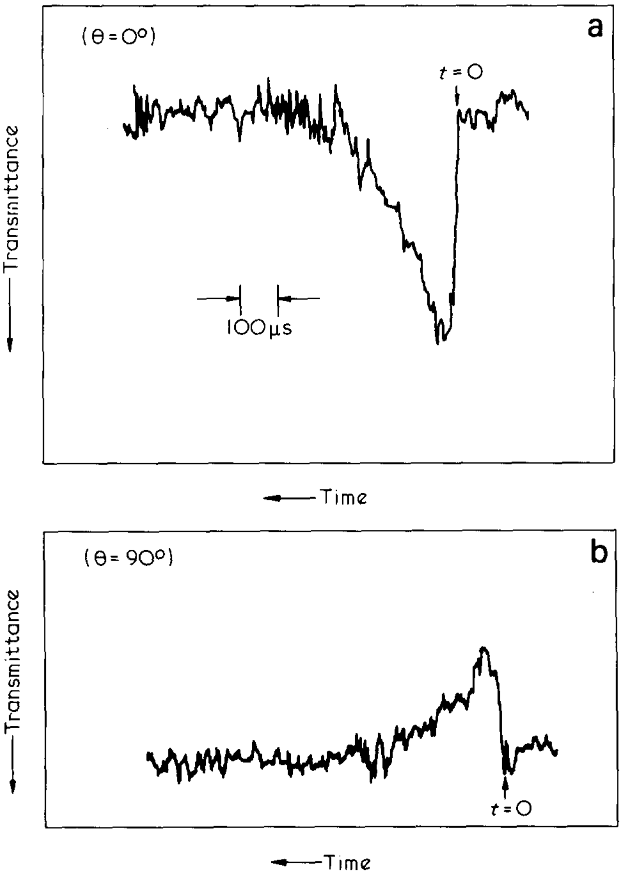

Figure 7 Transient signals observed during electric discharge about 0.2 s after mixing a Co(III)(αPAN)$_2^+$ and PDCML solution with a PSS solution. Final concentrations are [Co(III)(αPAN)$_2^+$] = 9 x 10^{-6} M, [PDCML] = 3.4 x 10^{-4} M and [PSS] = 6.9 x 10^{-5} M. $\theta = 0°$ (a), and 90° (b)

If this reaction proceeds by direct collision between a Co(III)(αPAN)$_2^+$·PDCML chain and a PSS chain, the forward bimolecular rate constants are extimated to be larger than 2×10^7 M^{-1} s^{-1}.

DISCUSSION

From the electronic spectra of a Co(III)(αPAN)$_2^+$ polyelectrolyte solution it can be seen that all of the polyelectrolytes except PLL bind a Co(III)(αPAN)$_2^+$ chelate in the appropriate pH region.

Since the sulphonate group in PSS and the carboxylate groups in PDCML, PAA and PLG completely lose a proton under these conditions, electrostatic attraction may be the main cause of the formation of a Co(III) chelate–polyelectrolyte complex. No attractive force is present in the case of PLL, since PLL has positive ε-NH$_3^+$ groups when the pH is below 10. The observed trend in the K_1 values in *Table 1* is in accord with this explanation.

K_1 values change by more than 10^4 going from PSS to PLG. One of the reasons for this is that the negative charge density along the polymer chain varies for the different polyelectrolytes (*Table 1*). However, this does not explain the exceptionally large K_1 for PSS, considering that PSS has the same charge density as PAA. The results suggest the presence of another kind of force in PSS, which we now discuss in connection with the electric dichroism results.

According to O'Konski *et al.*[13], the reduced linear dichroism, ρ in equation (3), is expressed as:

$$\rho = \frac{3}{4}(1 + 3\cos 2\psi)\cdot\Phi(E), \qquad (5)$$

where ψ is the angle between the electric field and the transition moment of an oriented chromophore, and $\Phi(E)$ is the orientation function. $\Phi(E)$ is the fraction of a polyelectrolyte whose axis is completely in direction of electric field. In deriving the above expression, the chromophore is assumed to adopt a fixed orientation to the polymer axis. $\Phi(E)$ varies from 0 to 1 with increasing electric field. Accordingly ρ may take a value between -1.5 and 3.0.

To the authors' knowledge, there is no theory describing the dynamic behaviour of a flexible polymer chain at high electric fields.

Kikuchi and Yoshioka, however, have studied the electric birefringence of an aqueous PSS solution[14] and found that Φ is a common function of E irrespective of the concentration and molecular weight of PSS. The dependence of $\Phi(E)$ on E resembles that for a rigid polymer with a permanent dipole moment. Their results were interpreted in terms of the saturation of the ion-atmosphere polarization around a charged polymer chain. Due to the interaction between this induced dipole moment and E, the polyelectrolyte stretches out in the direction of electric field.

Irrespective of its orientation mechanism, it is reasonable to conclude that ρ, extrapolated to infinite field strength and infinitely dilute solution (ρ^∞ at [P] = 0 in *Figure 6*) represents ρ at $\Phi(E) = 1$. This conclusion is based on the results in *Figure 2* of Ref. 14 which indicates that $\Phi(E)$ approaches 1 as E increases and as the polyelectrolyte concentration decreases. Under such conditions, the angle ψ is calculated by inserting the extrapolated ρ value in equation (4).

There are two difficulties in applying these procedures to our results. One is that the chromophore, Co(III)(αPAN)$_2^+$, has two transition moments arising from the two coordinating αPAN molecules. The other is that bound Co(III) chelate is free to move within some angle in space due to random motion of the side chain. This effect may be most prominent for PDCML, because it has a long [–(CH$_2$)$_4$–] side chain.

Since no precise mathematical method of overcoming the above difficulties is presently available we introduce two assumptions to derive at least a qualitative conclusion on the structural differences among the present metal chelate–polyelectrolyte complexes:

(i) the two αPAN molecules adopt the same angle ψ with respect to the oriented axis of a polymer segment; and

(ii) the motion of a side chain is taken into account by multiplying equation (4) by a factor, (sinΔ/Δ), where Δ is the amplitude of fluctuations of the angle ψ, affecting the transition moment of a bound chromophore experiencing random motion of a side chain[15].

Based on these assumptions, ψ is calculated from the experimental value of ρ^∞ at [P] = 0. The results are given in *Table 1*. Although the calculated ψ may involve a large error arising from the assumptions made here, the following comments, at least, are noteworthy.

In spite of the similarity in their electronic spectra, the Co(III) chelate–polyelectrolyte complexes have different structures depending on the nature of the residues. Among the chosen polyelectrolytes, PSS has the largest ψ value. Since the transition moment lies in the αPAN plane, a large ψ means that the αPAN plane is vertical to the polyelectrolyte chain. Most porbably, the results reflect situations where the aromatic rings in αPAN are pulled into the hydrophobic regions surrounded by the four or more styrene sulphonate residues in PSS. This interaction is also responsible for the largest K_1 obtained for PSS[16].

However, other polyelectrolytes have ψ values less than 55°. The results are explained by assuming that the αPAN plane becomes parallel to the polymer axis, when the central Co^{3+} ion is attracted close to the negative –COO$^-$ group.

The observed rapid change of dichroism after mixing a metal chelate and PDCML solution with a PSS solution provides direct proof for the transfer of a bound metal chelate from one polymer chain to another. It is hardly possible to reach the same conclusion from the electronic spectra only, because both Co(III)(αPAN)$_2^+$–PDCML and Co(III)(αPAN)$_2^+$–PSS complexes exhibit similar spectra.

Further application of electric dichroism may allow detection and identification of the binding states of a metal ion, when other conventional techniques like c.d. and e.p.r. are not applicable.

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16 The importance of the hydrophobic region in PSS has already been noted by other authors (see ref. 1, review by Ise)