Circular Dichroism Studies on the Interactions between Tris (L-alaninato)-cobalt(III) Complexes and Basic Polyelectrolytes in Solution

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The circular dichroism spectra of the poly-L-lysine complexes with *abc*(−), *abd*(−), and *abd*(+)Co(L-ala-*O*)$_3$ in water and isopropyl alcohol–water (1 :1), at different pH values and over a range of complex to polymer molar ratios, have been measured. Evidence of a specific site binding is presented for *abc*(−)Co(L-ala-*O*)$_3$ in the mixed solvent medium. Of the diastereoisomers used, only in the presence of this material does the macroion assume an α-helical conformation at pH values where the coil form normally predominates. The greater the complex to polymer ratio, the greater the proportion of material of α-helical conformation there is present. Directional modes in the binding, very likely involving hydrogen bonding interactions, are indicated by the changes in the visible c.d. spectra of *abc*(−)-Co(L-ala-*O*)$_3$ in PLL-50% isopropyl alcohol solutions. In contrast, the same complex destabilizes the α-helix structure of poly-L-ornithine. All these features are examined in the light of the structural characteristics of the interacting species. The influence of solvent composition is also considered. Implications of the different stereochemical features of *abd*-diastereoisomers on the association process with the polypeptides are discussed.

It is widely recognized that apolar interactions can play an important role in the binding process between small molecular ions [1,2] or complex ions [3,4] and polyelectrolytes in aqueous solution. Attention has recently been paid to hydrogen bonding interactions which, depending upon the structural features of the ionic species in solution, may enhance binding ability and eventually control specificity.[2,5] In all systems so far investigated, however, long-range electrostatic forces predominate. Furthermore, in very few instances have bound molecules been shown to affect the conformation of the polymeric substrate.[1,5–7]

We now describe an interesting effect exerted by tris-(L-alaninato)cobalt(III) complexes.† Thus, the addition of *abc*(—)Co(L-ala-*O*)$_3$ to poly-L-lysine (PLL) and poly-L-ornithine (PLO) in solution gives rise to a marked increase of α-helix structure in the former polypeptide but to a decrease in the latter, depending upon the solvent composition.

The present study was undertaken in order to investigate a system where the neutral character of one of the interacting species would make ion-pairing association minimal and it would be possible to investigate the role played by other kinds of interaction.

For this purpose *abc*(—)-, *abd*(—)-, and *abd*(+)-Co(L-ala-*O*)$_3$ complexes were used in conjunction with aqueous and isopropyl alcohol–water (1 : 1, v/v) solutions of the basic polyelectrolytes; the *abc*(+) isomer was insoluble in water and was not therefore used. These neutral complexes exhibit partial hydrophobic character and a great tendency to form hydrogen bonds *via* the NH_2 and CO groups.[8] In addition, it was thought that since other Co^{III} complexes are reported to have visible c.d. spectra which are very sensitive to outer-sphere co-ordination *via* hydrogen bonding,[3,5,9,10] such behaviour by the tris(L-alaninato)cobalt(III) compounds could be useful in studying the association process. On the other hand, the dissymmetric substrates also have H-bonding capacity and partially hydrophobic properties.

Spectral and optical results on complex–polymer solutions at different pH values and NaCl concentrations indicate that binding phenomena take place through forces of mixed nature. The occurrence of specific site binding between poly-L-lysine and *abc*(—)Co(L-ala-*O*)$_3$ in water–isopropyl alcohol solution, which is responsible for the marked ' renaturation ' effect on the charged polymer, will be discussed on the basis of some general considerations concerning the stereochemical features of the interacting species as well as the role of the solvent medium.

EXPERIMENTAL

Materials.—Poly-L-lysine hydrobromide (M = 95 000) and poly-L-ornithine hydrobromide (M = 30 000) were purchased from Miles–Yeda (Israel). Stock solutions were prepared by dissolving the polymer in double distilled water (conductivity less than $2{\cdot}5 \times 10^{-6}\ \Omega^{-1}\ \mathrm{cm}^{-1}$, 20 °C) and, when necessary, adding the appropriate amount of spectral quality isopropyl alcohol (Merck). They were stored at 5 °C and removed as needed. Concentration of polymers was determined by micro-Kjeldhal nitrogen analysis. Final concentrations were in the range of 2×10^{-4}—5×10^{-4}M (referred to monomeric unit).

The complexes were prepared according to known

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† In the following they are designated according to I.U.P.A.C. nomenclature, *e.g. abc* and *abd* refer to the positions of each co-ordinating oxygen atom in the octahedral co-ordination sphere, whilst the signs refer to the optical activity.

[1] I. M. Klotz, G. P. Royer, and A. R. Sloniewsky, *Biochemistry*, 1969, **8**, 4752; I. M. Klotz and J. U. Harris, *ibid.*, 1971, **10**, 923.

[2] F. Passero, E. J. Gabbay, B. Gaffney, and T. Kurucsev, *Macromolecules*, 1970, **3**, 158; E. J. Gabbay and R. Glaser, *Biochemistry*, 1971, **10**, 1665.

[3] V. Crescenzi and B. Pispisa, *J. Polymer Sci* (*A*), 1968, **6**, 1093; V. Crescenzi, F. Quadrifoglio, and B. Pispisa, *J. Chem. Soc.* (*A*), 1968, 2175.

[4] S. Brückner, V. Crescenzi, and F. Quadrifoglio, *J. Chem. Soc.* (*A*), 1970, 1168.

[5] F. Ascoli, M. Branca, C. Mancini, and B. Pispisa, *J.C.S. Faraday I*, 1972, **68**, 1213; IV International Biophysics Congress (Moscow), 1972, **2**, 250.

[6] J. M. Rifkind and G. L. Eichhorn, *Biochemistry*, 1970, **9**, 1753.

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[8] B. M. Fung and I. H. Wang, *Inorg. Chem.*, 1969, **8**, 1867.

[9] S. F. Mason and B. J. Norman, *Proc. Chem. Soc.*, 1964, 339; R. Larsson, S. F. Mason, and B. J. Norman, *J. Chem. Soc.* (*A*), 1966, 301; S. F. Mason and B. J. Norman, *ibid.*, p. 307.

[10] H. L. Smith and B. E. Douglas, *J. Amer. Chem. Soc.*, 1964, **86**, 3885; *Inorg. Chem.*, 1966, **5**, 784.

methods.[11,12] Elemental analyses were as follows: *abc*(−)-Co(L-ala-O)$_3$, Found: C, 34·0; N, 12·9; H, 6·0. Calc. for C, 33·45; N, 13·00; H, 5·57. *abd*(+)Co(L-ala-O)$_3$, $\frac{1}{2}$H$_2$O, Found: C, 32·6; N, 12·4; H, 5·9. Calc. for C, 32·51; N, 12·64; H, 5·72%). Solubility and optical properties of all aqueous complexes were in good agreement with those reported in the literature.[11,13] Their concentration was normally determined by u.v. absorption at $\lambda_{\text{max.}}$ 218 ($\varepsilon_{\text{max.}}$ 31 800) in H_2O and 220 nm ($\varepsilon_{\text{max.}}$ 29 500) in isopropyl alcohol for *abc*-Co(L-ala-O)$_3$ and at $\lambda_{\text{max.}}$ 221 ($\varepsilon_{\text{max.}}$ 24 700) in H_2O and 223 nm ($\varepsilon_{\text{max.}}$ 21 300) in isopropyl alcohol for *abd*-diastereoisomers.

Tris(hydroxymethyl)aminomethane (Sigma Chemical Corp., U.S.A.) was used as buffer in chloride form (tris buffer), at a concentration of 0·01M both in aqueous and in a mixed solvent medium. pH Values higher than 9 were obtained by adding standard base. Interaction between polymers and buffer was ruled out on the basis of preliminary measurements of rotatory power at 253 nm of solutions containing increasing amounts of buffer at a given pH. The same was true for the complexes, as evidenced by c.d. data at *ca.* 220 nm.

Measurements on complex–polymer solutions were performed at room temperature, samples being only occasionally shaken.

Apparatus.—Absorption measurements were carried out using a Beckman DK-2A spectrophotometer with quartz cells of 1, 0·5, or 0·1 cm path-length. The stray light of the instrument was found to be practically zero at 190 nm. Rotatory power data were obtained with a Perkin-Elmer 141 M apparatus and appropriate quartz cells. Circular dichroism (c.d.) and differential circular dichroism (d.c.d.) spectra were recorded on a Cary 61 instrument, at *ca.* 27 °C, unless otherwise stated, with quartz cells of 2, 1, or 0·1 cm path-length. pH Values were determined with a Radiometer 26 pH-meter and standard electrodes.

RESULTS

The use of a mixed solvent medium, *i.e.* isopropyl alcohol–water (1 : 1 v/v), throughout this work deserves some comment. It has been shown that poly-L-lysine undergoes a sharp transition in aqueous solution when electrostatic repulsion along the chain is minimized, *e.g.* near pH 10;[14,15] suppression of such repulsion is not sufficient however to stabilize the α-helix of poly-L-ornithine.[16,17] For instance, PLO in water is still largely in a coil form even at pH 12, although the pK values of both polyelectrolytes are similar.[17] On the other hand, tris(L-alaninato)cobalt(III) complexes slowly decompose at pH >10 (room temperature). Therefore the association between helical polypeptides and octahedral compounds could be investigated only by decreasing the coil to α-helix transition well below pH 10. This can be achieved either by employing a mixed solvent medium or aqueous perchlorate ions. In both cases the α-helix is stabilized at lower pH values.[16-19] The use of ClO_4^- or of certain other ions[19] was discarded since they could compete with Co(L-ala-O)$_3$ for association with the polyelectrolytes. In contrast, isopropyl alcohol–water (1 : 1) was found satisfactory since it reduces quite drastically the pH of transition and increases significantly the stability of the helical PLO. This effect is shown in Figure 1, where the fraction of α-helix (f_h), calculated in the conventional way, by assuming additivity of the ellipticities, $\Delta\varepsilon_{\text{obs}} = f_h\Delta\varepsilon_h + (1 - f_h)\Delta\varepsilon_c$ (c and h designate the coil and helical form, respectively), is reported as a function of pH.

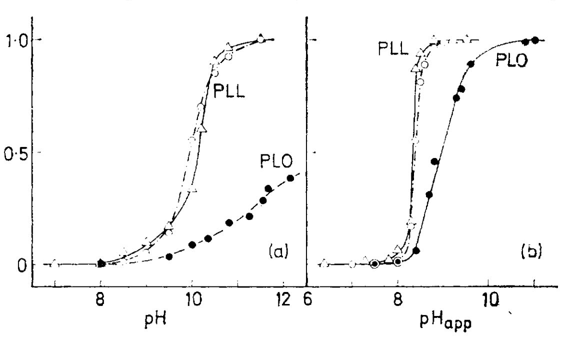

FIGURE 1 α-Helix fraction of poly-L-lysine (PLL) and poly-L-ornithine (PLO) as a function of (a) pH in aqueous solution and (b) apparent pH in isopropyl alcohol–water (1 : 1), from measurement of $[M]_{253}$ (○) and $(\varepsilon_L - \varepsilon_R)_{220}$ (△, ●); see the text. The data of PLO in water solution are taken from ref. 17

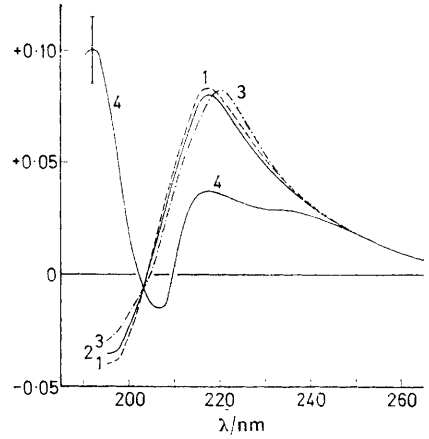

FIGURE 2 Ellipticity of *abc*(−)Co(L-ala-O)$_3$–PLL aqueous (pH = 9·2) and 50% isopropyl alcohol (pH$_{\text{app}}$ = 8·2) solution (curves 2 and 4, respectively) and sum of the ellipticities due to the pure components in aqueous and mixed solvent medium (curves 1 and 3), all referred to an optical path length of 1 cm. Polymer concentration: 2·10^{-4}M; complex to polymer molar ratio of 0·50

In addition, comparison between the results obtained in mixed solvent or water could give useful information on the type of interaction which eventually predominates such as, for instance, that based on hydrogen bonding or hydrophobic forces.

Ultraviolet D.c.d. Spectra.—C.d. spectral patterns in the u.v. region of the spectrum of poly-L-lysine–*abc*(−)Co(L-ala-O)$_3$ system in water (pH = 9·2) and in isopropyl alcohol–water (1 : 1) (pH$_{\text{app}}$ = 8·2) are shown in Figure 2, together

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[11] R. G. Denning and T. S. Piper, *Inorg. Chem.*, 1966, **5**, 1056.

[12] M. Mori, M. Shibata, K. Kyono, and M. Kanaya, *Bull. Chem. Soc. Japan*, 1961, **34**, 1837.

[13] E. Larsen and S. F. Mason, *J. Chem. Soc.* (*A*), 1966, 313.

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[15] Y. M. Mayer, *Macromolecules*, 1969, **2**, 624, and references cited therein.

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with the sum of ellipticities due to the pure components. At these pH values the helical fraction of PLL in both cases is comparable, being some 0·10 (Figure 1). A dramatic difference may, however, be noted: while the total dichroic spectrum in water is only slightly changed with respect to the sum of ellipticities, that in isopropyl alcohol–water (1 : 1) is markedly different when compared with the sum of ellipticities of pure components. In contrast, under the same experimental conditions, the total dichroic spectra of PLL-*abd*-diastereoisomer solutions show no significant variation with respect to the sum of ellipticities, in either of the solvent media.

Although tris(L-alaninato)cobalt(III) complexes have a strong c.d. band at *ca.* 220 nm (see later), the perturbation observed at this wavelength for the PLL-*abc*-isomer in isopropyl alcohol–water (1 : 1) is so marked that it must be essentially attributed to a change in conformation of the polymer. This hypothesis is consistent with the appearance of a band at 192 nm, typical of helical polypeptides, and is further supported by the results illustrated in Figure 3. Here the differential c.d. spectra of PLL-*abc*(−)-systems in the mixed solvent medium, monitored against the complex, are reported together with the c.d. spectra of pure PLL solutions, at different apparent pH values. In the presence of the complex the 217—218 ($R > 0$) and 196 nm ($R < 0$) c.d. bands of poly-L-lysine, typical of random coil, disappear even at those pH values where the unordered form normally predominates, while the 222 and 207—208 nm ($R < 0$) c.d. bands, typical of α-helix, appear or strongly increase, depending upon pH.

As shown in Figure 4, where the variation of ellipticity at 220 and 208 nm of poly-L-lysine in the presence of the octahedral compounds is plotted against pH (apparent), addition of *abd*-diastereoisomers, at the same complex to polymer molar ratio (C/P), does not apparently affect the molecular structure of PLL.

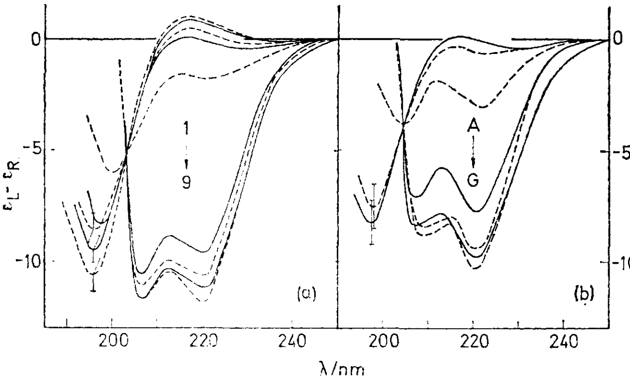

FIGURE 3 C.d. spectra of PLL (a) and d.c.d. spectra of PLL in the presence of *abc*(−)Co(L-ala-*O*)$_3$ (b), at different apparent pH values. Curve, 1, $\mathrm{pH_{app}} = 6{\cdot}4$; 2, 7·3; 3, 7·8; 4, 8·0; 5, 8·3; 6, 8·4; 7, 8·5; 8, 8·8; 9, 9·2—9·5; A, 6·8; B, 7·0; C, 7·5; D, 7·9; E, 8·0; F, 8·2; G, 9·1—9·3. Polymer concentration: $4{\cdot}70 \times 10^{-4}$M in 50% isopropyl alcohol solution containing tris buffer 0·01M; (C/P) ratio of 0·48

On the other hand, the lack of significant perturbations on the spectral patterns of the aqueous polymer compared with the sum of ellipticities of pure components (Figure 2) is reflected in the d.c.d. spectra of aqueous PLL-*abc*(−) as a little change in the rotational strength of the c.d. bands of the macroion, even at a C/P ratio as high as 0·70.

The relevance of these results is that in the mixed solvent medium only the *abc*-isomer has the ability to induce a conformational change in the poly(amino-acid). Therefore, in this case the occurrence of a specific site binding may be considered reasonable.

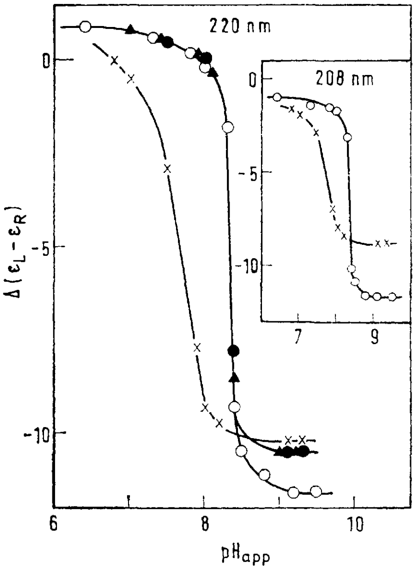

FIGURE 4 Variation of ellipticity at 220 and 208 nm of PLL in 50% isopropyl alcohol mixture (○) and of PLL in the same solvent but in the presence of *abc*(−) (×), *abd*(−) (●), and *abd*(+)Co(L-ala-*O*)$_3$ (▲), as a function of pH (apparent). All complex to polymer ratios were around 0·50 (see Figure 3)

From Figures 3 and 4 it can be seen that all the complexes, and in particular *abc*(−)-Co(L-ala-*O*)$_3$, give rise to a decrease in the rotational strength of the bands of helical PLL at 220 and 208 nm. This effect may be due either to a perturbation in the corresponding transitions of the polymer, owing to the association process, or to a change of the optical characteristics of the bound complexes, or to both. The c.d. bands of the octahedral compounds in the far-u.v. region (180—220 nm) originate not only from intramolecular charge-transfer but also from ligand-localized transitions.[20,21] Such complicating features make it difficult at present to discriminate with confidence between these factors. Since the positive c.d. band for both *abc*(−)- and *abd*(−)-Co(L-ala-*O*)$_3$ centred near 220 nm increases whereas the negative one of *abd*(+)Co(L-ala-*O*)$_3$ decreases (in absolute value) in the presence of helical PLL (see Figure 5), it is possible that a perturbation in the electronic transitions of the polymer is primarily responsible for the observed effect. A change in solvent distribution in the immediate environment of the helical chain caused by the bound complex [22,23] or coupling between the transition moments of the peptide chromophore in the macromolecule and the amino-acid ligands in the bound molecules [24] are both compatible with such a hypothesis. In any case, these results clearly indicate that *abd*-diastereoisomers interact with the polypeptide,

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[20] D. S. McClure, *Solid State Phys.*, 1959, **9**, 399.

[21] M. D'Alagni, B. Pispisa, and F. Quadrifoglio, *Ricerca sci.*, 1968, **38**, 475.

[22] F. Quadrifoglio and D. W. Urry, *J. Amer. Chem. Soc.*, 1968, **90**, 2760.

[23] U. P. Strauss and Y. Po Leung, *J. Amer. Chem. Soc.*, 1965, **87**, 1476.

[24] N. Amand, N. S. R. K. Murthy, F. Naider, and M. Goodman, *Macromolecules*, 1971, **4**, 564.

although they are not able to influence appreciably its conformation.

A further difference in the interaction of tris(L-alaninato)-cobalt(III) diastereoisomers with poly-L-lysine is illustrated in Figure 6. Here the ellipticity at 220 and 208 nm of charged PLL in 50% isopropyl alcohol in the presence of the complexes is reported as a function of C/P ratio, the corresponding spectral patterns being presented in Figure 7. The trend in the Figure suggests that in the case of the *abc*-isomer a co-operative process occurs. In contrast, with *abd*-diastereoisomers a smooth variation in the optical (and conformational) features of the polymer is observed only at relatively high values of C/P.

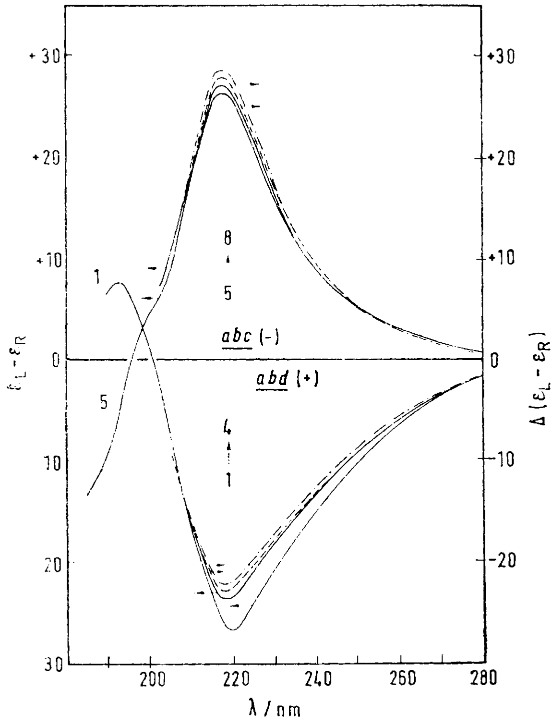

FIGURE 5 Far-u.v. c.d. spectra of pure complexes (full lines) and d.c.d. spectra of the same complexes in PLL–50% isopropyl alcohol buffered solution (broken lines); curves 1 and 5: in water solution; 2 and 6: in 50% isopropyl alcohol–water solution ($pH_{app} = 9$, tris buffer 0·01M); 3, 4, 7, and 8: in the presence of poly-L-lysine at the (C/P) ratio of 0·40 and 0·55, respectively. Polymer concentration: $4 \cdot 10^{-4}$M containing tris buffer 0·01M, $pH_{app} = 9$

If the association of *abc*(−)Co(L-ala-*O*)$_3$ is the main factor involved in the coil to α-helix transition of PLL, it would be of interest to know at what ratio of bound complex to polymer residue the conformational transition takes place. Unfortunately, equilibrium dialysis measurements were irreproducible, probably because of interaction between the isopropyl alcohol and the dialysis tube. Nevertheless, it was shown qualitatively that the affinity of charged poly-L-lysine for *abc*(−)Co(L-ala-*O*)$_3$ was higher than that for the *abd*-diastereoisomers. Furthermore, the same type of experiments (PLL 2×10^{-4}M in tris buffer 0·01M, $pH_{app} = 8$) indicated that the presence of 0·05M-NaCl strongly reduces the amount of bound complex. A parallel investigation of the d.c.d. spectra (similar to those reported in Figure 3)

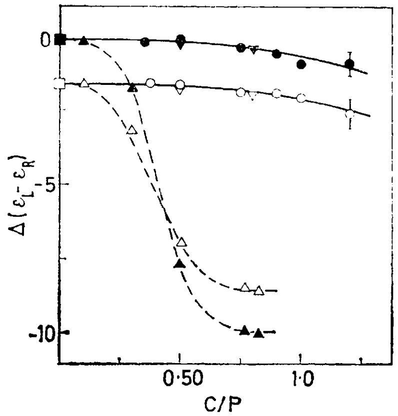

FIGURE 6. Variation of ellipticity at 220 (full symbols) and 208 nm (empty symbols) of PLL–50% isopropyl alcohol solutions ($pH_{app} = 7 \cdot 9$) in the presence of *abc*(—) (▲, △), *abd*(+) (●, ○), and *abd*(−)Co(L-ala-*O*)$_3$ (▼, ▽) diastereoisomers as a function of complex to polymer molar ratio (C/P)

for solutions containing the electrolyte, in the pH range 7·8—8·3, showed that the renaturating effect exerted by the *abc*-isomer on PLL was increasingly depressed, the higher the NaCl concentration.

Since the electrolyte is expected to shield the ionised side-chains, these results imply that electrostatic forces (very likely ion–dipole and ion–induced dipole) are involved in the association process under study which are favoured by the low dielectric constant of the mixed solvent medium. This in turn explains, at least partially, the lower affinity of charged PLL for the *abd*-diastereoisomers. The structural features of the latter complexes are such that two amino-acid ligands are oriented in a meridional fashion whilst with those of the *abc*-isomer are such that the three amino-acid ligands are oriented in a facial form.[11,13,25,26] The former molecules thus have a lower electrical polarity than the latter.

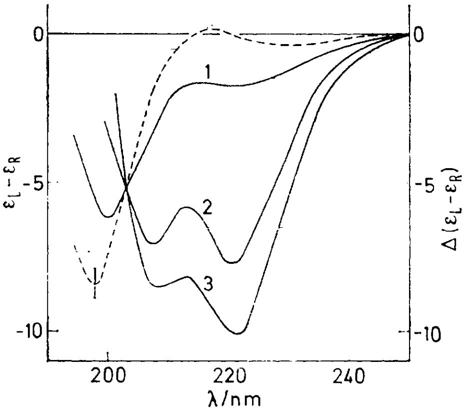

FIGURE 7. C.d. spectrum of PLL- (broken line) and d.c.d. spectra of PLL–50% isopropyl alcohol solutions (full lines) containing increasing amounts of the *abc*-isomer (see Figure 6); curve 1: (C/P) ratio of 0·30; 2 : 0·50; and 3 : 0·77. Polymer concentration: $4 \cdot 10^{-4}$M in tris buffer 0·01M, $pH_{app} = 7 \cdot 9$

The complexes are not strictly comparable however, since, under the same experimental conditions, the fraction of

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[25] B. E. Douglas and S. Yamada, *Inorg. Chem.*, 1965, **4**, 1561.

[26] M. G. B. Drew, J. H. Dunlop, R. D. Gillard, and D. Rogers, *Chem. Comm.*, 1966, 42.

bound molecules is different and this may well depend not only on their different electrical properties but also on their different stereochemical features, all of these being responsible for a diverse degree of binding.

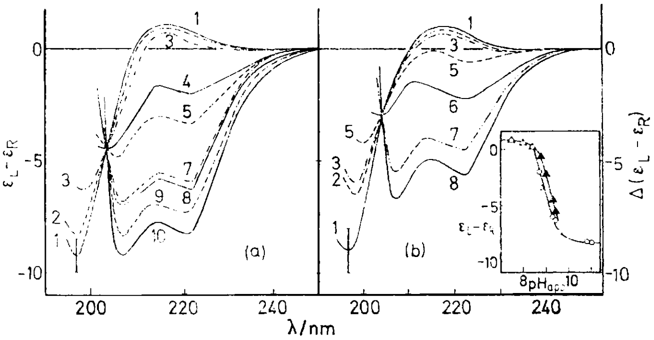

FIGURE 8. C.d. spectra of PLO (a) and d.c.d. spectra of PLO–50% isopropyl alcohol solutions in the presence of *abc*(−)Co(L-ala-*O*)$_3$ (b), at different apparent pH values. Curve 1, pH$_{app}$ = 7·5; 2, 8·0; 3, 8·4; 4, 8·7; 5, 8·8; 6, 9·0; 7, 9·3; 8, 9·4; 9, 9·6; 10, 10·8—11. Polymer concentration: 4 × 10^{-4}M; (C/P) ratio of 0·60. Insert: variation of ellipticity at 220 nm from the spectral patterns reported in (a) and (b) as a function of pH (apparent); empty symbols: PLO; full symbols: PLO in the presence of the complex

As far as the results for poly-L-ornithine are concerned, in Figure 8 the differential c.d. spectra of PLO-*abc*(−)Co-(L-ala-*O*)$_3$ in 50% isopropyl alcohol solution, monitored against the complex, are reported together with the c.d. spectra of PLO at the same apparent pH values.

Rather surprisingly, in this case the effect of the complex is the reverse of that expected in that it discourages the formation of the helical polymer structure. Thus, at pH$_{app}$ 9·3, the helix fraction of PLO is 0·74 whereas it is only about 0·58 in the presence of the *abc*-isomer (C/P = 0·60). The data in the insert of Figure 8, where the ellipticity at 220 nm is reported as a function of pH (apparent), clearly illustrate such a finding.

One final point must also be considered: it was observed that the spectral changes of PLL in 50% isopropyl alcohol caused by the bound *abc*-isomer (Figures 2—4, 6, and 7) are due to a slow kinetic process, lasting some days. An example of this behaviour is illustrated in Figure 9, where the ellipticity at 220 nm of PLL in the presence of *abc*(−)-Co(L-ala-*O*)$_3$, at different apparent pH values, is seen to vary with time, reaching a constant value only after several hours. Therefore, for consistency, all the spectral patterns reported here are those recorded after 7 days.

Rotational strength of the first ligand-field band of tris(L-alaninato)cobalt(III) complexes in different solvent media

Complex	Solvent medium	Circular dichroism λ$_{max}$/Å	 $(\varepsilon_L - \varepsilon_R)_{max}$	 $10^{40}R$ (c.g.s.)
abd(+)Co(L-ala-*O*)$_3$	90% TFE	5280	+4·79	+14·87
	Water	5300	+3·12	+9·68
	50% Isopropyl alcohol	5330	+2·58	+8·32
	50% Isopropyl alcohol (pH$_{app}$ = 7·9, tris 0·01M)	5330	+2·65	+8·47
	In PLL–50% isopropyl alcohol solution (pH$_{app}$ = 7·9, C/P = 1·3)	5330	+2·75	+8·79
abc(−)Co(L-ala-*O*)$_3$	Water	5300	−2·50	−8·01
	50% Isopropyl alcohol	5320	−2·21	−6·84
	50% Isopropyl alcohol (pH$_{app}$ = 7·9, tris 0·01M)	5320	−2·25	−6·95
	In PLL–50% isopropyl alcohol solution (pH$_{app}$ = 7·9, C/P = 0·77)	5320	−2·36	−7·47

Since blank experiments showed that at room temperature and below pH 10 the c.d. spectrum of the octahedral complexes remains practically unaltered for more than 15 days, this phenomenon may be ascribed to slow association between the polymer and the *abc*-complex, as a result of lack of a strong electrostatic driving force in the system considered. Alternatively, it might be due to a slow transition between two conformational states of the polylysine-*abc*(−)-Co(L-ala-*O*)$_3$ association complex, reminiscent of that observed with the same polyelectrolyte upon ion-pairing with 5′-nucleotides,[7] where a slow rearrangement of the bound molecules in an ordered fashion was thought to occur. In our case, however, stacking phenomena among bound molecules are very unlikely. We are inclined, therefore, to rule out the latter hypothesis, although additional experiments are needed to elucidate the nature of this effect.

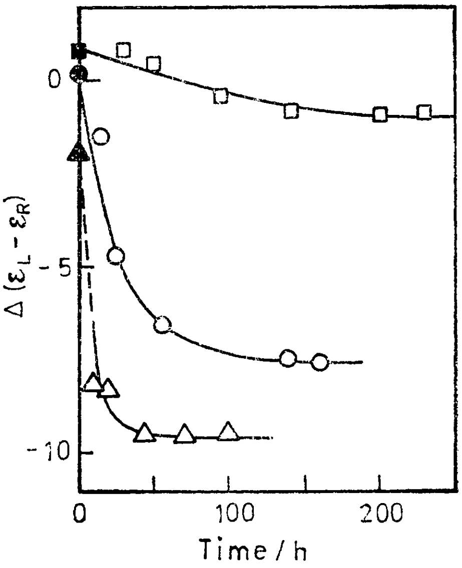

FIGURE 9. Variation of ellipticity at 220 nm of PLL–50% isopropyl alcohol solutions in the presence of the *abc*-isomer as a function of time. pH$_{app}$ = 7·4 (□), 7·9 (○), and 8·3 (△). Polymer concentration: 4 × 10^{-4}M, (C/P) ratio of 0·50. The full symbols refer to PLL solutions at the given pH values, in the absence of the octahedral complex

Visible and Near-ultraviolet C.d. Spectra.—Visible and near-u.v. c.d. spectra of tris(L-alaninato)cobalt(III) complexes in different solvent systems are presented in Figure 10 together with some typical spectra of the same complexes in PLL–50% isopropyl alcohol solutions. For clarity, those of *abc*(−)- and *abd*(+)-Co(L-ala-*O*)$_3$ only are reported.

All spectral patterns and, mainly, the low-frequency c.d. band of the octahedral T_{1g} transition of pure complexes, appear to be very sensitive to the solvent medium. For instance, the rotational strength of the band near 530 nm of *abd*(+)Co(L-ala-*O*)$_3$ markedly increases in going from 50% isopropyl alcohol to 90% trifluoroethanol–water mixture (v/v); this is quantitatively illustrated in the Table. A similar trend is observed for *abc*(−)Co(L-ala-*O*)$_3$. Since the corresponding absorption spectra (Figure 10a) show only minor variations with a change in the solvent system, it may be safely concluded that the enhancement of ellipticity is essentially ascribable to solvent–complex interactions rather than to the change in the dielectric constant of the medium.

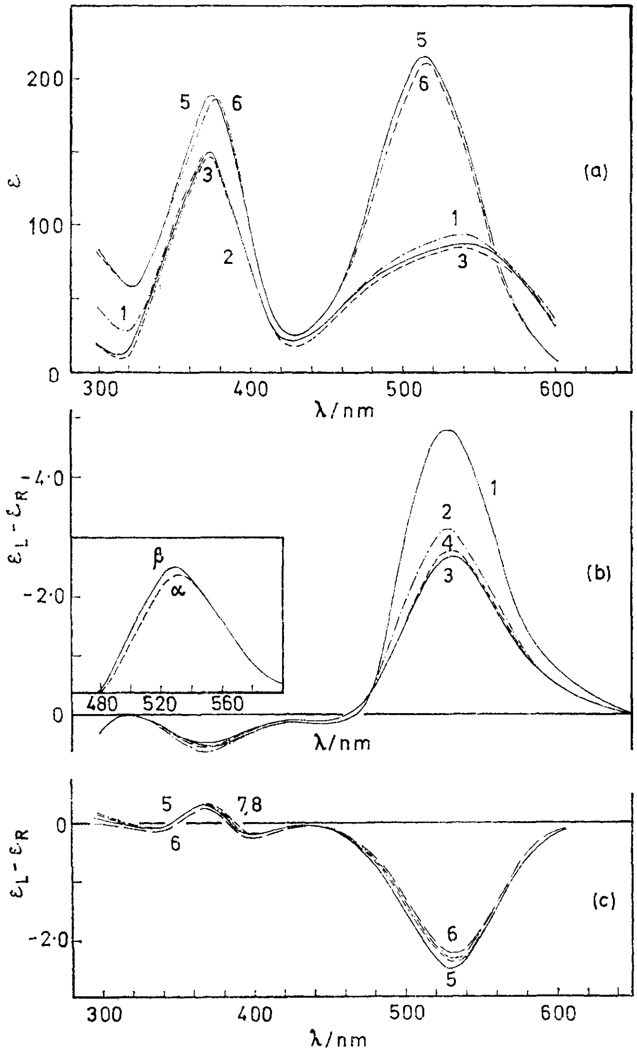

FIGURE 10. Absorption (a) and c.d. spectra of *abd*(+) (b) and *abc*(−)Co(L-ala-*O*)$_3$ (c) in the absence (curves 1—3 and 5, 6) and in the presence (curves 4, 7, and 8) of PLL; curve 1: in trifluoroethanol–water (1 : 1 v/v); 2 and 5: in water; 3 and 6: in isopropyl alcohol–water (1 : 1) at pH$_{\mathrm{app}}$ = 7·9 (tris buffer 0·01M); 4 and 7: in 50% isopropyl alcohol of PLL at pH$_{\mathrm{app}}$ = 7·9 and (C/P) ratios of 1·3 and 0·77, respectively; 8: in isopropyl alcohol solutions of PLL at pH$_{\mathrm{app}}$ = 9 and (C/P) ratio of 0·55. Polymer concentration: 4×10^{-4}M in 0·01M tris buffer. The complex concentration ranged from 2×10^{-4} to 6×10^{-4}M. Insert: effect of temperature on the rotational strength of the longer wavelength c.d. band of *abd*(+)Co-(L-ala-*O*)$_3$ in water solution; curve α: 70°, β: 20 °C

Stereochemical models as well as structural data on *abd*(+)Co(L-ala-*O*)$_3$ [26] indicate that the chelate rings in this type of complex are not planar although they are less puckered than those of trisdiamine–cobalt(III) compounds.[13] Therefore one may reasonably assume the existence in solution of an equilibrium among different conformers [13,27] which is likely to be perturbed by changing solvent composition.

Inspection of the Table and Figures 10b and c, shows that the rotational strength of the first ligand-field band rises as the H-bonding capacity of the solvent systems increases. This fact clearly suggests that in our work solvent–complex interactions primarily involve hydrogen-bonding forces, which may determine a loss of conformational mobility in the chelate rings leading to the observed trend. This hypothesis is consistent with recent p.m.r. data on aqueous tris(L-alaninato)cobalt(III) compounds, which suggest the formation of a second co-ordination sphere with solvent molecules by way of hydrogen bonding through the proton-donor NH$_2$ and the electron-donor CO groups of the ligands.[8]

The results in the insert of Figure 10 provide additional support for our hypothesis. The rotational strength of the longer wavelength c.d. band of aqueous *abd*(+)Co(L-ala-*O*)$_3$ is seen to decrease by some 7% in going from 20 to 70 °C, comparison being made first on heating and then on cooling the sample and thus taking into account racemization due to the heating (*ca.* 17%). According to the previous data, an increase of conformational mobility of chelate rings with temperature is compatible with this finding.

Addition of PLL to *abc*(−)Co(L-ala-*O*)$_3$–50% isopropyl alcohol solutions gives rise to a significant perturbation in the c.d. band of the complex (Figure 10c and Table) in the sense that the rotational strength rises throughout the pH (apparent) range explored. These results strongly suggest that the binding of the *abc*-isomer by PLL is predominantly directional in nature, very likely involving hydrogen-bonding interactions. The enhancement of rotational strength may be explained in fact in terms of increased rigidity of chelate rings in the bound molecules.

On the other hand, the less-significant changes in the low-frequency c.d. band of *abd*(+)Co(L-ala-*O*)$_3$ in PLL–50% isopropyl alcohol solutions (Figure 10b and Table) indicate that in this case directional interactions are reduced.

DISCUSSION

Very few examples of the behaviour of optically active complex ions in a solution of a dissymetric polyelectrolyte have been reported.[3,5] Even fewer are the studies on small molecule–macromolecule systems where ion-pair formation is not the dominant mode of binding. As far as we know, the work of Blauer *et al.* on aqueous haemin–poly-L-lysine association complexes represents the sole example in the field.[28] They prepared the so-

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[27] E. J. Corey and J. C. Bailar, jun., *J. Amer. Chem. Soc.*, 1959, **81**, 2620.

[28] G. Blauer, *Nature*, 1961, **189**, 396; *Biochem. Biophys. Acta*, 1964, **79**, 547; G. Blauer and A. Ehrenberg, *Acta Chem. Scand.*, 1963, **17**, 8; G. Blauer and Z. B. Alfassi, *Biochim. Biophys. Acta*, 1967, **133**, 206.

called ' red ' complex between the iron compound and helical (uncharged) PLL (pH > 10), whose absorption spectrum resembles that of ferricytochrome c,[28,29] as opposed to the ' green ' one formed by haemin with charged PLL (pH 8—10) or with poly-L-ornithine (pH 8—11). In no case however was a conformational change of the polymeric substrate found to accompany the binding process.

In contrast, the results described here demonstrate that major changes in the molecular structure of charged PLL in 50% isopropyl alcohol solution can occur upon interaction with *abc*(−)Co(L-ala-*O*)$_3$. Circular dichroism changes indicate a transition from the ' unordered ' conformation of the original PLL to an α-helix structure upon association of the poly(amino-acid) with the neutral octahedral complex; this contrasts with the behaviour observed when other diastereoisomers are used.

One of the main structural differences between *abc*- and *abd*-tris(L-alaninato)cobalt(III) compounds, most likely to be responsible for their different behaviour in poly-L-lysine solutions, is that the former complex, when viewed along the three-fold axis, presents all three CO groups of the ligands on the same face of the molecule whereas the latter has only two CO groups on one side of the molecule, the third position being occupied by an amino-group.[11,13,25,26] If one assumes the formation of hydrogen bonds between the CO groups of the complex and the ε-amino-groups of the polymer, these structural differences together with the different spatial orientation of the methyl groups of the ligands may become critical for the stability of the association complex. For example, on the basis of stereochemical models it may be easily seen that in the case of *abd*(+)-Co(L-ala-*O*)$_3$ the presence of the methyl groups with pseudo-equatorial orientation [11,26] allows only, in part, such an interaction, which in the most favourable case should involve only two CO groups per complex molecule, because of steric hindrance with the macromolecule.

When considering *abc*(−)Co(L-ala-*O*)$_3$, the simultaneous formation of three hydrogen bonds between the CO groups of the complex and the amino-groups of three side-chains of the polymer may be reasonably considered. According to stereochemical models, the ε-amino-groups of *a*, *b*, and *e* or *a*, *d*, and *e* residues are able to match the CO groups of the complex without any apparent strain, a relatively large portion of substrate being thus engaged by each bound molecule. Furthermore, since the methyl groups of the *abc*-isomer are in pseudo-axial position [11] they crowd the opposite face of the complex without interfering with the formation of the association complex.

This hypothesis is fully consistent with the experimental results presented for *abc*(−)Co(L-ala-*O*)$_3$–PLL solutions. In fact: (a) it justifies the marked ' renaturation ' effect exerted by the complex on charged poly-L-lysine (Figures 3 and 4), much larger than that found with *abd*-diastereoisomers even at much lower complex to polymer ratios (Figures 6 and 7); (b) it accounts for the characteristic perturbations in the c.d. band, of T_{1g} parentage, of the complex after addition of PLL (Figure 10c), which, as already pointed out, indicates predominantly directional modes in the binding; and (c) it explains why this effect is observed only when a mixed solvent medium, such as isopropyl alcohol–water (1 : 1), is used. Hydrogen bonds between complex molecules and polymer are, in fact, strengthened because of the lower dielectric constant of the medium. At the same time, however, ion–dipole and ion–induced dipole interactions are enhanced, whereas the second co-ordination sphere of the octahedral compound, formed by water molecules,[8] is depleted by the presence of the organic co-solvent.[30] The overall effect is such that the complex molecules are available for a more extensive and possibly stronger association than that in aqueous solution.

The data of Figure 6 are a further confirmation of our hypothesis. In the case of the *abc*-isomer the trend in the Figure may suggest some co-operative process, whereby bound molecules, the proportion of which is higher the greater the amount of complex in solution, facilitate the association of further molecules, with a resulting non-linear increase of α-helical structure in the polymer. This implies that helical PLL has a greater affinity for the octahedral compound than PLL in the coil form. This agrees with the fact that the overall flexibility of the helical structure is lower than that of the unordered form, the stereochemical requirements for the simultaneous formation of intermolecular hydrogen bonds being thus optimized.

Finally, the anomalous results obtained with poly-L-ornithine (Figure 8) provide additional support for the idea that hydrogen-bonding interactions are primarily involved in the association complex. Side-chains *ca.* 20% shorter than those in PLL make the approaching complex closer to the backbone chain such that the peptide groups may compete with the δ-amino-groups in the formation of hydrogen bonds with the complex molecules. Alternatively, structural stresses in the main chain may be thought to arise at the sites of the bound molecule because of the shorter side-chains, leading to the destabilization of the helical structure of PLO.

In conclusion, the results show the occurrence of specific site binding between *abc*(−)Co(L-ala-*O*)$_3$ and poly-L-lysine in isopropyl alcohol–water (1 : 1), which probably involve hydrogen-bonding interactions besides electrostatic ones. Specificity in such a process arises from the stereochemical characteristics of the octahedral compound suitable for multibonding interaction with the polyelectrolyte. This produces in turn a conformational transition in the polymer in terms of a marked increase of α-helical structure at those apparent pH values where the coil form normally predominates. The different

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[29] L. Stryer, *Biochim. Biophys. Acta*, 1961, **54**, 397; M. Tohjo and K. Shibata, *Arch. Biochem. Biophys.*, 1863, **103**, 401; T. E. King, F. C. Yong, and S. Takemori, *Biochem. Biophys. Res. Comm.*, 1966, **22**, 658.

[30] F. Franks and D. J. G. Ives, *Quart. Rev.*, 1966, **20**, 1; F. Franks in ' Hydrogen-bonded Solvent Systems,' eds. A. K. Covington and P. Jones, Taylor and Francis Ltd., London, 1968, p. 30.

stereochemical features of *abd*-diastereoisomers are responsible for a diverse degree of participation of these complexes in the binding process with charged PLL, which leaves the secondary structure of the macroion unaltered. The anomalous results obtained with poly-L-ornithine emphasize the influence of small structural variations on conformational phenomena which accompany association processes.

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