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# Interaction of Alginates and Pectins with Cationic Polypeptides

S. Bystrický, A. Malovíková, T. Sticzay

Institute of Chemistry, Centre for Chemical Research, Slovak Academy of Sciences, 842 38 Bratislava, Czechoslovakia

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*ABSTRACT*

*The interactions of alginates of various compositions and of pectins with basic polypeptides, namely, poly(L-lysine) and poly(Lys–Ala–Ala), have been studied by means of circular dichroism. The complexation efficiency of both types of anionic polysaccharides were compared and quantified on the basis of the extent of the induced α-helical conformation of the polypeptide. The alginate with a high content of guluronic acid (~ 75%) did not interact with poly(L-lysine). Pectins and alginates interacted with poly(Lys–Ala–Ala) rather intensively. The difference in efficiency of interaction of L-guluronan and D-galacturonan with poly(L-lysine) results from the difference in the conformational flexibility of their polyanionic chains in solution. L-Guluronan maintains the rigid two-fold symmetry in solution, and D-galacturonan is conformationally adaptable in the course of interaction.*

## INTRODUCTION

One of the most important properties of acidic polysaccharides is the ability to interact with positively charged counterions. The gelling processes whereby polygalacturonic and polyguluronic acids create similar stable stereospecific junction zones are well known. These are conditioned by a suitable stereochemical arrangement of the polysaccharide chain in solution. The rigid conformation of Ca-guluronan characterized by two-fold symmetry does not change even after drying up of the hydrated gel (Mackie, 1971; Mackie *et al.*, 1983). The two-fold symmetry of Ca-galacturonan has been ascribed only to the hydrated form. On drying of this Ca-gel, characteristic changes were observed in circular dichroism (CD) spectra (Gidley *et al.*, 1979; Morris *et al.*, 1982).



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The difference between the CD spectrum of Ca-galacturonan gel and that of solid Ca-galacturonan has been ascribed to the change in the chain conformation from two-fold to three-fold symmetry (Walkinshaw & Arnott, 1981; Alagna *et al.*, 1986).

Less knowledge is available about the interaction of acidic polysaccharides with polycationic macromolecules. The complexation of glycoaminoglycans of low concentration with poly(L-lysine) has been described most widely and reviewed by Hopfinger (1977). The changes observed in CD spectra reflect the inductive conformational transition of random coil → $\alpha$-helix of the polypeptide. A study of interactions of pectins of various degrees of esterification with a model polypeptide of the poly(Lys-Ala$_n$) type ($n = 0$–3) has shown that the interaction is governed by stoichiometric saturation of cationic and anionic groups (Bystrický *et al.*, 1985, 1986, 1988). The model of spatial structure of the complex was proposed on the basis of stereochemical compatible engagement of all anionic and cationic groups. This complex consists of an $\alpha$-helical polypeptide core surrounded by a superhelically oriented polysaccharide chain. There has not been, so far, any study of the extent to which the efficiency of this interaction is conditioned by the spatial arrangement of the charges on the polysaccharide in the solution.

The aim of the work reported in the present paper was to obtain an insight into this problem by studying and comparing the CD spectra of complexes of poly(L-lysine) and sequentially regular poly(Lys–Ala–Ala) with alginates of various compositions and pectins, respectively.

## EXPERIMENTAL

### Materials and methods

Poly(L-lysine).HBr was a commercial preparation provided by Sigma, USA. Its molecular mass, determined viscosimetrically, was 30 000–70 000. The sequentially regular poly(Lys-Ala-Ala).HBr was kindly donated by the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague. It was synthesized via polymerization of 1-hydroxysuccinimidyl ester of the appropriate tripeptide (Štokrová *et al.*, 1978). The molecular mass estimated from the sedimentation equilibrium according to the method described by Chervenka (1970) was 7900. The lysine content in freeze-dried samples used in the complexation experiments was established from the concentration of $Br^-$ ions determined by potentiometric titration with $AgNO_3$ (2 mmol litre$^{-1}$).

The pectin samples of various degrees of esterification of carboxyl groups ($E$) were prepared from a purified, commercially available citrus pectin (Genu Pectin, Medium Rapid Set, Type A, Københavns Pektinfabrik, Denmark) by partial and total alkaline de-esterifications, respectively, in 60% ethanol suspension with a dilute potassium hydroxide solution. The purified commercial pectin contained 88% of D-galacturonan as a dry substance. Characterization of pectin samples of different degrees of esterification is given in Table 1.

Alginates (1–3) of different compositions were commercial products (alginate 1 from BDH, UK, alginate 2 from Fluka, Switzerland, and alginate 3 from Carl Hartmann, Bremen, FRG) supplied by the Institute of Organoelement Compounds, Academy of Sciences, Moscow, USSR. The characteristic data of these alginates are presented in Table 2. The total content of the individual uronic acids and the content of D-mannuronic, L-guluronic, and mixed sequences were determined by the NMR technique (Penman & Sanderson, 1972). The alginate with a high content of L-guluronic acid (~75%, sample 4) isolated from the outer cortex of old stipes of *Laminaria hyperborea* was kindly given by Dr Skjak-Braek, University of Trondheim, Norway.

**TABLE 1**
Characterization of Pectin Samples

| *Sample* | | *Degree of esterification, E (%)* | *[η] $cm^3 g^{-1}$* |
|---|---|---|---|
| A | Potassium pectate | 0 | 122·0 |
| B | Potassium pectinate | 14·6 | 89·5 |
| C | Potassium pectinate | 25·2 | 91·0 |
| D | Potassium pectinate | 33·8 | 98·2 |

**TABLE 2**
Characterization of Alginates

| *Sample* | *Block composition (%)* | | | *D-ManUA content*[a] *(%)* | *[η] ($cm^3 g^{-1}$)* |
|---|---|---|---|---|---|
| | *MM* | *GG* | *MG* | | |
| 1 | 30 | 20 | 50 | 56·8 | 970·0 |
| 2 | 33 | 30 | 37 | 52·6 | 565·0 |
| 3 | 33 | 47 | 20 | 46·0 | 695·0 |

[a]Determined experimentally.

The alginates of different charge densities were prepared by esterification with diazomethane in 20% methanol (Markovič *et al.*, 1981).

The content of free and total carboxyl groups in pectate and pectinates, the content of D-galacturonan as a dry substance, and the degree of esterification were determined by the method based on precipitation of insoluble copper pectates and pectinates (Tibenský *et al.*, 1963; Kohn & Tibenský, 1965).

The degree of esterification of the esterified alginates and the concentration of free carboxyl groups of acidic polysaccharides for the complexation experiments were determined by potentiometric titration with KOH (0·05 mol litre$^{-1}$).

The limiting viscosity number of acidic polysaccharides $[\eta]$ was estimated by means of an Ubbelohde viscometer in NaCl solution (0·15 mol litre$^{-1}$ or 0·10 mol litre$^{-1}$) at 25 ± 0·1°C.

The concentration of free carboxyl groups of the starting solutions of acidic polysaccharides used to prepare the mixtures for the investigation of complexation was 0·6 mmol ($COO^-$) litre$^{-1}$ and that of ($NH_3^+$) of the polypeptide solutions was the same (0·6 mmol ($NH_3^+$) litre$^{-1}$). The mixtures were prepared in two ways. In one method, the solution of polypeptide corresponding to amounts of 20, 40, 60, 80, and 100% per free carboxyl group was added to the solution of acidic polysaccharide. The final concentration of the polysaccharide was adjusted by dilution to 0·3 mmol ($COO^-$) litre$^{-1}$ for the whole series. In the second method, the solution of the polysaccharide corresponding to amounts of 20, 40, 60, 80, and 100% per $NH_3^+$ group was added to the solution of polypeptide. The final concentration of the polypeptide was adjusted to 0·3 mmol ($NH_3^+$) litre$^{-1}$ for the whole series.

The CD spectra were recorded with a Jobin Yvon Dichrograph Mark III (France) spectrophotometer in 1- and 5-mm cells at 25°C.

A digital potentiometer Radiometer PHM 64 (Denmark), a silver electrode, an electrolytic bridge filled with 10% $KNO_3$ solution, and a combined electrode GK 2401C (Radiometer) were used.

## RESULTS AND DISCUSSION

The CD spectra of the individual acidic polysaccharides and polypeptides, respectively, are presented in Fig. 1. The spectral shape of alginates reflects the relative proportions of D-mannuronate, L-guluronate, and mixed sequences (Morris *et al.*, 1980). L-Guluronan itself is structurally a mirror image of D-galacturonan with an $\alpha(1\rightarrow4)$ diaxial bond, except for the configuration at C-3. Both these poly-

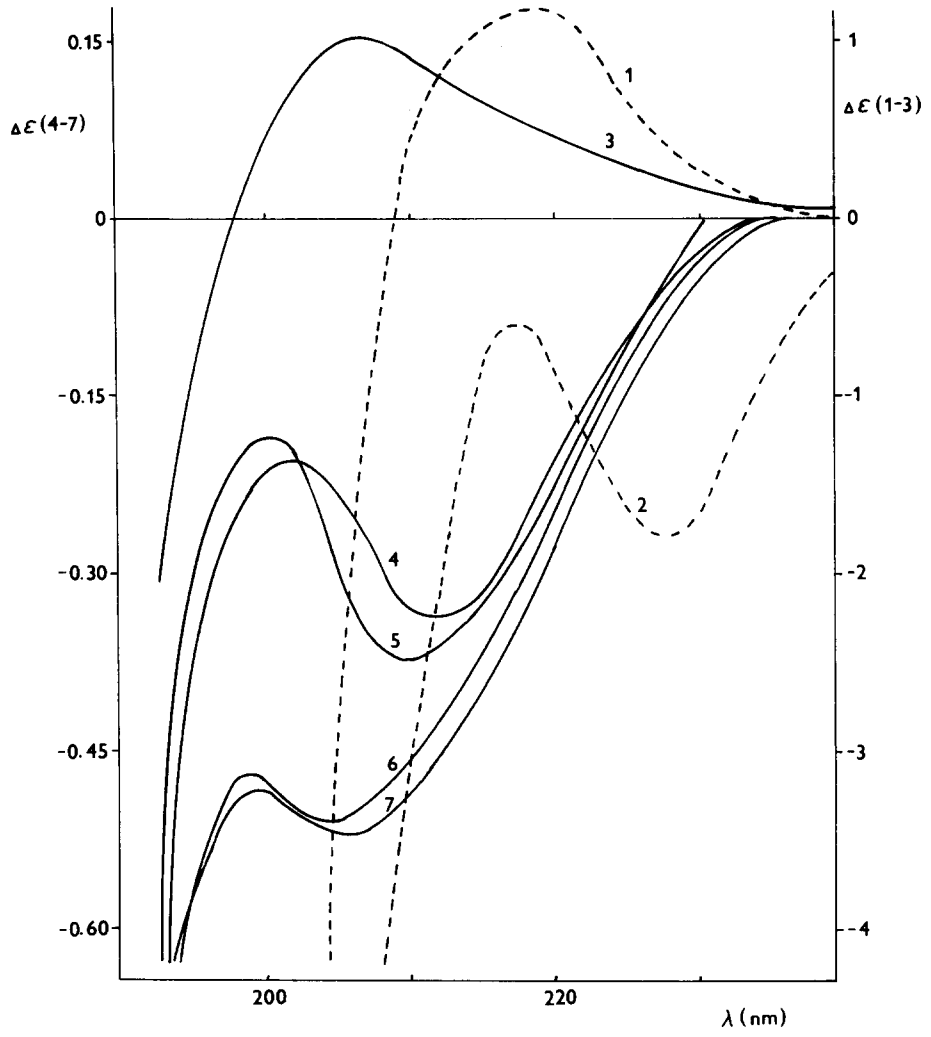


**Fig. 1.** The CD spectra of the individual acidic polysaccharides and polypeptides: 1, Poly(L-lysine); 2, poly(Lys-Ala-Ala); 3, potassium pectate; 4, alginate 1; 5, alginate 2; 6, alginate 3; 7, alginate 4.

saccharides are characterized in the CD spectrum by a simple band in the region of the $n-\pi^*$ transition. D-Mannuronan with a $\beta(1\rightarrow4)$ diequatorial bond differs from the previous ones structurally and also by its spectral pattern. Its CD spectrum in this region is characterized by a bisignate curve (Listowskij *et al.*, 1972; Morris *et al.*, 1975). Hence, with alginates of a higher D-mannuronate content, a marked difference between the ellipticity values of the negative maximum and minimum may be observed.

The CD spectrum of poly(L-lysine) shows a positive dichroic band corresponding to a disordered 'charged-coil' structure. The ellipticity values of poly(Lys–Ala–Ala) in the negative spectral region indicate an ability to adopt the regular helical structure readily.

Two methods of mixture preparation were used. Increasing amounts of the polypeptide were added to excess polysaccharide in solution, and, vice versa, increasing amounts of the polysaccharide were added to excess polypeptide. The CD spectra obtained were corrected by subtracting the CD of all the polysaccharide present in the solution and, additionally in the second series, the CD of the excess polypeptide in the charged-coil arrangement. The spectra corrected in this way (Figs 2 and 4) represent the CD of that part of the polypeptide in solution having an equivalent amount of carboxyl counterions on polysaccharide required for interaction.

The spectral shape of complexes with alginates 1 and 2 (Fig. 2(a), 2(b)) clearly indicates the presence of the $\alpha$-helical structure of poly(L-lysine). The apparent similarity to the CD of the $\beta$ conformation is illusive; for this conformation, the negative dichroic band at a lower wavenumber ($\sim$215 nm) is characteristic (Davidson & Fasman, 1967). By increasing the amount of the components added, the negative dichroic band in the region of 225 nm increases and shifts slightly batochromatically. The intensive negative circular dichroism in a short-wave region testifies to the presence of polypeptide not entering the interaction. The CD curves of complexes obtained in both methods of complexation are almost identical. This confirms that equivalent saturation of the ($COO^-$) and ($NH_3^+$) charges is typical for the interaction of poly(L-lysine) with alginates. The same conclusion was arrived at when pectin and poly(L-lysine) were the interacting components (Bystrický *et al.*, 1985).

The values of circular dichroic absorption in the 210–230-nm region with alginate 2 (Fig. 2(b)) are lower than those with alginate 1 (Fig. 2(a)). A marked decrease in value was observed with alginate 3 (Fig. 2(c)). Here, the positive circular dichroism demonstrates the presence of the prevalent portion of poly(L-lysine) in a disordered arrangement, i.e. polypeptide is not involved in the interaction. Alginate 3 contains the highest amount of L-guluronate when compared with alginates 1 and 2. The complexation was also studied with an alginate consisting of $\sim$75% L-guluronate (sample 4). As may be seen from Fig. 2(d), the CD spectra are practically identical with those of poly(L-lysine) in a disordered arrangement (Fig. 1). In this case, the interaction connected with inductive formation of the $\alpha$-helical structure has not taken place. Consequently, the L-guluronate sequence of alginates does not enter the complexation with poly(L-lysine).

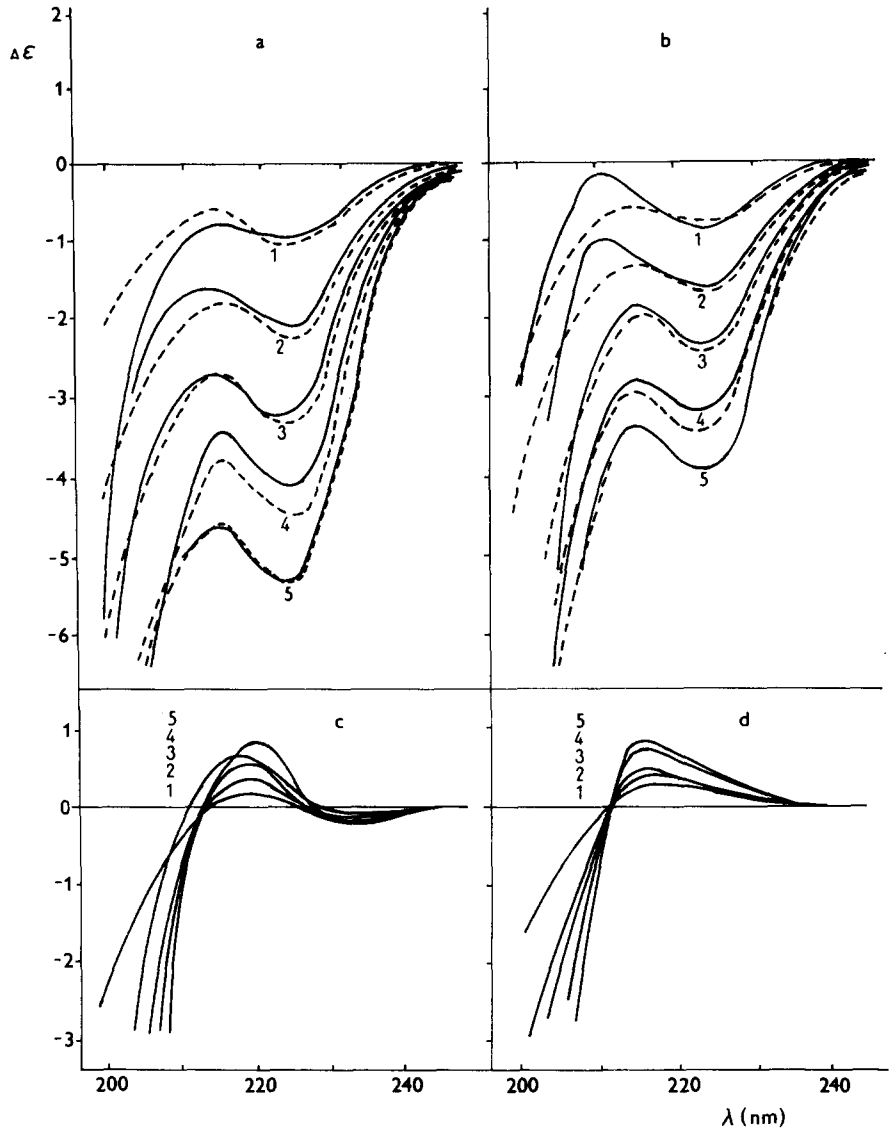


**Fig. 2.** The CD spectrum of poly(L-lysine) in the presence of alginates: a, alginate 1; b, alginate 2; c, alginate 3; d, alginate 4; --- alginate (0·3 mmol ($COO^-$) litre$^{-1}$) with addition of 20% (1), 40% (2), 60% (3), 80% (4), and 100% (5) poly(L-lysine); —— poly(L-lysine) (0·3 mmol ($NH_3^+$) litre$^{-1}$) with addition of 20% (1), 40% (2), 60% (3), 80% (4), and 100% (5) alginate.

With respect to the ionic nature of the interactions studied, the linear-charge density of the interacting components plays an important role in complex formation. It was shown that, on decreasing the charge density of pectin by partial esterification of carboxyl groups, the ability to interact decreased gradually (Bystrický *et al.*, 1985, 1986). The effect of the charge density was also studied with alginates 1 and 2 of three different degrees of esterification ($E$ 0–40%). The complexation was studied under identical conditions, and the CD spectra obtained were corrected in a similar manner as with unesterified polysaccharides. In order to obtain a clear picture of the efficiency of interaction, only the spectral values observed at 225 nm were used. At this wavenumber, the contributions of the individual components to CD are low. The values were further normalized to a uniform concentration of 0·3 mmol ($NH_3^+$) litre$^{-1}$ of poly(L-lysine). The results obtained in this way are summarized in Fig. 3. The complexation efficiency ($A$) is given as a percentage relative to the CD value of poly(L-lysine) at a pH of 11·3, where 100% of $\alpha$-helical conformation is assumed. The complexation efficiency achieved in the interaction of poly(L-lysine) with potassium pectate was 100% (Bystrický *et al.*, 1985). The values for complexation with pectins of different degrees of esterification are also presented in Fig. 3 for comparison. The complexation efficiency with alginates is lower and with increasing degree of esterification of the carboxyl groups decreases gradually as in the case of pectinates. The values of the complexation efficiency with alginates 2 are lower than those with alginates 1. Alginate 3 was not esterified, since the complexation efficiency with the original unesterified sample was nearly zero.

The alginates used differ from each other in the content of L-guluronate and mixed sequences. The content of D-mannuronate sequences in all alginates is almost identical. The lower complexation efficiency with alginate 2 than with alginate 1 and the nearly zero efficiency with alginate 3 are due to the presence of a considerable amount of non-complexing L-guluronate sequences in the alginate structure. The reason why L-guluronan does not enter the interaction, whereas the D-galacturonan interacts very intensively, lies in the spatial structure of both polysaccharides. The conformations of potassium L-guluronan and D-galacturonan in solution have not so far been determined. The extrapolation of the conformation found in the solid state to solutions is not reliable. The reason is evidently the already mentioned polymorphic-phase transition of calcium D-galacturonan (Rees, 1981). On the basis of the results of complexation achieved in this study, it may be suggested that the L-guluronan chain is more rigid, i.e. less adaptable to the changes in the surrounding medium than the D-galacturonan chain.

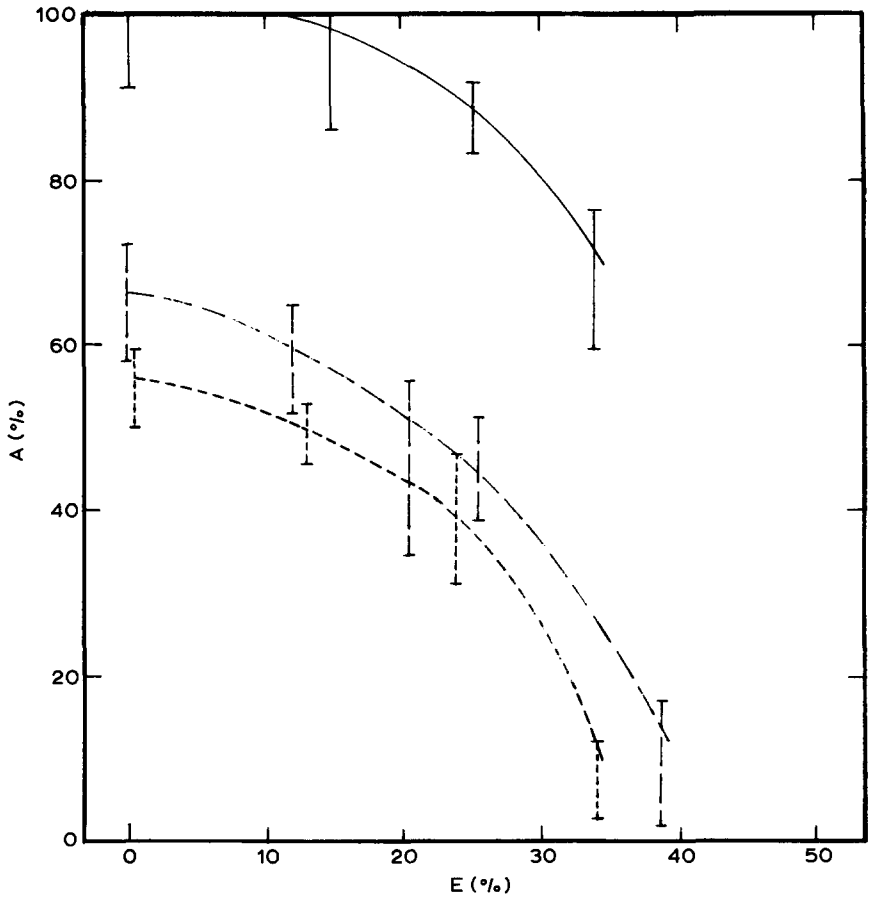


**Fig. 3.** The effect of degree of esterification ($E$) of acidic polysaccharides on the complexation efficiency ($A$,%) with poly(L-lysine). Scattering of values at various ratios of components in the mixture is not systematic and is marked as bars: —— pectin; - - - alginate 1; - - - alginate 2.

The influence of charge density of a polycation on complexation was studied with the sequentially regular poly(Lys–Ala–Ala), which is characterized by a charge density one-third that of poly(L-lysine). Moreover, the linear-charge density of poly(Lys–Ala–Ala) is close to that of the unesterified polysaccharides (pectate, alginate). It was found that the interaction of poly(Lys–Ala–Ala) was still effective up to 50% degree of esterification (Bystrický *et al.*, 1986). Poly(L-lysine) with a pectinate of this type ($E$ 50%) does not form complexes at all (Bystrický *et al.*, 1985). It means that unesterified uronans with two-fold symmetry, where the charges of carboxyl groups are located on the opposite sides of the polysaccharide chain, would interact with poly(Lys–Ala–Ala)

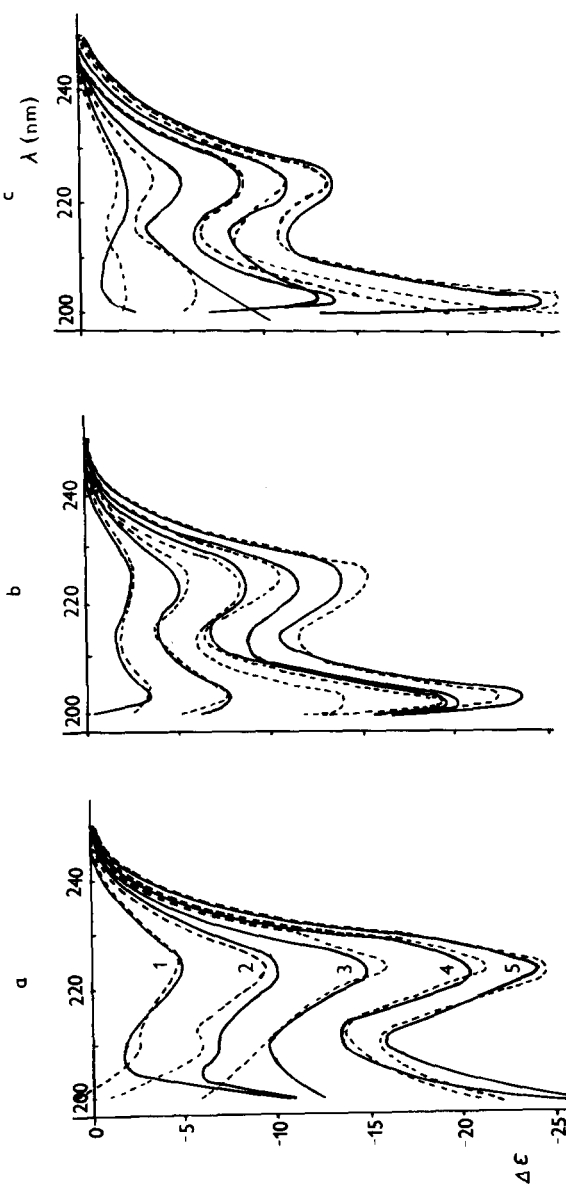


**Fig. 4.** The CD spectrum of poly(Lys–Ala–Ala) in the presence of acidic polysaccharides: a, potassium pectate; b, alginate 3; c, alginate 4; - - - acidic polysaccharide (0·3 mmol ($COO^-$) $litre^{-1}$) with addition of 20% (1), 40% (2), 60% (3), 80% (4), and 100% (5) poly(Lys–Ala–Ala); —— poly(Lys–Ala–Ala) (0·3 mmol ($NH_3^+$) $litre^{-1}$) with addition of 20% (1), 40% (2), 60% (3), 80% (4), and 100% (5) acidic polysaccharide.

effectively. Figure 4 shows the CD spectra of poly(Lys–Ala–Ala) in the presence of potassium pectate and alginates rich in L-guluronate (samples 3 and 4), respectively. The complex formation was accompanied by the opalescence of the solutions becoming more intensive on the saturation of charges. On achieving the equivalence of charges, i.e. when the amount of the component added was 100%, precipitation of complexes took place. In the spatial model proposed, the polysaccharide molecule is linearly oriented along the surface of the helical polypeptide structure (Bystrický *et al.*, 1986). The saturation of charges on the opposite side of the cylindrical surface, achieved by interaction with other polyanionic molecules, leads to the formation of aggregates.

As may be seen from Fig. 4, alginates with a significant portion of L-guluronate interact with poly(Lys–Ala–Ala). However, the extent of interaction does not achieve the values observed with pectate, which is most likely due to a different adaptability of the polysaccharide chain. We assume that, in the case of alginates, the interaction proceeds as a longitudinal association of macromolecules owing to the probable two-fold symmetry of alginates rich in L-guluronate. Consequently, the complex formed is less stable than that in the case of pectate with a three-fold symmetrical structure, where each polypeptide macromolecule is surrounded on three sides by macromolecules carrying opposite charges.

The fixed two-fold symmetry of alginates mentioned, is, however, unsuitable for the interaction with poly(L-lysine). In this case, a certain flexibility of the polysaccharide chain is necessary for the equivalent saturation of charges. Hence, the only part of the alginate structure that enters the interaction with poly(L-lysine) is that which contains mixed sequences of L-guluronic and D-mannuronic acids where the rigid two-fold symmetry is improbable.

The inefficient interaction of L-guluronate-rich alginates with poly(L-lysine) indicates that, in aqueous solution, L-guluronan itself maintains a rigid two-fold screw symmetry.

The most noticeable difference in complex formation between L-guluronan and D-galacturonan is obviously connected with the orientation of the hydroxyl group at the C–3 atom. Further attention will be devoted to this problem.

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