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Interaction of Acidic Polysaccharides with Polylysine Enantiomers. Conformation Probe in Solution

S. Bystrický, A. Malovíková, T. Sticzay

Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Czechoslovakia

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ABSTRACT

The interactions of D- *and* L-*enantiomers of polylysine with pectate and alginates with high contents of* L-*guluronate and* D-*mannuronate, respectively, were studied by means of circular dichroism (CD).* L-*guluronate arrangement that does not enter the complexation with poly(*L-*lysine) interacts with poly(*D-*lysine) very weakly.* D-*galacturonate arrangement interacting with poly(*L-*lysine) effectively, interacts with poly(*D-*lysine) only weakly.* D-*mannuronate arrangement interacting weakly with poly(*L-*lysine) enters the complexation with poly(*D-*lysine) effectively. On the basis of such enantioselectivity the handedness of the natural polysaccharide conformation may be deduced.*

INTRODUCTION

The study of interactions of acidic polysaccharides with model basic polypeptides has revealed that the complex formation is governed by the stoichiometric ratio of the charged groups (COO^-, NH_3^+) (Bystrický *et al.*, 1985, 1986, 1988). Thus the complex-forming interaction is regulated by stoichiometric compatibility of the charge densities. The ability of polysaccharide to take up the suitable orientation for the complementary saturation of charges plays a substantial role. In the course of interaction the conformation of polypeptide changes from a disordered to a compact α-helical arrangement. The complex is formed by winding up polysaccharide round the α-helical polypeptide core. The study of the interaction of pectinates with poly(Lys-Ala$_n$) ($n = 0, 1, 2, 3$) has shown that the complexation efficiency decreases with the decreasing charge density of pectinates used (Bystrický *et al.*, 1985, 1986,

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1988). Moreover, the study of complexation of alginate rich in L-guluronate has pointed at another factor important in interaction of polysaccharides having an $\alpha(1-4)$ diaxial glycosidic bond. While the efficiency of interaction of pectate with poly(L-lysine) is almost 100%, the foregoing alginate practically does not form any complex (Bystrický *et al.*, 1990). On the other hand pectate and alginate rich in L-guluronate interact with divalent cations similarly. The information obtained about different interactions with polypeptides indicates that the complexation efficiency is influenced by the rigidity of the polysaccharide conformation in solution. In the studies presented so far only polypeptides of the L-type have been used. In this respect it seemed to be desirable to examine the behaviour of polysaccharides in interaction with other optical isomers, e.g. poly(D-lysine). As polysaccharide components potassium pectate and potassium alginates with high L-guluronate and D-mannuronate contents, respectively, were used.

EXPERIMENTAL

Materials and methods

Poly(L-lysine).HBr and poly(D-lysine).HBr were commercial samples provided by Sigma, USA. Their molecular masses determined viscometrically were 30 000–70 000. The lysine content in 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}).

Potassium pectate was prepared from a purified, commercially available citrus pectin (Genu Pectin, Medium Rapid Set, Type A, Københavns, Pektinfabrik, Denmark) by total alkaline deesterification in 60% ethanol suspension with a dilute potassium hydroxide solution. This sample contained 88% of D-galacturonan in dry substance.

The alginate with high content of L-guluronic acid (~75%) was isolated from the outer cortex of old stipes of Laminaria hyperborea and alginate with a high content of D-mannuronic acid (~90%) was isolated from the fruiting bodies of *Ascophyllum nodosum*. Both samples were kindly given by Dr Skjak-Braek, University of Trondheim, Norway. The content of the individual uronic acids in both alginates was determined by n.m.r. analysis.

The concentration of carboxyl groups of acidic polysaccharides for the complexation experiments was determined by potentiometric titration with KOH (0·05 mol litre^{-1}).

The method of preparation of mixtures for complexation studies has been described in detail in our previous work (Bystrický *et al.*, 1990).

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

RESULTS

The CD spectra of components used for complexation are presented in Fig. 1. Poly(D-lysine) in neutral solution has a spectrum corresponding to a 'charged coil' structure, while at pH = 11·3 the spectrum is characteristic of a complete left-handed helical arrangement. The CD spectra of alginates reflect the relative proportion of L-guluronate, D-mannuronate and mixed sequences. The bisignate curve for alginate with high D-

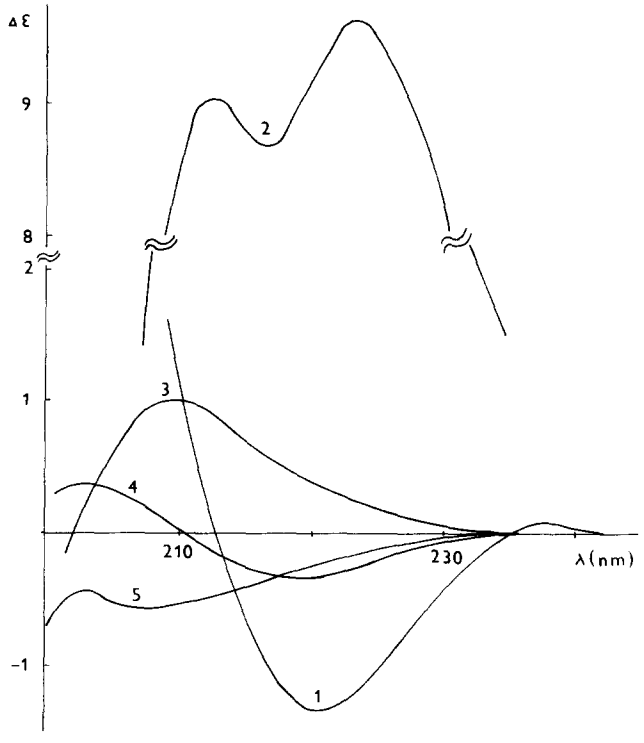

Fig. 1. The CD spectra of the individual acidic polysaccharides and polypeptide: (1) poly(D-lysine) at neutral pH; (2) at pH 11·3; (3) potassium pectate; (4) alginate rich in D-mannuronate; (5) alginate rich in L-guluronate.

mannuronate content as well as the negative CD of L-guluronate rich alginate are in agreement with the effective CD analysis (Morris *et al.*, 1980).

The complex-forming interaction was studied in diluted solutions so that the polypeptide was added stepwise to the polysaccharide solution of constant concentration. Moreover, in the case of alginate rich in D-mannuronate the polysaccharide was added stepwise to the polypeptide solution of constant concentration. The complex formation is connected with the change of polypeptide conformation which is reflected in the CD spectra. The CD spectra obtained were corrected by subtracting the CD of all polysaccharide present in solution. Additionally in the series with excess polypeptide, the CD of this excess polypeptide in the charged-coil arrangement was subtracted. The spectra corrected in this way (Figs 2–5) represent the CD of that part of the polypeptide in solution having an equivalent amount of carboxyl counterions on polysaccharide required for interaction.

Figure 2 shows the CD of poly(D-lysine) in the presence of alginate rich in L-guluronate. While the disordered structure of poly(D-lysine) is characterized by the negative sign of CD (Fig. 1), the complex formation, inducing regular arrangement, is reflected in the positive sign of CD. This shift, more distinct with increased concentration of polypeptide, indicates that part of poly(D-lysine) interacts with alginate. However, the spectral shape and low values of the CD spectra show that the major part of the polypeptide has not entered the complexation.

Figure 3 shows the CD spectra of poly(D-lysine) added stepwise to the excess of potassium pectate. As seen from the spectra the shift to the positive region is, similarly as in the preceding case, surprisingly small. In contrast to previous studies of poly(L-lysine)–pectate interaction (Bystrický *et al.*, 1985) where a very intensive conformational change of polypeptide connected with the maximum complex formation has been observed, such a small change of poly(D-lysine) conformation caused by very low complexation efficiency has not been noticed so far.

The CD spectra of both enantiomers D- and L-polylysine in the presence of alginate rich in D-mannuronate are shown in Figs 4 and 5. As seen the spectra are not a mirror image as would be expected for enantiomer structures of polylysine. In the case of interaction with poly(D-lysine), marked bands characteristic of an α-helical structure are observed in the positive region of the spectrum. Here the complex-forming interaction is effective. On the other hand, with poly(L-lysine) the CD in the negative region is of low intensity. The shape and the low values of CD testify that only a small part of poly(L-lysine) is involved in the interaction inducing regular helical arrangement. For completeness

Figs 4 and 5 present the CD spectra of mixtures prepared in both ways. The resemblance of CD curves evidences that the complex-forming interaction is governed by stoichiometric ratio of the charged groups (COO^- of polysaccharide and NH_3^+ of polylysine), i.e. it is independent of the excess of either component.

DISCUSSION

In our previous work dealing with interactions of alginates with poly(L-lysine) it was found that L-guluronate rich alginate did not interact with this polypeptide (Bystrický *et al.*, 1990). However, the present experiments with poly(D-lysine) revealed a certain weak interaction (Fig. 2). Substitution of L- for D-enantiomer of the polypeptide component resulted in the fact that a small part of alginate was able to enter the complexation. The alginate used contained besides the dominating

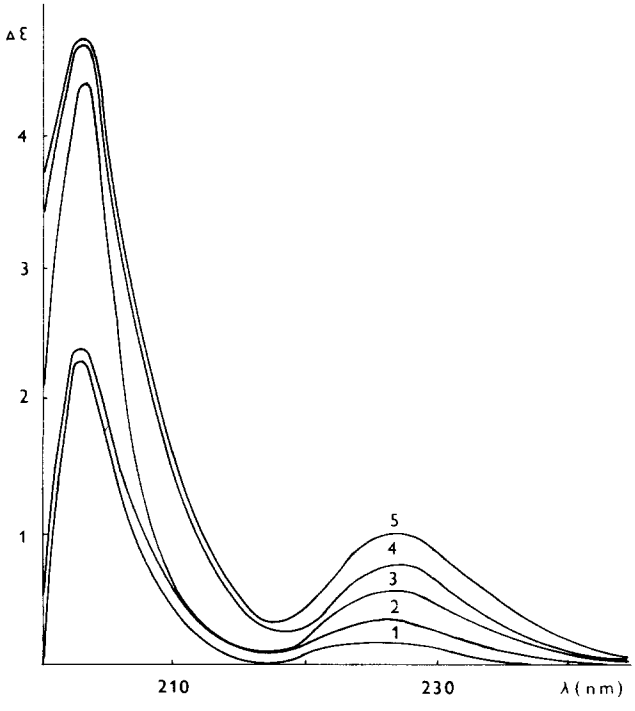

Fig. 2. The CD spectrum of poly(D-lysine) in the presence of alginate rich in L-guluronate: alginate (0·3 mmol (COO^-) $litre^{-1}$) with addition of 20% (1), 40% (2), 60% (3), 80% (4) and 100% (5) polylysine.

L-guluronate also a minor portion of D-mannuronate in homopolymeric and mixed sequences, respectively. From Figs 4 and 5 it is obvious that D-mannuronate rich alginate has a tendency to interact rather with poly(D-lysine) than with poly(L-lysine). With regard to this fact it is highly probable that only the D-mannuronate component of alginate is involved in the weak interaction with poly(D-lysine). It is known that L-guluronan in the solid state adopts a strictly two-fold helical conformation (Mackie, 1971; Mackie *et al.*, 1983). The polysaccharide with such conformation is not able to interact with polylysine effectively due to incompatibility of charge densities. The inability of alginate rich in L-guluronate to interact with either poly(D-lysine) or poly(L-lysine) confirms that the structure of L-guluronan itself is rigid also in water solution.

The CD spectra of poly(D-lysine) in the presence of potassium pectate reflect a markedly low degree of complexation. The prevalent part of

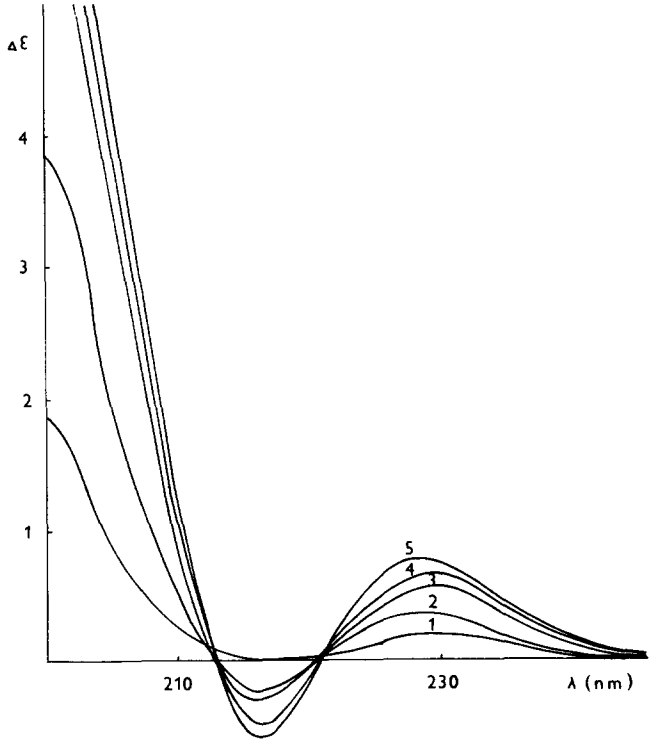

Fig. 3. The CD spectrum of poly(D-lysine) in the presence of potassium pectate: potassium pectate (0·3 mmol (COO^-) $litre^{-1}$) with addition of 20% (1), 40% (2), 60% (3), 80% (4) and 100% (5) poly(D-lysine).

poly(D-lysine) remains unchanged in random coil arrangement. The extent of complexation may be judged from comparison with the CD spectrum of poly(D-lysine) at pH = 11·3 (Fig. 1). Quantitative estimation of complexation is rather problematical as the contribution of the CD change of polysaccharide entering the interaction is unknown. According to the CD changes observed (Thom *et al.*, 1982) we assume that it is maximum 10% of the value of CD change of polylysine in its transition to helical conformation.

It was stated earlier that the complexation efficiency of poly(L-lysine) with pectate is almost 100% (Bystrický *et al.*, 1985), while with poly(D-lysine)) it is only about 10%. This evident difference in the interaction of pectate with enantiomers of polylysine points at a new, until now unobserved factor, i.e. the chiral discrimination of adaptable conforma-

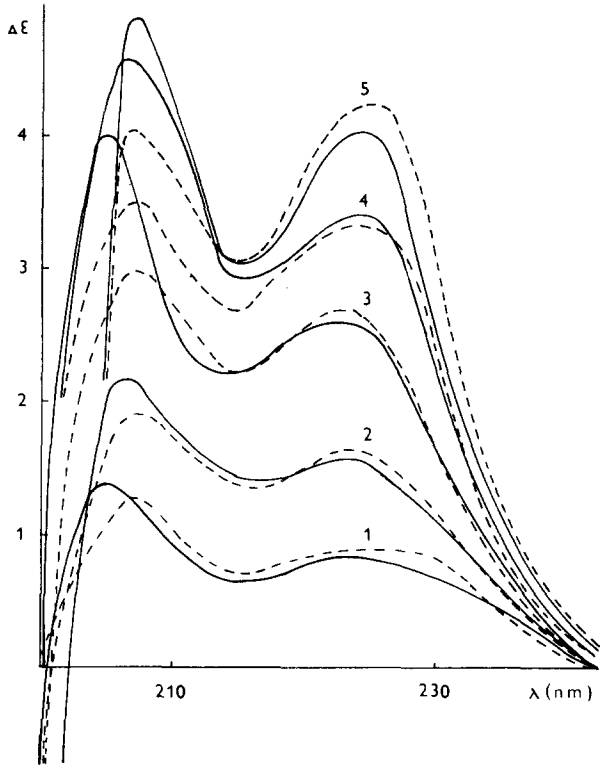

Fig. 4. The CD spectrum of poly(D-lysine) in the presence of alginate rich in D-mannuronate: (– – –) alginate (0·3 mmol (COO^-) $litre^{-1}$) with addition of 20% (1), 40% (2), 60% (3), 80% (4) and 100% (5) poly(D-lysine); (——) poly(D-lysine) (0·3 mmol (NH_3^+) $litre^{-1}$) with addition of 20% (1),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

tion of polysaccharide in solution. Pectate, basically $\alpha(1-4)$ D-galacturonan randomly interrupted by rhamnose units, behaves in the polyelectrolytic interactions studied markedly differently from $\alpha(1-4)$ L-guluronan contained in alginate. So far, the conformation of D-galacturonan in solution has not been determined precisely. While in the case of L-guluronan only a two-fold backbone symmetry has been stated (Mackie *et al.*, 1983), conformational polymorphism allows for D-galacturonan also a three-fold helical symmetry. However, its chiral handedness has not been convincingly confirmed even in the solid state (Perez, 1988). In an older X-ray study of sodium pectate, left-handed three-fold symmetry was suggested (Palmer & Hartzog, 1945). Later

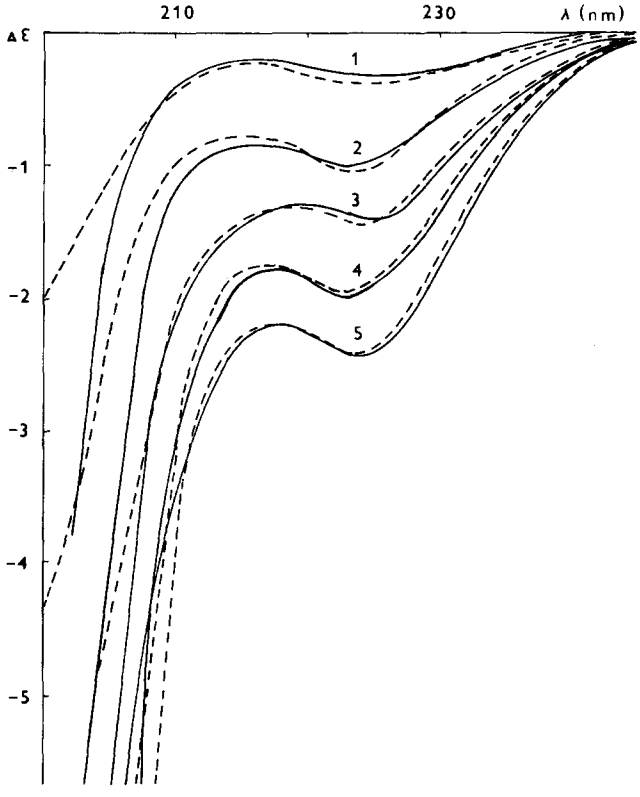

Fig. 5. The CD spectrum of poly(L-lysine) in the presence of alginate rich in D-mannuronate: (– – –) alginate (0·3 mmol (COO^-) litre^{-1}) with addition of 20% (1), 40% (2), 60% (3), 80% (4) and 100% (5) poly(L-lysine); (——) poly(L-lysine) (0·3 mmol (NH_3^+) litre^{-1}) with addition of 20% (1), 40%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

results of model building study endorse the opinion of a right-handed symmetry (Rees & Wight, 1971). The experimental method of the interaction of enantiomers presented herein may distinguish the handedness of the polysaccharide backbone sensitively. The conformational freedom around the glycosidic bond is not symmetric, usually it is constrained one-sided. The conformational transition from two-fold to three-fold symmetry, i.e. the change of ϕ, ψ angles of glycosidic bond is a one-way process directed to the energetically most favourable coordinate. This direction of unrestricted conformational freedom determines simultaneously those conformational possibilities of polysaccharide which are suitable for entering the complexation with polypeptide. The screw-sense of natural conformation of polysaccharide in solution is consistent with that in the complex.

The third polysaccharide type used for the interaction with polylysine enantiomers was alginate rich in D-mannuronate. The induced conformations presented in Figs 4 and 5 are not a mirror image. In this case, the interaction with poly(D-lysine) appears to be more effective (approximately 50%). The spectral shape confirms the helix-forming interaction. The complexation efficiency with enantiomeric poly(L-lysine) is about 20% only. This difference, smaller than with pectate, reflects the fact that the D-mannuronate arrangement in alginate is rather flexible due to a $\beta(1-4)$ diequatorial bond. It was found by an X-ray method for the salts of the D-mannuronan three-fold helical structure (Mackie, 1971) that was quoted as left-handed (Atkins, 1977). Here the enantiomeric effect on the interaction studied was shown to be reversed when compared with pectate. While pectate effectively interacted with the L-form of polylysine, alginate rich in D-mannuronate interacted more effectively with the D-form. On the basis of the results obtained we have arrived at the conclusion that D-mannuronan in water solution adopts a conformation of screw-sense in reverse to that of D-galacturonan.

The results presented here pointed out that complementarity of chiralities of the induced polypeptide conformation and of the polysaccharide backbone is the prerequisite for effective complexation. From comparison of complexation efficiencies of acidic polysaccharides with polylysine enantiomers the sense of conformational freedom of the polysaccharide may be deduced. Our experiments have clearly shown that D-mannuronan in solution tends to adopt a left-handed helical structure, while D-galacturonan may be characterized by conformational freedom in a right-handed sense.

Generally, for acid polysaccharides having each pyranose unit charged, the following chiral discrimination is valid: polysaccharides naturally adopting a right-handed helical structure readily enter the

complex-forming interaction with poly(L-lysine), while with poly(D-lysine) they interact very weakly or not at all. Polysaccharides with a left-handed helical arrangement interact strongly with the D-form of polylysine and weakly with the L-form.

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