Conformational Study of the Diastereomeric Pairs in Poly(lysine)−Pectate Complexes

Gaio Paradossi,*,† Ester Chiessi,† and Anna Malovìkovà‡

Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma "Tor Vergata", Via della Ricerca Scientifica, 00133 Rome, Italy; and Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovakia

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ABSTRACT: The complex formation in aqueous solution between the polyelectrolytes poly(lysine) and pectate was studied focusing on the conformational changes of the polysaccharidic moiety. The findings suggest that the pectate adopts a superhelical conformation around the α helix of the poly(L-lysine). A threshold value of the degree of polymerization of the pectate chain enabling the transition of the peptide was determined by chromatographic and circular dichroism methods. Conformational analysis results are in agreement with the experimental findings and support the superhelical topology of the polyelectrolyte poly(L-lysine)−pectate complex.

Introduction

The interactions between biopolymers are the basis for many biological processes, and lately their mimicking has been an important issue in biomedical technologies. Moreover, the concept of drug has undergone a deep change, including in this class also macromolecular species as DNA sequences, proteins, and vaccines.[1−5]

As the definition of drug is moving toward active molecules with increasing conformational complexity, the scenario for the drug delivery systems is also changing. This strategy includes drug bioavailability enhancement as the main issue.[6−10] It can be obtained, for example, by a more efficient passage of the active molecule through the cellular membrane by complexing the active molecule with another conformationally suited macromolecule.

In this context, the study of the interactions in some charged biopolymer complexes is a mandatory step toward the formulation of new drug delivery systems. In our laboratory, we have been working on the study of the interaction of two oppositely charged polyelectrolytes in solution, namely pectate and poly(lysine) which can be considered a good model system for studying the interaction pattern, existing in biological or biocompatible macromolecules of relevance in the drug delivery research.[11−13]

Pectate is a polysaccharide contained in the peel of several fruits. It can be considered as a poly(1 → 4)-α-D-galacturonate with a degree of methylesterification which depends on the source. In solution, this polysaccharide is a wormlike coil.[14−18] The other partner of the investigated complex, poly(lysine), is a thoroughly studied synthetic polypeptide that can be obtained with both enantiomers of lysine. Recently, the activity of poly(L-lysine) in gene therapy has been reported in the literature.[19−22]

The mixing of poly D-galacturonate with two enantiomeric forms poly(L-lysine) and poly(D-lysine) gives rise to two complexes, DL and DD, with different conformational features. At neutral pH in the couple DL, the peptide chain undergoes a transition from a random coil to a α helix whereas in the case of the couple DD the poly(lysine) remains in the disordered state. The chiral discrimination makes this complex an interesting in vitro example of the recognition processes occurring at cellular level, and it opens new perspectives in the science of the drug delivery.

In a recent paper,[23] we have focused on the role played by pectate in the interaction with poly(lysine). The hypothesis of a rearrangement of the saccharidic chain in addition to the already ascertained conformational transition of the polypeptide moiety was proposed after the findings obtained by measuring the heat of mixing of the DL and DD complexes. The comparison of the results in the two cases indicated a less exothermic process in the formation of the couple DL with respect to that one relative to the couple DD.

Considering the presence of a random coil → α-helix transition actuated by the poly(L-lysine) in the diastereomeric couple DL, this is a quite surprising result, and it can be explained by a concerted conformational rearrangement of the pectate chain. This interpretation is, at our knowledge, not yet substantiated by other experimental findings.

The possibility to move toward higher energy conformation can be done only by using up the amount of energy delivered by the partner chain.

The same situation cannot be achieved by the DD diastereomer as the peptidic moiety does not move from the disorder state. The direct observation of the described behavior is quite difficult to gather as the solubility of the poly(lysine)−pectate complexes is quite low for the methods useful in this case: proton NMR and scattering techniques in solution require a concentration two to five times higher, whereas in UV circular dichroism the strong ellipticity of the polypeptide overlaps and covers the contribution belonging to the carboxyl chromophore of the polysaccharidic chain.[24]

In this study, we present further evidences supporting the hypothesis of a concerted order−disorder transition of the two chains in the case of the DL diastereomer. Combined use of visible and UV circular dichroism with conformational energy calculations is made in order to

* Corresponding author: paradossi@stc.uniroma2.it. INFM − Roma 1.
† Università di Roma "Tor Vergata".
‡ Slovak Academy of Sciences.

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highlight and possibly to explain the specific formation of an ordered macromolecular complex between poly(L-lysine) and pectate.

Experimental Section

Materials. Potassium pectate was prepared by a complete alkaline deesterification of commercial citrus pectin. The sample used in this study had a uronate content of 90.3%, as determined by potentiometric titration. The number-average molecular weight determined by osmometry was 19 000.

Samples of poly(L-lysine) and poly(D-lysine) in hydrobromide salt forms were Sigma product used without further purification. Their molecular weights, determined by LALLS, were 29 800 and 17 100, respectively

Methylene blue, 3,7-bis(dimethylamino)phenotiazinium chloride from Sigma, was used without further purification.

Ion Exchange Phases. Throughout this study, the following ion exchange phases were used: (i) CM Sepharose Fast Flow (Pharmacia Biotech) for cation exchange; (ii) DEAE Sepharose Fast Flow (Pharmacia Biotech) for anion exchange; (iii) Amberlite IR-120 (Merck) for strong cationic exchange; (iv) Sephadex G-10 (Pharmacia Biotech) for desalting.

For the enzymatic degradation of pectate, the pectinase (EC 3.2.1.15) from *Rhizopus sp.* (Sigma) was used.

p-Hydroxybenzoic acid hydrazide (Sigma) and D-galacturonic acid (Aldrich) were used without further purification.

Acetic acid, sodium hydroxide, hydrochloric acid, anhydrous sodium sulfate, and trihydrate barium perchlorate were Carlo Erba reagent grade products.

Methods. Titrations of the Pectate/MB Complex with Poly(lysine). In a typical experiment, 2 mL of an aqueous solution containing 6×10^{-4} monomol/L of pectate and MB 1×10^{-4} M is titrated with additions of 60 μl of poly(L-lysine) or poly(D-lysine) solution with starting concentration 2.1×10^{-3} monomol/L. After each addition of polypeptide, the absorption and circular dichroism spectra were carried out in the region 400−750 nm.

Enzymatic Degradation of Pectate. To obtain fractions of pectate with a useful range of degree of polymerization two different protocols of degradation were followed. The hydrolysis was carried out for 30 min with a pectinase concentration of 0.32 g/L at 25 °C (degradation A) or for 10 min with a 10-fold lower enzyme concentration (degradation B), respectively. The concentration of pectate solution was 10 g/L, and both degradations were performed in 50 mM acetate buffer at pH 4.5. To quench the degradation the reaction mixture was heated at 80 °C for 30 min. After filtration, the solution was dialyzed exhaustively against Milli-Q water to remove the buffer using a membrane with molecular weight cutoff of 500 (Spectra/Por).

Ion Exchange Chromatography. The solution containing the degraded pectate was concentrated and loaded (2 mL) on a column (XK 16/20 Pharmacia Biotech) for anion exchange chromatography. Sodium sulfate solutions with concentrations ranging from 0.05 to 0.3 M were used as eluent at a flow rate of 0.79 mL/min.

In Figure 1 it is shown the elution profile of the material hydrolyzed under more degradative conditions (degradation A). The column was eluted with three different concentrations of sodium sulfate solution, 0.05, 0.1, and 0.2 M, respectively. Among the oligomers eluted with the lowest ionic strength, i.e., sodium sulfate 0.05 M, only the peak at higher elution volume was collected as the others were oligosaccharidic species with very low degree of polymerization. The elution pattern with 0.1 M sodium sulfate consisted of two peaks, whereas the elution with the 0.2 M solution gave one peak only. The pooling was carried out as indicated in Figure 1 by the vertical bars. Pectate hydrolyzed under milder conditions (degradation B, see "enzymatic degradation of pectate") was chromatographed with sodium sulfate 0.05, 0.2, and 0.3 M solutions as eluent. Only the fraction eluted with 0.2 M was collected (Figure 2). The peaks eluted with 0.05 and 0.3 M were identified as very short oligosaccharidic chains and intact

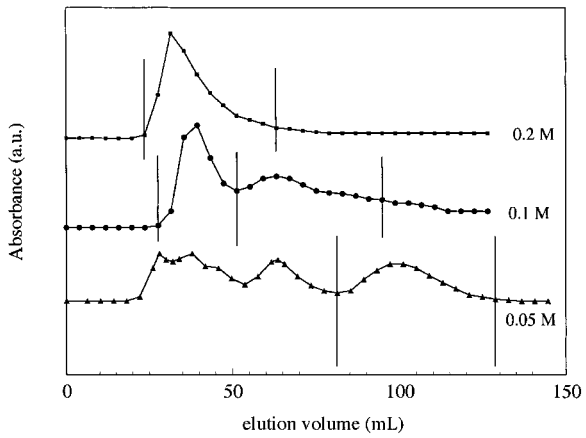

Figure 1. Anion exchange chromatography of products of degradation A: (▲) vertical lines delimit the elution volumes relative to fraction IA; (●) vertical lines delimit the elution volumes relative to fractions IIA and IIIA, respectively; (■) vertical lines delimit the elution volumes relative to fraction IVA.

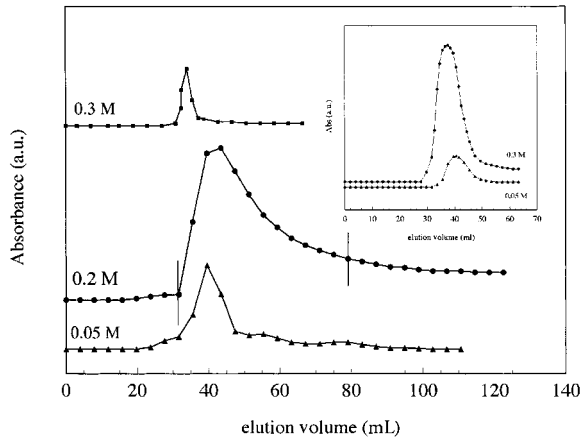

Figure 2. Anion exchange chromatography of products of degradation B: (●) vertical lines delimit the elution volumes relative to fraction IB. In the inset, an anion exchange chromatography run of intact pectate aqueous solution is shown: the elution volumes of the maxima at 0.05 and 0.3 M are coincident with the fractions from degradation B eluted with the same buffers.

pectate, respectively, according to a chromatographic blank experiment of the nondegraded polysaccharide (see insert of Figure 2).

The DP values of the different fractions were evaluated as the ratio between the moles of charges determined by potentiometric titration and the moles of reducing ends determined by PABA assay (see below).

Desalting of the collected fractions was carried out on a Sephadex G-10 in a column of a height of 40 cm and a diameter of 0.8 cm. The fractions were stored as aqueous solutions for further use. The concentrations of the fractions in solutions were determined by dry weight analysis.

Oligomers' Degree of Polymerization. The determination of the DP of the oligomers was achieved with a titration of the acid form of the oligomer and with the number of reducing ends present in the same solution.

The solution of fraction under investigation was percolated through a strong cationic exchange resin (Amberlite IR-120 H-form) and then titrated with a standardized sodium hydroxide solution. The reducing end group analysis was carried out with a colorimetric PABA assay[25] often used for carbohydrate solutions. It is based on the reaction between the hydrazide of the *p*-hydroxybenzoic acid and the reducing sugar. We used galacturonic acid as a standard for the calibration curve. After the reaction the absorbance of the solutions

containing the standard or the pectate oligomers was monitored at $\lambda = 410$ nm. The degree of polymerization of the oligomers was obtained as the ratio between the moles of charged groups found by titration and the number of reducing end groups obtained from the PABA assay.

Circular Dichroism (CD) and Absorption Spectroscopy. The CD and absorption spectra were carried out with a JASCO J600 spectropolarimeter and a JASCO J7850 spectrophotometer equipped with HELLMA quartz cells with optical length of 0.1, 0.5, and 1 cm.

Molecular Modeling. All simulations were carried out with a workstation XP1000 (Compaq Alpha station).

Molecular mechanics calculations were performed using the version c26β1 of the CHARMM package.[26]

No cutoff for nonbonded interactions was used, and the dielectric constant was taken to be equal to 4. The hydrogen bonding interaction was treated by adjusting the atomic partial charges and van der Waals parameters for the hydrogen bond donor and acceptor atoms.[27]

Both saccharidic and peptidic chains in the complex were modeled in an all-atom representation, starting with Cartesian coordinates obtained from the internal coordinates data of the corresponding CHARMM topology files. Lysine−galacturonate complexes were modeled with charged aminic and carboxylic groups, notwithstanding the fact that the overall net charge was taken to be equal to zero.

The NH_3^+−(L-LYS)$_{24}$−COO^- oligomer was initially built in an "all-trans" conformation of the peptidic backbone and of the side chains, with the aminic groups in the protonated state. The right α helix conformation was obtained by setting the 22 (ϕ, ψ) dihedral couples ($\phi = [C_{i-1}-N_i-C_{\alpha i}-C_i]$, $\psi = [N_i-C_{\alpha i}-C_i-N_{i+1}]$) to the values of (−65°, −40°), respectively. The resulting structure was then subjected to complex conjugate minimization to a derivative value of 0.01 kcal/Å or to an iterative decrease in energy less than 0.002 kcal/mol, by fixing in place all atoms of the peptidic backbone in order to avoid any "denaturated state". The L-lysine oligopeptide was finally oriented with the main moment of inertia along the *z* axis and used as a peptidic moiety in the DL complex, while in the DL2 complex the peptidic chain was rotated by 180° around the *x* axis, reverting to the head-to-tail direction.

The D-lysine segment was obtained by reflecting the L-lysine oligomer of DP 24 with respect to the *xz* mirror plane, obtaining the enantiomeric molecule with all C_α atoms in a D-configuration and a left-handed α-helical conformation.

The oligogalacturonate chain with DP = 24 was built by 1 → 4 linking residues of α-D-galacturonate in $^4C_1$ chair conformation. An elongated structure was obtained when the 23 couples of glycosidic dihedral angles ϕ_g, ψ_g, the former being the [O5−C1−O1−C4′] and the latter the [C1−O1−C4′−C5′], were set to 80 and 100°, respectively, corresponding to the main minimum in the potential energy map of the disaccharide.[18,28] A helical arrangement of the pectate chain was produced by varying the conformations of some glycosidic linkages. The dihedral couples (ϕ_g, ψ_g) between residues 1−2, 4−5, 6−7, 9−10, 11−12, 14−15, 16−17, 19−20, and 21−22 were set to values of (100°, −40°), which correspond to a higher energy minimum in the conformational map of the disaccharide.[18] The resulting molecule was energy minimized, but maintained in place all anomeric oxygen atoms. Alignment with the main momentum of inertia along the *z* axis and rotation of 120° around the *y* axis were then carried out. This pectate structure results in a turn of right-handed pseudohelix with pitch of about 44 Å and with a linear charge density along the *z* axis equal to that of poly(lysine) in an α-helical conformation. The helical structure of pectate had a radius suitable for accommodating the α-helical peptide moiety.

The geometry of the oligosaccharide−oligopeptide complex was optimized by analyzing the potential energy for different positions of the lysine chain.

The peptide was translated along the *z* axis from −18 to 18 Å, step 1 Å, and rotated around *z* from 10 to 360°, step 10°, exploring a 36 × 36 position grid. A constrained complex conjugate minimization was carried out for each of the obtained structures, to optimize the orientation of the lysine side chains.

The same energy analysis was performed for four kind of complexes in which the pectate oligomer was coupled with parallel and antiparallel D- and L-enantiomers of poly(lysine) originating the diastereomers DD, DL, DD2, and DL2.

Intermolecular interactions were evaluated by adopting as geometric definition a distance between the two atoms H and Y less than 2.4 Å and a X−H···Y angle greater than 120° for hydrogen bonding and a distance between ions less than 3.5 Å for ion pair occurrence.

Results and Discussion

Investigation of the Conformation of Pectate in the Pectate/Poly(L-lysine) Complex. The investigation on the conformational state of pectate was carried out by means of the probe methylene blue, hereafter called MB. Both constituents of the complex bear groups acting as chromophores in the same UV region around 220 nm as a consequence of the n → π^* electronic transition of the peptidic and carboxylic moieties of poly(lysine) and of pectate, respectively. In a solution containing both macromolecular species, these chromophoric groups, embedded in chiral matrices such as the polypeptide and polysaccharide backbones, contribute to the overall circular dichroic signal. In CD spectra of the macromolecular complex originated from the association of poly(lysine) with pectate, the deconvolution of the contributions of the two macroions is difficult as the polypeptide ellipticity is much more intense than pectate one, making unfeasible any direct evaluation of the conformation of the saccharidic chain interacting with the peptide.

For these reasons, we made use of a dye as conformational probe to monitor the behavior of the polyanionic pectate chain. It is known that the cationic phenothiazinium dye MB, interacts with oppositely charged polyelectrolytes and colloidal dispersions.[29−31] In the absence of negatively charged macromolecular species the dye has the tendency to form aggregates in equilibrium with the monomeric form. Its spectrum is characterized by an absorption band (α-band) centered at 664 nm, corresponding to the monomer, together with a shoulder at 610 nm (β band) assigned to the face-to-face dimeric form,[32] whose intensities are governed by an equilibrium constant of about 6000 M^{-1}.[32] An increase of dye concentration favors the formation of dimeric or multimeric stacks, the latter aggregates displaying a broad absorption band around 570 nm.

We have studied[22] by means of visible absorption spectroscopy the behavior of MB adsorbed on pectate during the titration with poly(lysine). The formation of the macromolecular poly(lysine)−pectate complex causes the displacing of the probe from the charged surface of pectate. The progressive detachment of the MB molecules was indicated by the recovering of the same spectrum of the dye in solution as was in the absence of polyanions. In this way it was shown also that a binding process is present both in the case of a titration with poly(L-lysine), PLL, and with poly(D-lysine), PDL, although no significant differences in the visible absorption behavior of MB were found during the formation of the two macromolecular pairs DL and DD.

In this study, we have investigated the ellipticity of the absorption bands of MB bound to pectate during the addition of poly(L-lysine) and of poly(D-lysine), with the aim to probe differences in the conformational behavior

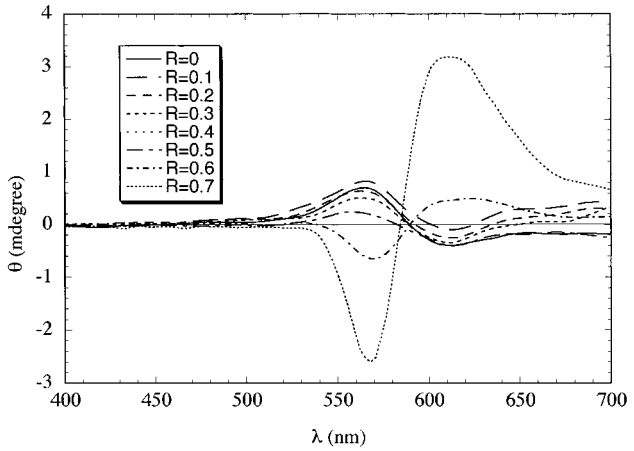

Figure 3. Circular dichroic spectra of the complex pectate-MB titrated with poly(L-lysine) for different mixing ratios.

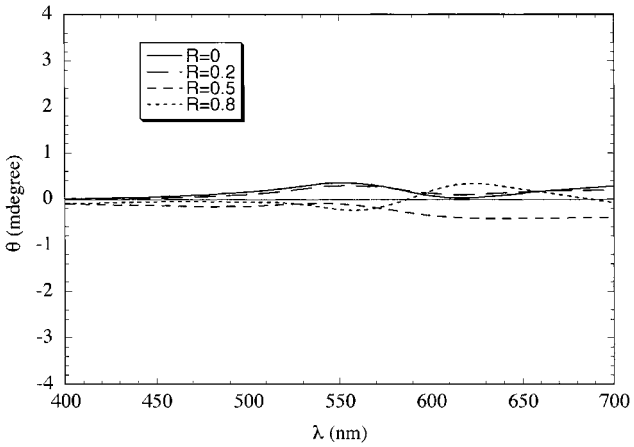

Figure 4. Circular dichroic spectra of the complex pectate-MB titrated with poly(D-lysine) for different mixing ratios.

of the pectate chain in the DL and DD pairs, respectively, as monitored by the dye. In Figures 3 and 4, the circular dichroic behavior in the region from 400 to 700 nm is shown at different molar ratio *R* of poly(L-lysine)/pectate and of poly(D-lysine)/pectate, respectively. In the case of the DL pair, an increase in the CD is detected at ratio of about 0.6, whereas for the DD pair the signal remains low for any value of *R*. This is in contrast with the behavior of the visible absorption bands of the dye, indicating for both pairs a progressive detachment of the probe stacks from the pectate domain as a consequence of the associative process of oppositely charged polyelectrolytes. The trend of the circular dichroic signal described above does not take into account the decrease of ellipticity due to the displacement of the probe from pectate domains. We have therefore normalized the dichroic signal at 570 nm by the absorption intensity at the same wavelength. The results, shown in Figure 5 as a function of *R* for the pairs DD and DL, indicate a very sharp decrease of the quantity $(\theta/\mathrm{Abs})_{570}$ when the complex pectate−methylene blue is titrated with poly(L-lysine). The end of the titration is determined by the limited solubility of the complex at mixing ratio, *R*, larger than 0.7 where a phase separation process takes place. On the contrary an almost constant value of $(\theta/\mathrm{Abs})_{570}$ is observed during the DD pair formation up to values of *R* equal to 0.8. This findings support the hypothesis proposed by us about a conformational rearrangement of the pectate chain when the interacting poly(lysine) has a L-chirality.

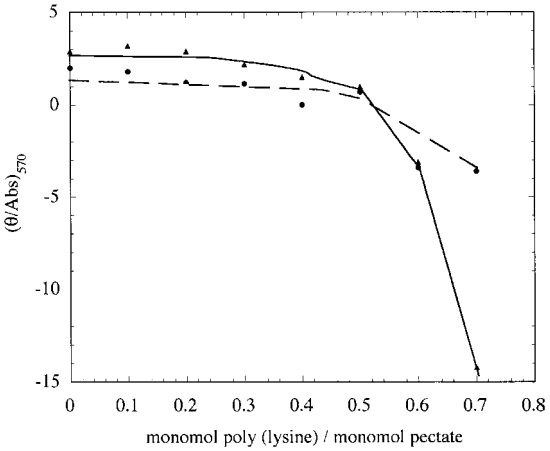

Figure 5. Circular dichroic signal at 570 nm normalized by the absorption value at the same wavelength as a function of the mixing ratio, *R*: titration of the MB−pectate complex with poly(L-lysine) (▲) and with poly(D-lysine) (●).

Table 1. Summary of the Fractions Collected by Anion Exchange Chromatography

degradation procedure	fraction collected	eluent: Na_2SO_4 (M)	elution vol (mL)	degree of polymerization[a]
A	IA	0.05	81.4	6
	IIA	0.1	28.4	13
	IIIA	0.1	51.3	20
	IVA	0.2	29.2	22
B	IB	0.2	34	25

[a] Determined by titration of charges on pectate chain and PABA assay.

Determination of the Minimal Length of Pectate Necessary for the Conformational Transition of Poly(L-lysine). To study the binding process and to determine the minimal length needed for inducing a conformational rearrangement of the pectate chain, first we have carried out an enzymatic hydrolysis of the pectate followed by separation and purification steps in order to obtain a set of pectate fractions. Anionic exchange chromatography coupled with a G-10 chromatographic desalting was found to be a convenient separation method of the digested polysaccharidic material.

Two degradation processes were carried out (see Experimental Section) for obtaining fractions with degrees of polymerization interesting for this study.

In Table 1, the results of the degradation and of the chromatographic separation are summarized. All the oligomers were tested in aqueous solution in the UV region by absorption spectroscopy and circular dichroism. The concentrations of the fractions stocked in solution were determined by dry weight analysis.

The obtained oligomers of pectate were used for studying the influence of the DP on the α-helical content of PLL in the complex formation. We have investigated the effect of DP in a molar excess of oligomers with respect to PLL equal to 3. This study, see Figure 6, indicates that oligomers of pectate with DP $\leqslant$ 22 are not able to induce any conformational changes on PLL chain as the CD spectra are clearly indicating the peptidic chain in a random coil arrangement. Only when poly(L-lysine) is mixed with a pectate oligomer with DP = 25 is a change in the CD signal obtained. In these conditions the CD spectrum is the result of a mixture of an equal amount of random coil and of α-helix conformation. Moreover, if we increase the molar mixing

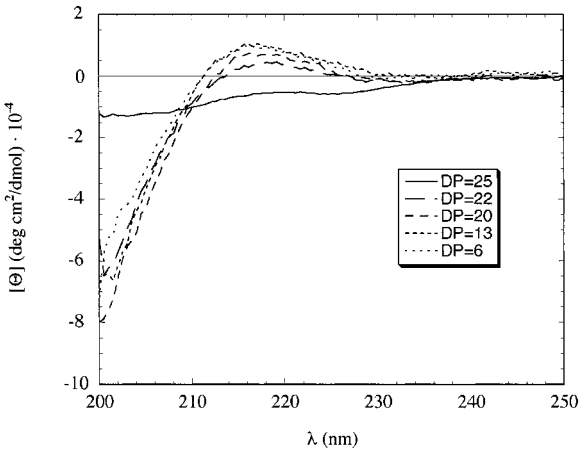

Figure 6. Circular dichroism spectra in the UV region of poly(L-lysine) in the presence of pectate oligomers with different degrees of polymerization at fixed mixing ratio $R = 3$.

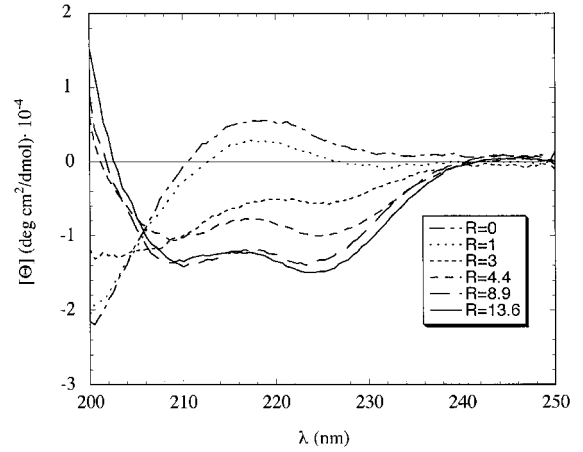

Figure 7. Circular dichroism spectra in the UV region of poly(L-lysine) in the presence of the pectate oligomer of DP = 25 as a function of the mixing ratio, R.

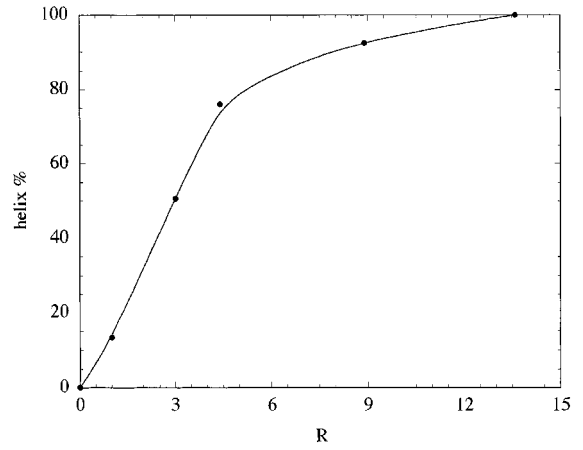

Figure 8. Poly(L-lysine) helix percentage for different mixing ratio with pectate oligomer of DP = 25.

ratio R to values larger than 3, using the pectate oligomer of DP = 25, we can recognize in Figure 7 the progressive buildup of the α-helical circular dichroism spectrum. In Figure 8 it is shown that about the 90% of this ordered peptidic conformation is reached for $R = 7$. It is worthwhile to note that with low pectate DP fractions, for example DP = 13, the α-helical conformation of PLL is not induced for any value of the mixing ratio R (not shown).

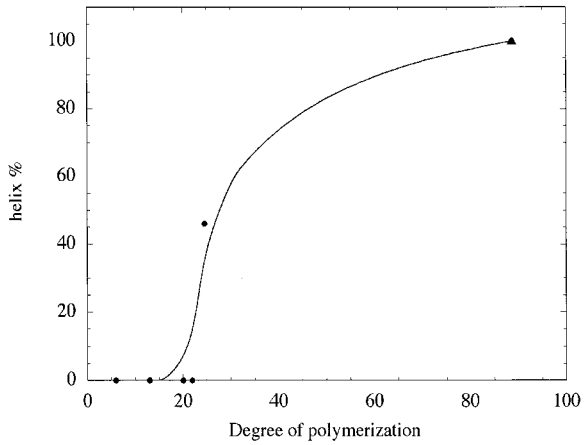

Figure 9. Poly(L-lysine) helix percentage as a function of DP for pectate oligomers. Mixing ratio $R = 3$. Key: (▲) intact pectate.

Molecular Modeling. The results shown in Figure 9 for $R = 3$ demonstrate that the conformational transition of poly(L-lysine) is triggered by a pectate oligomer with DP equal to 25. In light of this finding, we carried out simple geometrical considerations where the helical path length with pitch, p, on a cylinder surface with radius r is given for n turns by

$$L_n = n\sqrt{(2\pi r)^2 + p^2} \tag{1}$$

A poly(lysine) α-helix has a linear charge density, z_L, of 6.78 charges·nm^{-1} while for pectate in the extended configuration the linear charge density, z_P, is equal to 2.17 charges·nm^{-1}. Imposing the electroneutrality of the complex, i.e., $nz_PL = nz_Lp$, the pitch of a superhelix with radius r and the helical path length, L, is given for $n = 1$ by

$$p = \frac{2\pi r}{\sqrt{\left(\frac{z_L}{z_p}\right)^2 - 1}} \tag{2}$$

and

$$L = 2\pi r\sqrt{1 + \frac{1}{\left(\frac{z_L}{z_p}\right)^2 - 1}} \tag{3}$$

where r is the sum of the radius of the poly(L-lysine) α helix including the side chains and of the radius of the pectate chain.

From direct inspection of the geometrical model of the complex, built by available library structural data, a value of 16 Å is measured for the overall radius of the complex with an "all trans" conformation of the poly(lysine) side chains.

The resulting p and L parameters are 36 Å and 107 Å, respectively.

This latter value can be converted to the number of residues of the pectate chain by means of the linear charge density of pectate, z_p, yielding a number of residues *per* superhelix turn of 24.

This result is in very good agreement with the experimental finding and supports the hypothesis of a superhelical arrangement of pectate chain interacting with poly(L-lysine).

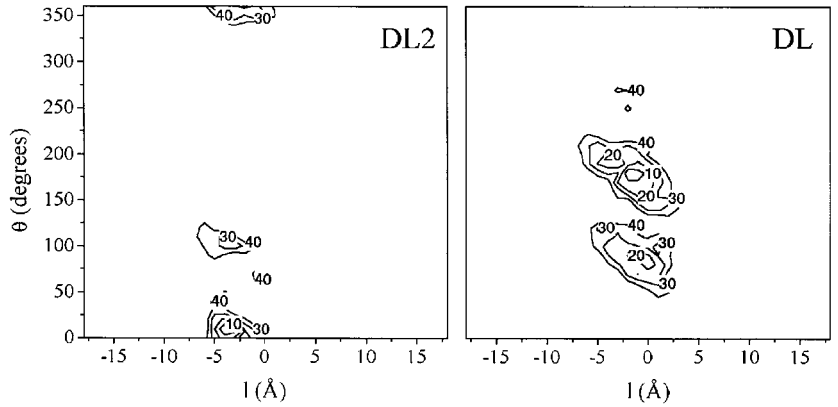

Figure 10. Potential energy maps of the poly(L-lysine)/pectate complexes as a function of the peptide position. *l* is the translation of the lysine center of mass along *z* (the superhelix axis), and θ is the rotation around the same axis. Right: head-to-tail parallel arrangement (DL). Left: head-to-tail antiparallel arrangement (DL2). Contour levels are given in kcal/mol of complex, above the minimum of each map (see Table 2).

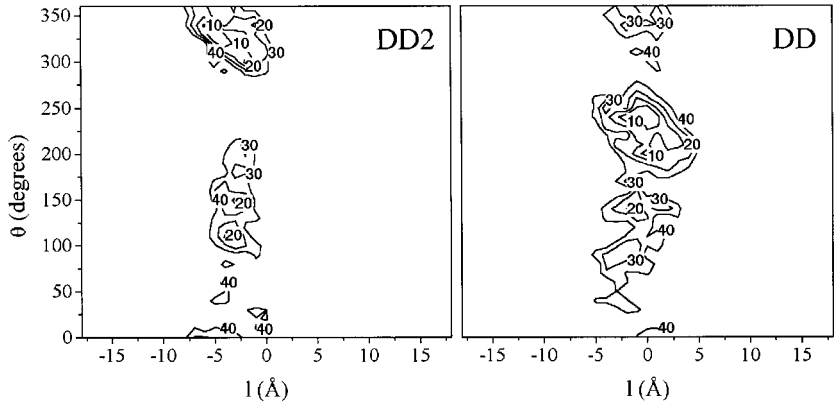

Figure 11. Same as Figure 10, but for DD (right) and DD2 (left) complexes.

We tried to validate this approach with more detailed molecular calculations. The structure of this polyelectrolytic assembly was studied by means of conformational minimization methods in order to shed light on the driving forces involved in the complex formation and in its stereoselectivity.[33]

Four diastereomeric couples were considered. Besides the DD and the DL couples containing parallel chains of pectate and poly(lysine), DD2 and DL2 were originated by coupling a 24-residues pectate chain with antiparallel D- and L-peptidic chains, respectively (see Methods in the Experimental Section).

The energy maps obtained for the four topologies are shown in Figures 10 and 11. In the DL2 case, a main steepest gradient potential well was found as other relative minima were at too high energy levels. Therefore, a more tightly bound peptidic chain has to be expected in the DL2 couple on the basis of its energy map. In Table 2, the total potential energies and their components are reported for the lowest energy structure of each topology. Both DL and DL2 structures are energetically preferred with respect to DD and DD2. It is also noticeable that for the former couples the discriminating contribution to the overall energy is the electrostatic term. The small difference in energy of topology DL2 with respect to DL is mainly due to a more favorable geometry in terms of angle and dihedral energy of lysine side chains. The main interchain hydrogen bond distances for DL, DD, DL2, and DD2 are reported in Table 3. The two electronegative atoms involved in the hydrogen bond are reported, the former belonging to pectate molecule, the latter to the lysine molecule. The residue number is indicated in parentheses, distances being in angstrom units, starting from the nonreducing end and from the N-terminus for pectate and lysine, respectively. O61 and O62 are carboxylic oxygen atoms, whereas NZ are ϵ-aminic nitrogen atoms. Analogously in Table 4 the main interchain ion pair distances are reported, being worthwhile to note that the larger number of ion pairs is displayed by the DL2 topology, whereas DL contains a larger number of hydrogen bonds (see Table 3). As far as the glycosidic dihedrals of the 24 galacturonate residues forming the pectate chain are concerned, the energies of 14 glycosidic linkages are within the main minimum of the dimer conformational map, corresponding to ϕ_g and ψ_g mean values of 80° and 101°,[14,15] respectively. Nine dihedrals belong to a higher energy minimum, with ϕ_g and ψ_g mean values of 97° and −49°,[15] respec-

Table 2. Summary of the Simulation Results for Minimum Energy Complexes[a]

	DL	DD	DL2	DD2
l_{min} (Å)	−2	−1	−4	−5
θ_{min}(deg)	180	240	10	330
total energy	243	253	235	250
bond	72	71	71	71
angle	252	248	248	251
Urey−Bradley	38	36	37	37
dihedral	−742	−745	−746	−748
improper	0	0	0	0
van der Waals	74	74	72	69
electrostatic	551	569	552	570

[a] All energy terms are in kcal/mol of complex.

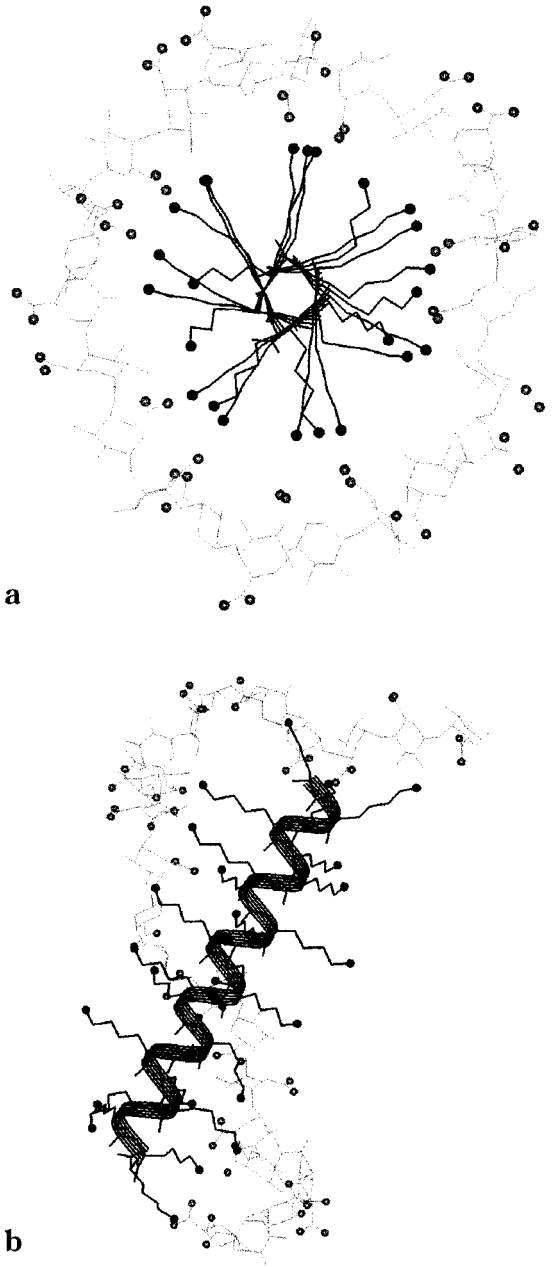

Figure 12. DL2 lowest energy structure. The pectate chain is drawn in gray and the carboxylic oxygens and ϵ-aminic nitrogen atoms are evidenced for an easier view of the structure: (a) projection along the *z* axis; (b) side view.

tively. The corresponding structure is shown in Figure 12, parts a, top view, and b, side view. Radius and pitch of the pseudosuperhelix are about 17 and 44 Å, respectively. This pitch is a little higher than the α-helix height of poly(L-lysine) due to the tilted direction of the terminal peptidic side chains. The overall dimension of the DL2 topology results in a radius of gyration of 16 Å. The charge distribution on the superhelical surface has a patchy pattern as only 11 charge pairings out of a total 24 theoretically possible ones are achieved.

Concluding Remarks

We have reported in this paper a study evidencing a structural rearrangement of the pectate chain around the polypeptidic α helix. This study aims at a deep understanding of the factors playing a role in the building up of macromolecular assemblies. The main issues gathered in this study are as follows:

Table 3. Intermolecular Hydrogen Bonds

DL	DD	DL2	DD2
O61(2)−NZ(3) 3.17	O61(6)−NZ(7) 2.91	O61(2)−NZ(24) 3.00	O61(2)−NZ(22) 2.81
O61(6)−NZ(7) 2.91	O62(6)−NZ(3) 3.02	O61(6)−NZ(20) 3.06	O61(6)−NZ(19) 2.84
O61(7)−NZ(7) 2.99	O62(7)−NZ(3) 2.83	O62(6)−NZ(23) 3.04	O62(6)−NZ(23) 3.01
O62(7)−NZ(4) 3.02	O61(11)−NZ(13) 2.93	O61(7)−NZ(19) 2.90	O62(7)−NZ(23) 3.00
O2(10)−NZ(11) 3.16	O61(12)−NZ(13) 3.06	O62(7)−NZ(23) 2.86	O61(11)−NZ(13) 2.83
O61(11)−NZ(11) 3.06	O62(16)−NZ(16) 3.06	O61(11)−NZ(15) 2.86	O61(17)−NZ(7) 3.07
O62(11)−NZ(8) 2.89	O61(17)−NZ(19) 2.76	O61(16)−NZ(4) 3.04	
O61(16)−NZ(19) 2.76	O61(21)−NZ(22) 2.83		
O61(17)−NZ(19) 2.97			
O2(19)−NZ(23) 3.22			
O61(22)−NZ(24) 3.06			

Table 4. Ion Pairs

DL	DD	DL2	DD2
O61(7)−NZ(4) 3.15	O61(11)−NZ(9) 3.18	O62(2)−NZ(24) 3.48	O62(6)−NZ(19) 3.09
O61(16)−NZ(16) 3.45	O62(11)−NZ(9) 3.41	O62(2)−NZ(20) 3.38	O61(7)−NZ(20) 3.12
O61(21)−NZ(24) 2.91	O61(16)−NZ(16) 3.02	O61(7)−NZ(16) 3.46	O61(12)−NZ(13) 2.86
	O62(18)−NZ(23) 3.48	O61(11)−NZ(12) 3.09	O62(12)−NZ(13) 3.15
	O61(22)−NZ(22) 3.43	O62(11)−NZ(15) 3.45	O62(16)−NZ(10) 3.18
		O61(12)−NZ(15) 3.22	O61(17)−NZ(3) 2.95
		O62(12)−NZ(15) 3.28	
		O61(17)−NZ(4) 3.16	
		O61(17)−NZ(7) 2.94	
		O61(21)−NZ(3) 2.87	

(i) Conformational plasticity of the pectate chain is observed which is able to form a superhelical complex complying to the helical pathway of poly(L-lysine).

(ii) Stereospecificity is a characteristic of the interaction of poly(lysine) with pectate.

(iii) There is a threshold value of DP of pectate for the complex to achieve the superhelical topology.

(iv) On the basis of the conformational analysis, the preferred topology is an antiparallel arrangement of the two chains forming the complex where the electrostatic contribution is the main stabilization factor.

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