Interactions of Pectin with Cationic Polypeptides

S. BYSTRICKÝ and A. MALOVÍKOVÁ

Institute of Chemistry, Slovak Academy of Sciences, CS-842 38 Bratislava

Received 19 October 1990

Dedicated to Associate Professor Ing. Dr. R. Kohn, DrSc., in honour of his 70th birthday

The CD measurement was shown as a very effective tool to investigate the complex-forming interactions of acidic polysaccharides with basic polypeptides. The review summarizes the progress achieved in the last five years in investigation of systems comprising different pectin macromolecules and model polypeptides. Conclusions on complexation effectiveness are derived from the observed conformational changes of polypeptides. The strict stoichiometry of complexation enables to propose the most probable stereochemical structure of the complex. The influence of charge densities of both interacting components on the complex structure is also presented. The detailed analysis of the stereochemical factor finally revealed the nonequivalent enantiomeric interaction, leading to recognition of the inherent conformation of the polysaccharide chain in solution.

Pectin, an acidic plant polysaccharide, occurs mainly in fruits and vegetables. At present, it is considered an essential substance in human nutrition with important functional and physiological properties [1]. Its ability to bind cations, basic organic substances of low-molecular nature, basic polypeptides, proteins, and enzymes is especially significant. Considering the binding of cations, pectin is functioning as a prophylactic agent against poisoning with toxic cations. Physicochemical rules governing the interaction of carboxyl groups of pectin with bivalent cations (Ca^{2+}, Sr^{2+}, Pb^{2+}, Cd^{2+}, and Zn^{2+}) [2—5] were studied in detail with pectins of various structural features. The degree of esterification of carboxyl groups of pectin *E*, which reflects the linear charge density of the macromolecule, is the parameter influencing significantly the interaction. With decreasing *E*, *i.e.* with increasing charge density, the strength of binding of bivalent cations increases. In the region of esterification degree around 0.4 the intramolecular electrostatic binding changes to intermolecular bonds under formation of chelates. The conclusion about the intermolecular binding of calcium and strontium ions to fully deesterified pectin, *i.e.* pectate [2] is in accordance with the "egg-box" binding model suggested for binding of calcium cations in solutions of L-guluronan and D-galacturonan [6]. It is connected with the change of the conformation of macromolecule probably from three-fold screw symmetry in solutions containing monovalent cations to two-fold screw symmetry of solid calcium salts of these polyuronates.

The electrostatic interactions of acidic polysaccharides with basic macromolecules, leading to formation of a complex, are also accompanied by conformational changes of the interacting components. Biologically important interactions of polysaccharides with cationic polypeptides have not been sufficiently clarified. Formation of the complex is generally associated with change of the secondary structure of polypeptides in the sense of a transition from less ordered to regular structures. The interaction, which is mainly electrostatic, is influenced by stereochemical character of both components, ionic strength of the solution, type of the ionic groups, and charge density of the macromolecules.

Circular dichroic measurements (CD) of dilute solutions were shown to be an extremely sensitive method for studying the extent of complex-forming interaction, as complexation is accompanied by expressive changes of polypeptide conformation. We have studied the interactions of potassium pectate and potassium pectinates of various esterification degree with model polypeptides (polylysine, poly(Lys-Ala-Ala)) by means of CD. Studies were focused on determination of complex-forming efficiency of the interaction and on elucidation of stereochemical aspects of this process. In order to find out the handedness of the interacting pectate, the interaction of this polysaccharide with polylysine enantiomers has been studied.

METHODS

Pectin and peptides are materials with dissymmetric structures convenient for direct probe by circularly polarized light. Alternating right and left circularly polarized light, produced in CD spectrometers, is differently absorbed by optically active substances. Circular dichroism provides information about the nature of the chromophore containing residues and it is also sensitive to their relative orientations in the polymer chain.

Mono- and poly(D-galacturonic acids) show a single positive band at $\lambda \approx 212$ nm corresponding to $n—\pi^*$ transition of the carboxyl group. The distance of the chromophores of polyuronate is too great for the exchange of energy between them, so that CD spectra are likely not sensitive to chain conformation. However, direct interactions between residues can be observed with the peptide structures where the component amide chromophores are sufficiently close to allow exchange of energy. Here the interactions are extremely sensitive to the chain conformation. For regularly ordered chain conformations such as α-helix or β-sheet distinctive characteristic bands in the range of λ = 205—240 nm are observed. Namely polylysine after neutralization of NH_3^+ charges (pH > 11) forms α-helical conformation. CD spectrum of α-helix is characterized by two negative maxima at λ = 222 nm ($n—\pi^*$ transition) and 205 nm ($\pi—\pi^*$ exciton), and by a positive one at 190 nm ($\pi—\pi^*$ exciton). β-Sheet conformation is characterized by one negative maximum at $\lambda \approx 215$ nm, whereas the irregular "charged coil" form is represented by a positive low-intensive band in the same region.

The CD spectra of mixtures of polypeptides with pectin and oligogalacturonates, respectively, were recorded at various ratios of both components. Interactions were examined in two series of samples according to the excess of one component. Series *I*. Solution of polypeptide in amounts increasing from 0 % to 100 % per the content of carboxyl groups of pectin was added to the solution of pectin and/or oligogalacturonate. Series *II*. Solution of pectin or oligogalacturonate in amounts increasing from 0 % to 100 % per the content of amino groups of polypeptide was added to the solution of polypeptide. The final concentration of the component in excess was always adjusted by dilution to 0.3 mmol dm^{-3} The CD spectra obtained were corrected by subtracting the CD of pectin and/or oligogalacturonate present in the solution. Additionally, in the series with excess polypeptide, the CD of this in the charged-coil arrangement was subtracted. The CD spectra corrected in this way represent the CD of that part of the polypeptide in solution having an equivalent amount of carboxyl counterions on pectin and/or oligogalacturonate required for interaction.

PECTIN AND POLY(L-LYSINE)

The main chain of pectin molecule is almost exclusively formed by homopolymeric D-galacturonan with a diaxial *trans*-glycosidic (1 → 4)-α bond randomly disconnected by L-rhamnose units in minor representation. The pectin samples contained 83 % to 91 % of the partially esterified D-galacturonan and 8 % to 16 % of neutral saccharides (D-galactose, L-arabinose, D-xylose, D-glucose) in the form of short side-chains.

The pectin samples of various esterification degree were prepared by a partial alkaline deesterification of a highly esterified pectin [3]. This reaction results in a random, with proceeding deesterification more or less regular, distribution of free and esterified carboxyl groups in the molecule. Conformational freedom of the pectin molecule chain is considerably restricted [7—9]. The CD spectra of pectic and pectinic acids of various esterification degree and their sodium and potassium salts are characterized by a simple dichroic band of positive sign at λ = 202—210 nm (Fig. 1, curve *1*) similarly as found with their monomer [9—11].

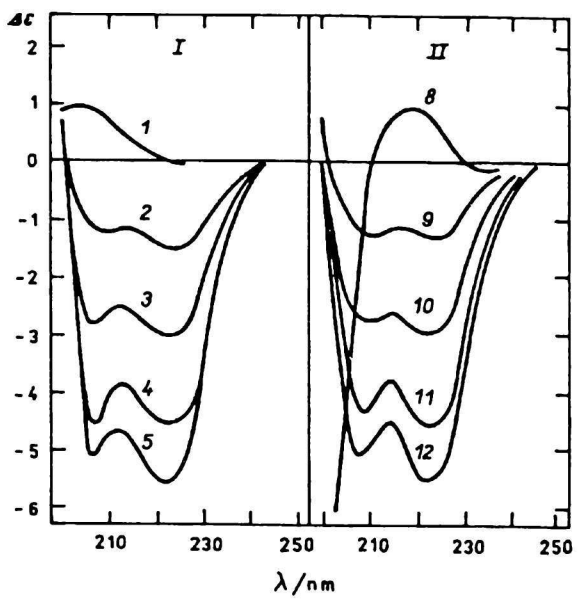

Fig. 1. The CD spectrum of poly(L-lysine) in the presence of potassium pectate. Series *I* — pectate solution ($c(COO^-)$ = 0.3 mmol dm^{-3}), addition of 0 % (*1*), 20 % (*2*), 40 % (*3*), 60 % (*4*), and 70 % (*5*) of poly(L-lysine). Series *II* — poly(L-lysine) solution ($c(NH_3^+)$ = 0.3 mmol dm^{-3}), addition of 0 % (*8*), 20 % (*9*), 40 % (*10*) , 60 % (*11*) , and 70 % (*12*) of pectate.

CD spectrum of poly(L-lysine) chloride in a neutral solution is shown in Fig. 1, curve *8*. This spectrum represents the conformation of the ionic form of this polypeptide in a random coil. The macromolecule became regularly arranged after neutralization of the macromolecule charge. Measurements in both series clearly show a regular increase of CD value corresponding to the increasing amount of the constituent added [12]. The shape of the corrected spectrum is characteristic of the α-helical structure of poly(L-lysine). Series *I* (an excess of pectate) is presumed to have all the poly(L-lysine) in the complex, *i.e.* in the helical conformation. Series *II* had the CD curves identical with those of series *I*. In spite of the excess of poly(L-lysine) also in this case the helical component is formed by the very amount of polypeptide, equivalent to that of the polyanion added. The interaction of poly(L-lysine) with pectate is a quantitative one, the 1 : 1 complex was formed regardless of the excess of either component. Unified CD spectra of both experimental series provide a proof for stoichiometrical equivalence of $-COO^-$ groups of pectate and $-NH_3^+$ groups of poly(L-lysine) in the complex.

The values of CD spectra of poly(L-lysine) in the presence of potassium pectinate of esterification degree E = 0.206, corrected by subtraction as in the preceding case, are lower than those of the unesterified pectate. Shapes of the CD curves and ellipticity values ε in both series were no more equal. Diminution of the helical form of the complex is due to a partial esterification of carboxyl groups of the pectinate added. These findings are even more backed by the CD spectra obtained with potassium pectinate of E = 0.460 and E = 0.637. Pectin with the highest value of esterification degree E = 0.861 does not form a complex with poly(L-lysine). The measured CD spectrum is a simple addition of CD spectra of both components in solution.

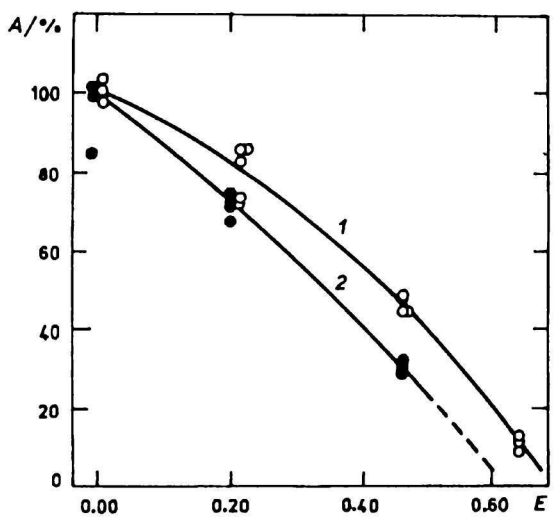

Fig. 2. The effect of esterification degree of pectin on formation of its complex with poly(L-lysine) . A – the amount of complex formed (%). *1*. The series *I* with an excess of pectin in solution; *2*. the series *II* with an excess of poly(L-lysine) in solution.

A general view on the ability of pectin to evoke formation of a regular helical conformation of poly(L-lysine) in the complex is best exemplified by comparing the ellipticity values at λ = 225 nm. The results obtained with pectin of various esterification degree at different experimental conditions are summarized in Fig. 2. The expression of the amount of the helical form of poly(L-lysine) A in % refers to a mean value of ellipticity as determined with the unesterified pectate; in this case A is considered as 100 %. Series *I*, where the concentration of poly(L-lysine) in solution changes, has the ellipticities unified to the concentration 0.3 mmol dm^{-3} of NH_3^+ (opened circles); series *II*, where the concentration of pectin changes, has the ellipticity unified to the concentration 0.3 mmol dm^{-3} of COO^- and $COOCH_3$ (full circles). Both experimental arrangements show that formation of the complex strongly decreases with increasing esterification degree of pectin. Points of series *II* lie somewhat lower than those of series *I*; this documents a lower efficiency of the esterified pectin added in series *II*. Really effective are only the ionized carboxyl groups in the complex-forming interaction.

Considering the afore-mentioned facts one can propose the stereomodel of the complex. Shape of the CD spectra evidences that poly(L-lysine) forms a regular α-helical structure in the complex. Experiment with the unesterified pectate (E = 0) is considered fundamental; open remains the conformation of this macromolecule in the complex. The found equivalence of charges of both polyelectrolytes in the complex has to be taken into account when interpreting the results. The linear charge density of pectate is by approximately three times lower than that of poly(L-lysine) in the α-helical form. The complex-forming engagement of all anionic and cationic groups can be achieved by a suitable winding of one pectate macromolecule around the helical structure of poly(L-lysine) or by interaction of some parallel pectate chains with one chain of poly(L-lysine).

To interpret the obtained results it must be presumed that the interaction does not take place in couples cation–anion, but the charge of one $-NH_3^+$ group is considered as a mean plane charge on the surface of a cylinder given by terminal $-NH_3^+$ groups of side chains of the poly(L-lysine) helical structure. The pectate molecule con-

sequently acts as oppositely (negatively) charged chain with a certain charge density. The interaction results in a complemental formation of both helical structures. The winding of pectin, *i.e.* formation of a superhelical structure should not considerably affect its basic secondary structure. The presumption of a complete saturation of charges from a suitable distance enables to propose geometrical parameters for the superhelix. Parameters of the superhelix were adduced taking the van der Waals radii of the single atoms into account. We characterized the molecule of poly(L-lysine) α-helix as a cylinder of radius r_L; its surface represents the planar distribution of all charges of $-NH_3^+$ groups. The linear molecule of pectin (D-galacturonan) is represented by a cylinder of radius r_P the surface of which contains all ionized carboxyl groups. Our rationalization was based upon the following data: one turn of the α-helix of poly(L-lysine) 0.54 nm high comprises 3.66 L-lysine units [13]. The linear side chains are oriented in the plane of the peptidic C—N—C(O) group and have a zig-zag conformation. This spatial arrangement offers the greatest distance between terminal $-NH_3^+$ groups. Provided that the linear D-galacturonan molecule has a three-fold screw symmetry one monomer unit corresponds to 0.437 nm [14]. We obtained following values: r_L = 0.94 nm, r_P = 0.55 nm. The resulting supermolecular system is characterized by the sum of radii of both helical components $r_L + r_P$ = 1.49 nm. Should the α-helix of poly(L-lysine) be wound up by one pectate chain, then the superhelix formed would contain roughly 23 D-galacturonic acid units in one turn of 3.4 nm height. The geometry of the superhelix is only approximation since the interaction of both polyions can comprise the effect of solvation shells of the macromolecule, their change during interaction, the influence of short side-chains of the pectin molecule consisting of neutral saccharides, the effect of esterification of carboxyl groups, *etc.*

Upon interaction of several parallel chains of pectate with poly(L-lysine) the distance between turns does not change; nevertheless, the steepness of helix (the distance between turns along the helix axis) will be greater. In a complex composed of two parallel chains of pectate the double superhelix turn is 2.53 times greater. The complex containing three parallel chains of an unesterified pectate is the limit case having the chains parallelly oriented along the α-helix axis of poly(L-lysine).

Partially esterified pectins prepared from the preparation with E = 0.0, do not completely form a complex. The decrease of charge density of esterified pectins causes that either a certain deformation in the sense of a compression of turns in the superhelical structure, or enhancement of number of interacting pectinate molecules oriented parallelly along the helix axis is needed for a complete neutralization of charges.

A small compression of the superhelix turns in case of the pectinate sample of E = 0.20 could be anticipated. A greater compression of the superhelical structure is, however, unlike, since it would require great change of torsion angles in the D-galacturonan chain.

Complexation of some parallel pectin chains on the surface of the poly(L-lysine) α-helix would presume an increase of the number of chains needed for a full saturation of charges with the increasing esterification degree of pectin, *i.e.* with the decreasing linear density of the negative charge. The number of chains arranged parallelly along the α-helix axis should be for various values of E as follows: three chains for 0, four for 0.25, five for 0.40, six for 0.50 and seven for 0.57. The maximum number eight chains for E = 0.62 is limited by spatial possibilities of the model under consideration. Such an arrangement of pectin chains should lead to a quantitative formation of the complex even with samples of a higher esterification degree of carboxyl groups. A strong decrease of the complex-forming efficacy of pectin with an increasing esterification degree indicates a parallel arrangement of some pectin chains around the poly(L-lysine) α-helix to be less probable. Therefore, the structure of complexes is better represented by the model composed of an α-helix of the polycation and a superhelix of the polyanion.

PECTIN AND POLY(Lys-Ala-Ala)

The CD spectrum of poly(Lys-Ala-Ala) in neutral aqueous solution in a charged coil arrangement is shown in Fig. 3 (curve *1*).

The CD spectra of a mixture of potassium pectate or pectinate with poly(Lys-Ala-Ala) were recorded at various ratios of both components [15]. Samples of low-esterified pectin revealed opalescence, which successively disappeared with increasing esterification degree of pectin. Fig. 3 shows the corrected CD spectra of poly(Lys-Ala-Ala) in the presence of potassium pectinate for some selected samples of pectin of various esterification degree. Concentration of both components of mixtures, *i.e.* $-NH_3^+$ groups of the polypeptide and all the carboxylato groups of pectin ($-COO^-$ and $-COOCH_3$) was identical, 0.3 mmol dm^{-3} Spectra showing two peaks in the negative region indicate the presence of

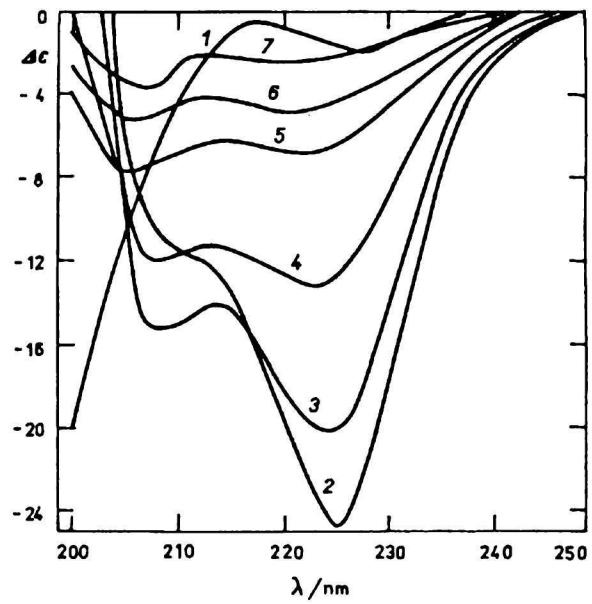

Fig. 3. The CD spectrum of poly(Lys-Ala-Ala) ($c(NH_3^+)$ = 0.3 mmol dm^{-3}) in the presence of pectin ($c(COO^- + COOCH_3)$ = 0.3 mmol dm^{-3}) with various esterification degree. *1*. Without pectin; *2*—*7*. addition of pectin of *E*: 0.0, 0.216, 0.441, 0.653, 0.747, and 0.826.

the polypeptide in an α-helical conformation. Intensity of dichroic bands decreases with increasing esterification degree of pectin; this evidences the effectiveness of only ionized carboxyl groups —COO^- in the complex-forming interaction. The sample with the unesterified pectate is characterized by a short-wavelength shoulder only at an intense dichroic band of the n—π^* transition. The corrected CD spectrum of polypeptides interacting with pectinates of esterification degree $E \geq 0.44$ has already a typical shape for α-helical structure.

The special shape of CD curves with an insignificant short-wavelength negative dichroic band, observed with low-esterified pectinate complex cannot be classified into known manifestations of regular protein structures [16]. Certain similarity could be encountered with the CD curve for β-structure. The sequence copolymer poly(Lys-Ala-Ala) does not form a β-structure [17]. The β-structure is indicative of only one negative dichroic band with the maximum shifted towards lower wavelength ($\approx$ 215 nm). In contrast, maximum of the negative dichroic absorption in our sample was shifted towards higher wavelengths with the decreasing esterification degree. A possible rationalization for the unusual shape of the CD spectrum could be the fact that the reduced intensity in the short-wavelength region of the helical conformation spectrum could be due to an opalescence of samples. The extent of the complex-forming interaction was investigated at λ = 225 nm, *i.e.* at reference value of ellipticity ($\Delta\varepsilon_{225}$), where the effect of opalescence is no more so significant. The ellipticity values, normalized to the unified concentration of —COO^- groups of the pectinate (0.3 mmol dm^{-3}) were employed for correlation of the efficiency of the helix-forming interaction (*A*) and the linear charge density of the pectinate (Fig. 4). The linear charge density of the pectin macromolecule is directly proportional to the ratio of free ionized carboxyl groups to their total content, *i.e.* expression (1 – *E*)/100. The value *A* expresses the amount of poly(Lys-Ala-Ala) passing into the helical form in the complex at a normalized addition of ionized carboxyl groups.

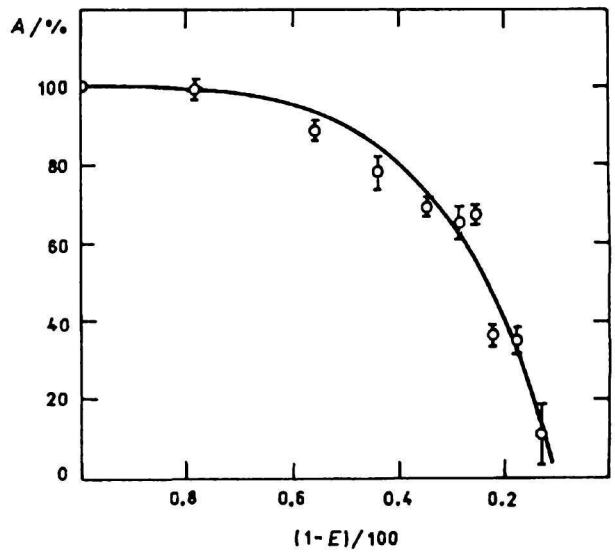

Fig. 4. The effect of the linear charge density (1 – *E*)/100 of pectin on the formation of the complex with poly(Lys-Ala-Ala).

This dependence documents the ability of poly(Lys-Ala-Ala) to enter the interaction with pectinates under formation of a helical structure. The helix-forming effect of the pectinate remains significant even at a considerable drop of the linear charge density of pectinate at E = 0.563. It is noteworthy that the poly(L-lysine) has not interacted with pectin of E = 0.64 at all. This difference in the action of both polypeptides reflects their different charge densities and proves the importance of complementarity of linear charge densities of both interacting polyions in the complex structure.

The above-mentioned pectinates induce the same α-helical structure with the sequential poly(Lys-Ala-Ala) and the homopolymeric poly(L-lysine). It remains open, whether the proposed models of superstructures are actual even for the

investigated complexes with poly(Lys-Ala-Ala). An important presumption tells that the mutual interaction need not take place at distinct points. Charges of $-NH_3^+$ groups of the polypeptide form a diffuse superficial electric field. The polyanionic pectate molecule surrounds this structure as an oppositely charged conductor. The linear charge density of the helical poly(Lys-Ala-Ala) structure is very close to that of the unesterified pectate. The distance between the neighbouring $-NH_3^+$ groups in the poly(Lys-Ala-Ala) molecule in the perpendicular projection on the helix axis is 0.443 nm, that between the neighbouring $-COO^-$ groups of the pectate is 0.437 nm. In other words, a condensation of the pectate charges along the polypeptide helix is not necessary for mutual saturation of charges by winding its chain round the polypeptide core to form a superhelix as with the pectate–polylysine complex. The macromolecule of an unesterified pectate is here stretched parallelly with the helix axis of poly(Lys-Ala-Ala). This arrangement faces, at the same time, the problem of a full saturation of the charge field of the polypeptide on its cylindrical surface. It is obvious that complete saturation of charges by the stretched pectate polyanion is impossible. Residual unsaturated charges of the polypeptide at the remote side of the cylinder surface of the helix can bring about a decrease of the thermodynamic stability of the complex. On the other hand, they enable electrostatic interaction with further polyanionic molecules. It is obvious that neither the pectin charges are here fully saturated. At an excess of the polypeptide an interaction of pectin with further molecules of this polycation can occur. Such a formation of aggregates including several molecules might be the reason for the opalescence observed.

Increase of the esterification degree of pectin evokes a situation requiring a condensation of charges of the pectin chain to compensate the charge densities. The formation of superhelical structure of the pectinate means some change in torsion angles of the glycosidic bond. The required change for lower esterification degrees of pectin involving a very gentle winding is minute; this deformation has to be considered at higher esterification degrees, where also a greater compression of turns of superhelical structure is necessary. The distance of the superhelix turns d corresponding to the equivalence of charges of both complex components was calculated for single samples of pectin of various esterification degree. The calculated distances of turns d of the superhelix are given on the abscissa in Fig. 5. The plot characterizes the dependence of the complexation effectiveness (A) on the distance

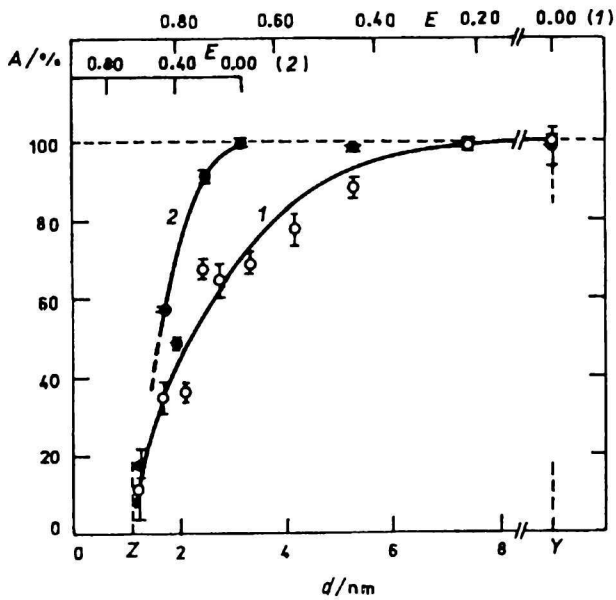

Fig. 5. The correlation between the complex-forming efficacy of pectin of various esterification degree and the distance of turns of its macromolecule *1*. Complex with poly(Lys-Ala-Ala); *2*. complex with poly(L-lysine). Point *Y* on the abscissa refers to the structure of the complex without turns, point *Z* to the limit contact distance of turns.

of turns (d) of pectinate necessary for a full saturation of charges of the superhelix model. Curve *1* for the complexes with poly(Lys-Ala-Ala) shows that at an esterification degree of pectin $E > 0.56$ the distance between turns is great and therefore not enabling a perfect spatial saturation of the polypeptide charges. An increase in the complexation effectiveness at an excess of poly(Lys-Ala-Ala) is here substantiated by formation of aggregates of several molecules. The compression of turns of the superhelix at $E > 0.56$ means an improvement of the spatial saturation of charges, but on the other hand, it brings about an already not negligible torsion of glycosidic bonds. At a greater approach of turns the electrostatic repulsive forces between single turns associated with carboxylato groups of pectin will come into effect. Fig. 5 (curve *2*) shows for comparison purpose this dependence also for the complex with poly(L-lysine).

Both curves successively approximate with the increasing esterification degree of pectin and limit to the value Z (d = 1.1 nm) characterizing the limit of the geometrical model of the superhelix and the direct contact of turns of the pectin macromolecule. Basing on the same point of zero complex-forming effect (Z) one can consider a similar spatial model of both polypeptide types. The necessity to saturate mutually and fully the charges of both polyions found with poly(L-lysine)

also holds for poly(Lys-Ala-Ala). The energetic stability of the complex at the same torsion of the glycosidic bond is influenced by an unequal electrostatic effect, which consists of attraction of oppositely charged polyions, mutual repulsion of the pectinate turns, as well as of repulsion of terminal $-NH_3^+$ groups. Results shown in Fig. 5 document this effect to be more favourable for formation of the poly(L-lysine) complex under assistance of pectin molecule with a three times higher linear charge density.

The examination of model revealed that the dominant factor for the complex-forming interaction of acidic polysaccharides with peptides is the complementarity of charge densities on the surface of presumed conformations of both components. This is exemplified by pectin of $E = 0.65$, which induces in a predominant measure the helical structure of poly(Lys-Ala-Ala), but this ability is no more manifested with poly(L-lysine) of a high density. In favour of this statement is the fact that the α-helix of poly(L-lysine) was not formed on a contact with hyaluronic acid, desulfated chondroitin and keratin sulfates, having the linear charge density analogous with pectin of $E \approx 0.50$. The above-mentioned glycosaminoglycans do already exhibit the helix-forming interaction with polyarginine, the helical structure of which has a greater radius and consequently, a lower superficial charge density. Polyornithine, having a higher superficial charge density, requires a polysaccharide with a high charge density for the helix-forming interaction. Really, of all hitherto investigated glycosaminoglycans [18] this ability is shown only by heparin which is characterized by the highest content of sulfate and carboxyl groups in the macromolecule.

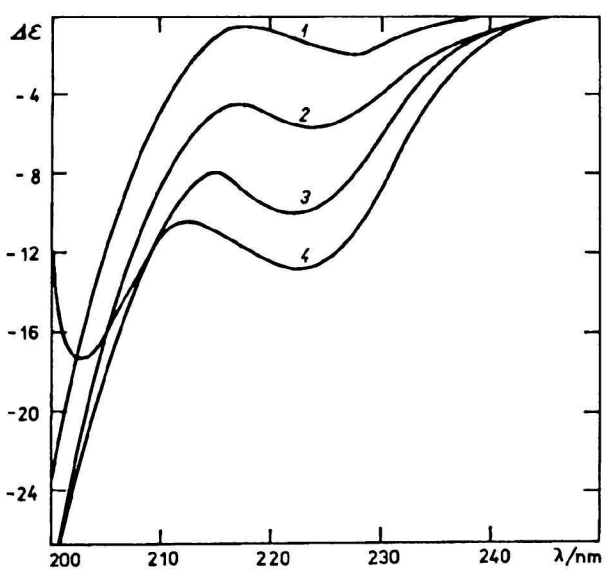

Fig. 6. The CD spectrum of poly(Lys-Ala-Ala) ($c(NH_3^+)$ = 0.3 mmol dm^{-3}) in the presence of oligogalacturonates ($c(COO^-)$ = 0.3 mmol dm^{-3}) *1*. Without oligogalacturonates; *2—4*. addition of oligogalacturonates of polymerization degree 2, 3, and 4.

OLIGOGALACTURONATES AND POLY(Lys-Ala-Ala)

The effect of the chain length of saccharide component on the formation of complex was examined with oligogalacturonates of shorter chain ($n \leq 9$) and poly(Lys-Ala-Ala) [19].

The chiroptical response of sodium oligo- and polygalacturonates has been examined [9]. The positive Cotton effect at $\lambda \approx 204$ nm undergoes a characteristic change depending on the polymerization degree n.

The CD spectra of mixtures of potassium oligogalacturonates with different excess, or with an equivalent amount of poly(Lys-Ala-Ala) show negative peaks above 200 nm indicative of formation of an α-helical structure of the polypeptide.

Figs. 6 and 7 display the corrected CD spectra of poly(Lys-Ala-Ala) in the presence of oligogalacturonates of various polymerization degree at equivalent charge ratios of both components. Provided that the CD of oligogalacturonates does not change during the interaction, the curves represent the CD spectrum of the polypeptide potentially able to participate in the complex-forming interaction. The shown dichroic curves differ in their shape in the short-wavelength negative dichroic band region ($\lambda \approx 205$ nm). The monomer did not raise formation of complexes. The dimer and trimer (Fig. 6, spectra *2* and *3*) have this band overlapped by a considerably rising dichroic absorption, reflecting the presence of a great portion of the polypeptide in a charged coil arrangement, *i.e.* not participating in the complex-forming interaction. The above-mentioned

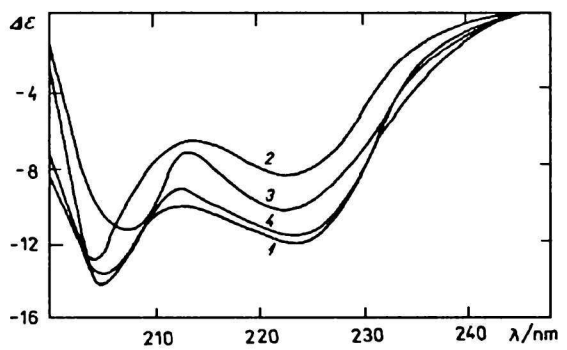

Fig. 7. The CD spectrum of poly(Lys-Ala-Ala) ($c(NH_3^+)$ = 0.3 mmol dm^{-3}) in the presence of oligogalacturonates ($c(COO^-)$ = 0.3 mmol dm^{-3}) of polymerization degree 6, 7, 8, and 9 (curves *1*, *2*, *3*, and *4*).

second dichroic band is clearly observable with higher oligomers (n = 4—9; Fig. 6, spectrum *4* and Fig. 7, spectra *1—4*).

The first long-wavelength dichroic band, which is not affected by the CD of oligogalacturonates, makes it possible to quantify the extent of the helix-forming interaction. The values at λ = 225 nm, from which the CD of the total original content of poly(Lys-Ala-Ala) was subtracted, were considered as the reference values. The spectra were further normalized and were employed for expression of the complex-forming efficacy of oligogalacturonates in relation to the maximum (100 %) effectiveness of polymeric D-galacturonan as determined for interaction with a fully deesterified pectin.

Fig. 8 shows the correlation between the complexation efficacy of oligogalacturonates (A/%) and their polymerization degree (n). The respective curves correspond to various ratios of interacting components. The extent of complexation considerably alters with the change of polymerization degree. It is evident that this change is not monotonous. The maximal values of complexation were found at n = 4 and 6. The amount of the oligosaccharide added is of significant effect on the complex-forming interaction. The highest efficacy of oligomers (curve *1*) was reached at the highest excess of the polypeptide (*i.e.* at a 40 % addition of the oligosaccharide).

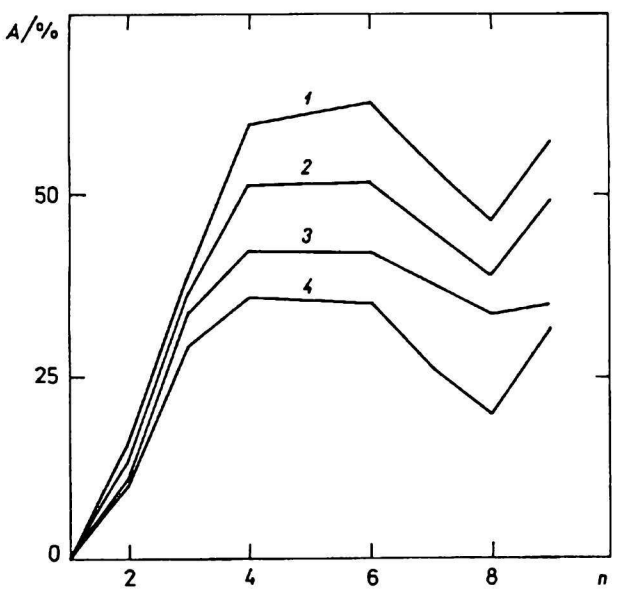

Fig. 8. The effect of polymerization degree n of oligogalacturonates on the formation of the complex with poly(Lys-Ala-Ala) ($c(NH_3^+)$ = 0.3 mmol dm^{-3}) at various ratios of components. A = 100 % is efficacy of polymeric D-galacturonan. *1—4*. The poly(Lys-Ala-Ala) containing 40 %, 60 %, 80 %, and 100 % of oligogalacturonate, respectively.

The efficacy decreases with the increase of the oligosaccharide content in the mixture. This finding evidences that the complexation does not proceed here as a complementary interaction of two equivalent ionic structures, as was the case with pectin preparations.

The interaction takes gradually place by a local mode: the short chains of oligoanions become located between pairs of NH_3^+ ions belonging to various turns of helical structure. The shortest distance between —NH_3^+ groups at two adjacent turns estimated from geometric parameters deduced previously is approximately 1.7 nm. The second shortest distance between two —NH_3^+ groups, which are vertically closest to each other in the direction of the helix axis separated by three turns, is approximately 2.4 nm. These distances are maximally approached by the lengths of oligoanions of four or six D-galacturonate units.

The effectiveness of the complex-forming interaction of these oligogalacturonates is low with respect to polymeric D-galacturonan (deesterified pectin), because only a part of the oligomer added enters the complex. A decisive factor lowering their efficacy is the mutual repulsion of oligoanionic molecules.

PECTIN AND POLY(D-LYSINE)

The parallel study of complexation of alginate rich in L-guluronate has pointed at another factor important in interaction of polysaccharides having a (1 → 4)-α diaxial glycosidic bond [20]. While the efficiency of interaction of pectate with poly(L-lysine) is almost 100 %, the foregoing alginate practically does not form any complex. On the other hand, pectate and alginate rich in L-guluronate interact with bivalent cations similarly. The information obtained about different interactions with polypeptides indicated 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 potassium pectate (E = 0.0) in interaction with other optical isomers, *e.g.* poly(D-lysine).

The complex-forming interaction was studied in diluted solutions so that the polypeptide was added stepwise to the polysaccharide solution of constant concentration [21]. Corrected CD spectra are presented in Fig. 9.

As seen from the spectra, the shift to positive region is surprisingly small. In contrast to previous studies of poly(L-lysine) and pectate interaction where 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 prevalent part of 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. 9, curve *6*). Quantitative estimation of complexation is rather problematical as the contribution of CD change of polysaccharide entering the interaction is unknown. According to the CD changes observed [22] we assume that it is maximum 10 % of the value of CD change of polylysine in its transition to helical conformation.

As it was stated earlier the efficiency of complexation of poly(L-lysine) with pectate is almost 100 %, while with poly(D-lysine) it is only about 10 %. This evident difference in the interaction of pectate with enantiomers of polylysine points at new, until now unobserved factor, *i.e.* the chiral discrimination of adaptable conformation of polysaccharide in solution. Pectate, basically (1 → 4)-α D-galacturonan randomly interrupted by rhamnose units, behaves in the polyelectrolytic interactions studied markedly differently from (1 → 4)-α 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 [23], conformational polymorphism allows for D-galacturonan also a three-fold helical symmetry. However, its chiral handedness has not been convincingly confirmed even in solid state. In an older X-ray study of sodium pectate left-handed three-fold symmetry was suggested [14]. Later results of model building study endorse the opinion of a right-handed symmetry [7]. The experimental method of the interaction of enantiomers presented herein may distinguish the handedness of the polysaccharide. The conformational freedom around the glycosidic bond is not symmetric, usually it is constrained to be 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 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 efficiences of acidic polysaccharides with polylysine enantiomers the sense of conformational freedom of polysaccharide may be deduced. Our experiments have clearly shown that D-galacturonan (pectate) in solution tends to adopt a right-handed helical structure.

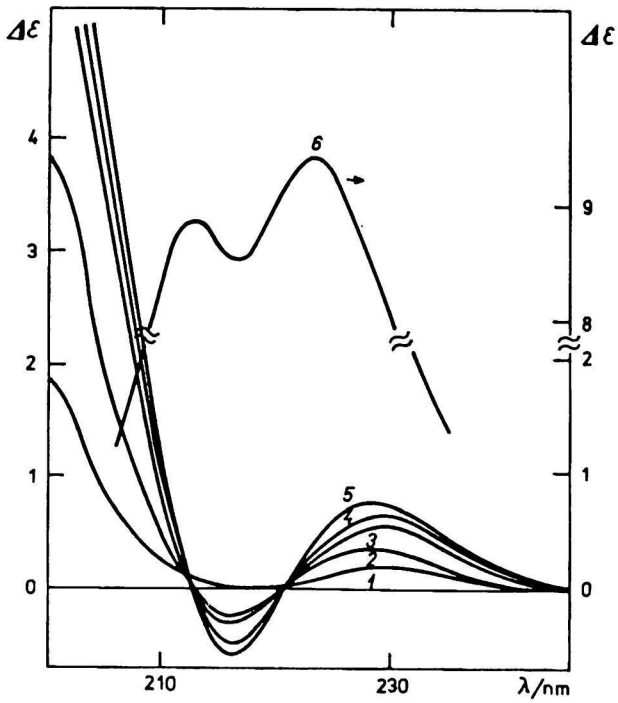

Fig. 9. The CD spectrum of poly(D-lysine) in the presence of potassium pectate ($c(COO^-)$ = 0.3 mmol dm^{-3}). *1—5*. Addition of 20 %, 40 %, 60 %, 80 %, and 100 % of poly(D-lysine), respectively; *6*. without pectate at pH = 11.3 ($c(NH_3^+)$ = 0.3 mmol dm^{-3}).

REFERENCES

1. Kohnová, Z. and Kohn, R., *Chem. Listy 75*, 1051 (1981).
2. Kohn, R., *Pure Appl. Chem. 42*, 371 (1975).
3. Malovíková, A. and Kohn, R., *Collect. Czechoslov. Chem. Commun. 44*, 2915 (1979).
4. Malovíková, A. and Kohn, R., *Collect. Czechoslov. Chem. Commun. 47*, 702 (1982).
5. Malovíková, A. and Kohn, R., *Collect. Czechoslov. Chem. Commun. 48*, 3154 (1983).
6. Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J. C., and Thom, D. J., *FEBS Lett. 32*, 195 (1973).
7. Rees, D. A. and Wight, A. W., *J. Chem. Soc., B 1971*, 1366.
8. Yathindra, N. and Rao, V. S. R., *J. Polym. Sci., A-2 10*, 1369 (1972).
9. Bystrický, S., Kohn, R., and Sticzay, T., *Collect. Czechoslov. Chem. Commun. 44*, 167 (1979).
10. Plaschina, I. G., Braudo, E. E., and Tolstoguzov, V. B., *Carbohydr. Res. 60*, 1 (1978).

11. Morris, E. R., Rees, D. A., Sanderson, G. R., and Thom, D. J., *J. Chem. Soc., Perkin Trans. 2 1975*, 1418.
12. Bystrický, S., Kohn, R., Sticzay, T., and Bláha, K., *Collect. Czechoslov. Chem. Commun. 50*, 1097 (1985).
13. Kalous, V. and Pavlíček, Ž., *Biofyzikální chemie.* (Biophysical Chemistry.) P. 34. Publishers of Technical Literature, Prague, 1980.
14. Palmer, K. J. and Hartzog, M. B., *J. Am. Chem. Soc. 67*, 2122 (1945).
15. Bystrický, S., Kohn, R., Sticzay, T., and Bláha, K., *Collect. Czechoslov. Chem. Commun. 51*, 1772 (1986).
16. Manavalan, P. and Johnson, W. C., *Nature 305*, 831 (1983).
17. Yaron, A., Tal, N., and Berger, A., *Biopolymers 11*, 2461 (1972).
18. Hopfinger, A. J., *Intermolecular Interactions and Biomolecular Organization*, p. 240. J. Wiley, New York, 1977.
19. Bystrický, S., Sticzay, T., Kohn, R., and Bláha, K., *Collect. Czechoslov. Chem. Commun. 51*, 2919 (1986).
20. Bystrický, S., Malovíková, A., and Sticzay, T., *Carbohydr. Polym. 13*, 283 (1990).
21. Bystrický, S., Malovíková, A., and Sticzay, T., *Carbohydr. Polym. 15*, 299 (1991).
22. Thom, D. J., Grant, G. T., Morris, E. R., and Rees, D. A., *Carbohydr. Res. 100*, 29 (1982).
23. Mackie, W., *Biochem. J. 125*, 89 (1971).

Translated by A. Malovíková