FORMATION OF THE POLY(L-LYSINE) COMPLEX WITH PECTIN OF VARIOUS ESTERIFICATION DEGREE

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The circular dichroic (CD) measurement was chosen the tool to investigate the complex formation of poly(L-lysine) with potassium pectate or pectinate of various esterification degree (E). The polycation–polyanion (pectate, E 0%) 1 : 1 complex was quantitatively formed by an electrostatic interaction independently upon the excess of either component. The partial esterification of pectin resulted in a decrease of complex formation; this drop is at a 20% esterification degree low, at higher esterification degrees (46% and 64%) considerable, and at an esterification degree 86% the complex has not been formed at all. The polypeptide chain was present in the complex in an α-helical arrangement characterized by the CD spectrum; the potential spatial arrangements of the complex were proposed. The α-helical polypeptide component constitutes a core surrounded by a superstructure of D-galacturonan chains.

The electrostatic interaction of acid polysaccharides with proteins is responsible for the formation of specific complexes involved in vital processes. Complexes prepared from synthetic precursors can be employed as biomembranes. Formation of polyelectrolyte complexes has been widely investigated on model systems. Poly(L-lysine) possessing a simple structure and a flexible chain forming secondary structures well characterized by CD spectra[1] was the basic component in these systems. Various types of naturally occurring or synthetic polyacids (RNA, DNA ref.[2]), mucopolysaccharides[3–5], cellulose sulfates[6], polyacrylic acids[7,8] *etc.*) were the acid components. Formation of the complex is generally associated with change of the secondary structure in the sense of a transition from a less ordered to regular structures. The degree of complexation depends on the chain length, type of the anionic group, linear charge density of the molecule, ionic strength of the solution, and on the stereochemical character of components. The latter becomes evident with acid polysaccharides, where the rigid conformation of the macromolecule chain restricts to some extent the orientation of the ionizable groups[4,5].

Investigation of complexes of poly(L-lysine) with polyanions, which meet requirements for stoichiometry and are flexible enough[3–5], resulted in a concept that the structure of this complex is based on an α-helix of poly(L-lysine) forming a core

for the superhelix of the polyanion. The recently reported[9] CD spectra of mixtures of polymeric sodium D-galacturonan with poly(L-lysine) added at various ratios clearly show the formation of an α-helixal structure of poly(L-lysine). These authors[9] further suppose that the spectra also reflect the conformation adapted by the polysaccharide. This conformation should be helical in the sense of a superhelix around the peptide chain.

The aim of this CD investigation is to contribute to the knowledge on formation and stereochemistry of the complex of poly(L-lysine) with pectin, representing a natural polyelectrolyte. For this purpose a series of defined pectin samples was selected; these had various esterification degrees of carboxyl groups with methanol, *i.e.* various linear charge density.

EXPERIMENTAL

Chemicals

The nona-D-galacturonic acid was a chemically pure preparation employed in the previous paper[10]. Poly(L-lysine) hydrogen chloride of molecular mass $\bar{M}_r$ 65 000 was obtained by polymerization of N^{ε}-benzyloxycarbonyl-L-lysine-N-carboxylic anhydride in dioxane, followed by a removal of protecting groups on the polymer by 20% HBr in the acetic acid–dichloroacetic acid 1 : 1 mixture. The final product was dialyzed and freeze-dried. The 0·05 mol l^{-1} KOH was carbonate-free. For experiments a freshly boiled and cooled redistilled carbonate-free water was used.

Pectin Preparations of Various Esterification Degree

Pectin preparations of various esterification degree E were obtained from a commercially available citrus pectin (Genu Pectin, Medium Rapid Set, Type A, Københavns Pektinfabrik, Denmark). Pectin was washed with hydrochloric acid acidified ethanol (5 ml of concentrated HCl in 100 ml of 60% ethanol), then with a neutral 60 and 96% ethanol, respectively, and ether; finally, it was dried at a temperature not exceeding 60°C (Table I, sample 4). This pectin was esterified to a preparation of E 95% with methanolic 1 mol l^{-1} H_2SO_4 at 3°C for 3 weeks[11]. Preparations of pectin of various esterification degrees (E 86% to 0%) were obtained from the highly esterified pectin and the purified starting sample by a partial alkaline deesterification in suspension in 60% ethanol with dilute potassium hydroxide in 60% ethanol). The products were successively washed with 60% and 96% ethanol and ether, and dried as already mentioned.

Preparation of Mixtures of Pectin with Poly(L-lysine)

The solution of pectin of a total carboxyl groups concentration roughly 3 mmol (—COOK + + —$COOCH_3$) l^{-1} was centrifuged at 20 000*g* for 10 min and percolated through a column packed with Dowex 50W × 2 (H^+) cation exchanger. Neutralization of the eluate with 0·05 mol . . l^{-1} KOH to pH ~7·2 afforded the potassium pectate or pectinate of a known concentration; dilution of this solution yielded the starting solution (A) of a 0·600 mmol (—$COO^{(-)}$ + + —$COOCH_3$) l^{-1} concentration.

Poly(L-lysine) hydrogen chloride was also dissolved to a 0·600 mmol (—$NH_3^{(+)}$) l^{-1} concentration (B) by stirring the suspension at an ambient temperature for 4—6 h; this solution

was left to stand overnight and centrifuged if containing particles of gel. In this case the poly(L-lysine) was determined in the supernatant, and the required concentration of the solution was adjusted by dilution.

Interaction of poly(L-lysine) with pectin of various esterification degree (E) was examined in two measurement series: *I*) Solution of poly(L-lysine) (B) in amounts increasing from 0% to 100% per the content of all carboxyl groups ($-COO^{(-)} + -COOCH_3$) of pectin was added to the solution of pectin (A). The final concentration of pectin was adjusted by dilution for the whole series to 0·300 mmol ($-COO^{(-)} + -COOCH_3$) l^{-1}. *II*) The solution of pectin (A) in amounts representing 0% to 100% per the content of ($-NH_3^{(+)}$) groups of poly(L-lysine) was added to the solution of poly(L-lysine) (B). Concentration of the pectin solution relates also to the content of all carboxyl groups ($-COO^{(-)} + -COOCH_3$). The final concentration of poly(L-lysine) in solution was 0·300 mmol ($-NH_3^{(+)}$) l^{-1} for the whole series.

The 0·3 mmol ($NH_3^{(+)}$) l^{-1} solution of poly(L-lysine) hydrogen chloride had pH = 6·3 ± 0·2 Pectin solutions of the total carboxyl groups concentration 0·3 mmol l^{-1} had roughly the pH = = 6·8 ± 0·3. The pH of mixtures of these solutions varied in the 6·2 to 7·1 range depending on the ratio of both components and on the esterification degree E of pectin. The pH values at an excess of pectin were in the higher, at an excess of poly(L-lysine) in the lower scope of the given interval. The pH of mixtures was determined after taking the CD spectra.

Analytical Methods

Content of free and total carboxyl groups in pectin, content of partially esterified D-galacturonan in dry substance and the esterification degree of carboxyl groups with methanol (E) were determined by the precipitation method of copper pectates and pectinates[12,13] with a ±0·5% error. The content of copper in the precipitate was determined chelatometrically by titration with a 0·01 mol l^{-1} Complexon IV solution in a weakly basic (ammonia) medium using murexide as an indicator and a spectrophotometric indication of the point of equivalence (interference filter Zeiss, Jena, IF 600 nm).

The limit viscosity number $[\eta]$ was estimated in solutions of potassium pectate and pectinates (pH ~7) by means of an Ubbelohde viscometer at 25·0 ± 0·1°C in 0·15 mol l^{-1} NaCl—0·005 mol . l^{-1} sodium oxalate.

TABLE I
Pectin samples

Sample	Esterification degree, E %	Content[a] %	$[\eta]$ ml g^{-1}	$\bar{M}_r$
1	0·0	88·9	117	26 000
2	20·6	85·5	211	41 000
3	46·0	83·3	327	57 000
4	63·7	85·9	503	78 000
5	86·1	90·9	232	44 000

[a] Partially esterified D-galacturonan (H^+ form).

Content of poly(L-lysine) hydrogen chloride in the freeze-dried preparation and in solution was determined from the concentration of Cl^- ions as found by argentometry (potentiometric titration with a 0·002 mol l^{-1} $AgNO_3$ and silver electrode; error ± 0·5%, the salt bridge was filled with 10% KNO_3 solution). Used were: a digital pH-meter Radiometer PHM 64 (Denmark), a glass electrode G 202 B, and a saturated calomel electrode K 401 (Radiometer).

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

RESULTS AND DISCUSSION

Characterization of Pectin and Poly(L-lysine) Samples

Characteristic data of samples of pectin of various esterification degree E are presented in Table I. The main chain of pectin molecule is almost exclusively formed by homopolymeric D-galacturonan with a diaxial *trans*-glycosidic $\alpha(1\rightarrow4)$ bond randomly disconnected by L-rhamnose units in a minor representation. The content of L-rhamnose in the sample of potassium pectate was <1% and therefore, it was not considered when interpreting the CD spectra. The pectin samples contained 83% to 91% of the partially esterified D-galacturonan and 16% to 8% of neutral saccharides (D-galactose, L-arabinose, D-xylose) in form of short side chains. The limit viscosity number $[\eta]$ was reduced to the relative molecular mass ($\bar{M}_r$) according to the Owens and collaborators'[14] equation, which is conventionally most utilized.

The pectin samples of various esterification degree E were prepared by a partial alkaline deesterification of a highly esterified pectin. This reaction results in a random, with proceeding deesterification more or less regular, distribution of free and esterified carboxyl groups in the pectin molecule due to mutual repulsion of $OH^{(-)}$ and carboxylate ($—COO^{(-)}$) ions. Conformational freedom of the pectin molecule chain is considerably restricted; this is backed by calculation of conformational energy maps of D-galacturonan macromolecule[15,16], by viscosity measurements[17] and by regularity of conformation of the D-galacturonan[18,19] and pectin[20] macromolecules in solution. The CD spectra of pectic and pectinic acids of various esterification degree E and their sodium and potassium salts are characteristic of a simple dichroic band[21,20] of positive sign at 202–210 nm (Fig. 1 to 4, curves *1*) similarly as found with their monomer[22,18].

The relative molecular mass of poly(L-lysine) hydrogen chloride was found to be 65 000; its CD spectrum 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. The α-helical arrangement is characterized by a CD spectrum with two negative maxima at 222 nm ($n\rightarrow\pi^*$) and 205 nm ($\pi\rightarrow\pi^*$ exciton), and by a positive one at 190 nm ($\pi\rightarrow\pi^*$ exciton)[23].

Formation of the Poly(L-lysine)–Potassium Pectate (E 0%) Complex

In series *I* increasing amounts of poly(L-lysine) were added to an excess of pectate in solution; in series *II* increasing amounts of pectate were added to an excess of poly(L-lysine). The experimental CD spectra comprise, however, the contribution of components not entering the interaction (free polyions) and the CD spectrum of the complex. So far, it is not known whether and by what manner the CD of pectate is being changed by its introduction into the complex with poly(L-lysine). Therefore we subtracted from the experimental spectrum of series *I* both the spectrum corresponding to the excess of pectate in solution (*a*) providing the stoichiometric interaction of polyions (contribution of the CD spectrum of pectate to the complex is regarded as zero) and the spectrum corresponding to total concentration of pectate

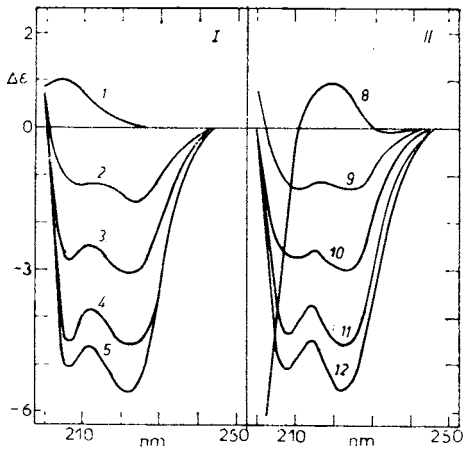


FIG. 1

The CD spectrum of poly(L-lysine) in complex with potassium pectate (*E* 0%). I, *1* the 0·3 mmol ($-COO^{(-)}$) l^{-1} pectate solution; *2*, *3*, *4*, *5*, the pectate solution with addition of 20%, 40%, 60% and 70% of ion-equivalent amount of poly(L-lysine). II, *8* the 0·3 mmol ($-NH_3^{(+)}$) l^{-1} poly(L-lysine) solution; *9*, *10*, *11*, *12* the poly(L-lysine) solution containing 20%, 40%, 60% and 70% of ion-equivalent amount of pectate

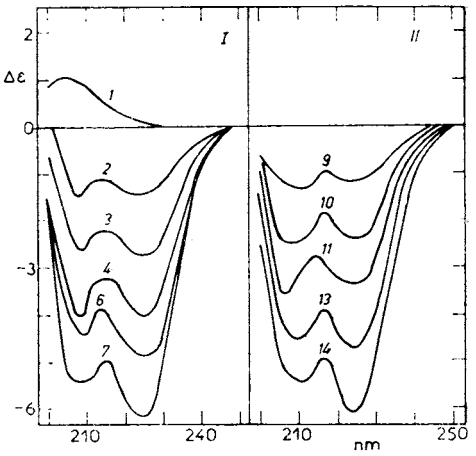


FIG. 2

The CD spectrum of poly(L-lysine) potentially able fo form a complex with potassium pectinate of esterification degree *E* 20·6%. I, *1* the 0·3 mmol ($-COO^{(-)}$ + $-COOCH_3$). l^{-1} pectinate solution; *2*, *3*, *4*, *6*, *7* the pectinate solution containing 20%, 40%, 60%, 80%, and 100% of poly(L-lysine) equivalent to total carboxyl groups content. II, *9*, *10*, *11*, *13*, *14* the 0·3 mmol ($-NH_3^{(+)}$) l^{-1} poly(L-lysine) solution containing 20%, 40%, 60%, 80%, and 100% of the equivalent amount of pectinate, with respect to total carboxyl groups content

in solution (*b*) providing that the spectrum of pectin in the complex with poly(L-lysine) is identical with that of the pectin not entering the complex. To judge which subtraction is more appropriate the spectra were put on a uniform poly(L-lysine) concentration 0·3 mmol $(—NH_3^{(+)})\,l^{-1}$. It has been ascertained that both corrections lead to good accordance of unified spectral curves with a scattering less than 10% in most cases; correction (*b*) gave somewhat better results. It could be, therefore, assumed that the circular dichroism of pectate undergoes a little change upon interaction with the basic polypeptide. Consequently, the CD spectra of total pectin in solution were always subtracted from the experimental CD spectra of poly(L-lysine)–pectin mixtures. In series *II* the CD spectrum (random coil) corresponding to the excess of poly(L-lysine) was further subtracted regarding the concentration of carboxylate groups $(—COO^{(-)})$ in solution. The corrected spectra present the CD of this particular fraction of poly(L-lysine) having the polyanion counterpart in solution and being, therefore, potentially able to take part in the complex-forming interaction. At an excess of pectin (series *I*) the solutions had a very weak opalizing turbidity passing after a greater period of time (more than 6 h) to a coagulate which sedimented. On the other hand, the solutions remained clear at an excess of poly(L-lysine).

Measurements in both series clearly show (Fig. 1)* a regular increase of CD values corresponding to the increasing amount of the constituent added. Shape of the corrected spectrum is characteristic of the α-helical structure of poly(L-lysine). The very intense CD band in the positive region at 190 nm, also corresponding to the complex formed, has not been considered when interpreting the spectra for a minor exactness of measurement in this wavelength region. 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 identity of the corresponding spectra of both series further evidences that formation of a very weak opalizing turbidity, taking place at an excess of pectate in solution (series *I*), was not manifested in the CD spectrum. Comparison of the corrected spectra (Fig. 1) after their unification shows the scattering of CD values at 225 nm not to exceed 5% (excepting curve *9* corresponding to the least addition of pectate to poly(L-lysine). The interaction of poly(L-lysine) with pectate is a quantitative one, the 1 : 1 complex was formed regardless the excess of either component. The presented spectra of series *I* refer to a constant content of pectate in solution, spectra of series *II* to a constant content of poly(L-lysine). The accordance of thus unified CD spectra of both experimental series provides a proof for stoichiometrical equivalence of carboxylate groups $(—COO^{(-)})$ of pectate and $(—NH_3^{(+)})$ groups of poly(L-lysine) in the complex.

* Since the curves in all figures are uniformly numbered, the same number always corresponds to equal composition of the pectin–poly(L-lysine) mixture.

The complex-forming effect was also investigated with potassium mono-D-galacturonate and oligomeric potassium nona-D-galacturonate. Poly(L-lysine) was added in successive amounts to the above-mentioned substances in solution. No changes in CD were observed in either systems, which would indicate formation of a regular structure of the complex. These spectra corresponded to a simple addition of those of separate components. The chain of the oligogalacturonate of polymerization degree *DP* 9 is still not long enough to influence the conformation of poly(L-lysine) macromolecule. Influence of the polymerization degree of the polyanion upon formation of a regular structure of poly(L-lysine) was studied in detail at its interaction with oligomers of L-glutamic acid[9]. As shown, the β-structure of poly(L-lysine) has been formed when the polymerization degree of poly(L-glutamic) acid $DP \geqq 23$. The complex-forming efficiency of carboxyl anion is generally subject to its binding to a polymeric carrier and proceeds, therefore, as a co-operative process of two polymeric structures.

Formation of the Poly(L-lysine) – Potassium Pectinate (E 21%–86%) *Complexes*

Fig. 2 shows the CD spectra of poly(L-lysine) in the presence of potassium pectinate of esterification degree *E* 20·6%, corrected by subtraction as in the preceding case (according to method (*b*) subtracting the contribution to CD corresponding to total pectin concentration in solution). The concentration of pectinate refers to the content of all carboxyl groups ($-COO^{(-)} + -COOCH_3$) in solution. The CD values are lower than those of the unesterified pectate (*E* 0%) (Fig. 1) in the 225 nm region diagnostic of the helical structure of poly(L-lysine). Drop of the complex-forming ability of the partially esterified pectinate becomes evident mainly in the series *I*, where lower values of ellipticity were observed for all CD curves even though a sufficient excess of anionic groups ($-COO^{(-)}$) was available. Shapes of the CD curves and ellipticity values in both series were no more completely equal. The ellipticity values for series *II* are somewhat lower in the characteristic region at about 225 nm than found in the experimental arrangement of series *I*. Diminution of the helical form of the complex is due to a partial esterification of carboxyl groups of the pectinate added. Really effective are only the ionized carboxyl groups ($-COO^{(-)}$) in the complex-forming interaction.

These findings are even more backed by the CD spectra obtained with potassium pectinate of esterification degree *E* 46·0% (Fig. 3). The ellipticity values at 225 nm are noticeably lower than those of the corresponding curves obtained with pectin preparations of higher linear charge density of the macromolecule (*E* 20·6 and 0%). Shape of the CD curves of series *I* (Fig. 3) differs from that of curves recorded for series *II*; the negative ellipticity values for series *I* are in the 225 nm region considerably higher than those for series *II*. Comparison of these spectra with those

of the unesterified pectate *E* 0% (Fig. 1) shows a change in the intensity rates of maxima of both chiroptic bands.

The highly esterified pectin (*E* 63·7%) was examined in series *I* only (Fig. 4). The CD spectra are, in contrast to preceding findings, substantially changed. The little significant negative maximum is shifted towards a higher wavelength (230 nm); the following intense negative maximum appears at a lower wavelength (200 nm). Shape of the CD curve reminds that of poly(L-lysine) in a random coil. The wavelength region below 215 nm evidently reflects the high prevalence of the non-complexing poly(L-lysine). Pectin with the highest esterification degree *E* 86·1% 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.

A general view to the ability of pectin to evoke formation of a regular helical conformation of poly(L-lysine) in the complex is best exemplified by comparing

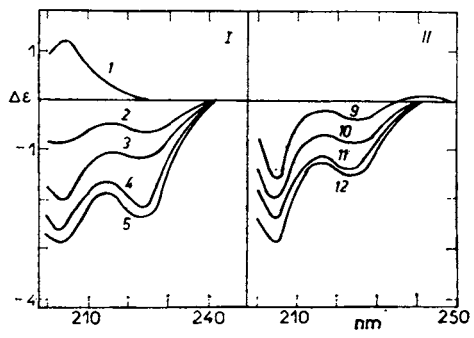


FIG. 3

The CD spectrum of poly(L-lysine) potentially able to form a complex with potassium pectinate of esterification degree *E* 46·0%. I, *1* the 0·3 mmol ($-COO^{(-)} + -COOCH_3$) . $.1^{-1}$ pectinate solution; *2*, *3*, *4*, *5* the pectinate solution containing 20%, 40%, 60% and 70% of poly(L-lysine) equivalent to total carboxyl groups content. II, *9*, *10*, *11*, *12* the 0·3 mmol ($-NH_3^{(+)}$) poly(L-lysine) solution containing 20%, 40%, 60%, and 70% of the equivalent amount of pectinate, with respect to total carboxyl groups content

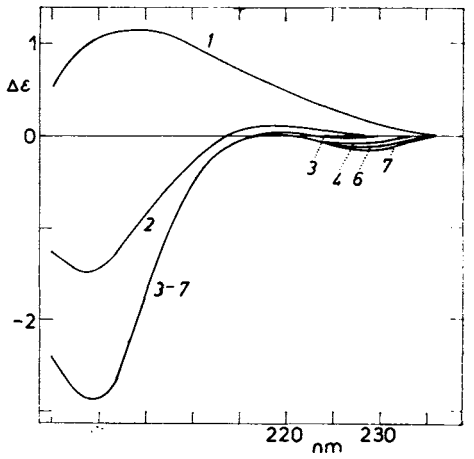


FIG. 4

The CD spectrum of poly(L-lysine) potentially able to form a complex with potassium pectinate of esterification degree *E* 63·7%. *1* the 0·3 mmol ($-COO^{(-)} + -COOCH_3$) . $.1^{-1}$ pectinate solution; *2*, *3*, *4*, *6*, *7*, the pectinate solution containing 20%, 40%, 60%, 80% and 100% of poly(L-lysine) equivalent to total carboxyl groups content

the ellipticity values at 225 nm (only the relatively low ellipticity values of both components in solution, which have not entered an interaction, are seen in this wavelength range). The results obtained with pectin of various esterification degrees E upon different experimental conditions are summarized in Fig. 5. The expression of the helical form of poly(L-lysine) (A) in % refers to a mean value of ellipticity as determined with the unesterified pectate (E 0%) in series I at various ratios of polyanions and polycations. In the last case the total content of poly(L-lysine) is considered to be in form of an α-helix (100%). Series I, where the concentration of poly(L-lysine) in solution changes, has the ellipticities unified to the 0·3 mmol ($-NH_3^{(+)}$) . . l^{-1} poly(L-lysine) concentration (opened circles); series II, where the concentration of pectin changes, has the ellipticity unified to the 0·3 mmol ($-COO^{(-)}$ + + $-COOCH_3$) l^{-1} concentration (full circles). Since the scaterring of ellipticity values determined at various ratios of reacting components is random, the ratio of both components in solution is not given for the individual points in Fig. 5.

The presented values for series I express the per cent of the poly(L-lysine) added passing to the helical form in the complex at various esterification degrees of pectin. The unesterified pectin (E 0%) has all points of both series cumulated excepting

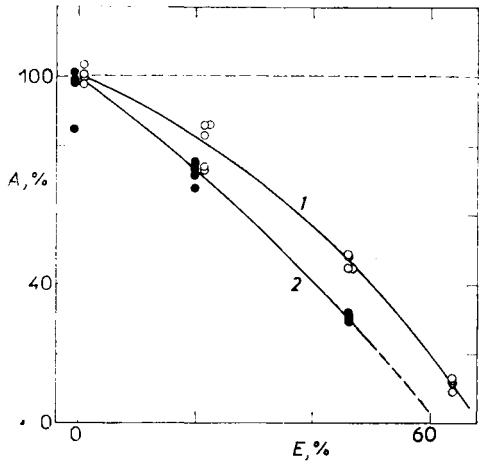


FIG. 5

The effect of esterification degree of pectin E on formation of its complex with poly(L-lysine). A the amount of complex formed (%); for details see the text; *1* the experimental series I with an excess of pectin in solution, *2* the experimental series II with an excess of poly(L-lysine) in solution

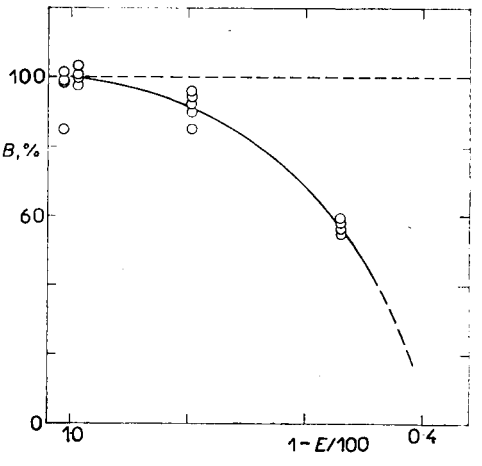


FIG. 6

The effect of the linear charge density of the pectin molecule on the formation of its complex with poly(L-lysine). B the amount of complex formed (%) relating to the unified addition of ionized carboxyl groups (series II); *1*, $1 - E/100$ *cf.* the text

the addition of 20% pectate to poly(L-lysine); this fact proves the stoichiometrically complete formation of the complex. Both experimental arrangements show that formation of the complex strongly decreases with increasing esterification degree E 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*.

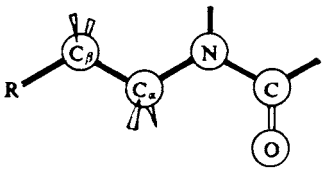
Should the efficiency of pectin in the series *II* be expressed per ionized carboxyl groups only (B %) and not per total carboxyl groups content, then one gets information on the influence of the linear charge density of the pectin macromolecule (inductor) on formation of the complex (Fig. 6). The linear 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 values B (%) are unified to a 0·3 mmol $(—COO^{(-)})\,l^{-1}$ concentration. The B values from both series obtained with the unesterified pectate (E 0%), where formation of the complex is independent on the arrangement of the experiment, are plotted in Fig. 6. Formation of the complex decreases with the decreasing linear charge density of the macromolecule. In accordance with the course of curves in Fig. 5 it is evident that pectin of esterification degree E 60% – 65% completely looses the ability of complex-forming interaction.

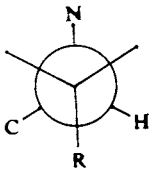
Spatial Arrangement of the Complex

Considering the afore-mentioned facts one is entitled to 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 and spatial possibilities for changing angles φ and ψ of the glycosidic diaxial $\alpha(1\rightarrow4)$ bond of pectin have to be taken into account when interpreting the results. Should the linear model of pectate molecule be maintained, a disproportion in the charge interactions takes place. The linear charge density of pectate is by approximately three times lower than that of poly(L-lysine) in the α-helical form. In other words, if all carboxylate groups are engaged, still two thirds of $(—NH_3^{(+)})$ groups do not interact, *i.e.* favour the random-coil arrangement. The complex-forming engagement of all anionic and cationic groups can be achieved *a*) by a suitable winding of the one pectate macromolecule around the helical structure of poly(L-lysine), *b*) by an interaction of some parallel pectate chains with one chain of poly(L-lysine).

To interprete the obtained results it must be presumed that the interaction does not take place in couples cation–anion, but the charge of one ε-$NH_3^{(+)}$ group is considered as a mean plane charge at the surface of a cylinder given by terminal $(—NH_3^{(+)})$ groups of side chains of the poly(L-lysine) helical structure. The pectate molecule consequently acts as opposite (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 following data: one turn of the α-helix of poly(L-lysine) 0·54 nm high comprises 3·66 L-lysine units[24]. The linear side chains are oriented in the plane of the peptidic $C_\alpha—N—C(O)$ group and have a zig-zag conformation. This spatial arrangement offers the greatest distance





between terminal ($—NH_3^{(+)}$) groups. The linear D-galacturonan molecule has a three-fold screw symmetry; one monomer unit corresponds to 0·437 nm, ref.[25]. We obtained following values: $r_L = 0{\cdot}94$ nm, $r_P = 0{\cdot}55$ nm. The resulting supermolecular system is characterized by the sum of radii of both helical components $r_L + r_P = 1{\cdot}49$ nm. Should the α-helix of poly(L-lysine) be wound up by one pectate chain, then the superhelix formed contains roughly 23 D-galacturonic acid units in one turn of 3·4 nm height. The geometry of the superhelix is only an 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 pitch of the double superhelix is 2·53 times greater. The complex containing three parallel chains of an unesterified pectate is the limit case having the chains paralelly oriented along the α-helix axis of poly(L-lysine).

Partially esterified pectins $E > 0\%$ do not completely form a complex. Part of the poly(L-lysine) added in series *I*, as well as part of pectate added in series *II* do not enter the helical structure of the 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 parallely along the helix axis is needed for a complete neutralization of charges.

A small compression of the superhelix turns with the pectinate sample of E 20% could be anticipated, as discloses the relationship of the complex-forming ability of pectin on its linear charge density (Fig. 6). 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 at 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 (E), *i.e.* with the decreasing linear density of the negative charge. The number of chains arranged parallely along the α-helix axis is for various values E as follows: three chains for $E \sim 0\%$, four chains for $E \sim 25\%$, five chains for $E \sim 40\%$, six chains for $E \sim 50\%$ and seven chains for $E \sim 57\%$. The maximum number eight chains for $E \sim 62\%$ is limited by spatial possibilities of the model under consideration. Should we, however, consider the superhelical structure of pectin with deformation of torsional angles in limits allowed according to conformational energy maps[15,16], the saturation of charges can be achieved by some less number of pectin chains. 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 encountered in this paper is better represented by the model composed of an α-helix of the polycation and a superhelix of the polyanion.

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