FORMATION OF THE POLY(LYS-ALA-ALA) COMPLEX WITH POTASSIUM OLIGOGALACTURONATES

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The helix-forming interaction of poly(Lys-Ala-Ala) with potassium oligogalacturonates of various polymerization degree ($n = 1-9$) was investigated by the circular dichroism spectra. The complex-forming effect of these oligomers is considerably lower than that of the polymeric D-galacturonan. The highest efficiency was observed at $n = 4$ and 6, where the chain lengths of oligogalacturonates approximate to the distance of the most nearly located $-NH_3^{(+)}$ groups of the α-helical polypeptide conformation.

Our preceding papers[1,2] dealt with the interaction of anionic pectin of various esterification degree of carboxyl groups with cationic polypeptides*, with poly(lysine) and sequentially regular poly(Lys-Ala-Ala). Both polypeptides underwent a conformation change from charge coil to α-helical one, which is quantitatively observable on circular dichroism spectra. Formation of the complex is directed by the stoichiometric saturation of charges.

The mutual compatibility of charge densities of components entering the interaction plays the main role. Complexation of pectinates with poly(Lys-Ala-Ala) proceeds approximately by a three-times higher effectiveness than that with poly (lysine). The derived spatial model of the complex consists of the polypeptide in an α-helical conformation; this polypeptide is surrounded by a suitably oriented acid poly-saccharide chain. We presume the most favourable orientation to be the superhelical structure of pectin macromolecule. Neither potassium monogalacturonate, nor the nonagalacturonate enter the complex with poly(lysine)[1].

Formation of β-structures was observed[3] upon interaction of poly(lysine) with poly(glutamate) of a low polymerization degree ($n \geqq 20$). Therefore, it was of interest to examine the complex-forming effect of oligogalacturonates of shorter chain ($n \leqq 9$) with poly(Lys-Ala-Ala). So far, no complex-forming interaction has been observed with anionic oligosaccharides.

* All aminoacids and their derivatives encountered in this paper had L-configuration.

EXPERIMENTAL

The sequentially regular poly(Lys-Ala-Ala) hydrogen bromide with an L-configuration of amino-acids was prepared according to[4]. The relative molecular mass 6 800 ± 500 was estimated from the sedimentation equilibrium. The lysine content was determined from the concentration of $Br^{(-)}$ ions by potentiometric titration with $AgNO_3$ 2 mmol l^{-1}.

Potassium oligogalacturonate samples were prepared by a partial acid hydrolysis of purified pectic acid (Genu Pectin, Københavns Pektinfabrik, Denmark) and by rechromatography of the oligomeric fragments on Sephadex-packed columns. Their preparation, characterization and polymerization degree estimation were already published[5,6]. Potassium oligogalacturonates were chemically pure substances.

The mixed solutions were obtained from stock solutions of poly(Lys-Ala-Ala) (concentration of $(-NH_3^{(+)})$ groups 0·6 mmol l^{-1}) and oligo(D-galacturonate) (concentration of $(-COO^{(-)})$ groups 0·6 mmol l^{-1}). Solution of potassium oligogalacturonate was gradually added to solution of poly(Lys-Ala-Ala) in such an amount as the respective concentrations of carboxyl groups corresponded to 40, 60, 80, and 100% concentration of $(-NH_3^{(+)})$ groups. The final concentration of $(-NH_3^{(+)})$ in solutions was uniformly adjusted to 0·3 mmol l^{-1}. The pH of solutions varied within 5·9–6·4 depending on the ratio of components in the mixture.

Employed were: digital potentiometer Radiometer PHM 64 (Denmark), silver electrode, electrolytic bridge filled with a 10%-solution of KNO_3, glass electrode G 222B, and a saturated calomel electrode K 401 (Radiometer). The circular dichroism spectra were recorded with a Dichrograph Mark III (Jobin Yvon, France) in 1 and 0·5 mm cells at 25°C.

RESULTS AND DISCUSSION

The character of conformation changes "disorder–order" of the poly(tripeptide), poly(Lys-Ala-Ala), observed on the circular dichroism spectra (CD) has already been reported[2,7]. The CD spectrum of the poly(Lys-Ala-Ala) itself is represented by the curve *1* (Fig. 1). Oligogalacturonates consist of D-galacturonic acid units in a $^4C_1$ conformation linked by a diaxial *trans*-glycosidic bond α(1→4). The chir-optic response of sodium oligo- and polygalacturonates has been examined[8]. The positive Cotton effect at about 204 nm undergoes a characteristic change depending on the polymerization degree.

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 (mean value of two measurements). To be quantitatively evaluable, the spectra were corrected by subtraction of both the intrinsic circular dichroism of the amount of oligogalacturonate added and the circular dichroism of the excess of poly(Lys--Ala-Ala) corresponding to the excess of $(-NH_3^{(+)})$ groups per $(-COO^{(-)})$ groups.

Figs 1 and 2 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 the CD of oligogalacturonates do 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 (~205 nm). The monomer did not raise formation of complexes. The dimer and trimer (Fig. 1, 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 charge coil arrangement, *i.e.* not participating in the complex-forming interaction. The above-mentioned second dichroic band is clearly observable with higher oligomers ($n = 4-9$), Fig. 1, spectrum *4* and Fig. 2, 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 $\Delta\varepsilon$ values at 225 nm, from which the CD of the total original content of poly(Lys-Ala-Ala) was subtracted, were considered the reference values. The spectra were further normalized to a uniform concentration of carboxyl groups of oligogalacturonates ((—$COO^{(-)}$) 0·3 mmol l^{-1}). The obtained values 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[2].

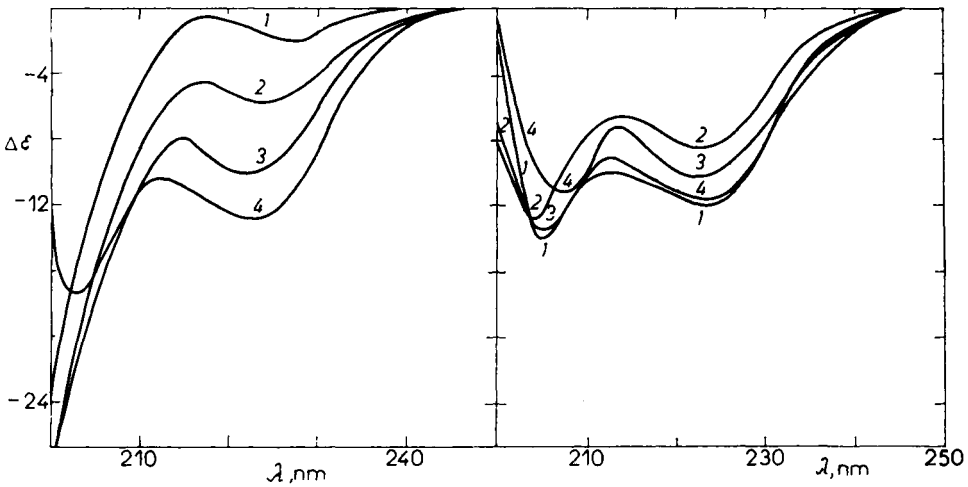

FIG. 1

The CD spectrum of poly(Lys-Ala-Ala), (—$NH_3^{(+)}$) 0·3 mmol l^{-1} (curve *1*), and its mixture with oligogalacturonates, (—$COO^{(-)}$) 0·3 mmol l^{-1}), of polymerization degree $n = 2$, 3, and 4 (curves *2*, *3*, and *4*)

FIG. 2

The CD spectrum of poly(Lys-Ala-Ala), (—$NH_3^{(+)}$) 0·3 mmol l^{-1}, in the presence of oligogalacturonates, (—$COO^{(-)}$) 0·3 mmol l^{-1}, of polymerization degree $n = 6$, 7, 8, and 9 (curves *1*, *2*, *3*, and *4*)

Fig. 3 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 in the polymerization degree range under investigation (up to $n = 9$) were found at $n = 4$ and 6; at $n = 7$ and 8 the efficacy is lower at $n = 9$ again higher. The amount of the oligosaccharide added is of significant effect on the complex-forming effect. 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). 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 the polyuronic pectin preparations[1,2]. The electrostatically induced formation of helical structure of the polypeptide is directed by the possibility of the added oligoanions to access and to be distributed at the surface of the polypeptide molecule. The mutual repulsion of the oligogalacturonate molecules becomes evident. Their spatial distribution is given by the chain length. The chains of oligomeric molecules are shorter than is the circumference of a cylinder formed by ($—NH_3^{(+)}$) groups of helical structure. 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 ($—NH_3^{(+)}$) ions at two adjacent turns estimated from geometric parameters deduced in previous papers[1,2] is approximatelly 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 4 or 6 D-galactu-

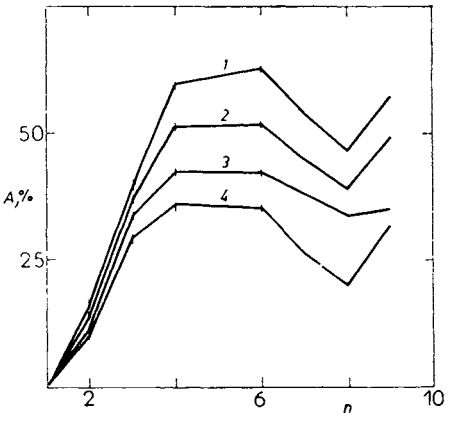

FIG. 3

The effect of polymerization degree (n) of oligogalacturonates on the formation of the complex with poly(Lys-Ala-Ala) at various ratios of components. The values are normalized to the same concentration of ($—COO^{(-)}$) groups in solution; A the complex-forming efficacy (%) of oligogalacturonates with respect to maximum (100%) effectiveness of polymeric D-galacturonan; *1*, *2*, *3*, *4* the poly(Lys-Ala-Ala) solution (concentration of ($—NH_3^{(+)}$) 0·3 mmol l^{-1}) containing 40, 60, 80, and 100% of the equivalent amount of oligogalacturonate

ronate units. Orientation of the oligogalacturonate chains of a higher polymerization degree ($n = 7-9$) is not univocal, the molecules are probably directed along the axis of the polypeptide helix. Oligosaccharides having longer chains ($n > 7$), like the polymeric D-galacturonan form solutions of complexes, which become opalizing; this could be due to the ability of an unsaturated oligoanion charge to interact with further polypeptide molecule under formation of aggregates.

The effectiveness of the complex-forming interaction of these oligogalacturonates is low with respect to polymeric D-galacturonan (deesterified pectin[2]), because only a part of the oligomer added enters the complex. A decisive factor lowering their efficacy is the mutual repulsion of oligoanionic molecules.

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