BASIC POLYPEPTIDES AS HISTONE MODELS: INFLUENCE OF THE ε-GLYCYLATION ON PROPERTIES OF LYSINE-CONTAINING POLYPEPTIDES

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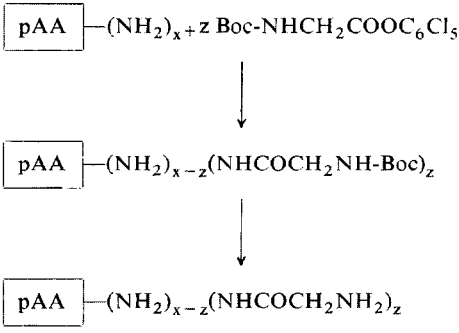
Grafting of side chains of the lysine residues with one glycine residue results in a decreased formation of the α-helix in the case of poly-L-lysine. In the random copolymer $(Lys^{30}, Ala^{70})_n$ with the Gly/Lys ratio equal to 0·12—0·96, the ε-glycylation has only a small effect on the conformation of the polypeptide. Grafting up to the Gly/Lys ratio 0·5 does not affect the structure of complexes with DNA.

In previous papers, we have reported the synthesis of various sequential and random lysine-containing copolymers along with utilisation of these polymers as histone models in the formation of complexes with DNA (*cf.*[1-4]). The properties of polypeptides and their complexes depend on the amino acid composition of polypeptides. The composition of particular complexes, *i.e.*, the content of DNA and the polypeptide, has not been so far examined in detail. The method applied to characterisation of complexes, circular dichroism, makes it possible to estimate particularly the DNA component. The direct quantitative detection of the peptide component could be advantageously effected with the use of a labelled model substance, *e.g.*, a grafted polymer obtained by ε-glycylation of the lysine side chains. In the present paper, we have examined whether this ε-glycylation affects properties of fundamental model peptides.

The influence of ε-glycylation has been examined with the use of both poly-L-lysine and a random copolymer $(Lys^{30}, Ala^{70})_n$ of the molecular weight 19000, the synthesis of which has been reported earlier[4]. This copolymer imitates conformational properties of some histones (*e.g.*, H 2B) and chiroptical properties of its complexes with DNA resemble to a certain degree the properties of respective histone complexes. In the literature, there are several examples of similar grafting experiments. The most

similar to our intention is the synthesis of some immunochemically interesting grafted polyamino acids starting from poly-L-lysine[5,6].

Two procedures were used in our grafting experiments. In procedure *A*, tert-butyloxycarbonylglycine or 2-nitrobenzenesulfenylglycine was attached to free amino groups of the copolymer in aqueous acetonitrile using the Woodward reagent K (2-ethyl-5-phenylisoxazolium-3'-sulfonate) in the condensation step[7]. In procedure *B*, an activated ester was used for the direct condensation. Procedure *B* is clearly superior in yielding a purer product and a higher degree of substitution of lysine residues (if desired). Poly-L-lysine was successfully glycylated by the same procedure. Grafted copolymers $(\text{L-Lys(Gly)}_z, \text{L-Lys}_{x-z}, \text{L-Ala}_y)_n$ with the z/x ratio equal to 0·12–0·96 were prepared according to Scheme 1.*



SCHEME 1

Boc, tert-butyloxycarbonyl; pAA —$(NH_2)_x$, lysine-containing polypeptide.

EXPERIMENTAL

Materials

Poly-L-lysine (Miles, Rehovot, Israel), m.w. 30 000 (sedimentation equilibrium). The copolymer[4] $(Lys^{30}, Ala^{70})_n$, m.w. 19 000 (sedimentation equilibrium). Substances were dried under diminished pressure at room temperature over phosphorus pentoxide and potassium hydroxide pellets. The amino acid analysis was performed on an Automatic Amino Acid Analyser (Developmental Workshops, Czechoslovak Academy of Sciences, Prague) with samples after hydrolysis in 6M-HCl (105°C/20 h) in ampoules sealed at 1 Torr.

* For symbols see ref.[8]. All amino acid residues are of the L-configuration.

Circular Dichroism

The circular dichroic spectra of polypeptides were measured on a Cary 61 apparatus in the 260—195 nm region in cells of optical pathways 0·05 and 0·01 cm. The concentration of solutions was about 0·1%. The pH values of solutions were adjusted by additions of the appropriate amount of 0·1M-NaOH to the original solution (0·02M-NaF). The final pH value was determined with an accuracy of 0·1 pH units. The circular dichroism is expressed in molar ellipticities $[\Theta]$ (deg . cm^2 . . dmol^{-1}) taking into account the average molecular weight of the residue for the given polypeptide.

The CD spectra of the complexes were measured on a Roussel Jouan Dichrograph CD 185 in 0·5 cm cells. Circular dichroism was expressed in specific ellipticities $[\psi]$ (deg . cm^{-2} . dg^{-1}).

Preparation of DNA–Polypeptide Complexes

The DNA preparation was from calf thymus[2]. Complexes of polypeptides with DNA were prepared by mixing both components in a given Lys/DNA (lysine/nucleotide) ratio in 2M-NaCl buffered with 0·013M sodium phosphate (pH 6·8), followed by flow dialysis against a linear gradient of NaCl molarity[2,3,9] to 0·15M-NaCl with 0·013M sodium phosphate (pH 6·8).

ε-Glycylation of the (Lys^{30}, Ala^{70}) Copolymer

Procedure A. The Woodward reagent K (25·9 mg; 0·1 mmol) was added to a solution of tert-butyloxycarbonylglycine (17·8 mg; 0·1 mmol) in acetonitrile (1·5 ml) and 0·25M N-ethylpiperidine in acetonitrile (0·41 ml). The mixture was shaken for 30 min and the resulting solution was added to a solution of the (Lys^{30}, Ala^{70})$_n$ copolymer hydrochloride (22·4 mg; 0·068 meq. of lysine residues) in water (5 ml) and 0·1M-NaOH (0·68 ml). The whole mixture was stirred at room temperature for 18 h and freeze-dried. The residue was washed with three 1 ml portions of ethanol, collected by centrifugation, dried under diminished pressure, and dissolved in trifluoroacetic acid (1 ml). After 15 min, the solution was evaporated under diminished pressure, coevaporated with five 1 ml portions of ether, washed with five portions of acetone, and dried under diminished pressure. Yield, 13 mg of the product; Gly/Lys, 0·38 (according to amino-acid analysis).

Procedure B. A stirred mixture of the (Lys^{30}, Ala^{70})$_n$ copolymer hydrochloride (37·9 mg; 0·12 mequiv. of lysine residues) and acetonitrile (4·6 ml) was treated with 0·5M-NaOH (0·10 ml) and a solution of tert-butyloxycarbonylglycine pentachlorophenyl ester[9] (7·3 mg; 0·017 mmol) in acetonitrile (0·46 ml). The mixture was shaken for 9 h and evaporated under diminished pressure. The residue (38·1 mg) was washed with eight 0·5 ml portions of ether by centrifugation, dried under diminished pressure, and the final residue (37·3 mg) dissolved in trifluoroacetic acid (2 ml). The solution was kept at room temperature for 15 min, evaporated under diminished pressure, and coevaporated with three 2 ml portions of ether. The material was then washed by trituration with five 0·5 ml portions of ether and centrifugation. After drying, the yield of the product was 44·1 mg; Gly/Lys, 0·12. Samples with the Gly/Lys ratio equal to 0·29, 0·33, 0·45, and 0·96 were prepared analogously.

ε-Glycylation of Poly-L-lysine

A stirred mixture of poly-L-lysine hydrochloride (33 mg; 0·2 mequiv of lysine residues) and acetonitrile (3 ml) was treated with 0·5M-NaOH (0·4 ml) and a solution of tert-butyloxycarbonylglycine pentachlorophenyl ester (42·4 mg; 0·1 mmol) in acetonitrile (2 ml). The mixture was processed

analogously to the preceding paragraph (Procedure *B*). Yield, 36·1 mg of the product; amino acid analysis: Gly/Lys, 0·41.

RESULTS AND DISCUSSION

The ε-glycylation of lysine residues in polymers may be performed by means of N-protected activated glycine esters. The extent of ε-glycylation may be preparatively controlled by the ratio of lysine residues to the acylation reagent taking into account an about 70% conversion.

The CD spectra were taken of the grafted poly(L-Lys(Gly)) with the Gly/Lys ratio equal to 0·41 and of several grafted random copolymers $(\text{L-Lys}^{30}(\text{Gly}), \text{L-Ala}^{70})_n$ with the glycine residue on the side chain of the lysine residue in Gly/Lys ratios equal to 0·12, 0·29, 0·33, 0·45, and 0·96 (pH range, 7–10·5). The results were compared with those shown by non-grafted parent polymers.

In the pair poly-L-lysine/grafted poly-L-lysine, the pH dependences of CD curves differ (Figs 1 and 2). The grafted polymer exhibits markedly lower molar ellipticity values at λ 222 nm in the alkaline pH region. Although the $[\Theta]_{208}/[\Theta]_{222}$ ratio is only a little lower with the non-grafted polymer (poly-L-lysine[10], 0·945) than with

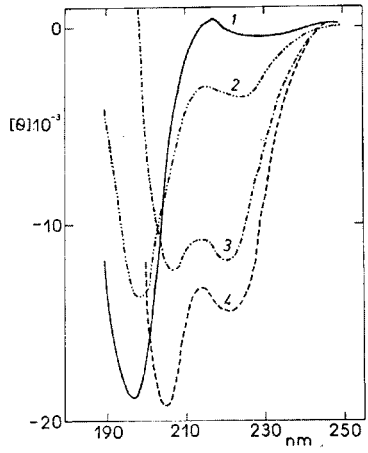


FIG. 1

CD Spectra of the ε-Glycylated Poly-L-lysine (Gly/Lys, 0·41)

1 pH 6·5; *2* pH 9·1; *3* pH 10·5; *4* CD spectrum of poly-L-lysine, pH 9·2.

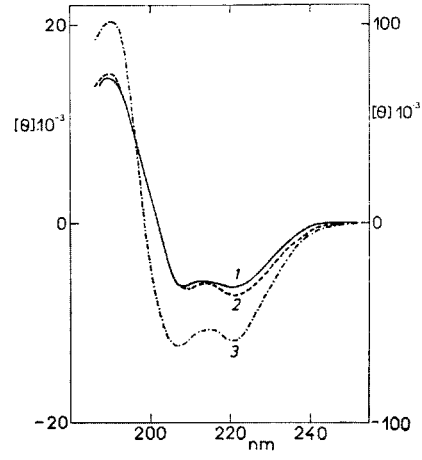


FIG. 2

CD Spectra

1 Poly-L-lysine (Miles, Rehovot; m.w. 30000, pH 11·0), scale on the right; *2* poly-L-lysine (ref.[11]; m.w. 120000, pH 11·1), scale on the right; *3* ε-glycylated poly-L-lysine (Gly/Lys, 0·41), pH 10·5, scale on the left.

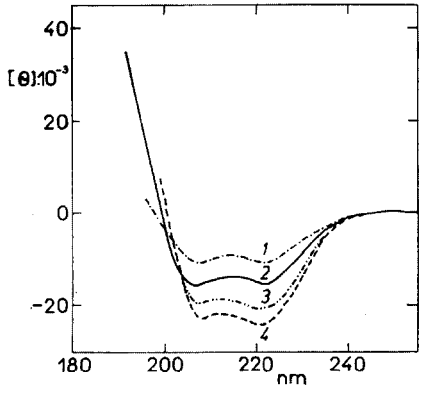


FIG. 3

CD Spectra of the $(Lys^{30}, Ala^{71})_n$ Copolymer, pH Dependence

1 pH 7·0; *2* pH 7·8; *3* pH 9·3; *4* pH 11·4.

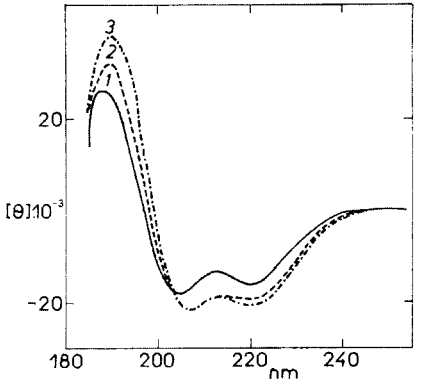


FIG. 4

CD Spectra of the $(Lys^{30}(Gly), Ala^{70})_n$ ε-Glycylated Copolymer (Gly/Lys, 0·12), pH Dependence

1 pH 7·0; *2* pH 8·7; *3* pH 10·2.

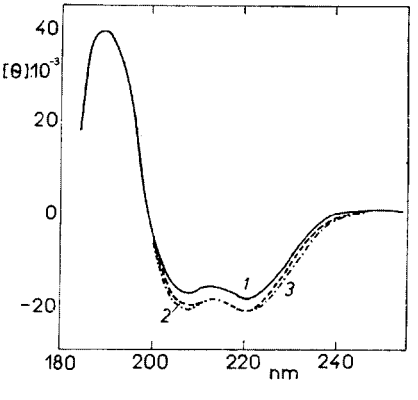


FIG. 5

CD Spectra of the $(Lys^{30}(Gly), Ala^{70})_n$ ε-Glycylated Copolymer (Gly/Lys, 0·96), pH Dependence

1 pH 6·7; *2* pH 9·3; *3* pH 10·4.

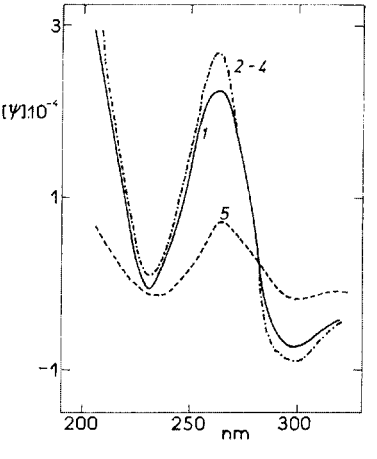


FIG. 6

CD Spectra of Complexes of Copolymers with DNA Lys/DNA, 0·65

Gly/Lys 0 (*1*), 0·12 (*2*), 0·29 (*3*), 0·45 (*4*), 0·96 (*5*).

the grafted polymer (1·033), the absolute values of molar ellipticities markedly differ in the case of the ε-glycylated poly-L-lysine with the Gly/Lys ratio equal to 0·41:

pH	6·5	9·1	10·5
$[\Theta]_{222}$	−200	−3500	−11800

In the neutral pH region (where an unordered conformation of the polymer should be assumed), the spectra of the two polymers differ in intensity ratio of the positive and negative maximum of n-π^* bands. Fig. 2 shows the difference between the spectra of the two polymers in the alkaline region where the conformation of an α-helix is assumed. The wavelength of zero ellipticity as well as the 222 nm band ellipticity of the grafted polymer indicate a lower population of the α-helical conformation[11]. The presence of a glycyl group in the side chain of the grafted polymer thus interferes to a certain degree with the formation of an α-helix.

The behaviour of the grafted copolymers $(Lys^{30}(Gly), Ala^{70})_n$ and that of the parent non-grafted copolymer is illustrated by Figs 3 to 5. While the non-grafted copolymer exhibits a marked pH dependence of the circular dichroism spectrum, the pH dependence of the almost 100% grafted copolymer is insignificant. The observed maximum value of molar ellipticities at 208 and 222 nm is comparable with those of the non-grafted copolymer (Fig. 5). There is little dependence of the $[\Theta]_{222}$ intensity on the ε-glycylation degree within a wide ε-glycylation range (12−96%); in neutral pH range, the values vary from 16 000 to 19 000 (Figs 4 and 5). At higher pH values, the maximum degree of helicity appears to be attained very soon and the conformation does not change with increased pH values. This behaviour could be explained by a decrease of the pK value of the amino group in the side chain by glycylation. It is however noteworthy that the behaviour of the glycylated poly-L-lysine does not fully correspond to this idea (*cf.* the pH 9 curves of the glycylated and non-glycylated poly-L-lysine, Figs 1 and 2).

As shown by CD spectral measurements, the glycylation of the side chain of the lysine residue has only a small effect (particularly in the case of the $(Lys^{30}, Ala^{70})_n$ copolymer) on the conformation of the copolymer and results in a shift of the coil-helix transition to lower pH values.

The CD spectra of complexes of DNA with polymers of the $(Lys^{30}(Gly), Ala^{70})_n$ type do not depend on the amount of the grafted glycine and are identical with the spectrum of the complex with the parent copolymer (Fig. 6). The only exception is the almost 100% grafted copolymer. It may be thus inferred that grafting up to the Gly/Lys ratio lower than 0·5 does not affect the structure of complexes.

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