A SYNTHETIC POLYPEPTIDE WITH A COMPACT STRUCTURE AND ITS SELF-ORGANIZATION

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1. Introduction

The compact structure of globular proteins is predominantly determined by hydrophobic interactions of bulky nonpolar side groups. Both from the point of view of modelling this main feature of globular protein structure, and also from the viewpoint of their evolutionary origin, it is of significant interest to clarify whether water-soluble random copolymers of polar and non-polar amino acids are able to form compact structures. Recently Miller and his collaborators proposed [1] using random copolymer gel-chromatography to isolate fractions with a compact structure and obtained evidence for the presence of such fractions in random copolymers consisting of Ala, Glu and Lys.

It has been shown in a previous paper [2] that a non-aggregating fraction can be isolated from random copolymers of Glu and Leu. The potentiometric titration curve of this fraction allowed one to presume the existence of a compact structure in a weakly ionized state. In the present communication it is reported that with a decrease of ionization of molecules of this fraction, they at first pass from a coil-like state into the helical with a subsequent intramolecular aggregation of helical regions, which may be of interest in the light of existing concepts on the mechanism of globular protein self-organization [3–5].

2. Materials and methods

Copolymerization of *N*-carboxyanhydrides of Leu and Glu was done in benzene with tri-*n*-butylamine as an initiator [6]. A random copolymer obtained from a 32% Leu + 68% Glu reaction mixture, contained 27% Leu and 73% Glu. Gel-chromatography of this copolymer on Bio-Gel A-5m at pH 7.0 gave a fraction which does not aggregate at these pH, i.e. in an ionized state. Repeated gel-chromatography of this fraction at pH 5.2 gave a fraction which does not aggregate even at this pH and contains 17% Leu (see [2]). This fraction was studied by potentiometric titration, optical activity dispersion (see [2]) and polarized luminescence. For comparison a study was also made of polyglutamic acid (PGA) and a Glu + Leu copolymer containing 4% Leu. All measurements were done in 0.2 M NaCl at room temperature. Polarized luminescence was studied on macromolecules, with covalently bound luminescent anthrylacyloxymethane groups [7] (1 label per 1000–5000 monomer residues). The binding of anthryldiazomethane (ADM) to carboxyl groups of macromolecules was carried out in a water-dioxane solution at 2–4°C. After the reaction was completed, the solution was centrifuged and passed several times through a G-10 column to separate low molecular weight ADM decomposition products. The 17% Leu copolymer was then once again chromatographed on Bio-Gel A-5m. The luminescence polarization, P, was measured on the instrument described in [8], and the luminescence excitation life-time τ_φ was measured on the GOI-IF-39 fluorimeter (USSR). The relaxation time τ_φ was determined from the dependence of $1/P$ on the solvent viscosity η, varied by addition of sucrose, at a constant temperature (25°C).

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3. Results and discussion

Curves of the Moffitt-Yang constant b_o (pH) dependence and potentiometric titration curves (in the coordinates of $pK_{eff} = pH - \log [\alpha/(1-\alpha)]$ versus α, where α is the degree of ionization of Glu residues) are given in figs.1 and 2. It follows from the figures that the helical degrees of all three samples nearly identically depend on pH, while the titration curve of the 17% Leu copolymer at $\alpha < 0.5$ significantly differs from the titration curves of the two other samples. Anomalously high pK_{eff} values at $\alpha < 0.5$ (i.e. anomalously low values of the effective ionization constant) are evidence of a great electrostatic interaction of Glu residues, which suggests the presence of a compact structure of this copolymer at acid pH [2].

To check this suggestion, polarized luminescence studies were made of all three samples with anthryl-acyloxymethane groups covalently bound to them, as this method was shown earlier [9–11] to have a high sensitivity to any processes structurizing the macromolecules. The dependences of relaxation times on pH, given in fig.3, show that τ_ω increases with a decrease of pH for PGA and the copolymer with 4% Leu (curves 1 and 2), reflecting the decrease of intramolecular mobility on transition of macromolecules

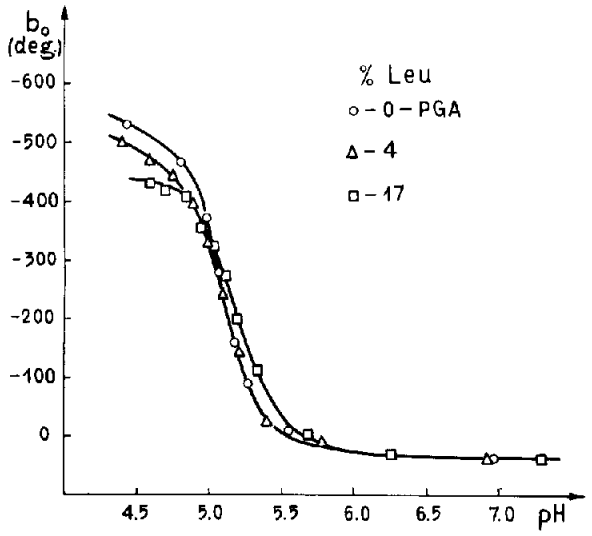

Fig.1. Dependences of b_o (pH) for PGA and copolymers with 4% and 17% Leu.

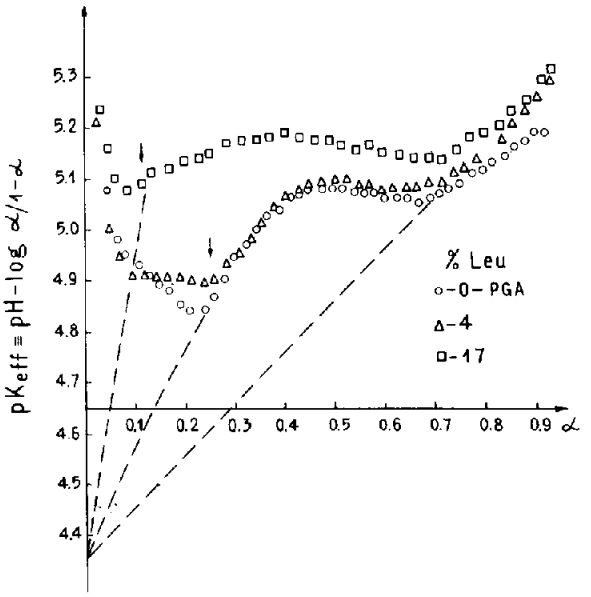

Fig.2. Potentiometric titration curves of PGA and copolymers with 4% and 17% Leu. The dashed line shows extrapolation of pK_{eff} (α) curves, the arrows indicate the beginning of aggregation.

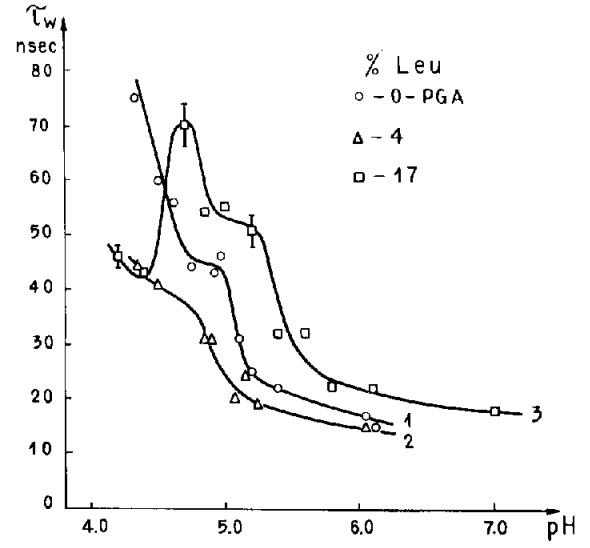

Fig.3. Dependences of τ_ω (pH) for PGA [η] = 0.5 dl/g, c = 0.04%) (curve 1), of copolymer with 4% Leu ([η] = 0.6 dl/g, c = 0.04%) (curve 2), and of copolymer with 17% Leu (η_{sp}/C = 0.08 dl/g, c = 0.01%) (curve 3).

into the helical state [9] and intermolecular aggregation of PGA. On the contrary, in the copolymer with 17% Leu (curve 3) the τ_ω (pH) curve reaches the maximum at pH 4.7 (i.e. at completion of the helix–coil transition, see fig.1), and then begins to decline (the low molecular weight of this copolymer seems to prevent intermolecular aggregation at c = 0.01%).

The presence of the maximum on the τ_ω (pH) curves for the copolymer with 17% Leu completely agrees with the assumed transition of this copolymer into the compact state. Indeed, for a macromolecule possessing both internal and external degrees of freedom, $\tau_\omega^{-1} = \tau_i^{-1} + \tau_0^{-1}$, where τ_i is the relaxation time connected with intramolecular mobility and τ_0 is the relaxation time of the macromolecule as a whole [10]. During the transition of macromolecules into the compact state τ_i increases due to a decrease of the intramolecular mobility and τ_0 decreases due to a drop in the dimension of the macromolecule, so that for sufficiently compact macromolecules $\tau_0 \ll \tau_i$ and $\tau_\omega \simeq \tau_0$. Therefore the transition of the τ_ω (pH) curve through the maximum means a decrease of the intramolecular mobility accompanied by a drop in dimensions of macromolecules, i.e. it directly reflects the transition of macromolecules into the compact state. It is important to underline that this transition begins at pH < 4.7, i.e. after completion of the helix–coil transition in these macromolecules. Thus it represents the intramolecular aggregation of the *pre-existing* helical regions.

4. Conclusion

The compact state of molecules of the copolymer studied (which evidently can be explained by hydrophobic interactions of Leu residues) does not, of course, mean that these molecules have a unique tertiary structure, similar to that of globular proteins. The irregular unique tertiary structure most likely demands a unique amino acid sequence, strategically selected for such a structure [12]. Nevertheless, studies of self-organization processes of the compact state of random polypeptides can be useful for understanding some general principles of globular protein self-organization.

It is not excluded that the intramolecular aggregation of the *pre-existing* helical regions established in this study reflects particular regularities connected with a gradual change in the degree of ionization of the polypeptide studied. However, there is also another possibility. It can be expected that in macromolecules with a *comparatively small* content of mutually attracted groups, the formation of a compact structure is possible only on the basis of pre-existing local structures. The pre-existence of such structures can lead to the spatial approach of these groups in each separate region of the chain and simultaneously provides for the co-operative interactions of clusters of several groups belonging to different regions. The α-helices, the length of which is only 1.5 Å per monomer, are in good agreement with this requirement. From this view-point the stages of self-organization of the compact state of the synthetic polypeptide studied can reflect general regularities of compact structure formation in heteropolypeptide chains. These regularities can also be important for self-organization of globular proteins where the content of bulky non-polar side groups does not usually exceed one third.

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