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The Conformation of Acid Form γ-Polyglutamic Acid

Takehiko WATANABE, Takafumi INA, Kozo OGAWA,* Takeshi MATSUMOTO,
Sumihiko SAWA** and Sôzaburo ONO

*Laboratory of Biophysical Chemistry and** Laboratory of Fermentational*
Chemistry, College of Agriculture, University of Osaka Prefecture, Sakai
** Radiation Center of Osaka Prefecture, Shinke-cho, Sakai*

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In the previous paper,[1] the dissolved state of γ-polyglutamic acid solution at different pH's was studied by means of rotatory dispersion, flow birefringence and intrinsic viscosity. And it was reported that the polymer molecule in neutral and alkaline media will be in the form of a highly expanded chain while that in acid medium in a contracted form having a some ordered chain conformation. Edelhoch and Lippoldt[2] reported that γ-poly-D-glutamate obtained from the capsule of *Bacillus anthracis* exists in a hydrogen-bonded hyper coiled form in water at low degrees of ionization. Later Rydon[3] suggested that the unionized acid form polymer possesses a helical structure from the optical rotatory dispersion measurement from 250 to 600 mμ.

In the present paper, the conformation of γ-polyglutamic acid in acid form was studied by rotatory dispersion and infrared spectra.

Experimental

Materials. γ-polyglutamic acid, or γ-PGA is a polymer of glutamic acid linked through the γ-carboxyl and α-amino group and is produced by the *Bacillus subtilis* No. 5E[4] from glucose and urea. γ-PGA is isolated by the NaCl-alcohol precipitation method[5] from the culture. The extracted γ-PGA was dissolved in 3% NaCl and 30—35% alcohol aqueous solution and centrifuged for the removal of cells, and then precipitated by pouring the centrifuged solution in alcohol. This procedure was repeated and the centrifuged solution was dialyzed against distilled water until free of chloride and the dialyzed solution was lyophilized. Thus obtained sample is the sodium salt of γ-PGA (Naγ-PGA) and its intrinsic viscosity obtained in 10% NaCl solution at 25°C is 4.71 d*l*/g and sedimentation constant at infinite dilution (in 10% NaCl solution) is 6.80×10^{-13}. The molecular weight calculated by Mandelkern and Flory equation[6] is 1×10^{6}. To obtain the acid form γ-PGA (Hγ-PGA), the Naγ-PGA was successively treated with ion exchange resin Dowex-50, Amberlite IRA-400 and Dowex-50 as the 0.2% aqueous solution and the treated solution was lyophilized. Both Naγ-PGA and Hγ-PGA were hydrolyzed with 6N HCl at 105—110°C for 15—24 hr. Degree of optical rotation of both hydrolyzed samples was coincided with that of the mixture of 75% D-glutamic acid and 25% L-anomer.

Measurements. Optical rotatory dispersion measurement was carried out with a Yanagimoto ORD-185. Mean residual rotation [R] was calculated as the correction factor $3\overline{M}/100\ (n_\lambda^2+2)\fallingdotseq 1$, where $\overline{M}$ is mean residual weight of glutamic acid and n_λ is the refractive index of solvent.

Infrared spectra was measured using a Perkin-Elmer Spectrophotometer Model 221.

Results and Discussion

The optical rotatory dispersion curves of Hγ-PGA at pH=9.0, 4.7 and 2.5 are shown in Fig. 1. The curves show a negative Cotton effect with a trough at 230 mμ[*1] and the depth of the trough becomes deeper with decreasing in pH. According to Sarkar and Doty[7] and Iizuka and Yang[8], the appearance of the trough at 230 mμ on optical rotatory dispersion curve suggests that the polymer dissolved in acid medium is in a β-form, not the helical structure which was proposed by Rydon[3] under the deficiency of the data obtained from the optical rotatory measurement at lower wavelength below 250 mμ, where the Cotton effect is

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[*1] In the previous paper (Ref. 1, Fig. 6), a deep trough due to Cotton effect was observed at 225 mμ and a shallow trough was observed on curves at the wavelength of 260—270 mμ. The discrepancies of the results from that of the present one may be brought about by the coexistence of unknown substance (in small amounts) which has a absorption peak at 260—270 mμ. The substance was almost perfectly eliminated from the γ-PGA in the course of the treatment with the ion exchange resins.

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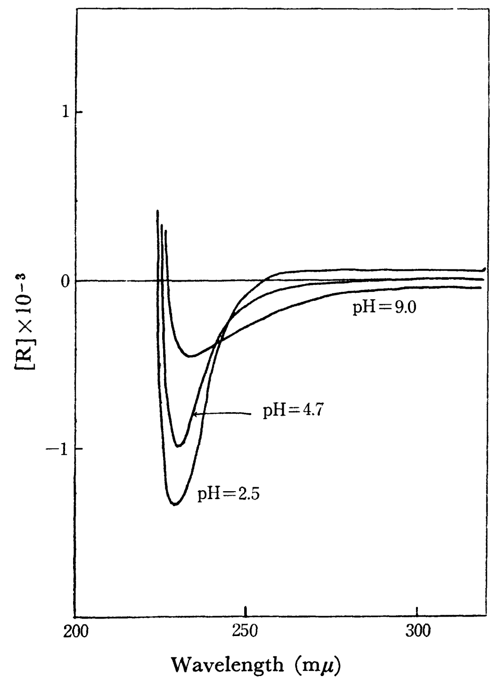

Fig. 1. Optical rotatory dispersion curves of Hγ-PGA at different pH's.

observed. With the value of [R], the negative value is obtained at 230 mμ and this result corresponds to the one reported in literature for α-linked L-polymer,[7,8] in spite of the presence of large excess amounts of D-polymer. The magnitude of the value of [R] expected for pure D-polymer at pH=2.5, is −1350 × 2= −2700, if we assume a compensative effect on the value of [R] between D- and L-polymer based on the composition ratio of D : L=3 : 1. However, this value of −2700 is far different from the value of [R]=−6 × 10^3 reported for α-linked L-polymer.[7,8]

The infrared spectra of Naγ-PGA and Hγ-PGA obtained with KBr disc method are shown in Fig. 2. With Hγ-PGA, the amide I bands were observed at 1630 cm^{-1} (strong) and 1650 cm^{-1} (weak) and the amide II bands were observed at 1530 cm^{-1} (strong) and 1550 cm^{-1} (weak), indicating that the Hγ-PGA is in the β-form, according to the results obtained with α-linked polymers by Miyazawa and Blout[9] and Masuda *et al.*[10] With Naγ-PGA, the amide I and II bands in disordered form (random coil)[9,10] were observed at 1655 and 1535 cm^{-1}, respectively, however these bands are quite diffuse due to the overlapping by the band due to the COO^- groups around 1600 cm^{-1}. The above result was obtained with the lyophilized polymer, however, the same conformation may be taken in solution, because the infrared spectrum obtained with acid form α-poly-D-glutamic acid (product of Miles-Yeda Ltd.) by KBr disc method shows the characteristic spectrum of α-helix.

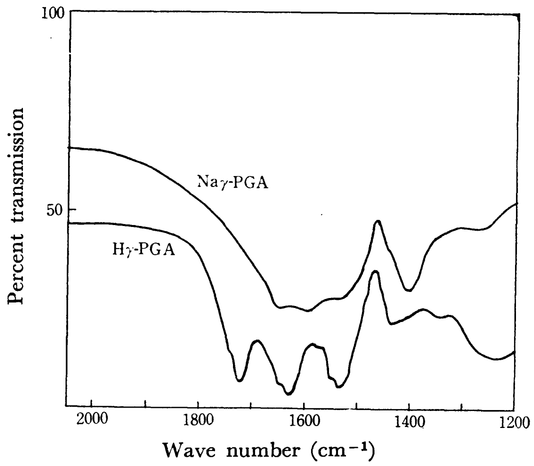

Fig. 2. Infrared spectra of Hγ-PGA and Naγ-PGA.

From the above result, γ-PGA molecule will be mostly in a β-form in acid medium and will take a contracted form with folding of the sheet of β-form molecule, as stated in the previous paper.[1]

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