# The Complex of Poly-α,L-glutamic Acid with Acridine Orange in the Neutral and Alkaline pH Region

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It is well known that an extrinsic optical activity is induced in the region corresponding to the absorption bands of acridine orange (AO) only when the dye combines with helical form of poly-α,-L-glutamic acid (PLGA).[1-3] Myhr and Foss,[4] and Eyring and Yang[5] reported that the Cotton effect could be induced for the complex even at pH 8.0, where the structure of PLGA is regarded to be a random coil form. The cause of the induced circular dichroism (CD) of the PLGA-AO complexes and their structures are, however, not yet completely clarified.

In this communication, we present preliminary results on the optical activities and structures of the complexes in the PLGA-AO system for *R*/*D* values smaller than 10. It was found that, in the cases of *R*/*D* of around unity, complexes with ordered structures can be formed in the neutral and alkaline pH region.

The complexes were prepared by adding an aqueous solution of AO to that of PLGA with pH of 4.5. The pH of the complex solutions was brought to the desired value by addition of NaOH. The total molarity of AO was fixed at $2\times10^{-5}$ M. The CD curves of these complexes were recorded at several *R*/*D* values for various pH. When *R*/*D* exceeds 4, the intensity of the induced CD was found to diminish in the neutral and alkaline pH regions. However, the induced CD spectra were observed even in the neutral and alkaline pH regions for *R*/*D* of around unity (Fig. 1).

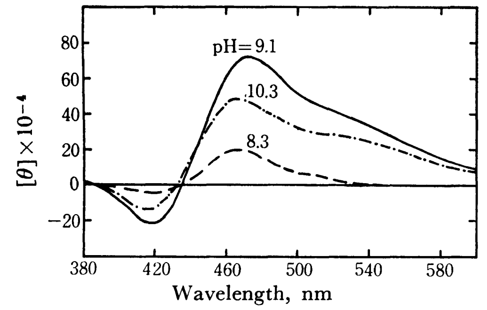

Fig. 1. pH dependence of CD spectra of PLGA-AO system at *R*/*D*=1.

As shown in Fig. 1, the induced CD which starts to develop at pH of about 6 reaches a maximum intensity at pH of about 9 and diminishes at pH of about 12. It is likely that the induced CD spectra developed in the neutral and alkaline pH regions for the case of *R*/*D* of around unity are due to the ordered structures of the complexes.

For the sake of confirmation, we also measured the specific viscosity $\eta_{sp}$ of PLGA with various degrees of polymerization (DP) at pH of 4.5 and those of PLGA-AO complexes of *R*/*D* of unity at pH of 9.0. By means of the following Simha equation, J values were calculated from specific viscosity.

$$\frac{\eta_{sp}}{\Phi}=\frac{J^2}{15(\ln 2J-1.5)}+\frac{J^2}{5(\ln 2J-0.5)}+\frac{14}{15} \qquad (1)$$

where J is the axial ratio of a postulated ellipsoid of the polymer and $\Phi$ is the volume fraction of the polymer in the solution. As shown in Fig. 2, *J* values of PLGA-AO complexes are proportional to the DP of PLGA. These results indicate that PLGA-AO complexes with *R*/*D* of unity at pH 9.0 have rigid rod-like structures, *e.g.*, helical structures.

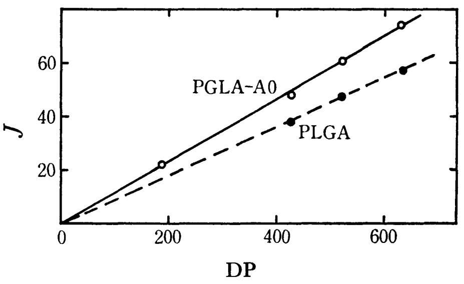

Fig. 2. Simha's J values as a function of degree of polymerization (DP) for PLGA in $H_2O$ (pH 4.5) and PLGA-AO complex of *R*/*D*=1 in MeOH:$H_2O$ (5:3) (pH 9.0).

In the case of *R*/*D* of unity, almost all available AO molecules can be bound to PLGA by ionic bonds between the cationic ring nitrogen atoms of AO molecules and ionized carboxyl groups of the side chains of PLGA in the neutral and alkaline pH regions. As a result of neutralization of the side chains of PLGA by the AO cations, PLGA can be compelled to form a helical structure with AO molecules around it. In the strong alkaline region above the $pK_a$ value of AO,[6] the molecules are deprotonated to be neutral. It seems to be difficult for AO molecules to be bound to PLGA and to stabilize the helical structure of PLGA. Ballard *et al.*[2] inferred, in the case of *R*/*D* larger than 20 at pH of 4.5, the presence of a left-handed super-helix of AO molecules around the right-handed helix of PLGA on the basis of a negative CD band polarized parallel to the helix axis at 470 nm. In comparing our results with theirs, we tentatively conclude that AO molecules bound to the carboxylate groups of the side chains of PLGA are arranged to form a right-handed super-helix surrounding the core of the right-handed helix of PLGA, since in this case a positive CD band at about 470 nm is observed.

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