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# Viscosity Behavior of Some Synthetic Polypeptides at Low Concentration

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ABSTRACT

The viscosity of poly($\gamma$-benzyl L-glutamate) (PBLG), poly-$\epsilon$-carbobenzoxy-L-lysine (PCBL), polyglycine (PG), and five tricopolypeptides $(glu^{x}\text{-}lys^{y}\text{-}gly^{z})_{n}$ was studied as a function of concentration at 25°C. The solvents used include two random coil solvents, a theta solvent and a helicogenic solvent. The Huggins plots of all the systems except polyglycine showed anomalously high reduced viscosity values at low concentration of solutions. A variation of 20°C in the temperature of some of the systems did not show any marked effect on the abnormality in the Huggins plots. Various factors likely to cause abnormality were considered; disentanglement of the polypeptide chains appeared to be the prime factor responsible for the anomalous rise in the reduced viscosity.

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## INTRODUCTION

Intrinsic viscosity measurements have been widely used in studies of several aspects of the conformation of proteins and synthetic polypeptides [1-4]. Usually, reduced viscosity is plotted as a function of solution concentration and the extrapolated value of the reduced viscosity at zero concentration is taken as intrinsic viscosity. In many experiments, with peptidic as well as nonpeptidic polymers, however, an anomalous feature was observed in the above kind of plots; at low concentration there was a steep rise of reduced viscosity with decrease in concentration. This type of behavior was reported by Streeter and Boyer [5] for polyethylene in benzene. A similar feature was observed with poly(vinyl chloride) in dioctyl phthalate and cyclohexane [6]. Bovine liver glutamate dehydrogenase was also reported by Reisler and Eisenberg [7] to exhibit the anomalous rise.

The abnormal behavior was sought to be explained in terms of the effect of (a) adsorption of solute on the capillary surface and consequent decrease in the capillary size [7, 8], (b) disentanglement of polymer chains as concentration of the system decreases [5], or (c) the charge on side chain groups [2b, 9] or more interestingly, on the backbone of the peptidic polymers [10, 11]. However, there does not appear to be a satisfactory explanation to account for all the known experimental facts. In the present work, experimental data were obtained on polypeptidic systems under varying experimental parameters of solvent temperature and composition of the polymer, with a view to elucidate the cause of the abnormality.



## EXPERIMENTAL

Solvents used were of commercial grade and were, therefore, purified and freshly distilled over suitable dehydrating agents before use. Urea and KCl were of AR grade (BDH). Amino acids were of reagent grade.

### Preparation of N-Carboxy Anhydrides

$\gamma$-Benzyl L-glutamate and $\epsilon$-carbobenzoxy-L-lysine were prepared by standard procedures [12, 13]; their corresponding N-carboxyanhydrides and glycine anhydride were prepared by Fuchs-Farthing method [14] by use of phosgene. The anhydrides were

TABLE 1. Composition and Intrinsic Viscosity of Polypeptides

| Sample | Composition (mole %) | | | $[\eta]$ in DCA at 25°C (dl/g) |
|---|---|---|---|---|
| | Glu | Lys | Gly | |
| PP3-1 | 37.4 | 35.2 | 27.3 | 0.146 |
| PP3-2 | 32.6 | 21.4 | 46.0 | 0.139 |
| PP3-4 | 20.7 | 21.5 | 51.8 | 0.150 |
| PP3-6 | 8.6 | 21.6 | 69.8 | 0.156 |
| PP3-7 | 42.9 | 39.9 | 17.2 | 0.240 |
| PBLG (fraction IV) | 100 | - | - | 0.176 |
| PG | - | - | 100 | 0.121 |
| PCBL | - | 100 | - | 0.117 |



purified by crystallizing twice from ethyl acetate and petroleum ether (60-80°C).

## Polymerization

Polymerization of appropriate mixtures of the three N-carboxy anhydrides was carried out in dioxane medium with n-butylamine as initiator (A/I=200) with continuous bubbling of nitrogen at 25°C. The polymerization was over in 3-4 days. The tricopolypeptide formed was completely precipitated by adding petroleum ether, washed with hot ethyl acetate, and dried.

The three homopolypeptides, poly($\gamma$-benzyl L-glutamate) (PBLG) poly-$\epsilon$-carbobenzoxy-L-lysine (PCBL) and polyglycine (PG), were prepared in a similar way. Further, the poly($\gamma$-benzyl L-glutamate) sample was fractionated by using Bio-glass 200 with dioxane as the effluent solvent. Four fractions were obtained.

The composition of the heteropolypeptides was determined using a Beckman Amino Acid Analyser. The composition of the polypeptides is given in Table 1.

## Removal of Protecting Groups

Polypeptides with free side-chain groups were obtained by debenzylation and decarbobenzoxylation by using a 36% (w/v) HBr

solution in glacial acetic acid; the bromide formed was converted to free base with the help of IRA-45 (OH) column.

## Viscosity

Experiments with tricopolypeptides were carried out in two random coil solvents: dichloroacetic acid (DCA) and an aqueous solution which is 8 M with respect to urea and 0.15 M with respect to KCl; the latter solvent was used with "deblocked" samples of polypeptides. The viscosity of PBLG was determined in a mixture of 2-diethylene glycol (DEG) and dichloroethane (DCE) (20:80 v/v), reported to be theta solvent [15] at 25°C for PBLG; it was also studied in the helicogenic solvent dimethylformamide (DMF). The viscosities of all the three homopolypeptides were determined in DCA as well.

Viscosity measurements were made by using an Ubbelohde type dilution viscometer (of Pyrex glass) in which 5 ml of solution could be diluted to 50 ml. The solvent and solutions were passed through a sintered glass crucible (G-4) to free them from any dust particles. Time of flow was recorded with two stop watches, reading directly to 0.1 sec, and the average of five measurements was taken. As the effluent time for the solvents was greater than 180 sec, no kinetic energy correction was made. The density of the solution was taken to be the same as that of the solvent.



# RESULTS AND DISCUSSION

Huggins plots of $\eta_{sp}/c$ versus c exhibited an abnormal feature in the low concentration region. The curves, in general, could be considered to be made up of two parts: a straight line part and a steeply ascending part. The intrinsic viscosity of the systems was obtained by extrapolating the linear part of the Huggins plots and some typical values obtained in DCA are incorporated in Table 1. If one were to assume the validity of Mark-Houwink equation for copolypeptide systems, and the values of constants a and k of Doty [16] for PBLG in DCA, the molecular weight range of the samples would be roughly 15,000-34,000.

The steeply ascending portion of the Huggins plots is only germane to the present discussion. Some typical plots are shown in Figs. 1 and 2. The abnormal behavior of poly($\gamma$-benzyl glutamate) and poly-$\epsilon$-carbobenzoxylysine in the random coil solvent DCA is depicted in Fig. 1 (curves 1 and 2); similar behavior of two tricopolypeptides,

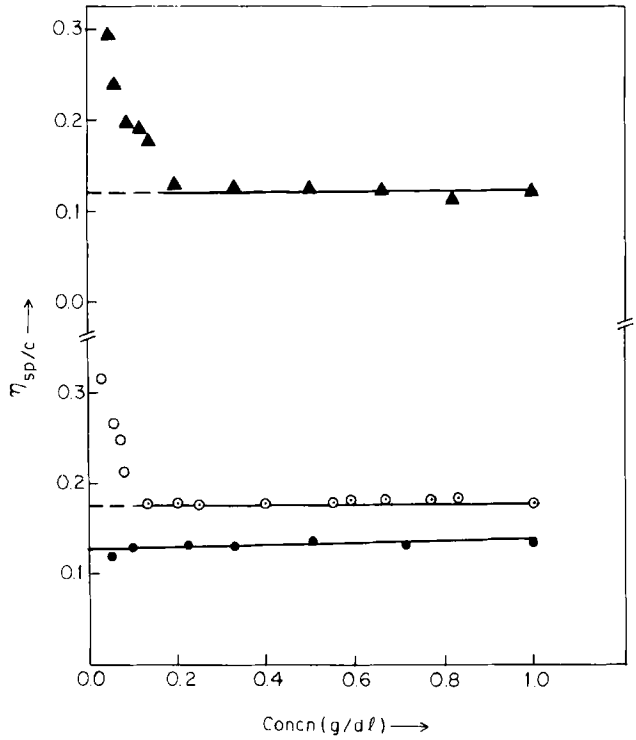


FIG. 1. Reduced viscosity of homopolypeptides as a function of concentration at 25°C in dichloroacetic acid (DCA): (⊙) poly($\gamma$-benzyl L-glutamate) (PBLG); (▲) poly-$\epsilon$-carbobenzoxy-L-lysine (PCBL); (●) polyglycine (PG).

PP3-1 and PP3-6, in the same solvent is shown in Fig. 2 (curves 1 and 2). The anomalous increase in reduced viscosity of two "deblocked" tricopolypeptides, PP3-7 and PP3-6, in 8 M urea-0.15 M KCl, can be seen from curves 3 and 4 of Fig. 2. Huggins plots of data of PBLG in the theta solvent DCE + DEG, as also in the helicogenic solvent DMF showed the anomalous feature. In fact, all the systems studied by us, except polyglycine (curve 3, Fig. 1) exhibited the abnormality whether the solvent is a helicogenic (poor), random coil (good) or theta solvent.

An analysis of the reduced viscosity data of the tricopolypeptides did not reveal any correlation between the amino acid composition of the polypeptides and the abnormal behavior. This is particularly striking in view of the rather high glycine content in some of the

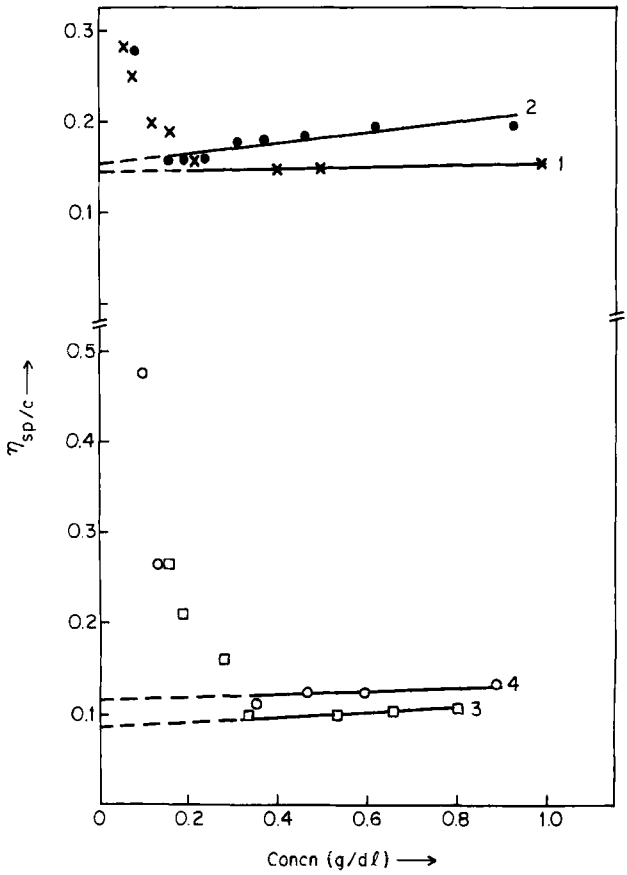


FIG. 2. Reduced viscosity of tricopolypeptides as a function of concentration at 25°C: (1) PP3-1 in DCA; (2) PP3-6 in DCA; (3) PP3-7 in 8 M urea-0.15 M KCl; (4) PP3-6 in 8 M urea-0.15 M KCl.

polypeptides; PP3-6 has as high a glycine content as 69.8%. Again, the concentration of the solution, at which the abnormality sets in, varied from system to system. However, the abnormality, in general, sets in at higher concentration for "deblocked" tricopolypeptides (in 8 M urea) as compared to blocked samples (in DCA).

According to Ohrn [8], the abnormality is due to adsorption of polymer molecules on the capillary of the viscometer. Adsorption, on one hand would lead to a small loss of polymer concentration, and

on the other hand, the adsorbed film on the wall of the capillary would decrease the capillary bore size. Reisler and Eisenberg [7] considered the latter effect, and showed that the decrease in capillary radius could be evaluated from the difference in flow time ($\Delta t$) between the flow time of pure solvent, and the extrapolated flow time at zero concentration of a plot of flow time against concentration of solution. By taking into account the adsorption effect on the radius of the capillary, they successfully eliminated the anomalous feature in the Huggins plot for bovine glutamate dehydrogenase. We adopted Reisler and Eisenberg's approach and modified our data suitably. We found, interestingly, that in a few cases there is some decrease in the abnormality, as for PP3-1 in DCA (curves 1 and 2, Fig. 3) but in other systems there was hardly any improvement (curves 3 and 4 for PP3-6 in 8 M urea-0.15 M KCl).

Unpublished results from our laboratories showed that there was no significant difference in the nature of the abnormality, even on a hundred per cent increase in the size of the capillary. Again, adsorption effects ought to be sensitive to temperature changes. However, even on increasing the temperature from 25 to 55°C, no significant change in the anomalous behavior was observed, for example, with PP3-1. The intrinsic viscosity of PP3-1, however, has a maximum value around 38°C. This is similar to the observation of Salahuddin et al. [17] on some globular proteins. It appears from the above that adsorption is not the deciding factor in causing the anomalous viscous behavior, at least for the systems under study.

It is well known that the presence of ionizable groups in a polymer would cause abnormal behavior at low concentration due to electrostatic repulsion, referred to as "polyelectrolyte effect." But as Booth [18] had shown, at an ionic strength of about 0.01, the surface charge on a protein would be effectively screened and the electroviscous effect would be suppressed for naturally occurring proteins. The polyelectrolytic effect, it should be noted, would not be expected to be observed in systems such as poly($\gamma$-benzyl glutamate) (PBLG) which has no ionizable groups. However, Bradbury [10, 11] suggested that the backbone of polypeptide chains, say of PBLG, would be charged in strong acids like DCA and TFA, and that this charging would lead to electroviscous effects. Electrophoretic experiments of Bradbury and Yuan [19] clearly showed that poly($\gamma$-benzyl glutamate) dissolved in TFA is protonated. Unfortunately, the extent of charging could not be determined. Hence, it is difficult to estimate the importance of this charge in determining the anomalous viscous behavior. Balasubramanian [9] also ascribed the abnormal behavior to polyelectrolytic effect, but claimed that the charge originates due to a hydrolysis of a very small number of side chain ester groups in DCA.

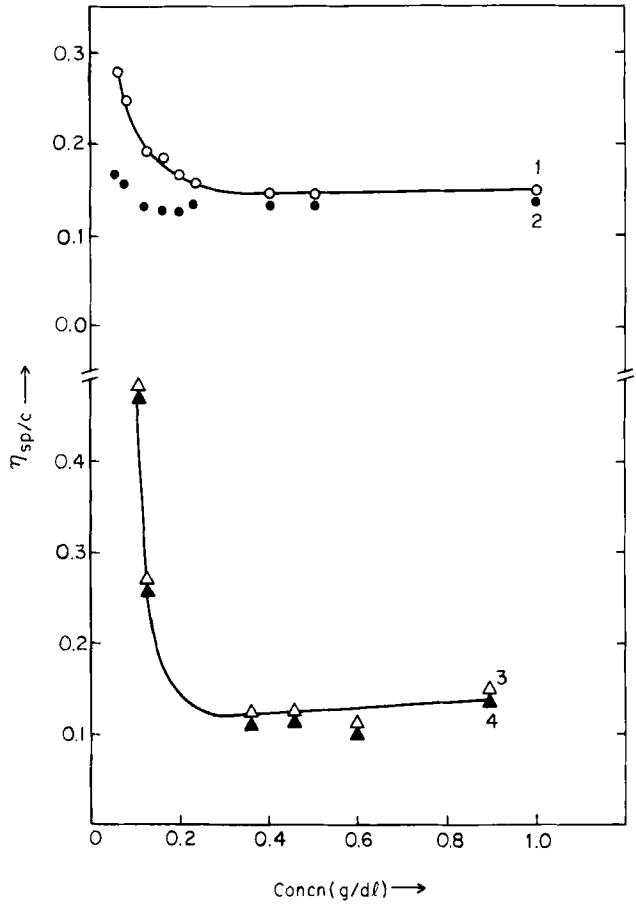


FIG. 3. Huggins plots for ( ○ ) PP3-1 in DCA and ( △ ) PPE-6 in 8 M urea-0.15 M KCl and modified Huggins plots for ( ● ) PP3-1 and ( ▲ ) PP3-6.

It is surprising, however, that even in presence of 0.15 M KCl, the abnormal behavior is shown (Fig. 2) by tricopolypeptides in 8 M urea-0.15 M KCl solutions. Here, the $\gamma$-COOH and $\epsilon$-$NH_2$ groups of the glutamyl and lysyl side chains are free, and the apparent pH of the systems is about 8. But the molarity of KCl is 0.15, which would give a concentration of ions (due to KCl) roughly ten times than that of ions arising out of an assumed complete ionization of

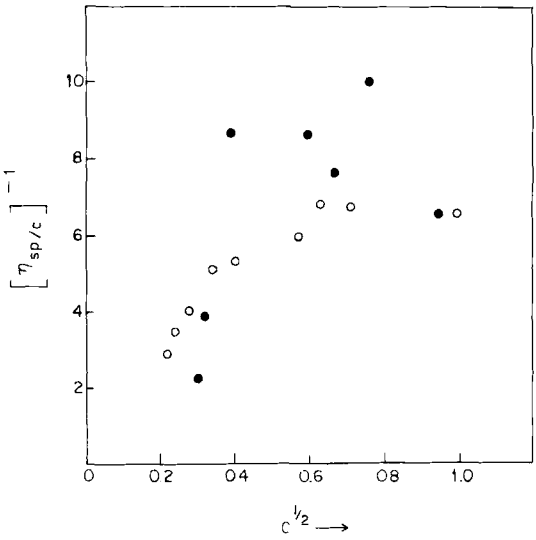


FIG. 4. Fuoss plots: (○) for PP3-1 in DCA and (●) for PP3-6 in 8 M urea-0.15 M KCl.

side chain groups of the polypeptides. The side-chain charges should then effectively be screened and, consequently, the electroviscous effect should not have been observed. Further, the experimental data in aqueous as well as nonaqueous systems, did not yield linear Fuoss [20] plots when $[\eta_{sp}/c]^{-1}$ is plotted against $c^{1/2}$; two typical plots are shown in Fig. 4. This clearly indicates that our systems do not conform to the behavior of polyelectrolytes. In view of the above, it is difficult to consider charging of the polypeptides as the cause of abnormal viscous behavior.

An understanding of the abnormal viscous behavior of the polymeric systems should perhaps then be sought in terms of the effect of concentration on the "packing" of macromolecules in solution. In the concentration range normally used for viscosity measurements (0.2-1.0 g/dl), the polymer molecules would be quite close and may even be touching each other. As the concentration is decreased there is scope for the expansion of the polymer coils. Further, the macromolecular chains would have more freedom to move independently of each other than at higher concentration and the resultant disentanglement of the chains [5] would cause a sharp rise in reduced viscosity. Our observation, that the concentration, at which the abnormal rise

commences is, in general, higher in DCA (when bulky side chain protecting groups are intact) than in aqueous urea-KCl medium, is in keeping with the concept of chain disentanglement.

Adsorption on the capillary and chain disentanglement at low concentration, we believe, are the factors which operate in all solvents, both polar and nonpolar. Of the two, chain disentanglement seems to be the prime factor responsible for the anomalous effect as the nature of the solvent, be it a poor, good, or theta solvent, has apparently, no influence on the occurrence of the phenomenon. The exceptional behavior of polyglycine seems to only support the above.

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