Intermolecular Association of Tetra-(γ-benzyl-L-glutamate)s in Ethylene Dichloride Solutions

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Synopsis

Light scattering from ethylene dichloride solutions of tetra-(γ-benzyl-L-glutamate)s has been measured and their association in solution is examined. One of the peptides is monodisperse *o*-nitrophenylthio-tetra-(γ-benzyl-L-glutamate) ethylamide prepared by a stepwise condensation method, and the other is low-molecular-weight poly(γ-benzyl-L-glutamate) prepared by the *N*-carboxyanhydride method with *n*-hexylamine initiation at $[A]/[I] = 4$ and fractionated by dissolution in formic acid. Concentration-dependent association of both peptides occurs noncooperatively, without giving critical micelle concentrations. The aggregate size is small: about 23 for the former tetrapeptide and about 7 for the latter polypeptide. While angular dissymmetry is close to unity, light scattering shows anomalous angular dependence, the intensity being symmetrically low with respect to the scattering angle of 90°. The observed angular dependence is interpreted in terms of the effect of optical anisotropy of peptide units. Formation of the anisotropic phase in concentrated solutions of these peptides is also examined briefly.

INTRODUCTION

Monodisperse oligopeptides can form aggregates in solution, if the solvent interacts weakly with peptide groups. The aggregation of oligopeptides is induced by hydrogen bonding between peptide groups, thus accompanying a conformational change of amino acid residues from the σ-form to the β-form. Our previous work[1-3] on *o*-nitrophenylthio-hexa-(γ-benzyl-L-glutamate) ethylamide demonstrated that the hexapeptide forms micellar aggregates in ethylene dichloride and dioxane, thus exhibiting a critical concentration for micelle formation, as well as for conformational change in each solvent.

We have recently shown by means of ir spectra that *o*-nitrophenylthio-tetra-(γ-benzyl-L-glutamate) ethylamide prepared by a stepwise condensation method[4] is subject to a concentration-dependent change of the conformation, from the σ- to the β-form in ethylene dichloride and chloroform, without giving any critical concentrations.[5] In the present work, we examine the accompanying aggregation of *o*-nitrophenylthio-tetra-(γ-benzyl-L-glutamate) ethylamide in ethylene dichloride by means of light scattering and viscosity measurements. We show that the aggregation of the tetrapeptide takes place noncooperatively, similar to the conformational change, and that the mode of aggregation of the tetrapeptide is very different from that of the hexapeptide.

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Some years ago, we investigated the aggregation of low-molecular-weight poly(γ-benzyl-L-glutamate) in ethylene dichloride, which was prepared by the polymerization of γ-benzyl-*N*-carboxy-L-glutamate anhydride initiated with *n*-hexylamine at $[A]/[I]$ ratio of 4.[6] In that work, we found a series of solvents for promoting both hydrogen bonding and aggregation of the polypeptide and demonstrated that ethylene dichloride is more effective for both intermolecular hydrogen bonding and aggregation than chloroform and dioxane.[6-8] The same series holds for the *o*-nitrophenylthio-oligo-(γ-benzyl-L-glutamate) ethylamides, as was shown previously.[1-5] In the present work, we measure the light scattering of low-molecular-weight poly(γ-benzyl-L-glutamate) hexylamide prepared at $[A]/[I]$ 4, dissolved in ethylene dichloride and investigate its mode of aggregation.

We compare results of the monodisperse tetrapeptide and the low-molecular-weight polypeptide. In addition, we observe anomalous angular dependence of light scattering from these peptide aggregates in ethylene dichloride, and we interpret these observations as the manifestation of optical anisotropy of γ-benzyl-L-glutamate residues. We also examine the behavior of concentrated solutions of these oligopeptides in ethylene dichloride with respect to the formation of optically anisotropic phases.

EXPERIMENTAL

Materials

The sample of *o*-nitrophenylthio-tetra-(γ-benzyl-L-glutamate) ethylamide was the same as previously used.[4,5] It was synthesized by the fragment condensation of two dipeptides followed by substitution of the carbonyl end. It consisted of yellow-colored crystals, and had an absorption band at 376 nm in ethylene dichloride, the molar extinction coefficient of which was 3737 L mol^{-1} cm^{-1} in the region of concentrations lower than 0.03×10^{-2} g cm^{-3}. Its formula molecular weight is 1075. Concentrations of the solution were determined by the measurement of absorption spectra.

The sample of low-molecular-weight poly(γ-benzyl-L-glutamate), prepared by polymerization with hexylamine initiation at $[A]/[I]$ 4, was the same as previously used.[6] It had a hexylamino group at the carboxyl end and a formic acid group at the amino terminal, after fractionation with formic acid. The number-average molecular weight of this polypeptide, determined by vapor pressure osmometry in dioxane and ethylene dichloride was found to be approximately equal to the formula molecular weight, 1008, and this value was taken as its molecular weight.

The sample of *o*-nitrophenylthio-hexa-(γ-benzyl-L-glutamate) ethylamide was the same as previously used.[1-3]

Ethylene dichloride was dried over CaH_2 and redistilled.

Methods

Light scattering was measured on a Shimadzu light-scattering photometer, PG-21, and the refractive index increment was measured on a Shimadzu differential refractometer, DR-3, both using unpolarized light of 546 nm from mercury lamps. Details of the procedure are described elsewhere.[3] The temperature was kept at 25 ± 0.05°C by circulating water of constant temperature through the cell jacket.

The viscosity of solution was measured at 25 ± 0.01°C by means of an Ubbelohde dilution-type viscometer having a flow time of 85 s for ethylene dichloride. The relative viscosity of a solution was obtained as the ratio of flow times for the solution and the solvent.

In order to examine the formation of the optically anisotropic phase in concentrated solutions of these oligopeptides in ethylene dichloride, solutions were sealed in a glass tube or a rectangular cell and incubated at 25 ± 0.01°C for a week or two. The optical anisotropy (birefringence) was observed at room temperature (25 ± 2°C) by an Olympus BH microscope with a crossed nicol.

CONCENTRATION DEPENDENCE OF LIGHT SCATTERING

Figure 1 shows the concentration dependence of the refractive index increment of a solution over the solvent, $\tilde{n} - \tilde{n}_0$, where the concentration is expressed by c (g cm^{-3}). While the tetrapeptide gives a straight line, the low-molecular-weight polypeptide shows a concave downward curve. The specific refractive index increment, $d\tilde{n}/dc$, is 0.108 cm^3 g^{-1} for the tetrapeptide, and it is comparable to the value, 0.107 cm^3 g^{-1}, for the corresponding hexapeptide. The refractive index increment is almost linear for the low-molecular-weight polypeptide up to 0.3 × 10^{-2} g cm^{-3}, giving a value of 0.141 cm^3 g^{-1}, where the light-scattering experiments are performed. Note that the concentration dependence of the specific refractive index increment was observed for the hexapeptide in dioxane in the region of the critical micelle concentration.[2] High-molecular-weight poly(γ-benzyl-L-glutamate) has a specific refractive index increment around 0.109 cm^3 g^{-1} in ethylene dichloride.[9]

The reduced intensity of light scattering in the 90° direction, R_{90}, for the tetrapeptide and the low-molecular-weight polypeptide in ethylene dichloride is shown in Fig. 2, together with the angular dissymmetry at 45°, z_{45}, as plotted against concentration. In contrast to the hexapeptide solutions[2,3] that have critical micelle concentrations, light scattering of the tetrapeptide solution gradually increases with increasing concentration, without a sudden rise. The low-molecular-weight polypeptide behaves similarly, but the increase in reduced intensity with increasing concentration is even lower. Similarly, the

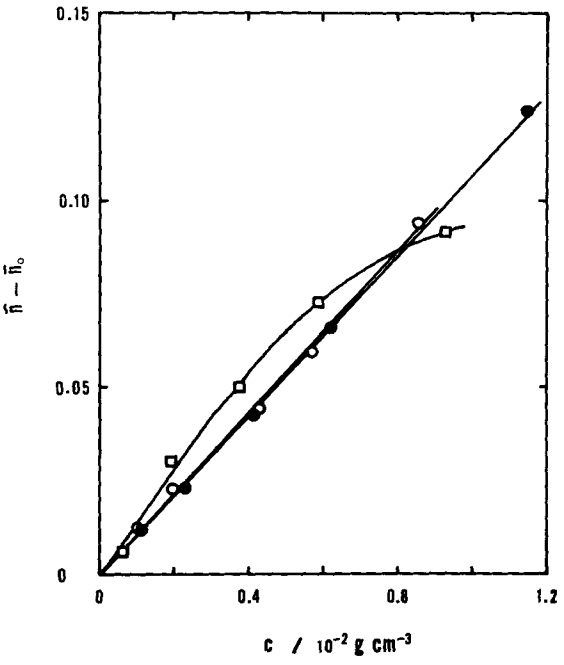

Fig. 1. Concentration dependence of refractive index increment of oligopeptides in ethylene dichloride. ○, Tetrapeptide; □, low-molecular-weight polypeptide; ●, hexapeptide.

angular dissymmetries of the tetrapeptide and the low-molecular-weight polypeptide solutions increase only slightly with increasing concentration, but the hexapeptide solution exhibits a sudden rise of angular dissymmetry above the critical concentration.[3]

These results suggest that, as their concentrations are increased, small aggregates are formed gradually by both the tetrapeptide and the low-molecular-weight polypeptide without any critical concentrations. The aggregates of the low-molecular-weight polypeptide are smaller than those of the tetrapeptide.

Light scattering of these solutions can be related to the molecular weight of solute particles by

$$\frac{Kc}{\Delta R_\theta} = \frac{1}{M_w' P'(\theta)} + 2B'c \tag{1}$$

where

$$K = 2\pi^2 \tilde{n}_0^2 \left(\frac{d\tilde{n}}{dc}\right)^2 \Big/ N_A \lambda^4 \tag{2}$$

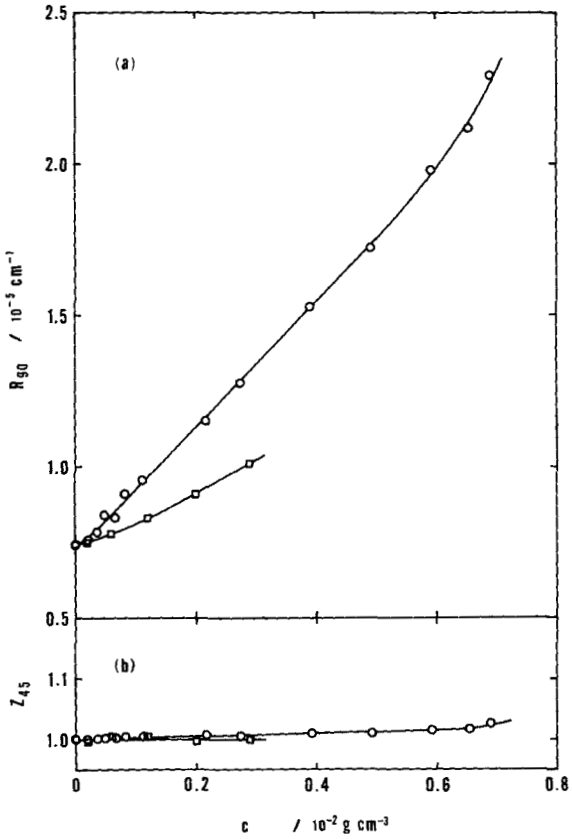

Fig. 2. Concentration dependence of light scattering from solutions of oligopeptides in ethylene dichloride. (a) R_{90}; (b) z_{45}. ○, Tetrapeptide; □, low-molecular-weight polypeptide.

and

$$\Delta R_\theta = R_\theta - R_\theta^0 \tag{3}$$

Here, M_w' is the weight-average molecular weight of all peptide species in solution, B' the corresponding second virial coefficient, and $P'(\theta)$ the corresponding average particle scattering factor. N_A is Avogadro's number, and λ is the wavelength of light *in vacuo* (546 nm). The reduced intensity at the scattering angle, θ, of a solution of concentration c is represented by R_θ; that of the solvent is R_θ^0. The reduced intensity is defined by

$$R_\theta = r^2 i_\theta / I_0(1 + \cos^2\theta) \tag{4}$$

where I_0 is the intensity of incident light, and i_θ is the intensity of scattered light at the distance, r, from the solution.

If the weight-average molecular weight of aggregates, excluding monomers, is represented by M_w, then the average molecular weight, M_w', at concentration c is given by

$$M_w' = w_1 M_1 + (1 - w_1) M_w \tag{5}$$

where M_1 is the molecular weight of monomer, 1075 or 1008 for the oligopeptides, and w_1 is its weight fraction. The monomer concentration is then given by

$$c_1 = w_1 c \tag{6}$$

The weight-average aggregation number of aggregates is expressed by

$$m' = M_w'/M_1 \tag{7a}$$

or

$$m = M_w/M_1 \tag{7b}$$

Figure 3 shows light scattering of the tetrapeptide and the low-molecular-weight polypeptide solutions in the 90° direction, plotted according to Eq. (1). The tetrapeptide associates into aggregates sharply with increasing concentration, and its average size is $M_w' = 25{,}100$, or $m' = 23$ at 0.69×10^{-2} g cm^{-3}, assuming $B' = 0.5 \times 10^{-4}$ cm^3 g^{-1} and $P'(\theta) = 1$. (The value of B' was taken from that found for the

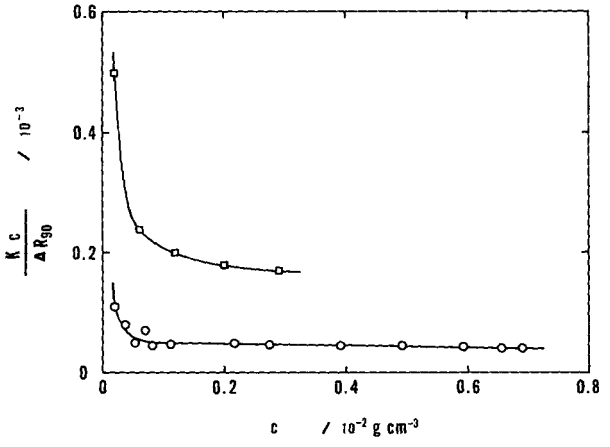

Fig. 3. Reciprocal reduced intensity, $Kc/\Delta R_{90}$, of the oligopeptides in ethylene dichloride as a function of concentration. ○, Tetrapeptide; □, low-molecular-weight polypeptide.

secondary micelles of the corresponding hexapeptide in ethylene dicloride,[3] and it is usually independent of self-association.) Later it is shown that the aggregate size derived here includes only a negligible contribution of monomers, less than 5%, at concentrations above 0.1 $\times$ 10^{-2} g cm^{-3}, so that the aggregation number of the aggregates, exclusive of monomers, is also 23.

We have previously shown that the hexapeptide in ethylene dichloride forms primary micelles having an aggregation number of 48 at the critical micelle concentration, 0.075 $\times$ 10^{-2} g cm^{-3}, and that, with an increase in micelle concentration, they further associate into secondary micelles having an aggregation number of 294.[2,3] Thus the aggregation number of aggregates of the tetrapeptide is less than half that of the hexapeptide.

The light-scattering plot for the low-molecular-weight polypeptide also decreases with increasing concentration, but less sharply, and the average size of the aggregates is given by M'_w = 6200 or m' = 6.2 at 0.29 $\times$ 10^{-2} g cm^{-3}, assuming B' = 0.5 $\times$ 10^{-4} cm^3 g^{-1} and $P'(\theta)$ = 1. As will be shown below, the concentration of monomer of the low-molecular-weight polypeptide remains low, i.e., less than 15%, at concentrations above 0.1 $\times$ 10^{-2} g cm^{-3}, so that the aggregation number of the aggregates, excluding monomers, is 6.6.

In spite of the low dissymmetry of light scattering, these solutions of oligopeptides exhibit anomalous angular dependence, as will be shown below. However, the aggregation numbers of their aggregates would not differ from the true values, even if this effect is taken into account. We may then state that aggregate size is largely lowered by the polydispersity of the peptide molecules.

According to Steiner's analysis,[10] the weight fraction of monomer, w_1, in a self-associating solute at a given concentration, c, can be calculated from the weight-average molecular weight, M_w', as

$$w_1 = \exp \int_0^c \left(\frac{M_1}{M_w'} - 1 \right) \frac{dc}{c} \tag{8}$$

Applying Eq. (8) to the present data of light scattering and carrying out graphical integration, the fraction of association, $1 - w_1$, and the monomer concentration, c_1, can be calculated as functions of concentration. Figure 4 shows the results for the tetrapeptide and the low-molecular-weight polypeptide in ethylene dichloride. The concentrations of monomers remain very low over the whole region examined. The fraction and concentration of monomer are lower for the tetrapeptide than for the low-molecular-weight polypeptide, possibly suggesting the effect of polydispersity of the peptide molecules on their aggregation.

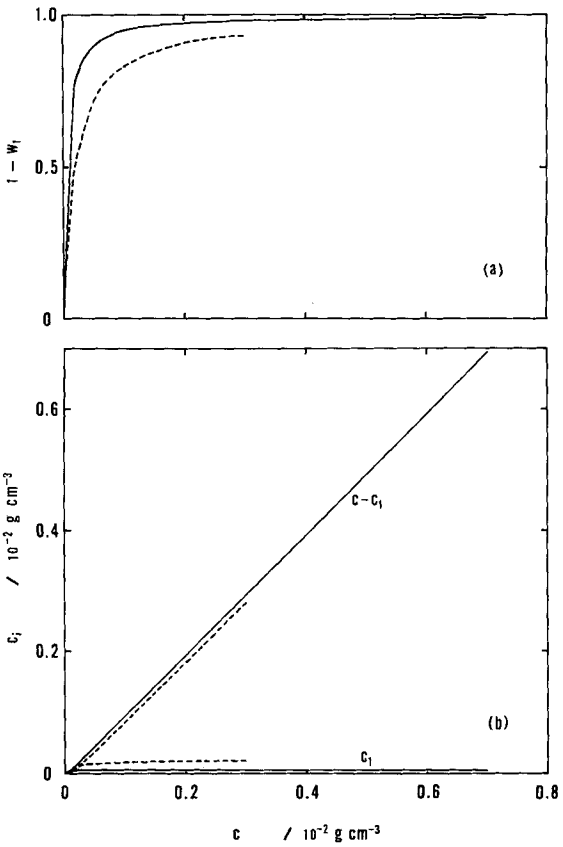

Fig. 4. Fraction of association, $1 - w_1$, and the monomer and aggregate concentrations, c_1 and $c - c_1$, of the oligopeptides in ethylene dichloride. (a) $1 - w_1$; (b) c_1 and $c - c_1$. Full line, tetrapeptide; broken line, low-molecular-weight polypeptide.

ANGULAR DEPENDENCE OF LIGHT SCATTERING

In spite of low angular dissymmetries of light scattering, the tetrapeptide and the low-molecular-weight polypeptide solutions exhibit strong angular dependence of light scattering (Fig. 5). The reciprocal scattering intensities have minima in the 90° direction and are symmetrically high at both low and high angles. Such a tendency is commonly observed at all concentrations of both peptides, and this type of angular dependence could be caused by the effect of optical anisotropy of constituent units.[11-14] As will be seen below, this effect is least in the 90° direction, and, consequently, the weight-average molecular weight derived above would not be significantly influenced by this effect; thus it will not differ appreciably from the true value.

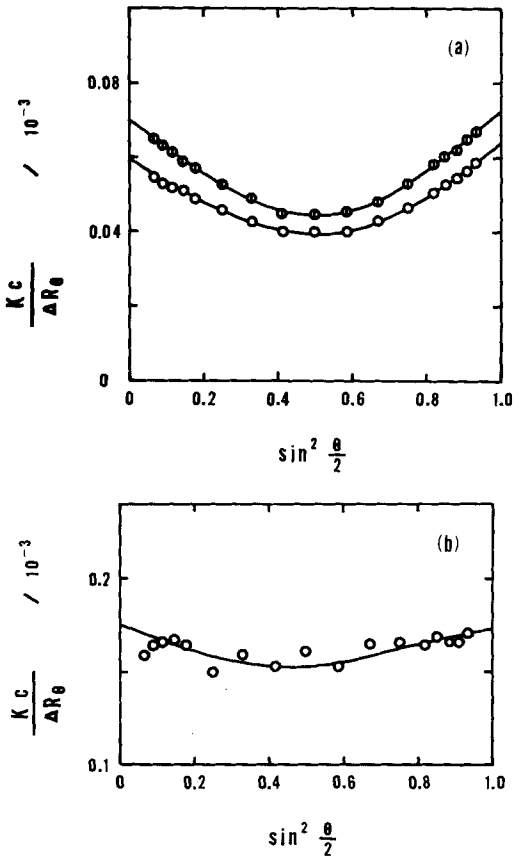

Fig. 5. Angular dependence of light scattering. (a) Tetrapeptide, ⊕, 0.493 × 10^{-2} g cm^{-3}; ○, 0.690 × 10^{-2} g cm^{-3}. (b) Low-molecular-weight polypeptide, ○, 0.291 × 10^{-2} g cm^{-3}.

The theory of light scattering from dilute solutions of optically anisotropic macromolecules has been developed by many investigators. The effect of optical anisotropy depends on the shape of macromolecules. So far, the rigid rod,[15-17] random coil,[16-20] and wormlike chain[14,21,22] have been treated. We have previously found that the tetrapeptide aggregates into a hydrogen-bonded network consisting of both free and hydrogen-bonded peptide residues.[5] The low-molecular-weight polypeptide also aggregates similarly, but to a lesser extent.[6,8] The content of β-structure or the fraction of intermolecularly hydrogen-bonded residues is less than 30% in the range of concentrations examined. Thus, we may imagine that the aggregates of these peptides are approximately spherical and behave like freely jointed random-coil chains, although the number of optically anisotropic units is somewhat too small: the number of units is 4 × 23 for the tetrapeptide

and 4×6.2 for the low-molecular-weight polypeptide, if the terminal groups are not included.

When the principal polarizabilities of each peptide unit are represented by α_1, α_2, and α_3, the optical anisotropy of the unit, δ_0, is defined by[21]

$$\delta_0^2 = \frac{(\alpha_1 - \alpha_2)^2 + (\alpha_1 - \alpha_3)^2 + (\alpha_2 - \alpha_3)^2}{2(\alpha_1 + \alpha_2 + \alpha_3)^2} \tag{9}$$

Introducing the (average) overall optical anisotropy of all the aggregates, including monomers, in solution in the approximation of freely jointed chains of units,[14,23] we have

$$\delta^2 = \delta_0^2/xm' \tag{10}$$

where x is the (average) degree of polymerization of peptides. It is noted that the definition of the overall anisotropy given by Eqs. (9) and (10) differs from that of Utiyama and Kurata, δ_{UK}[19,20]; that is, $\delta_{UK} = \delta^2/5$.

Light scattering from dilute solutions of spherically symmetric aggregates consisting of xm' anisotropic units is then given by

$$\frac{\Delta R_\theta}{KM_w'c} = P'(\theta)[1 - 2B'M_w' P'(\theta)c] + \frac{\delta^2}{5}\frac{13 + \cos^2\theta}{1 + \cos^2\theta} \tag{11}$$

provided that the incident light is unpolarized.[11,16,19,20] It is seen that the second term should have a magnitude comparable with the first term for the anisotropic effect of the constituent units to be manifest. According to Eq. (10), δ^2 decreases as the number of units increases for the aggregates of these oligopeptides, so that the effect of optically anisotropic units should be larger for smaller aggregates, whenever light scattering is measurable.

Equation (11) can be converted into a more convenient form[19,20]

$$\frac{\Delta R_\theta}{KM_w'c} = 1 - 2B'M_w'c - (1 - 4B'M_w'c)\frac{\mu^2}{3} + \frac{\delta^2}{5}\frac{13 + \cos^2\theta}{1 + \cos^2\theta} \tag{12}$$

where

$$\mu = \frac{4\pi\tilde{n}_0}{\lambda} R_G' \sin\frac{\theta}{2} \tag{13}$$

and R_G' is the average radius of gyration of solute particles. When

TABLE I
Overall Optical Anisotropy of Aggregates of Oligopeptides in Ethylene Dichloride

Oligopeptide	c (10^{-2} g cm^{-3})	δ^2
o-Nitrophenylthio-tetra(γ-benzyl-L-glutamate) ethylamide	0.493	0.30
	0.690	0.28
o-Nitrophenylthio-hexa(γ-benzyl-L-glutamate) ethylamide[a]	0.155	0.0070
Poly(γ-benzyl-L-glutamate) $[A]/[I] = 4$	0.291	0.11

[a] Contributions from the monomers are eliminated by substituting the micelle concentration, 0.080×10^{-2} g cm^{-3}, for c in Eq. (11) or (12), so that only the micelle properties, M_w, B, and R_G, are included.

applying Eq. (12) to the present data, we first plot the difference, $(\Delta R_\theta - \Delta R_{180-\theta})/KM'_w c$, against $1 - 2\sin^2(\theta/2)$ and obtain the value of $(1 - 4B'M_w'c)\,\mu^2/[3\sin^2(\theta/2)]$ from the initial slope. We can then plot $\Delta R_\theta/KM_w'c + (1 - 4B'M_w'c)\,\mu^2/3$ against $(13 + \cos^2\theta)/(1 + \cos^2\theta)$ and obtain the value of δ^2 from its slope. The values of δ^2 derived in this way for the tetrapeptide and the low-molecular-weight polypeptide are listed in Table I.

We have previously published the results of light scattering from micellar solutions of the corresponding hexapeptide in ethylene dichloride and found a similar anomaly in the angular dependence of light scattering in the region slightly above the critical micelle concentration.[3] This result could have occurred because the second term of Eq. (11) is comparable with the first term in this concentration region and the intensity of scattered light is sufficiently strong. Substituting the micelle concentration for the total concentration, c, in Eq. (12) and regarding all the average quantities as those for the primary micelles alone, we can similarly derive the value of δ^2, as given in Table I.

The overall optical anisotropy of aggregates of the tetrapeptide is not dependent on the concentration, but the values of optical anisotropy of these aggregates are different, depending on the peptide. Using Eq. (10), we can calculate the values of the optical anisotropy of each peptide unit, δ_0^2, some of which are larger than unity.

The optical anisotropy of a peptide unit has been estimated by the measurements of the Kerr effect of some simple amides in solution. Khanarian et al.[24] evaluated principal polarizabilities of *N*-methylacetamide in dioxane for the D-line as $\alpha_1 = 9.42$, $\alpha_2 = 11.6$, and $\alpha_3 = 5.37$ (10^{-24} cm^3 molecule^{-1}), which leads to an optical anisotropy, $\delta_0^2 = 0.0018$.

Before the present results on the optical anisotropy of the peptide unit are compared with those for the model amide, we must take into

account the contribution of the side-chain group, i.e., the benzyl ester, as well as that of terminal groups; furthermore, we must examine the validity of the approximation of peptide aggregates in terms of freely jointed chains, as well as the effect of solvent.

Similar large values of optical anisotropy can be found in the published data for random-coil polymers and rigid rodlike macromolecules. Schurz et al.[18] reported $\delta^2 = 0.068$–1.21 for Vinyon N (a copolymer of 40% acrylonitrile and 60% vinyl chloride) in dimethylformamide. Utiyama and Kurata[19,20] observed $\delta^2 = 0.0089$–0.267 for isotactic polystyrene in chlorobenzene. Some of these values also lead to values of δ_0^2 exceeding unity. For rigid-rod macromolecules, Eq. (10) must be replaced by $\delta^2 = \delta_0^2$; that is, the overall anisotropy of the macromolecule is equal to that of the constituent unit.[14,16,17] Horn[16] found $\delta^2 = 0.092$ for tobacco mosaic virus in aqueous solution, Berry[14] gave $\delta^2 = 0$–1 for different fractions of a heterocyclic ladder polymer in methane sulfonic acid, and Wong et al.[25] also reported $\delta = 0.3$–1.0 for some ladder polymers such as Kevlar in similar solvents.

It is likely that some of the anomalous angular dependence of light scattering might also be caused by external interference as well as the optical anisotropy of constituent units; this could hold for some other polymers.[26–29] The present oligopeptide aggregates have, however, molecular weights that are too small to cause external interference.

CONCENTRATION DEPENDENCE OF REDUCED VISCOSITY

Figure 6 shows the concentration dependence of reduced viscosity, η_{sp}/c, of the oligopeptide solutions in ethylene dichloride. The reduced viscosity of the corresponding hexapeptide has been measured up to a higher concentration in the present work than previously.[3] The reduced viscosity of the tetrapeptide is very low, and this is consistent with the smaller aggregate size deduced from light-scattering measurements. It indicates that the aggregates have a compact structure. On the other hand, the reduced viscosity of the low-molecular-weight polypeptide behaves rather anomalously, as previously found,[8] and a somewhat swollen structure was suggested to interpret this observation. These differences in reduced viscosity are consistent with the ir results that indicate a lower content of β-structure or a lower fraction of intermolecularly hydrogen-bonded residues for the low-molecular-weight polypeptide than for the tetrapeptide.[5,7]

The high reduced viscosity of the hexapeptide solution has been adequately explained by the formation of secondary rodlike micelles with an increase in micelle concentration.[3] The break in the viscosity curve of the hexapeptide could be induced by the initiation of short-range intermicellar interaction at higher concentrations.

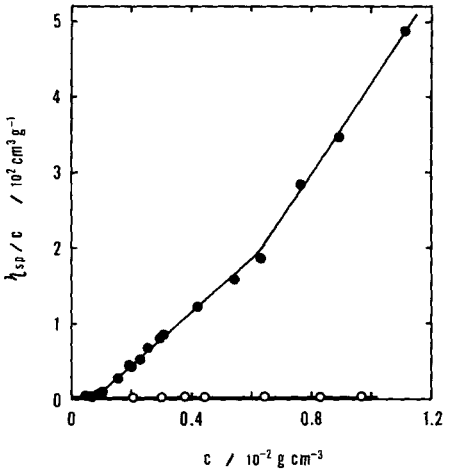

Fig. 6. Concentration dependence of reduced viscosity of the oligopeptides in ethylene dichloride. ○, Tetrapeptide; ●, hexapeptide.

FORMATION OF ANISOTROPIC PHASES

We have previously observed that the optically anisotropic (birefringent) phase develops in solutions of the low-molecular-weight polypeptide in ethylene dichloride, when the concentration is about 15–25% and the solutions are incubated for several days.[30]

In the present work, we have made similar observations on the tetrapeptide and hexapeptide in ethylene dichloride after incubation for a week or two. At concentrations of 4–19%, the tetrapeptide solutions contained a small amount of emulsion; at 55%, they formed an anisotropic phase having a fine, complicated texture, together with emulsion, under the crossed nicol [Fig. 7(a)]. On the other hand, in the hexapeptide solutions, the emulsion predominated over the anisotropic phase [Fig. 7(b)]. A higher content of β-structure in the hexapeptide could interfere with the formation of the anisotropic phase than the lower content of β-structure in the tetrapeptide.

Furthermore, the difference in the terminal group between the low-molecular-weight polypeptide and the tetrapeptide could also have a marked effect on the formation of the anisotropic phases.

CONCLUSION

The self-association in ethylene dichloride, of the monodisperse tetrapeptide and the low-molecular-weight polypeptide having average

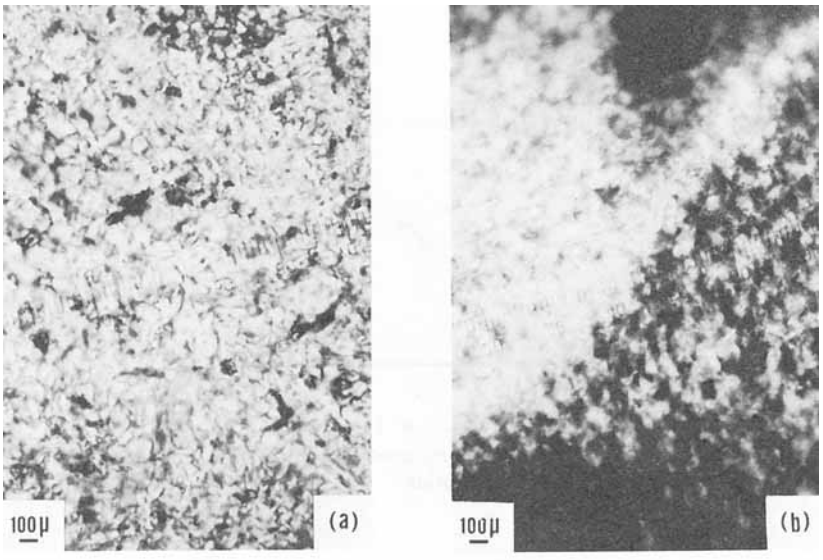

Fig. 7. Polarized micrographs of anisotropic phases formed by the oligopeptides in ethylene dichloride. (a) Tetrapeptide, 55.3 w/w %; (b) hexapeptide, 44.3 w/w %.

degree of polymerization of 4, was investigated by means of light-scattering and viscosity measurements. Both peptides form aggregates (aggregation numbers 23 and 7, respectively) as their concentrations are increased, but the former aggregates are more compact than the latter. The monodispersity of the peptide promotes its self-association to a higher aggregate size.

The aggregate size of the tetrapeptide, 23, is smaller than that of the hexapeptide, 48 (for the primary micelles), and this is a reason for the absence of any critical micelle concentration in the tetrapeptide solutions. For the formation of peptide micelles in weakly interacting solvents, more than four peptide groups must be present in a molecule; then, sufficiently large aggregates stabilized by intermolecular hydrogen bonds between peptide groups can be formed cooperatively.

It was found that the optical anisotropy of peptide groups revealed a strong effect on the angular dependence of light scattering because of the moderate size of the aggregates.

Concentrated solutions of the tetrapeptide and the low-molecular-weight polypeptide form optically anisotropic (birefringent) phases after long incubation, but they can do so more easily than those of the hexapeptide. The moderate content of β-structure of these two peptides would be suitable for the formation of anisotropic phases, compared with its high content in the hexapeptide.

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