Infrared Spectra and Conformation of Monodisperse Oligo-γ-Benzyl-L-Glutamates in Solution

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Synopsis

The ir spectra of o-nitrophenylthio-tetra- and hexa-γ-benzyl-L-glutamate ethylamides were measured at different concentrations in chloroform and ethylene dichloride. The molar extinction coefficients of two bands, each for the amide I and A modes, were observed as indicating the content of β structure and the fraction of hydrogen bonds; they were analyzed for elucidating the hydrogen-bonded state of peptide residues in the σ and β conformations of oligopeptides. While the content of β structure of the tetrapeptide increases with increasing concentration, the hexapeptide is in the β conformation above the critical concentration only. The fraction of hydrogen bonds remains finite even at infinite dilution or below the critical concentration, indicating the intramolecular hydrogen-bonding in the σ-form. As the fundamental structure of folded forms having only intramolecular hydrogen bonds, the 2_7 ribbon is most likely. With increasing concentration or above the critical concentration, the extended forms are stabilized by the intermolecular hydrogen bonds between residues of the β-form. The β-form is present only when intermolecular hydrogen bonds link two residues in an antiparallel way. Possible structures of the oligopeptides in the σ and β conformations in the two solvents are described briefly.

INTRODUCTION

Monodisperse oligopeptides have been good model compounds for elucidating the structure and properties of polypeptides and proteins in solution. We have synthesized monodisperse oligopeptides, o-nitrophenylthio-tetra- and hexa-γ-benzyl-L-glutamate ethylamides, by the stepwise condensation method and found by ir spectral measurements that both oligopeptides are in the β structure of antiparallel chains in the solid state.[1,2] We have further investigated the conformation, association, and structure of aggregates of the hexapeptide in solution by means of ir spectra, light scattering, and solution viscosity.[3,4] In dioxane and ethylene dichloride, the hexapeptide is in the σ conformation below the critical concentration, but is transformed into the β conformation and aggregated into micelles above that concentration. The micelles are composed of molecules which are hydrogen-bonded through their peptide residues. They can be classified into two types: the primary micelles are of a swollen structure, having a smaller number of intermolecular hydrogen bonds, while the secondary mi-

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celles, formed at higher concentrations in ethylene dichloride, have a compact structure consisting of the β structure of in-register form. In this paper and the forthcoming papers, however, we will show that the tetrapeptide does not form such micelles cooperatively but that they gradually change their conformation and aggregated state with concentration, without giving any critical concentration.

In this work, we report the results of ir spectral measurements on *o*-nitrophenylthio-tetra- and hexa-γ-benzyl-L-glutamate ethylamides, $o\text{-}NO_2\text{—}C_6H_4\text{—}S\text{—}[Glu(OBzl)]_x\text{—}NHC_2H_5$ with $x = 4$ and 6, in chloroform and ethylene dichloride. Both of these solvents induce the β conformation of low-molecular-weight poly(γ-benzyl-L-glutamate), but chloroform is weaker than ethylene dichloride in stabilizing the β conformation.[5] Our aim is to examine the ir spectra of both carbonyl (C=O) and amino (N—H) groups in peptide bonds exhibited in the amide I and A bands, respectively, and to distinguish the hydrogen-bonded states of these two groups, both in the σ and the β conformations.

EXPERIMENTAL

Materials

The samples of *o*-nitrophenylthio-tetra- and hexa-γ-benzyl-L-glutamate ethylamides were the same as previously prepared and used.[1,2] The tetrapeptide was prepared by condensing the *o*-nitrophenylthio-dipeptide with the HCl salt of dipeptide ethylamide or by converting the *o*-nitrophenylthiotetrapeptide *p*-nitrophenyl ester into its ethylamide. The hexapeptide was prepared by reacting the *o*-nitrophenylthiodipeptide with the HCl salt of tetrapeptide ethylamide.

Chloroform and ethylene dichloride were redistilled over CaH_2 and stored over the molecular sieve No. 4A. They were filtered before use. Solutions were prepared by dissolving the oligopeptide samples in these solvents, and ir spectra were measured after overnight.

Measurements

Infrared spectra were recorded on a Jasco ir spectrophotometer A-3 at room temperature (25 ± 2°C). The solutions or solvents were placed in cells with CaF_2 windows, having path lengths of 0.50 and 0.105 mm. The spectra were scanned over 5000–1500 cm^{-1}, and the spectrum of a solution was referred to that of the solvent, both recorded in the absorbance scale in the same cell. Then the spectrum of the oligopeptide was calculated in the optical-density scale in the amide A and I regions. Chloroform was almost clear in both the amide A and I regions, but ethylene dichloride was less transparent, having small absorption bands in these regions.

The molar extinction coefficient, ϵ_ν, at a given wave number, $\tilde{\nu}$, is expressed in the unit of 1 mol^{-1} cm^{-1}, on the basis of mol of oligopeptide, the molecular weights being 1075 for tetrapeptide and 1514 for hexapeptide.

RESULTS

IR Spectra

Figure 1 shows the ir spectra in the amide I region of the tetra- and hexapeptides in chloroform at various concentrations. Similar ir spectra are obtained for the tetrapeptide in ethylene dichloride at various concentrations. The amide I band around 1670 cm^{-1} of peptide bonds represents the C=O stretching mode and can be assigned to the σ-form, which was first designated by Blout and Asadourian[6] and is characteristic of unassociated or free molecules of short-chain poly(γ-benzyl-L-glutamate).[5] The band around 1630 cm^{-1} is ascribed to the amide I band of the β-form.[7,8] The σ-form is stable in more dilute solutions, while it is converted into the β-form at higher concentrations. The hexapeptide at 1.17×10^{-2} g cm^{-3} in chloroform exhibits a weak band at 1693 cm^{-1}, indicating that the oligopeptide molecules are arranged in an antiparallel way in the β conformation.[9,10] Table I lists the values of the wavenumbers of characteristic bands of the oligopeptides in chloroform and ethylene dichloride.

Figure 2 gives the concentration dependence of the molar extinction coefficient of amide I bands for the tetra- and hexapeptides in the two solvents. With increasing concentration, the coefficient of the amide

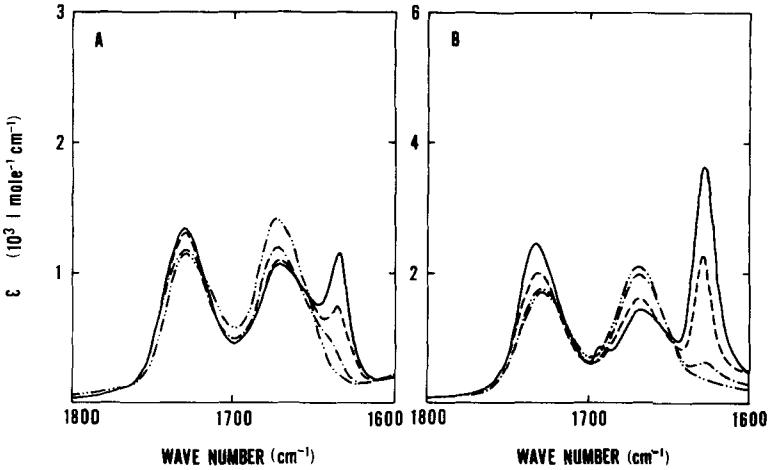

Fig. 1. Ir spectra in the amide I region of the oligopeptides in chloroform. Concentration (10^{-2} g cm^{-3}): (A) tetrapeptide: (– · · –) 0.35, (– · –) 1.33, (- - -) 2.38, (—) 2.96; (B) hexapeptide: (– · · –) 0.21, (– · –) 0.47, (- - -) 0.80, (—), 1.17.

TABLE I
Wave Number[a] of the Main IR Bands of Oligopeptides in Chloroform and Ethylene Dichloride

IR Band	Tetrapeptide		Hexapeptide
	Chloroform	Ethylene Dichloride	Chloroform
Amide A free	~3390	~3390	~3390
Amide A hydrogen-bonded	3300	3310	3290
Ester carbonyl	1730	1736	1733
Amide I σ	1673	1675	1669
Amide I $\beta(0, \pi)$			1693
Amide I $\beta(\pi, 0)$	1634	1632	1628

[a] In cm^{-1}.

I band of the β-form increases, while that of the σ-form decreases. The dependence is stronger in ethylene dichloride than in chloroform.

The molar extinction coefficients of amide I bands for the tetrapeptide gradually change with concentration, indicating gradual conversion between the σ- and the β-form. In contrast, the molar extinction coefficients of amide I bands for the hexapeptide remain constant up to the critical concentration, 0.43×10^{-2} g cm^{-3} or $2.84 \times 10^{-3}M$, and then the coefficient of the σ-form decreases and that of the β-form increases with further increasing concentration. This suggests that the hexapeptide molecules in chloroform associate into aggregates

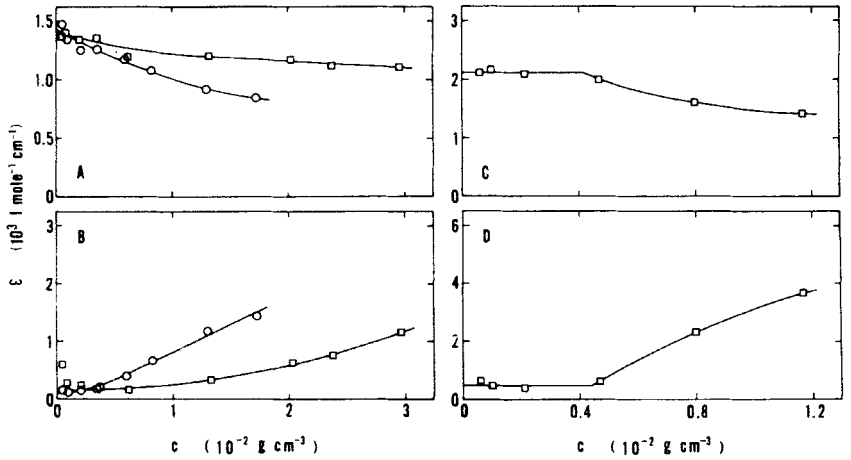

Fig. 2. Concentration dependence of molar extinction coefficients of amide I bands: (A) 1673 cm^{-1} for the tetrapeptide, (B) 1634 cm^{-1} for the tetrapeptide, (C) 1668 cm^{-1} for the hexapeptide, (D) 1626 cm^{-1} for the hexapeptide. Solvent: (□) chloroform, (○) ethylene dichloride.

cooperatively above the critical concentration, just like those in dioxane and ethylene dichloride.[2,3]

Figure 3 shows the ir spectra in the amide A region of the two oligopeptides in chloroform. The amide A band appears as two peaks, one around 3390 and the other about 3300 cm^{-1}. The amide A band represents the N—H stretching mode,[8,10] and its wave number changes, depending on the hydrogen-bonded state of N—H groups, whether they are free or hydrogen-bonded.[11-13] The amide A band shifts toward a lower wave number when the N—H group is hydrogen bonded. The amide A band around 3390 cm^{-1} can be assigned to the hydrogen-bond-free N—H groups, while the band appearing about 3300 cm^{-1} is due to the hydrogen-bonded N—H groups. Similar observation can be made for the tetrapeptide in ethylene dichloride.

The ratio of molar extinction coefficients of the two amide A bands, $\epsilon_{3300}/\epsilon_{3390}$, is plotted against concentration in Fig. 4, in order to see the concentration dependence of the hydrogen-bonded state of N—H groups. For these oligopeptides, the ratio increases with increasing concentration, but it should be noted that even at infinite dilution, the ratio does not disappear nor does it extrapolate to zero. For the tetrapeptide at infinite dilution, the ratio has values of about 1.2 in chloroform and 0.8 in ethylene dichloride, respectively, and it increases nearly linearly with increasing concentration. On the other hand, the hexapeptide in chloroform shows an almost constant value, 2.0, for the ratio below the critical concentration, and then it has values gradually increasing above that concentration.

Content of β Structure and Fraction of Hydrogen Bonds

If the C=O group of a peptide residue is hydrogen-bonded with the N—H group of another peptide residue and both these residues are arranged in an antiparallel way, then these residues could be assigned as the β-form and the amide I band appears around 1630 cm^{-1}. If a peptide residue is not in the β-form, it is in the σ-form and its amide

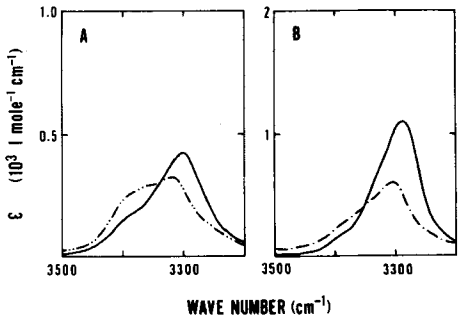

Fig. 3. Infrared spectra in the amide A region of the oligopeptides in chloroform: (A) Tetrapeptide in chloroform; concentration (10^{-2} g cm^{-3}): (-···-) 0.35, (—) 2.96, (B) Hexapeptide in chloroform; concentration (10^{-2} g cm^{-3}): (-·-) 0.47, (—) 1.17.

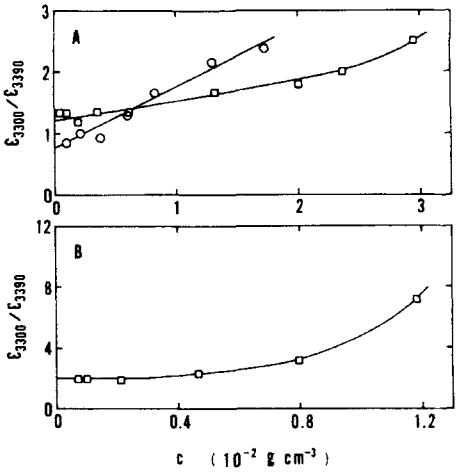

Fig. 4. Concentration dependence of the ratio of molar extinction coefficients of amide A bands: (A) tetrapeptide, (B) hexapeptide. Solvent: (□) chloroform, (○) ethylene dichloride.

I band appears around 1670 cm^{-1}. The fraction of residues in the β-form, that is, the content of β structure, f_β, can be obtained from the observed molar extinction coefficient, $\epsilon_{\tilde{\nu}_\sigma}$, as

$$f_\beta = 1 - f_\sigma \tag{1}$$

$$f_\sigma = \left(\epsilon_{\tilde{\nu}_\sigma} - \epsilon^\beta_{\tilde{\nu}_\sigma}\right) \Big/ \left(\epsilon^\sigma_{\tilde{\nu}_\sigma} - \epsilon^\beta_{\tilde{\nu}_\sigma}\right) \tag{2}$$

where f_σ is the fraction of residues in the σ-form, $\epsilon^\beta_{\tilde{\nu}_\sigma}$ and $\epsilon^\sigma_{\tilde{\nu}_\sigma}$ are the molar extinction coefficients of amide I bands of the residues in the β- and σ-forms, respectively, both at a wave number, $\tilde{\nu}_\nu$. Here $\tilde{\nu}_\sigma$ is the wave number of the amide I band around 1670 cm^{-1}.

For low-molecular-weight poly(γ-benzyl-L-glutamate) in dioxane, ethylene dichloride, and trimethyl phosphate–ethylene dibromide mixtures, it was previously shown[5] that an approximate relation

$$\epsilon^\beta_{\tilde{\nu}_\beta} = 2\,\epsilon^\sigma_{\tilde{\nu}_\sigma} \tag{3}$$

holds for the amide I bands of the residues in the β- and σ-forms, where $\tilde{\nu}_\beta$ is the wavenumber of the amide I band around 1630 cm^{-1}. From this relation it follows that, if the bandshapes are similar to each other, their tails also should have the relation

$$\epsilon^\beta_{\tilde{\nu}_\sigma} = 2\,\epsilon^\sigma_{\tilde{\nu}_\beta} \tag{4}$$

We assume that the same relation, Eq. (4), holds for the oligopeptides in chloroform and ethylene dichloride. Using the observed values for

$\epsilon^{\sigma}_{\tilde{\nu}_\beta}$ and $\epsilon^{\sigma}_{\tilde{\nu}_\sigma}$, the content of β structure, f_β, can be calculated from Eqs. (1), (2), and (4). The variation of the content of β structure with concentration is shown in Fig. 5.

If the N—H group of a peptide residue is hydrogen-bond-free, the amide A band will be located around 3390 cm^{-1}. If it is hydrogen-bonded with a C=O group at all, then the amide A band shifts toward a lower wavenumber; for the present oligopeptides it appears around 3300 cm^{-1}. It may be noted that Mizushima et al.[11,12] concluded that it was not possible to distinguish the intra- and intermolecular hydrogen bonding of peptide residues by the location of amide A bands.

If the fraction of hydrogen-bonded N—H groups, or the fraction of hydrogen bonds, is represented by f_H, then the molar extinction coefficients of amide A bands, $\epsilon_{\tilde{\nu}_F}$ and $\epsilon_{\tilde{\nu}_H}$, at the two wave numbers, $\tilde{\nu}_F$ and $\tilde{\nu}_H$, are expressed by

$$\epsilon_{\tilde{\nu}_F} = (1 - f_H)\epsilon^{F}_{\tilde{\nu}_F} + f_H \epsilon^{H}_{\tilde{\nu}_F} \tag{5a}$$

$$\epsilon_{\tilde{\nu}_H} = (1 - f_H)\epsilon^{F}_{\tilde{\nu}_H} + f_H \epsilon^{H}_{\tilde{\nu}_H} \tag{5b}$$

where $\epsilon^{F}_{\tilde{\nu}_F}$ and $\epsilon^{H}_{\tilde{\nu}_F}$ are the molar extinction coefficients of amide A bands of free and hydrogen-bonded residues, respectively, both at a wave number, $\tilde{\nu}_F$, and $\epsilon^{F}_{\tilde{\nu}_H}$ and $\epsilon^{H}_{\tilde{\nu}_H}$ are the corresponding coefficients of amide

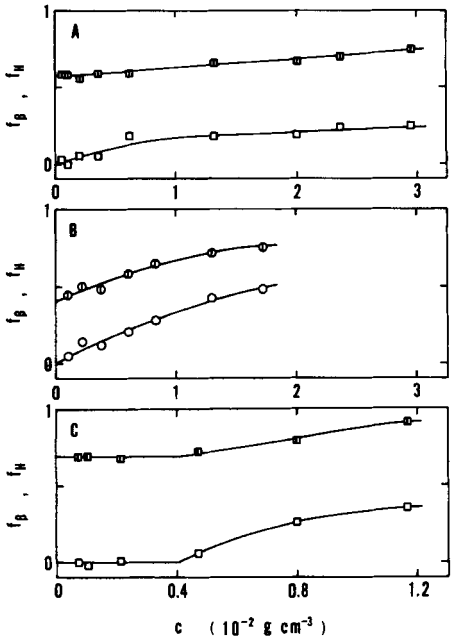

Fig. 5. Content of β structure and fraction of hydrogen bonds as functions of concentration: (A) tetrapeptide in chloroform, (B) tetrapeptide in ethylene dichloride, (C) hexapeptide in chloroform. (○,□), f_β; (●,■), f_H.

A bands at a wavenumber, $\tilde{\nu}_H$. Here $\tilde{\nu}_F$ and $\tilde{\nu}_H$ are the wave numbers around 3390 and 3300 cm^{-1}, respectively.

It is not easy to evaluate the values of $\epsilon^F_{\nu_F}$ and $\epsilon^H_{\nu_H}$ as well as $\epsilon^H_{\nu_F}$ and $\epsilon^F_{\nu_H}$, since the observed fraction of hydrogen bonds does not change appreciably but remains intermediate, as will be seen below. If the value of $\epsilon_{\nu_F} - \epsilon^H_{\nu_F}$ are plotted against those of $\epsilon_{\nu_F} - \epsilon^H_{\nu_H}$, assuming appropriate tail values for $\epsilon^H_{\nu_F}$ and $\epsilon^F_{\nu_H}$, we can draw an approximate straight line having a slope near -1 in the case of the tetrapeptide in ethylene dichloride. Thus we may take an equal value for the molar extinction coefficients of the two amide A bands

$$\epsilon^F_{\nu_F} = \epsilon^H_{\nu_H} \tag{6}$$

In addition, assuming similar bandshapes for the amide A bands, we have,

$$\epsilon^H_{\nu_F} = \epsilon^F_{\nu_H} \tag{7}$$

Then the fraction of hydrogen bonds is obtained as

$$f_H = \left(1 + \frac{\epsilon_{\nu_F} - \epsilon^H_{\nu_F}}{\epsilon_{\nu_H} - \epsilon^F_{\nu_H}}\right)^{-1} \tag{8}$$

The value of $\epsilon^H_{\nu_F} = \epsilon^F_{\nu_H}$ can be estimated from the observed molar extinction coefficient of the amide A band for the hydrogen-bonded residue at a wavenumber of the lower-wavenumber tail, that is, at $\tilde{\nu}_H - (\tilde{\nu}_F - \tilde{\nu}_H)$. The fraction of hydrogen bonds is given in Fig. 5 as a function of concentration. Even if a deviation from Eqs. (6) and (7) occurred, the factor would influence the second term of the denominator in Eq. (8). Since this term is generally smaller than unity, as seen in Fig. 4, the error arising from Eqs. (6) and (7) should be small.

Hydrogen-Bonding in the σ and β Conformations

As can be seen in Fig. 5, the tetrapeptide molecules are in the σ conformation at infinite dilution in both chloroform and ethylene dichloride, but, even at infinite dilution, about 58 and 41% of N—H groups are still hydrogen-bonded. Since the σ conformation is not associated intermolecularly, hydrogen-bonding must be intramolecular. On the other hand, the hexapeptide molecules are in the σ conformation below the critical concentration in chloroform. At these low concentrations, the fraction of hydrogen bonds is still as high as 69% and remains constant. The hydrogen bonding must be intramolecular.

If the side-chain groups are not concerned with such hydrogen-bonding, the N—H groups must form hydrogen bonds mainly with the C=O groups of peptide bonds in the same molecule. Then we have two kinds

of C=O groups of peptide residues in the σ conformation, one being hydrogen-bonded with an N—H group intramolecularly and the other being free. Both of these C=O groups exhibit an amide I band around 1670 cm^{-1}.

Since the hexapeptide consists of residues in the σ-form alone below the critical concentration, the hydrogen-bonding in the β-form should be intermolecular, which appears only above the critical concentration. Thus the hexapeptide molecule must be composed of residues of the σ- and β-forms. This result would also hold for the β-form of the tetrapeptide and its aggregates.

Summarizing the hydrogen-bonded state of peptide residues above, we can establish the relationship of oligopeptide conformations with residue forms, as shown in Table II.

The σ conformation of oligopeptide should have folded forms, since the hydrogen-bond-free residue would be disordered and the intramolecular hydrogen-bonding folds back the oligopeptide chain. The conversion of oligopeptide from the σ to β conformation alters its forms from folded to extended, holding two peptide residues together in an antiparallel way by intermolecular hydrogen bonds.

Combination of the concentration dependence of the content of β structure and the fraction of hydrogen bonds leads to the mechanism of the σ–β conversion of the oligopeptides in solution. We can now derive the fraction of hydrogen bonds of peptide residues in the σ-form, or the fraction of hydrogen-bonded σ-form, $\phi_{H/\sigma}$, by

$$\phi_{H/\sigma} = \frac{f_H - f_\beta}{f_\sigma} = \frac{f_H - f_\beta}{1 - f_\beta} \tag{9}$$

Figure 6 shows the fraction of hydrogen-bonded σ-form as a function of concentration. The fraction of hydrogen-bonded σ-form of a given oligopeptide in the σ conformation is defined by the solvent species,

TABLE II
Conformation and Hydrogen-Bonded State of Oligopeptides in Solution

Oligopeptide Molecule	Peptide Residue	Hydrogen-Bonded State	IR Spectra: Amide I (cm^{-1})	IR Spectra: Amide A (cm^{-1})
σ Conformation (unassociated)	σ-form	hydrogen-bond-free	1670	3390
		intramolecularly hydrogen-bonded	1670	3300
β Conformation (associated)	σ-form	hydrogen-bond-free	1670	3390
		intramolecularly hydrogen-bonded	1670	3300
	β-form	intermolecularly hydrogen-bonded	1630	3300

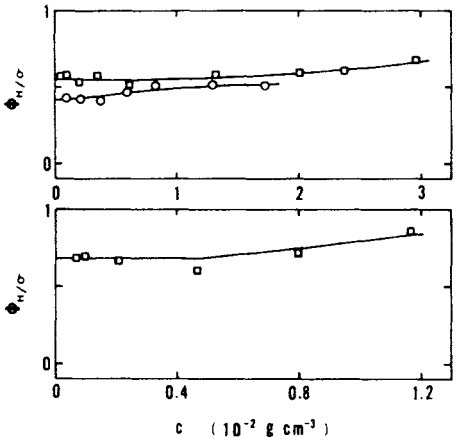

Fig. 6. Fraction of hydrogen-bonded σ-form as a function of concentration. Upper: (□) tetrapeptide in chloroform, (○) in ethylene dichloride. Lower: (□) hexapeptide in chloroform.

but it increases with increasing concentration above infinite dilution for the tetrapeptide and above the critical concentration for the hexapeptide. Thus the fraction of hydrogen-bonded σ-form in the β conformation should increase with increasing concentration.

As both the content of β structure and the fraction of hydrogen bonds increase with increasing concentration, more residues of hydrogen-bond-free σ-form are converted into the intermolecularly hydrogen-bonded β-form rather than residues of intramolecularly hydrogen-bonded σ-form are to the β-form. Indeed, the former increases the fraction of hydrogen-bonded σ-form, while the latter would decrease it or keep it unaltered.

It is not possible to evaluate the content of β structure in the β conformation of the tetrapeptide, since the concentration of aggregates other than unassociated molecules is unknown. For this purpose, at least, the weight- or number-average molecular weight of the tetrapeptide in solution must be measured as a function of concentration.

On the other hand, the hexapeptide forms micelles above the critical concentration in chloroform, which means that such a cooperative formation of micelles always accompanies a constant concentration of unassociated molecules; the monomer concentration is approximately equal to the critical concentration, c_0. Thus the micelle concentration is given by $c - c_0$, where c is the total concentration.

Then we can estimate the content of β structure of the hexapeptide micelles, $g_{\beta/m}$, and the fraction of hydrogen-bond-free residues in the micelle, $g_{F/m}$, by

$$g_{\beta/m} = \frac{c}{c - c_0} f_\beta \tag{10}$$

$$g_{F/m} = \frac{c}{c - c_0}(1 - f_H) - \frac{c_0}{c - c_0}(1 - f_{H,0}) \quad (11)$$

where $f_{H,0}$ is the fraction of hydrogen bonds at the critical concentration, that is, the fraction of hydrogen-bonded N—H groups in the σ conformation. In this way we can obtain the fraction of all the residue forms for the hexapeptide at a given concentration. Table III illustrates some of numerical values for the hexapeptide in chloroform, and also some in dioxane from the previous results.[3]

The content of β structure in micelles is almost constant, and the fraction of free residues in the micelle is very low. The micelles consist of the β structure of out-of-register form. The micelles in chloroform have a higher content of β structure and are, therefore, less swollen than those in dioxane.

DISCUSSION

The conformation of monodisperse oligopeptides in solution has been investigated by means of the nmr and also ir spectral measurements.[14-18] It has been generally agreed that at low concentrations, the folded form of oligopeptides is more stable and in equilibrium with the extended form, while at high concentration, the extended form is stabilized and aggregated by the intermolecular hydrogen bonds.

Many years ago, several possible structures by various workers have been proposed.[8,19-24] Among them, the $3n + 4$ family of Bragg et al.[20] would be most probable for the oligopeptides, where n is the number of residues in a hydrogen-bonded ring or in a helical pitch.

Pysh and Toniolo[15] suggested three folded forms for *t*-butyloxycarbonyl-tri-L-norvaline methyl ester in $CDCl_3$ at low concentrations. They are (a) the β-turn, which corresponds to a half-pitch of the helix with $n = 2$ or the 3.0_{10} helix, (b) the γ-turn, which is a half-pitch of the helix with $n = 1$ or the 2_7 helix, and (c) the sequence of the 2_7 ribbons, or the 2_7 helix itself.

Ribeiro et al.[16] investigated the conformation of *t*-butyloxycarbonyl-di- to hepta-L-methionine methyl esters in $CDCl_3$ and proposed hydrogen-bonded rings of the 2_7 helix as the folded form of tetra- to heptapeptides in dilute solutions. Ribeiro et al.[17] further extended their studies to carbobenzoxy-di- to hepta-γ-ethyl-L-glutamate ethyl esters in $CDCl_3$ and assigned a similar folded form at low concentrations.

Since the observed fractions of hydrogen bonds for the present tetrapeptide at infinite dilution and the hexapeptide below the critical concentration are as high as 58 and 69% in chloroform, respectively, the 3.0_{10} helix ($n = 2$) and the ω helix ($n = 4$) must be ruled out, for the fractions are much less. The α helix can also be excluded for the same reason. Furthermore, the β- and γ-turns cannot stand for the

TABLE III
Fraction of Residue Type of the Hexapeptide in Chloroform and Dioxane

Conformation	Residue Type		Fraction (%) of Residue Type at Concentration (10^{-2} g cm^{-3})			
			Chloroform		Dioxane	
			$c < 0.43$		$c < 0.40$	
σ	free	$1-f_H0$	31			
	intramolecularly hydrogen-bonded	f_H0	69			
			$c=0.8$	$c=1.2$	$c=0.8$	$c=1.2$
β	free	$g_{F/m}$	10	(−3)		
	intramolecularly hydrogen-bonded	$1-g_{F/m}-g_{\beta/m}$	34	48		
	intermolecularly hydrogen-bonded	$g_{\beta/m}$	56	55	29	35

folded form either, because both of them should always link to the residues of the intramolecularly hydrogen-bonded β-form, despite no β-form being present in the σ conformation. Thus we can propose the 2_7 ribbon or a half-pitch of the 2_7 helix as a fundamental structure of the folded form. (The 2_7a ribbon,[19,20] having the side chain nearly perpendicularly oriented to the plane of a hydrogen-bonded ring, will be more likely.) The 2_7 ribbon is in equilibrium with the hydrogen-bond-free residues in the same chain, both constituting the σ conformation. In the β conformation, the 2_7 ribbon may be in equilibrium with the residues of the β-form, which are hydrogen-bonded with those in the other chain.

It is seen that, even in the same solvent, e.g., in chloroform, the fraction of hydrogen bonds depends on the species of amino acid residues and the degree of polymerization. In contrast to the high values for the present oligopeptides, *t*-butyloxycarbonyl-tri- and tetra-L-norvaline methyl esters have fractions as low as 21 and 38% around $10^{-4}M$ in $CDCl_3$,[15] but *t*-butyloxycarbonyl-tri- and tetra-L-methionine methyl esters have 31–45 and 55–61% in $CDCl_3$.[16]

In order to build possible structure of the folded form of the oligopeptides in solution, we postulate that the N—H group on the N-terminal residue is hydrogen-bonded with the nitro group on the *o*-nitrophenylthio moiety, and that its amide A band is located indistinguishably from that for the peptide hydrogen bond.

Then the observed fractions of hydrogen bonds, 58 and 69%, for the tetra- and hexapeptide in the σ conformation in chloroform correspond to the structures in which two N—H groups of five for the tetrapeptide and four N—H groups of seven for the hexapeptide are hydrogen-bonded with the C=O groups. If the folded form is fundamentally the 2_7 ribbon, the perfect fold should consist of three and five hydrogen-bonded rings, respectively. Accordingly, the tetra- and hexapeptides in the σ conformation in chloroform are, on the average, composed of two and four rings, respectively, and the other one open or disordered form, in addition to the N-terminal hydrogen-bonded ring.

The tetrapeptide would also have the 2_7 ribbon as the fundamental fold at infinite dilution in ethylene dichloride. The observed fraction of hydrogen bonds, 41%, indicates that the tetrapeptide in the σ conformation in ethylene dichloride is, on average, composed of one ring of the 2_7 ribbon and two open or disordered forms. Following the same line, the hexapeptide in ethylene dichloride would have two or three rings of the 2_7 ribbon and three or two open or disordered forms below the critical concentration.

For the hexapeptide micelles in chloroform, the observed values of the fraction of hydrogen-bonded σ-form in the micelle, $1 - g_{F/m} - g_{\beta/m}$, around 40%, and the content of β structure in the micelle, $g_{\beta/m}$, about 55%, lead to the average structure of the molecule, in which one ring

or two of the 2_7 ribbon and three hydrogen-bonded residues in the β form are present, if the N-terminal hydrogen-bonded ring is reserved.

References

1. Okahashi, K. & Ikeda, S. (1979) *Int. J. Peptide Protein Res.* **13**, 462–472.
2. Okahashi, K. & Ikeda, S. (1979) *Biopolymers* **18**, 2105–2113.
3. Ikeda, S. & Okahashi, K. (1979) *Biopolymers* **18**, 2115–2126.
4. Imae, T., Okahashi, K. & Ikeda, S. (1981) *Biopolymers* **20**, 2553–2566.
5. Imae, T. & Ikeda, S. (1973) *Biopolymers* **12**, 1203–1221.
6. Blout, E. R. & Asadourian, A. (1956) *J. Am. Chem. Soc.* **78**, 955–961.
7. Ambrose, E. J. & Elliott, A. (1950) *Proc. R. Soc. (Lond.) A* **205**, 47–59.
8. Bamford, C. H., Elliott, A. & Hanby, W. E. (1956) *Synthetic Polypeptides*, Academic, New York.
9. Miyazawa, T. (1960) *J. Chem. Phys.* **32**, 1647–1652.
10. Miyazawa, T. (1962) in *Polyamino Acids, Polypeptides and Proteins*, Stahmann, M., Ed., The University of Wisconsin Press, Madison, WI, p. 201.
11. Mizushima, S., Shimanouchi, T., Tsuboi, M. & Souda, R. (1952) *J. Am. Chem. Soc.* **74**, 270–271.
12. Mizushima, S., Shimanouchi, T., Tsuboi, M., Sugita, T., Kurosaki, K., Mataga, N. & Souda, R. (1952) *J. Am. Chem. Soc.* **74**, 4639–4641.
13. Shields, J. E., McDowell, S. T., Pavlos, J. & Gray, G. R. (1968) *J. Am. Chem. Soc.* **90**, 3549–3556.
14. Goodman, M., Ueyama, N., Naider, F. & Gilon, C. (1975) *Biopolymers* **14**, 915–925.
15. Pysh, E. S. & Toniolo, C. (1977) *J. Am. Chem. Soc.* **99**, 6211–6219.
16. Ribeiro, A. A., Goodman, M. & Naider, F. (1979) *Int. J. Peptide Protein Res.* **14**, 414–436.
17. Ribeiro, A., Saltman, R. P. & Goodman, M. (1980) *Biopolymers* **19**, 1771–1790.
18. Goodman, M., Ueyama, N. & Naider, F. (1975) *Biopolymers* **14**, 901–914.
19. Huggins, M. L. (1943) *Chem. Rev.* **32**, 195–218.
20. Bragg, W. L., Kendrew, J. C. & Perutz, M. F. (1950). *Proc. R. Soc. London Ser. A* **203**, 321–357.
21. Shimanouchi, T. & Mizushima, S. (1947) *Kagaku* **17**, 24–26.
22. Shimanouchi, T. & Mizushima, S. (1948) *Bull. Chem. Soc. Jpn.* **21**, 1–8.
23. Zahn, H. (1947) *Z. Naturforsch. B* **2**, 104–108.
24. Ambrose, E. J. & Hanby, W. E. (1949) *Nature* **163**, 483–484.

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