Notes

Chain-Length Dependence for Secondary Structure Formation of Poly(ethylene glycol)-Bound Homooligopeptides of ϵ-Benzyloxycarbonyl-L-lysine in the Solid State and in Solution

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Introduction

In recent years there has been a great interest in the conformational preferences of monodispersed homooligopeptides,[2-4] the molecularly uniform lower members of a homologous series which do not yet fully adopt the secondary structure of the particular polypeptide.[5] It is known that in the case of homopolypeptides potentially capable of forming α helices (i) the corresponding oligopeptides can exist either in the unordered form or in the interchain β conformation, depending upon size and experimental conditions, and (ii) the monodispersed higher homologues can assume the fully α-helical structure if they have a chain length considerably longer than the critical chain length for the onset of that structure. These compounds, according to the nomenclature introduced by Zahn and Gleitsmann, should be referred to as pleionopeptides (longer peptides).[6] Critical chain lengths for α-helix formation of homooligopeptides reported thus far vary from 6 to 15 residues as a function of experimental conditions.[2,4,7-15] The synthesis of peptides having a large amount of α-helical character is difficult, owing to the onset of serious solubility problems characterizing the homologues of intermediate size (β-structure formation).[12] To overcome this problem, we recently undertook the preparation and study of a number of homologous peptide series covalently bound to the solubilizing polymer poly(ethylene glycol) (PEG) at their C-terminal ends. The critical chain lengths for interchain β structure and right-handed α-helix formation were determined for the $(L\text{-Ala})_n$, $(L\text{-Met})_n$, $(L\text{-Glu})_n$, and $[L\text{-Glu(OBzl)}]_n$ series.[4,7-13,16-18] In this note we describe the results of an investigation in the solid state and in solution (using solvents with diverse solvating properties) on the PEG-bound $[L\text{-Lys(Z)}]_n$ series (Z = benzyloxycarbonyl). High-molecular-weight poly$[L\text{-Lys(Z)}]_n$ adopts the right-handed α-helical form under structure-supporting conditions.[19]

While the conformational properties of the ϵ-unsubstituted $(L\text{-Lys})_n$ homooligopeptides were examined in some detail,[15,20] there is no report dealing with a complete series of corresponding ϵ-substituted derivatives. This is somewhat surprising in view of the interest in $(L\text{-Lys})_n$ oligopeptides as carriers of haptens,[21] drugs,[22] and mesogenic groups[23,24] in the form of side-chain conjugates.

Experimental Section

The details of the synthesis of the monodispersed, chemically and optically pure $[t\text{-Boc-L-Lys(Z)}]_n\text{-Gly-OCH}_2\text{-1,4-(2-NO}_2\text{C}_6\text{H}_3\text{)-CONH-PEG}$ (*t*-Boc = *tert*-butyloxycarbonyl) homopeptides to $n = 15$ by the liquid-phase method,[25] using bifunctional PEG of molecular weight 6000, will be described elsewhere.[26] A single Gly residue was incorporated at the C-terminal end of the peptide chain as an internal reference in the amino acid analyses.[27] The infrared absorption spectra were recorded with a Perkin-Elmer model 580 spectrophotometer. The band positions are accurate to ±1 cm^{-1}. The solid samples of the various peptides were prepared by precipitation from a CH_2Cl_2 solution with diethyl ether or by evaporation of a 2,2,2-trifluoroethanol (TFE) solution.

Circular dichroic spectra were recorded at 22 °C with a Cary Model 61 circular dichrometer or a Jasco Model J-500 A, equipped with a Jasco Model DP-501 N data processor. For experimental details see ref 8. The solvents used for the spectral measurements

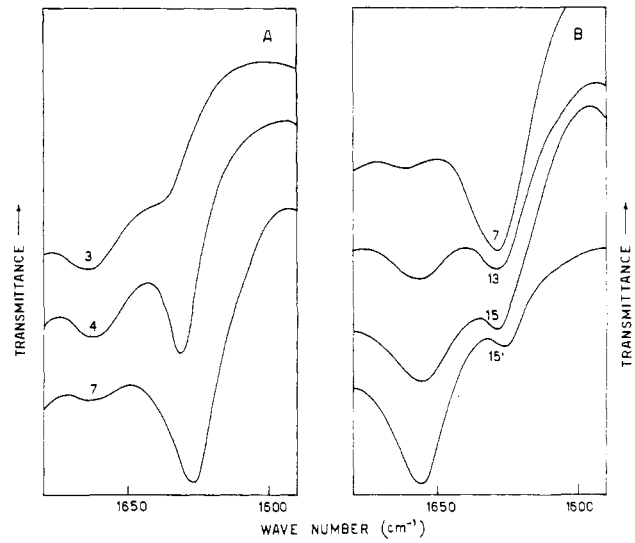

Figure 1. Infrared absorption spectra in the 1680–1590-cm^{-1} region of the PEG-bound-$[L\text{-Lys(Z)}]_n$ homopeptides in the solid state (from CH_2Cl_2/diethyl ether): (A) N_α-deblocked peptides; (B) N_α-blocked peptides.

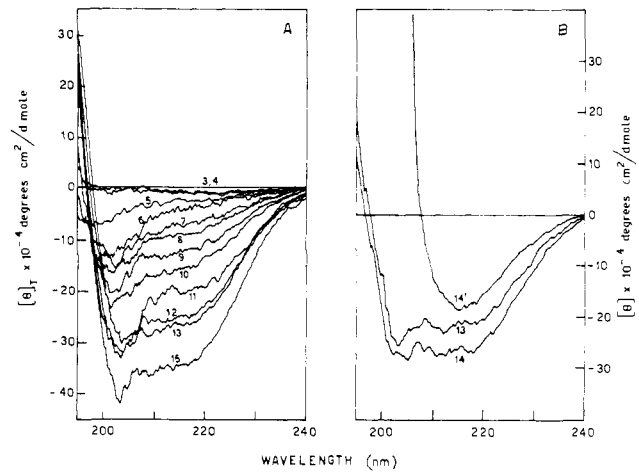

Figure 2. Original, computer-drawn circular dichroism spectra of the PEG-bound-$[L\text{-Lys(Z)}]_n$ homopeptides in TFE. The 14′ spectrum is that of the 14-peptide in a TFE/H_2O mixture [85% H_2O (v/v)]. (A) N_α-blocked peptides; (B) N_α-deblocked peptides.

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were doubly-distilled water and TFE (Fluka).

Results and Discussion

Figure 1 illustrates the results of the IR absorption measurements for some significant N_α-deblocked and N_α-blocked [L-Lys(Z)]$_n$ oligopeptides in the most informative spectral region for conformational assignments (1680–1590 cm^{-1}; amide I band of peptide groups).[28] The peptides with n = 1–3 adopt essentially aggregated, but unordered conformations, characterized by an absorption at 1665 cm^{-1}.[28] Peptides with n = 4–6 show this same band, but in ever decreasing amount with increasing n, while a second band at 1630 cm^{-1},[28] indicative of the onset of β structure, increases with n.

This β structure is (i) most probably of the antiparallel-chain type,[28] as inferred from the enhanced intensity of the shoulder at 1695 cm^{-1} (not shown in Figure 1), which overlaps with the broad and strong absorption in the 1725–1685-cm^{-1} region associated with the urethane moieties present in side chains,[7] or (ii) of the extended, interchain type;[7] since the present study utilizes *bifunctional* PEG as a macromolecular support, the possibility of the occurrence of intramolecularly hydrogen-bonded forms cannot be excluded.

The peptides having n = 7–12 essentially assume the β structure, the 1665-cm^{-1} band being almost negligible in these spectra. Peptides of n = 13–15, however, show a significant amount of α helix, which increases with increasing n, in addition to the β structure. The occurrence of the α-helical structure is revealed not only by an intense absorption at 1655 cm^{-1} [28,29] but also by the shift of the amide A band to higher frequency, from about 3290 to about 3305 cm^{-1} [7] (not shown in Figure 1).

Figure 1 also shows that no appreciable difference occurs in the spectra of the N_α-deblocked and N_α-blocked 7-peptides. This also holds true for the helical-forming 13-peptides (not shown). The CD curves of the N_α-blocked [L-Lys(Z)]$_n$ oligopeptides in TFE are presented in Figure 2A. The peptides with n = 3–5 exist essentially as statistically coiled forms.[30] In contrast, at the level of the 6-peptide, the CD spectrum indicates the onset of the right-handed α helix (to a low extent), particularly evident from the markedly increased intensity and the red shift of the $\pi \rightarrow \pi^*$ amide transition from well below 200 nm to 202 nm due to the exciton spliting of the peptide chromophores in a helical array.[30] The percentage of α helix increases regularly from 6-peptide (10%)[31] to the 15-peptide (70%),[31] the spectrum of which is characterized by two negative maxima at about 215 nm (amide n $\rightarrow \pi^*$ transition) and 203 nm (parallel component of the exciton-split amide $\pi \rightarrow \pi^*$ transition) and the crossover point at 195 nm.[30]

In the N_α-blocked series the amout of α helix in TFE decreases only slightly (compare the curves of the 13-peptide in Figures 2A and 2B). In Figure 2B the effect of addition of water to the TFE solution of the 14-peptide is visible. The conformational transition from α helix to β structure [negative maximum at 215 nm (n $\rightarrow \pi^*$ transition) and crossover point at 208 nm] induced by the presence of 85% water (v/v) seems to be due to the combined properties of water (less capable of solvating the peptide chain than TFE) and the side chains of the Lys(Z) peptide [relatively low polarity of the $C_6H_5CH_2OCONH(CH_2)_4$ moiety]. The tendency for β structure formation of the [L-Lys(Z)]$_n$ peptides in water is confirmed by the spectrum (not shown) of the N-deblocked tetramer, the highest water-soluble oligomer, which exhibits a negative maximum at 220 nm, a crossover point at 215 nm, and a positive maximum at 193 nm.

In summary, we were able to determine the critical chain length for development of interchain β structure (at n = 4) and α helix (at n = 13) for the L-Lys(Z)$_n$ oligopeptides in the solid state. These results indicate that the conformational transitions that take place in the solid state with increasing n in optically pure homopeptide series with a tendency to α-helix formation (in the corresponding polypeptide) are unordered (although aggregated) structure $\rightarrow \beta$ structure $\rightarrow \alpha$ helix. The critical size for the formation of the two ordered structures in the [L-Lys(Z)]$_n$ series compare well with those previously reported for other homooligopeptide series.[7,10] In the structure-supporting solvent TFE the critical chain length for development of the right-handed α helix in the [L-Lys(Z)]$_n$ series was found to be n = 6, again in agreement with the published results of other homooligopeptides series.[2,4,8,9,11,13,14] As in the [L-Glu(OBzl)]$_n$ series,[11] deblocking of the N_α-protecting group in a higher L-Lys(Z) oligopeptide does not induce the onset of the β structure, as observed in the (L-Met)$_n$,[8] (L-Ala)$_n$,[9] and (L-Val)$_n$[9] series. However, the formation of the aggregated β structure could be achieved by adding the nonsolvent water to the fluoro alcoholic solution of the L-Lys(Z) 14-peptide. The critical chain length for development of the β structure for the [L-Lys(Z)]$_n$ oligopeptides is found at n = 4 in 100% water.

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Registry No. t-Boc-[L-Lys(Z)]$_n$-Gly-OCH$_2$-1,4-(2-$NO_2C_6H_3$)-CONH-PEG, 83692-51-7.

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How To Separate Polydisperse Polyelectrolytes by Thermal Field Flow Fractionation Techniques

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Thermal field flow fractionation (TFFF)[1] allows for the separation of macromolecules over a broad range of molecular weights—from 4000 to several millions. A macromolecular solution is placed between two plates and heated from above. A laminar flow between the two plates $V_X(Z)$ is superimposed at right angles to the thermal gradient $\partial T/\partial Z$. Coils of different molecular weight have different concentration profiles $c(Z)$ in steady state under the thermal gradient. They are transported by the fluid at different velocities $V_X(Z)$ and separated. The analysis of TFFF data[1,2] is based on the distribution in the channel for one single species. Let us start with a monodisperse solution of macromolecules (degree of polymerization N). In the thermal gradient, the macromolecules migrate toward one plate (the cold plate in most cases). The steady-state concentration profile is given by the balance of this thermal flow (diffusion coefficient D_T) with the mass diffusion flow (cooperative diffusion coefficient D_M):

$$J = -cD_T\nabla T - D_M\nabla c = 0 \tag{1}$$

where c is the monomer concentration and J the monomer current. The profile deduced from eq 1 is controlled by the ratio $s = D_T/D_M$, called the Soret coefficient. If s is strongly dependent upon N, we expect a good separation of macromolecules of different molecular weight. If s is independent of N, no separation will occur.

Our aim here is to review the case for different types of macromolecules (coils, rods, and compact chains) and to discuss what happens if they become *charged*.

(1) Neutral Flexible Polymer in Dilute Solutions. The mass diffusion coefficient D_M is well-known, both experimentally and theoretically.[3,4,7] Quite generally, D_M is related to the mobility coefficient μ and to the osmotic pressure Π by the relation

$$D_M = \mu(\partial\Pi/\partial c) \tag{2}$$

[μ is the transport coefficient relating the velocity of a monomer to an applied external force ($v = \mu F$).]

In the dilute regime, $\Pi = (c/N)kT$. Kirkwood showed that backflow interactions control the sedimentation coefficient.[3] A polymer coil behaves hydrodynamically as a rigid sphere of radius R comparable (within a coefficient) to the radius of gyration. Then, $\mu = N/6\pi\eta R$, where η is the solvent viscosity. This leads to

$$D_M = kT/6\pi\eta R \tag{3}$$

In good solvents, $R \sim N^{0.58}$ and $D_M \sim N^{-0.58}$ in one typical experiment on polystyrene.[4]

For the thermal diffusion coefficient, de Gennes and Brochard have argued recently[5] that backflow does *not* lead to a long-range coupling between monomers. Thermally, the chain behaves as a Rouse chain and in a thermal gradient, a coil moves with the same velocity that would be seen with separated monomers. D_T is independent of N, as observed experimentally,[1,2,6] and of the order of 10^{-7} cm^2 s^{-1} K^{-1}:

$$D_T = D_{T_0}N^0 \tag{4}$$

From the results (3) and (4), we expect

$$s = D_T/D_M \sim N^{0.58} \tag{5}$$

This prediction is remarkably verified on polystyrene.[1] With these coils, the N dependence of D_M is entirely responsible for the separation of polymer chains. The solution of eq 1 is

$$c = C_0e^{-Z/l_s} \tag{6a}$$

$$l_s = s/\nabla T \tag{6b}$$

For a channel of thickness $d = 0.1$ mm and a temperature difference $T_2 - T_1 = 30$ °C, one can separate chains if $l_s < d$, i.e., for $N > 6000$. Note that one must constantly work with *dilute* solutions. In the semidilute regime, the cooperative diffusion coefficient becomes independent of N[7] and no separation occurs.

(2) Charged, Flexible Polymers. The discussion of D_T is unchanged and result (4) is valid. From experiment,[7] one knows that D_M is (1) very large and (2) independent of both N and concentration. Theoretically, D_M was first calculated by a RPA or Debye–Hückel theory[11] in the dilute regime. However, for ions of large charge, the RPA approach extended to semidilute solutions[12] does not explain the neutron scattering data[9,13] because the high electrostatic repulsions cannot be treated as a perturbation. By a more powerful scaling approach,[9] we have shown that in both dilute and semidilute regimes, one expects

$$D_M = kT/6\pi\eta a = D_{M_0}N^0 \tag{7}$$

where α is monomer size. The result (7) can be understood easily: in the dilute regime, the chain forms a rod of length $2R \sim Na$. The sedimentation coefficient per monomer is then roughly $\mu = N/6\pi\eta R$. The osmotic pressure is very large ($\Pi = ckT$). By using eq 2, we find result (7). The resulting Soret coefficient is then independent of N:

$$s = D_{T_0}/D_{M_0} \tag{8}$$

We conclude that (a) the concentration profile is completely independent of N and no separation is expected, (b) $s = s_0$ is very small (the concentration profile given by eq 7 is completely flat (l_s is larger than d) and the polyelectrolyte moves with the mean solvent velocity (zero retention)), and (c) in semidilute solutions, the equations for Π and for D_M are not qualitatively modified and the value of l_s (eq 6) is not changed: again we expect zero retention.

(3) Neutral Rods. The only difference with case 1 is the value of D_M. If L is the length of the rod, we have (ignoring weak logarithmic corrections)

$$D_M \simeq kT/6\pi\eta L \sim N^{-1} \tag{9}$$

This leads to a very large Soret coefficient

$$s = s_0N \tag{10}$$

and TFFF is expected to be very efficient for separating rigid polymers.

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