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Enhancement in helicity of an oligopeptide by its organization onto a dendrimer template

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An oligopeptide comprised of γ-benzyl-L-glutamic acid has been successfully assembled on an amido amine dendrimer surface by graft polymerization and the resultant dendrimer has shown drastic enhancement in helicity of the peptide segment.

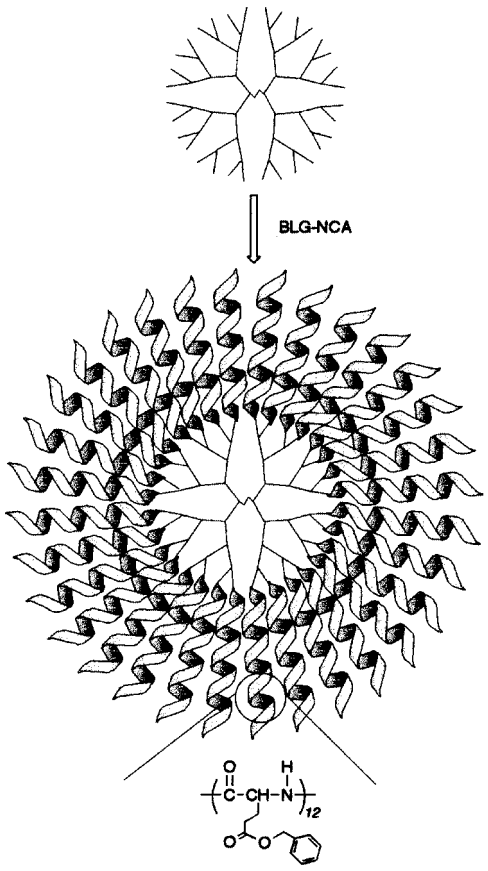
Here we describe the assembly of an oligopeptide onto the surface of an amine-terminated, poly(amido amine) (PAMAM) dendrimer and the enhancement in helicity of the oligopeptide.

Much effort has been dedicated to the molecular design and synthesis of model proteins to define interactions involved in protein folding[1,2] and to develop protein-based materials.[3] Protein tertiary structures can be looked upon as assemblies of secondary structural elements (α-helix, β-strand, reverse turn). This model has been the basis for the design of artificial proteins. A recent approach to protein design is to use a rigid template molecule.[4] Many artificial proteins have been prepared in aqueous solutions by attaching peptide blocks to templates that direct the component helices into a protein-like packing arrangement, *e.g.* a bundle structure of α-helices.[5] The amphiphilic properties of the helical blocks seem to be essential to stabilize such a bundle structure. We have devised a strategy in which purely synthetic polypeptides are aligned on two-dimensional media[6] such as on water and on Au surfaces. Poly(L-glutamic acid) has been chosen as a structural element because of its ease of synthesis and well-defined conformational characteristics in water. We now describe that complete surface modification of a three-dimensional dendrimer with an oligopeptide can be accomplished by graft polymerization of γ-benzyl-L-glutamate *N*-carboxy anhydride (BLG-NCA) and the resulting graft chains show interesting conformational properties.

Dendrimers are known to be hyperbranched macromolecules possessing a very high concentration of surface functional groups.[7] A variety of dendrimers have been developed by introducing functionalities into these terminal groups. For example, dendrimers terminated with an amino acid,[8] a sugar,[9] and a perfuluoroalky[10] or alkyl[11] chain show encapsulation functions for guest molecules. The other structural feature of dendrimers is the high degree of control over molecular weight and shape. The diameters of the spherical dendrimers range from 3 to 10 nm.[12] Taking account of these features in shape, the oligopeptide-attached dendrimer in this study may be a relevant candidate for model proteins.

The third-generation, amine-terminated PAMAM dentrimer (G3-NH_2) with branches and an ethylenediamine core was used as a template for assembly and an initiator for graft polymerization. As shown in Scheme 1, BLG-NCA (3.8 mmol) was polymerized with G3-NH_2 ([NH_2] = 0.30 mmol) in $CHCl_3$ at room temperature. A relatively short chain length of the peptide segment (degree of polymerization, n = 10–15) was employed because the number of amino acid units is appropriate for both characterization of the resultant dendrimer and elucidation of the effect of assembly. After stirring for 30 min, the reaction mixture was poured into a large excess of Et_2O and then purified and dried, giving a white powdery product (G3-PBLG) with a yield of 97%. The M_w/M_n value† determined by size exclusion chromatography was reasonably narrow (M_w/M_n = 1.06), indicating the absence of the polymerization catalyzed by the tertiary amine of the inner part of PAMAM dendrimer that produces a homopolymer of BLG-NCA with a broad molecular weight distribution.[9] ^{1}H and ^{13}C NMR spectroscopies (data not shown) supported the structure of G3-PBLG. In the ^{13}C NMR spectrum of G3-PBLG, no peaks assigned to the α- and β-carbons of the unreacted terminal amino groups of PAMAM dendrimer were observed, while those of G3-NH_2 appeared at δ_c 41 and 42, respectively. This means that the graft polymerization proceeded for all of the terminal amino groups located at the G3-NH_2 dendrimer surface. The n value of the grafted peptide segment was evaluated to be 12 on the basis of ^{1}H NMR analysis.‡

The secondary structure of G3-PBLG was investigated by means of circular dichroism (CD) and ^{1}H NMR spectroscopy. For comparison, Pr-PBLG, which possesses a propyl group at the C-terminus of the PBLG chain, was prepared by polymerization of BLG-NCA initiated with propylamine instead of G3-NH_2. The ^{1}H NMR analysis of Pr-PBLG showed that n for the PBLG segment was also 12. In the CD spectrum of this CH_2Cl_2 solution ([glutamate unit] = 1.0 mM), a trough appears at *ca.* 222 nm, indicating the exsitence of a right-handed α-helix



Scheme 1

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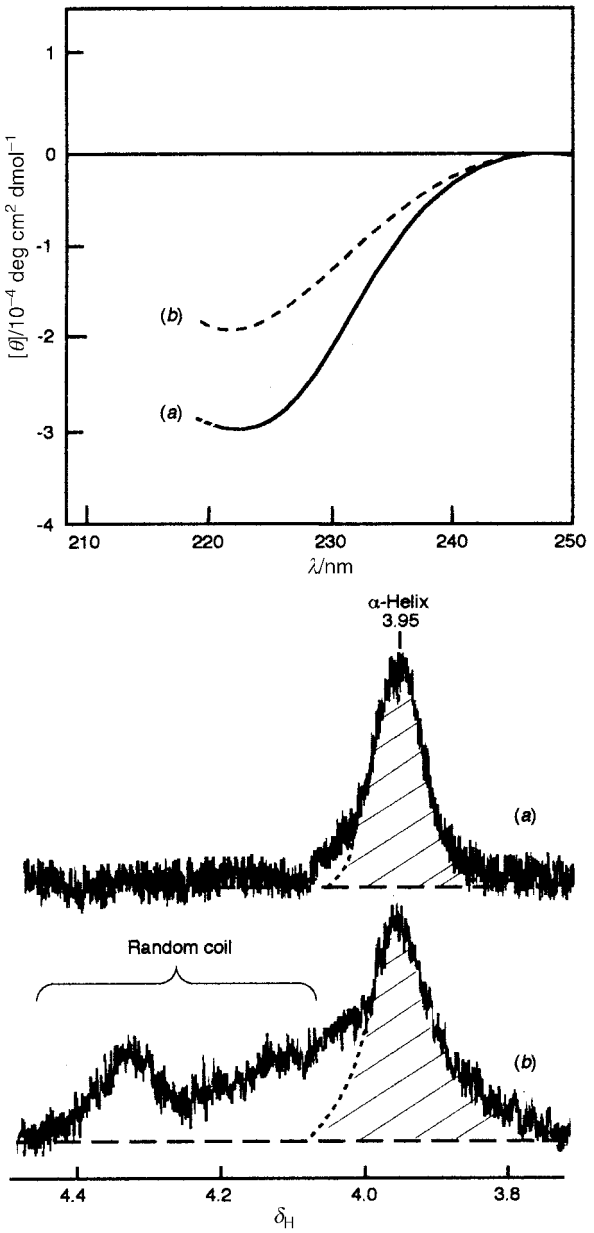

Fig. 1 CD and ^{1}H NMR spectra of G3-PBLG (*a*) and Pr-PBLG (*b*) in CH_2Cl_2 (for CD) or CD_2Cl_2 (for NMR) at 25 °C; [glutamate unit] = 1.0×10^{-3} M.

conformation (Fig. 1). The helix content of the Pr-PBLG solution is calculated to be 53% from the observed molar ellipticity $[\Theta]$ at 222 nm ($[\Theta] = 1.8 \times 10^{-4}$ deg cm^2 dmol^{-1}).§ This helix content is reasonable taking account of such a short segment length as $n = 12$.[13] On the other hand, the molar ellipticity of the G3-PBLG solution is found to be enhanced drastically, compared with that of the Pr-PBLG solution. Surprisingly, the helix content goes up to 92%. To obtain more quantitative information on the secondary structure, ^{1}H NMR spectra were measured in CD_2Cl_2 under the same conditions (Fig. 1). The α-C*H* resonance signal of the PBLG main chain was known to give a peak at δ_H 3.95 ascribed to the α-helix conformation, while giving an apparent lowfield shift on going from the helix to the random coil form.[14] From signal areas based on α-helix and random coil forms, the helix contents were evaluated to be 93 and 57% for G3-PBLG and Pr-PBLG, respectively. These values are consistent with those obtained by CD analysis. FTIR spectra (data not shown), in particular in amide II band region of the main chain, showed that β-sheet structures were absent for both PBLGs. It is clear from these spectral data that transfer and aggregation of the PBLG segment onto the dendrimer surface from bulk solution would cause such an enhancement in helicity since the PBLG segment lengths (*n*) of Pr-PBLG and G3-PBLG are the same. The enhancement of helicity due to aggregation of helices has sometimes been observed in aqueous solutions when a helix-bundle structure is formed by assembling with a template.[5] In those cases, it has been demonstrated that the hydrophobic effects among the side chain groups play an important role in stabilizing the helical conformation. Presumably, in our case, the driving force for causing such enhancement in helicity must also be the hydrophobic effect, resulting from the characteristic shape of the spherical dendrimer surface, at which the peptide segments are forced to assemble densely, which will require further exploration.

Notes and references

† M_w and M_n denote weight-average molecular weight and number-average molecular weight, respectively, determined by means of size exclusion chromatography, and the ratio of M_w/M_n is the polydispersity of the polypeptides prepared by polymerization of NCA. Size exclusion chromatography was performed in DMSO at 30 °C, with a Shimazu Model LC-5A high performance liquid-chromatograph apparatus (column, Shodex KD803 and 804).

‡ The *n* value was calculated by using the area ratio of the signal of CH_2 (benzyl) in the PBLG segment to that of CH_2 in the PAMAM dendrimer, observed in the ^{1}H NMR spectrum of G3-PBLG.

§ The helix content was calculated by using the following equation: helix content (%) = $([\Theta]_{222}/[\Theta]_h) \times 100$, where $[\Theta]_{222}$ and $[\Theta]_h$ are the molar ellipticity at 222 nm and −34 000 (deg cm^2 dmol^{-1} (ref. 15), respectively.

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