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Synthesis and structure of a vesicular assembly of dialkylammonium-type amphiphile carrying L-3-(3-N-ethylcarbazolyl)alanine

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Summary. A novel unnatural amino acid, L-3-(3-N-ethylcarbazolyl)alanine, was synthesized and used for synthesis of a chiral dialkylammonium-type amphiphile. The synthetic amphiphile formed a vesicular assembly in aqueous dispersion showing gel-liquid crystalline phase transition around 25 °C. The bilayer membrane composed of the amphiphile was nearly free from excimer as evidenced by fluorescence spectroscopy.

Keywords: Amino acids – Unnatural amino acid – Ethylcarbazolylalaline – Vesicular assembly – Photoenergy-harvesting system

Introduction

The syntheses of novel unnatural amino acids have attracted increasing attention of protein chemists, because modification of proteins by incorporation of an unnatural amino acid will pave the way for development of new proteins with improved properties, regulatory systems, and novel functions (Noren et al., 1989). Especially, unnatural aromatic amino acids are of interest in view of photo-sensitive properties. For example, D-9-anthrylalanine and L-1-pyrenylalanine were synthesized, and the polypeptides containing the unnatural aromatic amino acids were studied on the photo-physical properties of the chromophores regularly aligned along the α-helical backbone chain (Sisido et al., 1983, 1985). On the other hand, unnatural aromatic amino acids have been used for a constituent of synthetic amphiphiles forming a vesicular assembly in pursuit of an efficient photoenergy-harvesting organizate (Sisido et al., 1990; Sasaki et al., 1990). However, one of the difficulties for obtaining an effective organizate is the formation of excimer, which works as an energy-dissipating site and disturbs the efficient energy migration among the chromophores. In this paper, a novel unnatural aromatic amino acid, L-3-(3-N-ethylcarbazolyl)alanine, was synthesized, and a dialkylammonium-type

amphiphile was prepared from the amino acid. N-Ethylcarbazole is a suitable chromophore for a long-range interchromophoric interaction in membrane, because the electronic transition from the ground state to the lowest-excited singlet state is allowed. Besides, the N-ethyl group hinders the formation of carbazole excimers (Yokoyama et al., 1975).

Material and methods

Synthesis

L-3-(3-N-Ethylcarbazolyl)alanine was synthesized as follows. D,L-3-(3-N-Ethylcarbazolyl)alanine was prepared from hippuric acid and N-ethylcarbazolyl-3-carboxyaldehyde as shown in Fig. 1. The racemic amino acid was acetylated, and was subjected to acylase treatment. The precipitate obtained was dissolved in 1N NaOH aqueous solution and

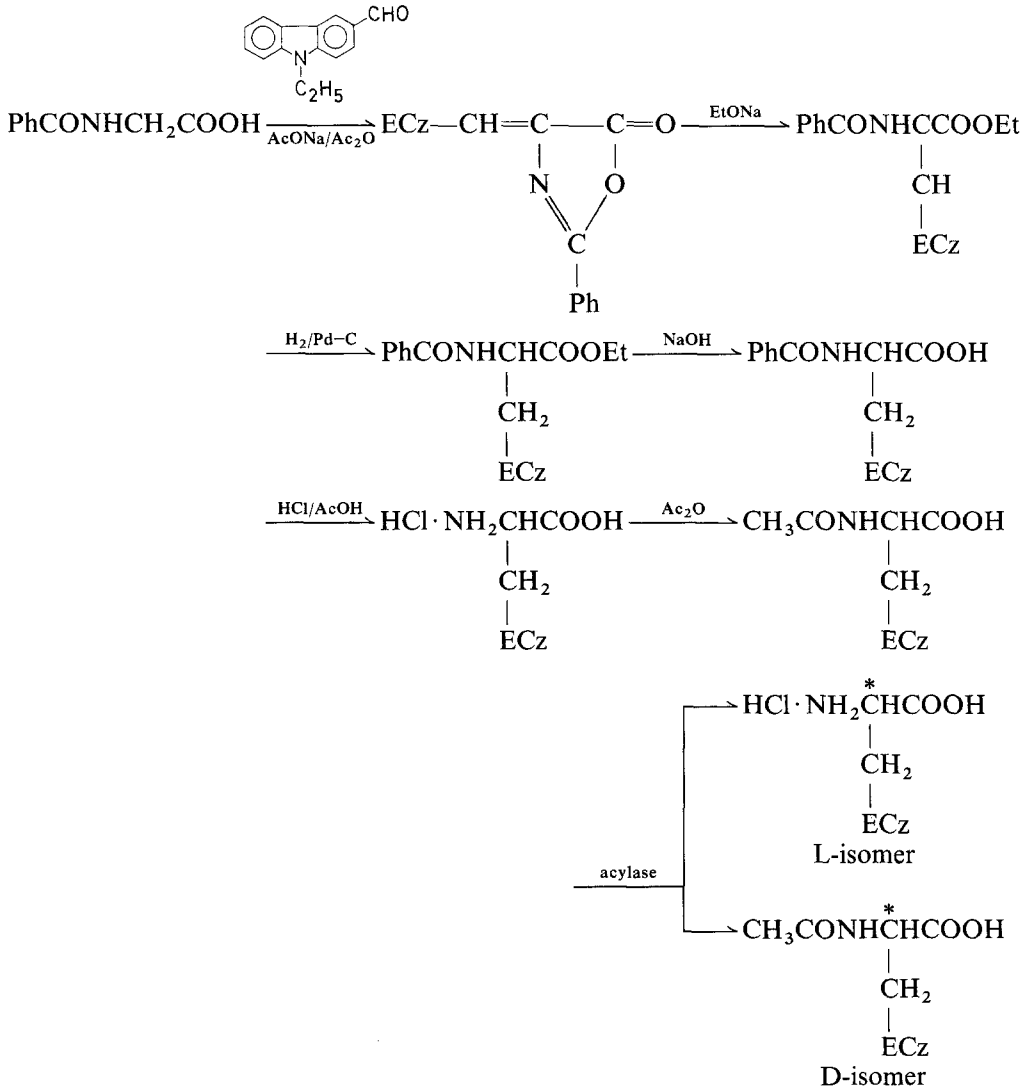

Fig. 1. Synthetic route of L-3-(3-N-ethylcarbazolyl)alanine

neutralized with hydrochloric acid to obtain L-3-(3-N-ethylcarbazolyl)alanine. The optical purity was checked by ^{1}H NMR of the amino acid methyl ester in the presence of a shift reagent, tris[3-((trifluoromethyl)hydroxymethylene)-d-camphorato]europium(III) (Egusa et al., 1986). Two peaks were present in the OCH_3 region in the case of racemic compound, while only a single peak was observed with L-3-(3-N-ethylcarbazolyl)alanine methyl ester (Fig. 2), indicating the complete optical resolution.

Dialkylammonium-type amphiphile containing L-3-(3-N-ethylcarbazolyl)alanine (5Cz18) was synthesized by a method similar to the previously reported one (Fig. 3, Sisido et al., 1990). L-3-(3-N-Ethylcarbazolyl)alanine was protected by a Boc group using di-t-butyldicarbonate, and coupled with dioctadecylamine by using dicyclohexylcarbodiimide. Anal. Calcd. for $C_{58}H_{99}O_3N_3 + 0.6H_2O$: C, 77.64; H, 11.26; N, 4.68. Found: C, 77.45; H, 11.25; N, 4.74. After removing the Boc group with HCl/dioxane, the N-terminal was reacted

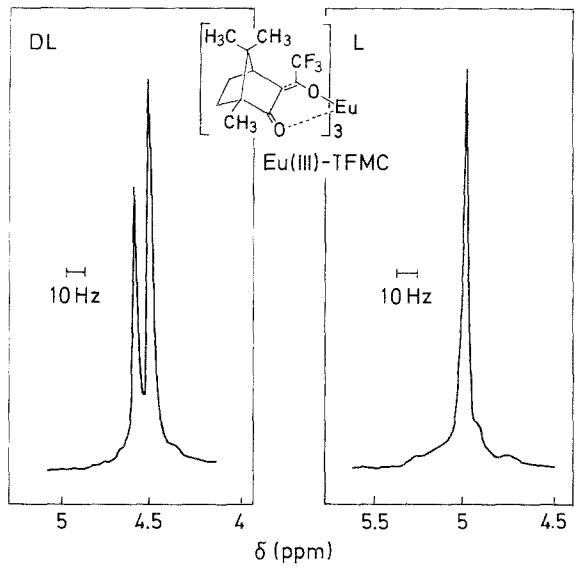

Fig. 2. ^{1}H NMR spectra of D,L-3-(5-N-ethylcarbazolyl)alanine mehtyl ester (left) and the prepared L-N-ethyl-3-carbazolylalanine methyl ester (right) in the presence of a chiral shift reagent

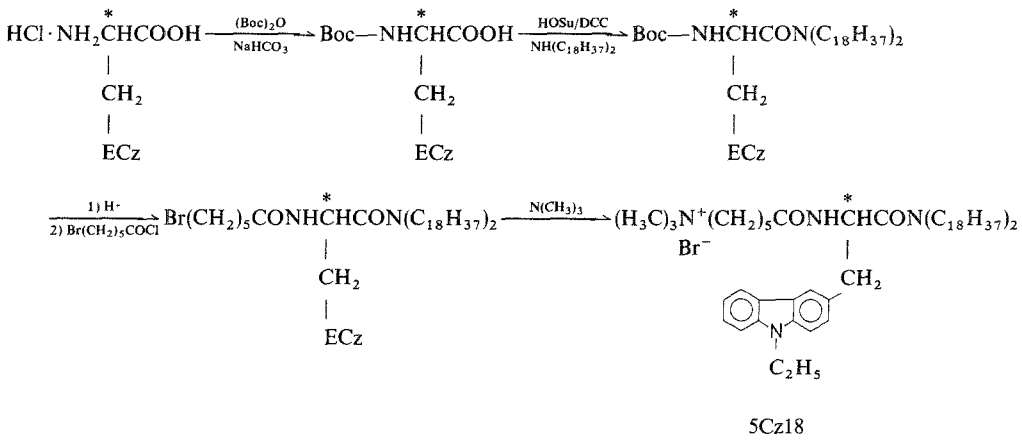

Fig. 3. Synthetic route of 5Cz18

with 6-bromohexanoylchloride. The product was recrystallized from chloroform/methanol. Anal. Calcd. for $C_{59}H_{100}O_2N_3Br + 1.5H_2O$: C, 71.55; H, 10.48; N, 4.24; Br, 7.98. Found C, 71.41; H, 10.43; N, 4.29; Br, 7.47. N-(6-Bromohexanoyl)-L-3-(3-N-ethylcarbazoyl) alanyldioctadecylamide was dissolved in chloroform containing trimethylamine, and the solution was left for standing at room temperature for 7 days. The product was purified by an LH-20 column using ethanol as eluant. Anal. Calcd for $C_{62}H_{109}O_2N_4Br + 0.5H_2O$: C, 72.19; H, 10.74; N, 5.46; Br, 7.75. Found C, 72.24; H, 10.84; N, 5.45; Br, 7.22.

Vesicle formation

5Cz18 (5 mg) was dissolved in chloroform, and evaporated to form a thin film. Distilled water (2 ml) was added and agitated strongly at 50 °C. The suspension became a nearly clear solution after sonication (Tomy Seiko Co., Ltd., UR-200P, irradiation power was 30W) for 1 min at 50 °C.

Measurements

The following instruments were used: transmission electron microscopy (TEM), Hitachi H-600S; differential scanning calorimetry (DSC), Daini-Seikosha SSC-580; fluorescence, Hitachi MPF-4.

Results and discussion

Synthesis of L-3-(3-N-ethylcarbazolyl)alanine

Racemic 3-(3-N-ethylcarbazolyl)alanine was synthesized through an oxazolone derivative of N-ethylcarbazole. Optical resolution of the amino acid was successful neither by diastereomeric complex formation with chiral amines nor direct enzymatic resolution of the benzoyl derivative. However, an enzymatic deacetylation of N-acetyl-D,L-3-(3-N-ethylcarbazolyl)alanine was found to be useful for the resolution. It should be noted that the deacetylation method was not effective for preparation of L-3-(1-pyrenylalanine) and L-3-(9-anthryl)alanine.

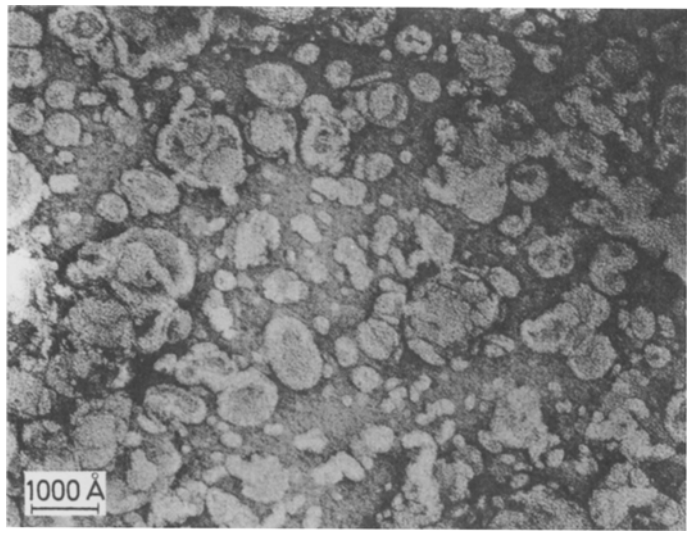

Fig. 4. Electron micrograph of the aqueous dispersion of 5Cz18 after a brief sonication. The sample was negatively stained with uranyl acetate

Observation of the vesicular structure

The morphology of the 5Cz18 assembly in aqueous dispersion was observed by TEM using uranium acetate as a negative stain. It was shown that small vesicles with diameters between 200 and 800 Å were formed in the aqueous dispersion of 5Cz18 after sonication (Fig. 4). The vesicular structure has been observed for other amphiphiles carrying naphthyl (1N18 and 2N18) and anthryl groups (5A18), in which L-3-(3-N-ethylcarbazolyl)alanine of 5Cz18 is replaced with L-3-(1-naphthyl)alanine, L-3-(2-naphthyl)alanine, and D-3-(9-anthryl)alanine, respectively (Sisido et al., 1990; Sasaki et al., 1990a). On the other hand, the amphiphile carrying a D-3-(1-pyrenyl)alanine (1P18) has showed a lamellar structure in aqueous dispersion (Sasaki et al., 1990b). Taking these results together into consideration, the amphiphiles carrying a chromophore, which

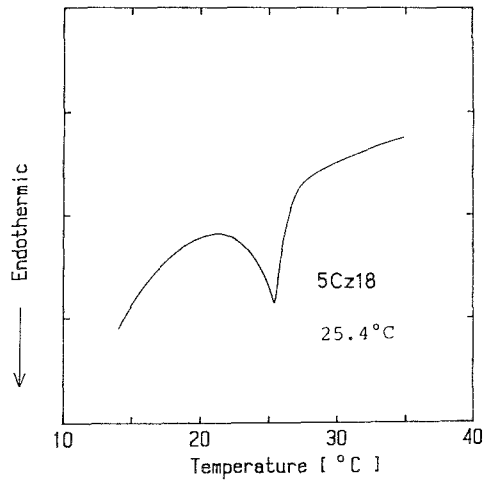

Fig. 5. DSC thermogram of the sonicated aqueous dispersion of 5Cz18

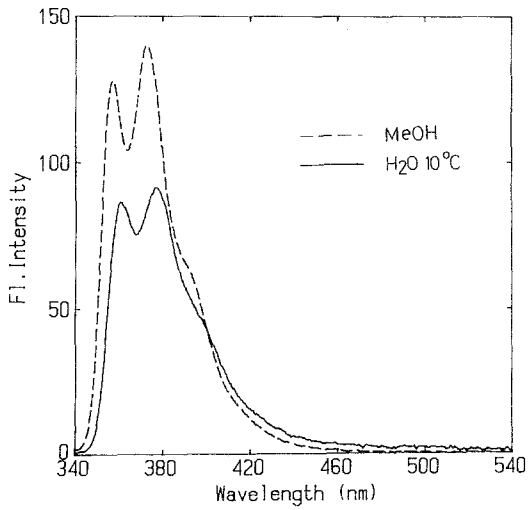

Fig. 6. Fluorescence spectra of 5Cz18 in aqueous dispersion and methanol

molecular size is smaller than anthracene or carbazole, will form a vesicular structure.

DSC thermogram

A phase change of the 5Cz18 vesicular assembly was followed by DSC (heating rate 2 °C/min). An endothermic peak was observed at 25.4 °C, which is attributed to a gel-liquid crystalline transition of the bilayer membrane (Fig. 5). The phase-transition temperature of 5Cz18 is similar to those of other amphiphiles, 1N18, 2N18, and 5A18. Therefore, the size effect of chromophore on the alkyl-chain packing in the vesicular structure should not be significant.

Fluorescence spectra

Fluorescence spectra of 5Cz18 are shown in Fig. 6. Obviously, monomer emission is prevailing in the fluorescence spectrum of the vesicular assembly below the phase-transition temperature, which is similar to that in methanol except a few nm red-shift in the wavelength of the maximum intensity. This result is in contrast to 1N18, 2N18, and 5A18 where a significant excimer emission was observed (Sisido et al., 1990; Sasaki et al., 1990). Since all these amphiphiles have the same molecular frame, the difference is attributed to different properties of the chromophores. The assembly free from excimer formation is expected to function as an efficient photoenergy-harvesting system.

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