Hemispherical synthesis of dendritic poly(L-lysine) combining sixteen free-base porphyrins and sixteen zinc porphyrins

Naoki Maruo,[a] Motonori Uchiyama,[a] Tamaki Kato,[a] Toru Arai,[a] Hideo Akisada[b] and Norikazu Nishino*[a]

[a] *Department of Applied Chemistry, Faculty of Engineering, Kyushu Institute of Technology, Kitakyushu 804-8550, Japan. E-mail: nishino@che.kyutech.ac.jp*

[b] *Department of Environmental Chemistry, Faculty of Engineering, Kyushu Kyoritsu University, Kitakyushu 807-8585, Japan*

Received (in Cambridge, UK) 10th August 1999, Accepted 7th September 1999

Dendritic poly(L-lysine) combining sixteen free-base porphyrins and sixteen zinc porphyrins hemispherically at the fifth generation was successfully synthesised and showed intramolecular fluorescence energy transfer in DMF.

Before Tomalia proposed the 'dendrimer', a new type of macromolecule,[1] Aharoni *et al*. had described the synthesis and properties of dendritic poly(L-lysine)s up to tenth generation.[2] Unlike the original concept of the dendrimer described Tomalia, the amino groups of L-lysine are not of course symmetrical. However, the chirality of L-lysine is advantageous in the design of functional dendrimers. In addition, the L-lysine residue is useful in combining a special functional group in large numbers at the desired generation. At the fifth generation, there are thirty two side chain amino groups where functional group-carrying L-lysines can be combined. More advantageously, peptide synthetic chemistry can be conveniently applied for design and synthesis of functionalized dendritic poly(L-lysine) by incorporation of photochemically interesting groups such as porphyrin rings.

Porphyrin rings have been formed into clusters using dendritic approaches by several research groups.[3] The most recent clusters involve nine porphyrin rings with designed metallation. However, we hoped to utilise a totally different type of design and synthesis of macromolecules combining a large number of porphyrin rings in any appropriate arrangement. We introduced a number of porphyrin rings (eight, sixteen or thirty-two) into a dendritic poly(L-lysine) in almost the same stratum.[4] The porphyrins showed extremely strong split circular dichroism (CD) ($[\theta]_{428} - [\theta]_{407} = 2.0 \times 10^6$ deg cm^2 dmol^{-1}) under certain conditions (toluene–DMF = 9:1, v/v), while they were silent in DMF. In the present study, we attempted to introduce a crowd of free-base porphyrins and a crowd of zinc porphyrins in separate hemispheres of dendritic poly(L-lysine) (Fig. 1).

The Boc and Fmoc[5] chemistries were alternately applied to build the desired **1** by hemispherical synthesis (Fig. 2). The synthesis was started by the condensation of N^{α},N^{ε}-di-*tert*-butoxycarbonyl-L-lysine [Boc-L-Lys(Boc)-OH] and hexamethylenediamine with benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP) and 1-hydroxybenzotriazole (HOBt). The resulting half-amine [Boc-L-Lys(Boc)-NH$(CH_2)_6$NH$_2$] was condensed with N^{α},N^{ε}-difluoren-9-ylmethoxycarbonyl-L-lysine [Fmoc-L-Lys(Fmoc)-OH]. TFA was used to remove the Boc group (0 °C, 1 h), then the Boc hemisphere was expanded by a second condensation with 2 equiv. of Boc-L-Lys(Boc)-OH. The Fmoc protection was removed with 20% piperidine in DMF, then the Fmoc hemisphere was expanded by a second condensation with 2 equiv. of Fmoc-L-Lys(Fmoc)-OH. Thus, Boc and Fmoc chemistries were repeated alternately up to the fourth generation. The condensation reaction proceeded quantitatively (room temperature, 1 h). The completion of the condensation was confirmed at each step by a Kaiser test.[6] At the fifth generation, sixteen Boc-L-Lys(Por(2H))-OH moieties were introduced to the Boc hemisphere of the dendritic poly(L-lysine) intermediate. Expansion of Boc hemisphere to the seventh generation was carried out to bury the free-base porphyrins appropriately in a stratum of the dendritic poly(L-lysine).[4] Subsequently, another half side was condensed with sixteen Fmoc-L-Lys(Por(Zn))-OH moieties with the aid of *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and 1-hydroxy-7-azabenzotriazole (HOAt). The hemispherical elongation was repeated to give **1**, whose surface is completely covered by seventh generation of Boc-L-Lys(Boc) groups.

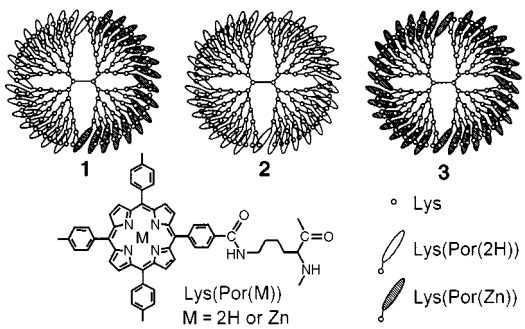

Fig. 1 Dendritic poly(L-lysine)s combining sixteen free-base porphyrins and sixteen zinc porphyrins separately (**1**), thirty-two free-base porphyrins (**2**) and thirty-two zinc porphyrins (**3**) at the fifth generation and covered by an additional two generations. The end-protecting group is *tert*-butoxycarbonyl.

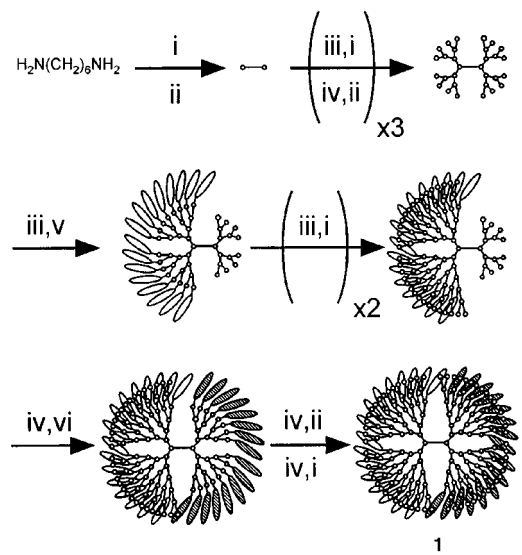

Fig. 2 Synthetic route for dendritic poly(L-lysine) combining hemispherically separated free-base and zinc porphyrins. *Reagents and conditions*: i, Boc-L-Lys(Boc)-OH, BOP, HOBt; ii, Fmoc-L-Lys(Fmoc)-OH, BOP, HOBt; iii, TFA; iv, 20% piperidine in DMF; v, Boc-L-Lys(Por(2H))-OH, BOP, HOBt; vi, Fmoc-L-Lys(Por(Zn))-OH, HATU, HOAt.

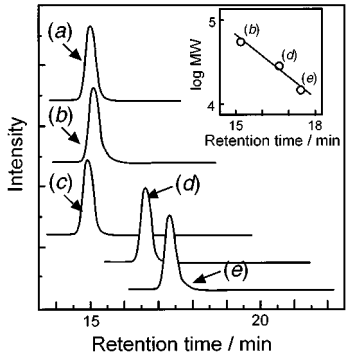

Fig. 3 Analytical size-exclusion chromatograms of dendrimers **1** (*a*), **2** (*b*) and **3** (*c*) and two smaller size homologues (*d*), (*e*) of **2** in DMF. The molecular weights are 56 047, 55 033, 57 061, 27 446 and 13 653 respectively.

Species **1** was purified using a Sephadex LH-60 column (1.8 × 70 cm, DMF) and analyzed by size exclusion chromatography [TSKgel G3000H_{XL} (7.8 × 300 mm) column, with DMF, 2 ng of sample, detected by absorption at 420 nm]. Species **1** was eluted at 14.97 min, the retention time being very close to that (15.16 min) of dendritic poly(L-lysine) combining thirty-two free-base porphyrins (**2**), which was synthesized as previously reported elsewhere.[4] The dendritic poly(L-lysine) with thirty-two zinc porphyrins (**3**) was prepared from **2** and $Zn(OAc)_2$ in CH_2Cl_2–MeOH (3:1, v/v). The formation of **3** was monitored by the disappearance of the Qx(0,0) band of **2** at 650 nm in the absorption spectrum within 3 h. Species **3** showed a single peak at 14.81 min in the size exclusion chromatograph.

The retention times of **1**, **2** and **3** were compared with smaller dendritic poly(L-lysine)s combining eight and sixteen free-base porphyrins[4] at the third and fourth generations, respectively. As shown in the inset of Fig. 3, there is a linear relation between the retention times (17.41, 16.74 and 15.16 min) and the logarithms of the molecular weights (13 653, 27 446 and 55 033) of the dendritic poly(L-lysine)s combining eight, sixteen and thirty-two free-base porphyrins.

Three types of dendritic poly(L-lysine) with thirty-two porphyrins, hemispherically separated free-base and zinc porphyrins (**1**), all free-base porphyrins (**2**) and all zinc porphyrins (**3**), were further characterized by UV-VIS absorption, CD and fluorescence spectra measurements. The absorption spectra in DMF of **2** and **3** were similar to the monomeric free-base and zinc porphyrins respectively. The dendrimer with hemispherically mixed porphyrins (**1**) showed slight deviation (data not shown) from the 1:1 additive spectrum of those of free-base and zinc porphyrins (**2** and **3**). The CD spectra of dendrimers in DMF showed no Cotton effect at the Soret band, whether the porphyrins were free-base or metallated. After addition of toluene (toluene–DMF = 9:1, v/v), **2** with only free-base porphyrins showed an intense split CD ($[\theta]_{428} - [\theta]_{407} = 2.0 \times 10^6$ deg cm^2 dmol^{-1}) for the Soret band as previously observed.[4] However, **1** showed a much weaker CD ($[\theta]_{438} - [\theta]_{421} = 1.8 \times 10^5$ deg cm^2 dmol^{-1}) under the same conditions, while **3** with all zinc porphyrins showed a weak CD ($[\theta]_{440} - [\theta]_{427} = 1.1 \times 10^5$ deg cm^2 dmol^{-1}). The chiral arrangement of free-base porphyrins induced by nonpolar solvent was obviously affected by the crowd of zinc porphyrins.

The fluorescence spectra of the dendrimers are shown in Fig. 4. The dendrimers **2** and **3** in DMF gave typical fluorescence emission upon excitation at 560 nm.[7] A mixed solution of **2** and **3** gave an almost additive spectrum corresponding to both porphyrins. The hemispherical dendrimer **1** gave a fluorescence spectrum in which emission at 610 nm from the zinc porphyrin was significantly quenched. By calculation, about 43% of the

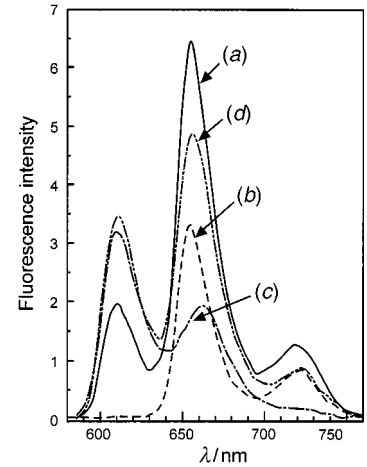

Fig. 4 Fluorescence spectra of dendritic poly(L-lysine)s combining (*a*) hemispherical free-base and zinc porphyrins (**1**, 5.8 μM), (*b*), all free-base porphyrins (**2**, 2.9 μM), (*c*) all zinc porphyrins (**3**, 2.9 μM) and (*d*) a mixture of **2** and **3** (2.9 μM, respectively) in DMF. Excitation at 560 nm.

fluorescence energy of the excited zinc porphyrins was transferred to the free-base porphyrins on the other hemisphere. This fact suggests that about seven of the sixteen porphyrins in each hemisphere might locate on the boundary of said hemisphere, resulting in close contact. The efficiency of the energy migration was slightly increased to 46% by the addition of toluene (toluene–DMF = 9:1, v/v), suggesting that the energy transfer occurs mainly at the hemispherical boundaries and with almost no relation to the mobility of the chromophores.

In conclusion, we have succeeded in the synthesis of dendritic poly(L-lysine) combining separate crowds of free-base and zinc porphyrins, and preliminarily observed fluorescence energy transfer from zinc porphyrins to free-base porphyrins probably due to close contact at the boundaries of the hemispheres. We will further study light harvesting[8] by porphyrin systems arranged in a stratum of the dendrimer. Although they are too primitive to model the antenna chlorophyll membrane proteins revealed recently,[9] it should be possible to mimic the effect of accumulation of porphyrin rings in the compact zone.

Notes and references

1 D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder and P. Smith, *Polym. J.*, 1985, **17**, 117.
2 S. M. Aharoni, C. R. Crosby III and E. K. Walsh, *Macromolecules*, 1982, **15**, 1093.
3 D. L. Officer, A. K. Burrell and D. C. W. Reid, *Chem. Commun.*, 1996, 1657; C. C. Mak, N. Bampos and J. K. M. Sanders, *Angew. Chem., Int. Ed.*, 1998, **37**, 3020; A. Nakano, A. Osuka, I. Yamazaki, T. Yamazaki and Y. Nishimura, *Angew. Chem., Int. Ed.*, 1998, **37**, 3023; T. Norsten and N. Branda, *Chem. Commun.*, 1998, 1257; S. L. Darling, C. C. Mak, N. Bampos, N. Feeder, S. J. Teat and J. K. M. Sanders, *New J. Chem.*, 1999, **23**, 359.
4 N. Maruo and N. Nishino, *Kobunshi Ronbunshu*, 1997, **54**, 731.
5 J. Meienhofer, *Biopolymers*, 1981, **20**, 1761; G. B. Fields and R. L. Noble, *Int. J. Peptide Protein Res.*, 1990, **35**, 161.
6 E. Kaiser, R. L. Colescott, C. D. Bossinger and P. I. Cook, *Anal. Biochem.*, 1970, **34**, 595.
7 N. Nishino, R. W. Wagner and J. S. Lindsey, *J. Org. Chem.*, 1996, **61**, 7534.
8 D-L. Jiang and T. Aida, *Nature*, 1997, **388**, 454.
9 G. McDermott, S. M. Prince, A. A. Freer, A. M. Hawthornthwaite-Lawless, M. Z. Papiz, R. J. Cogdell and N. W. Isaacs, *Nature*, 1995, **374**, 517.

Communication 9/06501F