Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

Synthesis of a Novel Water-Soluble Polyazobenzene Dendrimer and Photoregulation of Affinity Toward DNA

T. Nagasaki [a], K. Atarashi [a], K. Makino [a], A. Noguchi [a] & S. Tamagaki [a]

[a] Department of Bioapplied Chemistry, Faculty of Engineering, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka, 5588585, Japan

Version of record first published: 24 Sep 2006

To cite this article: T. Nagasaki, K. Atarashi, K. Makino, A. Noguchi & S. Tamagaki (2000): Synthesis of a Novel Water-Soluble Polyazobenzene Dendrimer and Photoregulation of Affinity Toward DNA, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 345:1, 227-232

To link to this article: http://dx.doi.org/10.1080/10587250008023923

Mol. Cryst. and Liq. Cryst., 2000, Vol. 345, pp. 227-232
Reprints available directly from the publisher
Photocopying permitted by license only

Synthesis of a Novel Water-Soluble Polyazobenzene Dendrimer and Photoregulation of Affinity Toward DNA

T. NAGASAKI, K. ATARASHI, K. MAKINO, A. NOGUCHI and S. TAMAGAKI

Department of Bioapplied Chemistry, Faculty of Engineering, Osaka City University, 3–3–138 Sugimoto, Sumiyoshi-ku, Osaka 5588585, Japan

A novel water-soluble polyazobenzene dendrimer modified with L-lysines at the periphery was synthesized. The results from light scattering and gel filtration chromatography showed that the particle size is controllable upon UV or visible light irradiation. Furthermore, a gel shift assay with plasmid DNA and an electrophoretic light scattering analysis demonstrated that the affinity of this cationic dendrimer toward DNA is photo-controllable on the basis of zeta potential alteration on dendrimer's surface.

Keywords: dendrimer; diazo-compound; photoregulation; zeta potential; transfection

Polyamidoamine dendrimers are a new class of highly branched spherical polymers that are highly soluble in aqueous solution and has a unique surface of primary amino groups.[1] This class of dendrimers have been used successfully as a vehicle for transferring nucleic acids into mammalian cells.[2] On the other hand, we have synthesized and evaluated novel photochromic dendrimers including polyazobenzenes in the branch structure.[3] Polyazobenzene dendrimers are photochemically size-controllable. If the size-change of cationic

polyazobenzene dendrimers induced by UV irradiation cause the alteration of the cation density on the surface, the affinities of these cationic compounds toward polyanionic nucleic acid are expected to be variable. In this paper we report synthesis of a highly water-soluble polyazobenzene dendrimer and the photo-regulation of the interaction between the polyazobenzene dendrimer and plasmid DNA in aqueous solution.

Synthesis of H-Lys-G2. The structure and acronym of the compound used in this study are given in chart 1. The similar strategy described previously[3] was taken to prepare the novel polyazobenzene dendrimer (**H-Lys-G2**) modified with L-lysines at the periphery in order to increase the water–solubility. The theoretical molecular

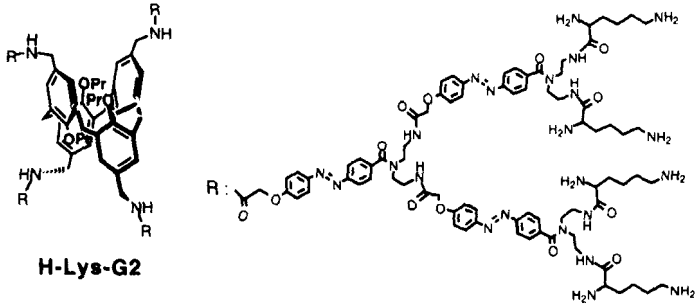

Chart 1.

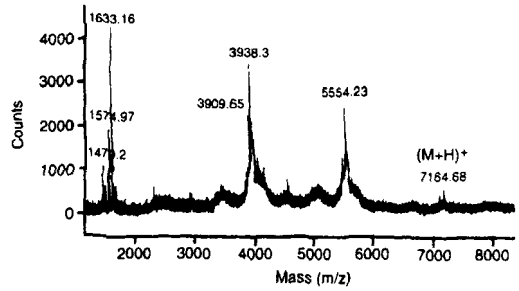

Figure 1. MALDI-TOF mass spectrum of **H-Lys-G2** using sinipinic acid as the matrix.

weight of **H-Lys-G2** is computed to be 7163.95 for $C_{368}H_{504}N_{96}O_{56}$ whose MALDI-TOF mass spectrum (Figure 1) exhibits a distinct molecular ion peak (m/z 7164.68). The structure of **H-Lys-G2** was also identified by IR, ^{1}H-NMR and amino acid analysis. **H-Lys-G2** contains 12 azobenzene units and 32 peripheral primary amino groups.

Photochromic properties and size-regulation. The photo-isomerization of azobenzene upon irradiation of UV light or upon standing in the dark was examined. The changes in the UV-vis spectra upon irradiation with 365 nm UV light for **H-Lys-G2** are shown in Fig.2. UV irradiation yields a 56/44 mixture of trans/cis conformer in TE buffer (pH7.4). The regulation of particle size by an on-off UV light switch was confirmed using a gel filtration chromatography (GFC) in aqueous solution (Fig. 3) and a dynamic light scattering in organic solvent. GFC showed that the retention time of UV irradiated **H-Lys-G2** eluted later than before irradiation. That is to say, the decrease in

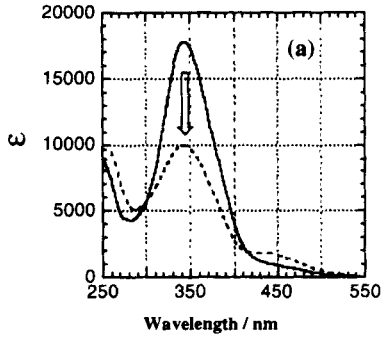

Fig. 2. UV-vis spectral change of 0.86 μM **H-Lys-G2** solution at 25 °C in 0.05 mM TE buffer (pH7.5) upon 365 nm UV light irradiation.

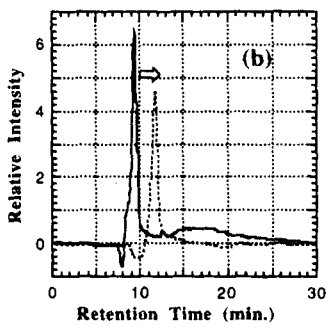

Fig. 3. Gel filtration chromatograph of **H-Lys-G2** before (solid line) and after (dotted line) UV irradiation. Chromatography was performed using YMC Diol-120 column at 25 °C in H_2O/MeCN (8:2): flow rate 1.0 ml/min: detected at 350 nm.

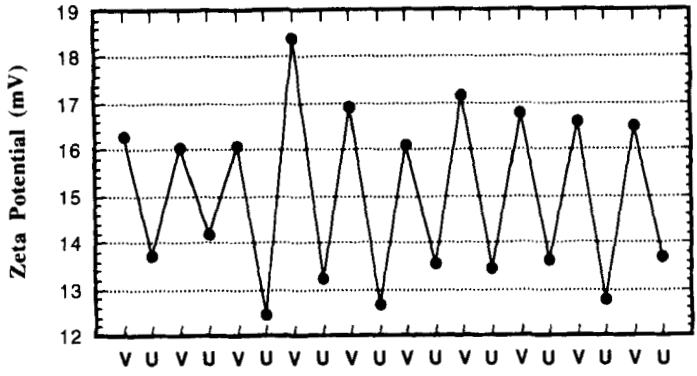

Fig. 4 Reversible photoinduced zeta potential change of **H-Lys-G2** at 10 °C in 10 mM tris buffer (pH7.5). **V** indicates visible light irradiation; **U** indicates UV irradiation.

hydrodynamic radius occurred by UV irradiation. Dynamic scattering were measured at 35 °C in EtOH with Otsuka Electronics ELS-800 by using a hydrophobic derivative of **H-Lys-G2** in which amino groups were protected with Boc group in order to disperse each molecule in an organic solvent. This experiment also supported that UV irradiation caused the contraction from 7.3 nm to 5.6 nm. Furthermore, electrophoretic light scattering revealed that zeta potential of **H-Lys-G2** was decreased upon UV irradiation in aqueous solution. When **H-Lys-G2** was irradiated with 365 nm UV light, the zeta potential was decreased from 16.7±1.7 mV to 13.3±0.9 mV. The switching of zeta potential by UV or visible light irradiation was confirmed repeatedly (Fig. 4). This result indicates that the larger **H-Lys-G2** has higher cationic density on the surface than the smaller one. The decrease of cationic density is ascribable to the deprotonation of primary amines due to the increase of electric repulsion.

Interaction between DNA and H-Lys-G2. The plasmid pCH110 (Pharmacia, 7128 bp) was used for characterization of complex

formation. The interaction between pCH110 and **H-Lys-G2** were evaluated by dynamic light scatter analysis and electrophoresis. Ratios of the dendrimer to DNA were based on the calculation of the electrostatic charge present on each component; e.g., the number of terminal amino groups on the dendrimer vs. the number of phosphate groups in DNA. From the dynamic light scattering result, a relatively small particle (250 nm) was observed in the range of 0 to 1 charge ratio. On the other hand, a larger complex (900 nm) formed and enlarged with times to a particle size of 5000 nm in one hour under the amino group excess condition. 0.6% agarose gels were electrophoresed for 80 minutes at 120 V in 1X TAE and stained with ethidium bromide. Complexes were formed by varying dendrimer-to-pCH110 (100 ng) stoichiometry in TE buffer, pH 7.4. The rapidly migrating bands due to the free DNA was observed when the expanded **H-Lys-G2** prepared by standing in the dark overnight was used as compared to the contracted one generated by UV irradiation (Fig. 5). This result indicates that the photo-isomerzation of azobenzenes changes the affinity of **H-Lys-G2** to DNA. From the determination of zeta potential of DNA complexes in the presence of excess **H-Lys-G2** (24:1 charge ratio) in 28% glycerol-

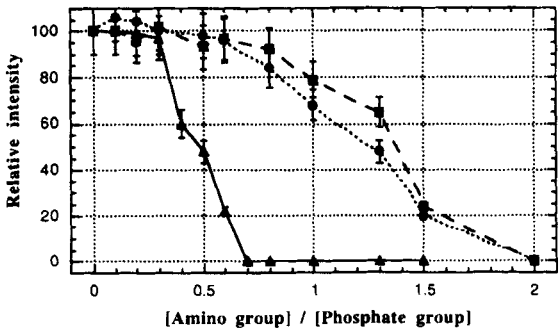

Fig. 5. Relative fluorescent intensity of free plasmid (pCH110) on electrophoresis with various equivalent polyamines : polyethylenimine (solid line), H-Lys-G2 before (broken line) and after (dotted line) UV irradiation.

H_2O solution, UV irradiation decreased zeta potential of pCH110 complex from 6.74±1.2 mV to 2.99±2.6 mV. Consequently, it was found that the DNA-complex with the highly charged cationic **H-Lys-G2** is more stable than the complex with the low charged form.

Transfection. Transfer of pCH110 containing LacZ encoding β-galactosidase into the COS-1 cells was estimated using an ONPG assay. By varying the ionic strength of an incubating solution on complex formation, the driving force on polyion complex was confirmed. The fact that the maximum activity was attained at the 28% glycerol addition also supported that the electro-static interaction is worked dominantly. Although excess amount of **H-Lys-G2** (24:1 charge ratio) is required for effective transfection, the efficiency with **H-Lys-G2** is comparable to a commercial transfection kit. Interestingly, UV light irradiation after the incorporation of DNA-complex with **H-Lys-G2** into a cytoplasm significantly caused a fifty- percent increase on the transfection efficiency. In the cytoplasm, UV irradiation promoted the dissociation of the complex, therefore, the transcription from a free gene was carried out favorably. The detail of transfection will be described elsewhere.

Acknowledgement. This research was supported by a Grant-in-Aid for Scientific Research (C), No. 09680573, from the Ministry of Education, Science, Sport and Culture of Japan.

References

[1] G. R. Newkome, C. N. Moorefield, F. Vögtle, *Dendritic Molecules,* VCH, Weinheim(1996).
[2] J. Haensler, F. C. Jr Szoka, *Bioconj. Chem.*, **4.** 372(1993).
[3] (a) T. Nagasaki, S. Tamagaki, K. Ogino, *Chem. Lett.*, **1997**,717. (b) T. Nagasaki, A. Noguchi, T. Matsumoto, S. Tamagaki, K. Ogino, *An. Quim. Int. Ed.*, **93,** 341(1997).