Synthesis and properties of a new type DNA dendrimer

Yukinao Suzuki, Toshiya Otomo, Hiroaki Ozaki and Hiroaki Sawai
Faculty of Engineering, Gunma University, 1-5-1 Tenjin-cho, Kiryu, Gunma 376-8515, Japan

ABSTRACT
We have designed a new type of a DNA dendrimer which has rigid branched structure. The branching molecule was prepared from 1,3,5-tribromobenzene. The dendrimer unit, in which three oligonucleotide-chains, two molecules of T15 and one molecule of A15, linked to the branching molecule, was synthesized by an automated DNA synthesizer. The properties of the dendrimer unit and dendrimer formation by inter-molecular association of T15 and A15 chains of the dendrimer unit will be presented.

INTRODUCTION
A dendrimer molecule has a branched or dendritic structure as show in Fig.1. A dendrimer can posses many functional groups outside of one molecule. Thus, a DNA dendrimer bearing many labelling agents such as fluorophores will be useful as a high sensitive DNA probe (Fig.1(a)). A few DNA dendrimers with or without fluorescent agents have been reported 1-3. These reported DNA dendrimers are composed of covalent bond for constructing the dendritic structure.

Figure 1. Summary of DNA Dendrimer
If two molecules of oligodeoxyribonucleotides (ODN) and one molecule of the complementary ODN link to a branching molecule and hybridization between the complementary ODNs takes place inter-molecularly, the dendrimer structure will be formed reversibly depending on the change of temperature (Fig.1(b)).

The branching molecule will be also applicable as a building block for making DNA dendrimer composed of the covalent bond as shown in Fig1(a).

In this report, we present synthesis and properties of a new type of a DNA dendrimer unit which has a rigid branched-structure. The dendrimer formation from this unit will be also described.

**RESULTS AND DISCUSSION**

We designed a trisubstituted benzene derivative 3 as a core of a branching molecule. The compound 3 has a rigid bulky structure which will prevent intramolecular double-stranded DNA formation in the dendrimer unit. The compound 3 was prepared according to Scheme 1. Thus, reaction of tribromobenzene with trimethylsilyl acetylene by palladium complex catalyst gave 1 in a high yield. The compound 1 was treated with aqueous sodium hydroxide in ethanol for hydrolysis of a trimethylsilyl group, and was subsequently condensed with ethyl p-iodobenzoate using palladium complex catalyst yielding the compound 2. Ethyl esters of the compound 2 was hydrolyzed with aqueous lithium hydroxide in THF. The resulting tri-acid was reacted with thionyl chloride forming the corresponding tri-acid chloride which condensed with aminooxyethanol or aminohexanol yielding the branching compound 3.

Two terminal hydroxyl groups of the compound 3 was protected with a dimethoxytrityl group and the third terminal hydroxyl group was phosphitylated by the usual manner giving a unit (5) for the synthesis of DNA dendrimer unit.

The dendrimer unit, in which one molecule of A 15mer and two molecules of T 15mer were linked to the branching core, was prepared by an automated DNA synthesizer. Adenylate 15mer was synthesized on a CPG support at first, and the branching unit 5 was introduced into the 5'-terminal hydroxyl group of the adenylate 15mer. After removal of other two trityl groups, two thymidylate chains (each 15mer) were successively introduced from 5' to 3' into the branching portion giving the dendrimer unit 6 on the CPG support. After removal of protecting groups and cleavage from the CPG support by treatment with conc. ammonia solution, products were analyzed and purified by a reversed-phased HPLC. The branched DNA dendrimer unit (45mer) was formed along with the linear failure product (30mer). The products were characterized by enzyme digestion, followed by HPLC analysis. The denaturing 7M urea-polyacrylamide gel electrophoretic (PAGE) showed that the branched 45mer had a different electrophoretic mobility compared to that of the linear DNA. The small mobility of the branched 45mer under the native PAGE condition indicates that the branched 45mer unit hybridized each other to form possibly the dendrimer structure.

**REFERENCES**

